GSICA
THE ITALIAN SCIENTIFIC GROUP
OF FOOD PACKAGING

DSA e DIMP
UNIVERSITY OF NAPLES
"FEDERICO II"

IMCB
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SHELF LIFE INTERNATIONAL MEETING

SLIM 2008

Shelf-life International Meeting

Ischia, June 25-27th 2008

Edited by

GIOVANNA G. BUONOCORE & ELENA TORRIERI

Special Issue

ITALIAN JOURNAL
OF
FOOD SCIENCE

CHIRIOTTI EDITORI

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In cooperation with

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This Special Issue of the Italian Journal of Food Science collects the presentations given at the "SLIM 2008, Shelf Life International Meeting" organized by GSICA, National Research Council – IMCB, University of Naples – DSA and DIMP, held at Ischia on June 25-27th 2008.
These papers were reviewed by the Scientific Committee of the congress before their presentation but they did not undergo the conventional reviewing system of the Italian Journal of Food Science.

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INTRODUCTION

This volume collects the contributions to Shelf life International meeting (SLIM) 2008, the unique congress dealing with the various aspects connecting with studing of the shelf life of food products.

The congress has been organized by the Italian Scientific Group of Food Packaging (GSICA) in co-operation with the Institute of Composite and Biomedical Materials (CNR), the Department of Food Science (DSA), and the Department of Materials and Production Engineering (DIMP) of the University of Naples Federico II. The Third Shelf Life International Meeting (SLIM 2008) was held in Ischia (Naples, Italy) and was attending by 150 partecipants coming from all around the world, in particular, researchers coming from outside Italy passed from 20% to 30% respect to SLIM 2006.

The volume reports the research results presented as oral and poster presentations, following the scheme of the conference sessions, addressing, respectively:

Shelf Life Modelling and Prediction: Prediciton by means of mathematical models of shelf life and quality decay of food and beverages, prediction of barrier and protective properties of packaging materials, study of the kinetics of food quality degradation, prediction of sensorial indexes decay, predictive microbiology

New Technologies for Shelf Life Extension: New materials, active and intelligent packaging, new packaging devices, new food processing technologies, new food preservatives alternative to the traditional ones, new techniques for risk reduction

Shelf Life Testing: Non-invasive analytical techniques, sensorial techniques, new freshness indicators, selection and validation of reliable quality indexes, evaluation of performances of packaging materials.

Prediction, testing and extension of the Shelf Life require a multi-disciplinary approach, which involves analytical chemistry, microbiology, food processing, food packaging, material science as well as physical chemistry. In this framework, due to the diversity of attendees, SLIM 2008 provided an international forum for presenting fundamental aspects of current developments and future directions for research and applications on the shelf life of packaged foodstuff.

For the first time, during the meeting, GSICA organized a Workshop and a Poster competition. The Workshop **New analytical and regulatory approaches for safe food packaging** presented the latest developments in regulation and mainly in compliance testing of food packaging materials from the leading opinions of two key figures of the food packaging world: the Plastic Packaging Industry and the Control Authority. Thanks to workshop, scientific discussions was promoted and the need for a safer and more effective food packaging was highlighted. The poster competition recognised research activity in the field of Food Packaging and Shelf Life. The award, consisting in a certificate, has been given to the Authors of nine posters (three for each session) for their original contributions. Thanks to this competition, posters session has been enhanced and it was effective in giving positive stimuli to young researchers.

The GSICA has programmed SLIM 2010 which will be held in Saragoza (SPAIN) in cooperation with the Department of analytical chemistry and the Aragon Institute for Engineering Research-University of Saragoza.

Giovanna G. Buonocore & Elena Torrieri

SESSION I

"Shelf Life Modelling and Prediction"

Chairmen:

D.S. Lee (Kyungnam University, South Korea)
G. Mensitieri (University of Naples, Italy)

PREDICTION OF SHELF-LIFE OF BEVERAGE STORED IN PET BOTTLES WITH PASSIVE AND ACTIVE WALLS

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ABSTRACT

The aim of this paper is to provide a framework whereby gas permeation rates through plastic packaging walls, and hence food shelf life, may be estimated. Two situations are considered: when the walls simply provide a passive resistance to the flux (as is the case for standard PET or PET blended with some other low permeability material) and when an active gas scavenger is incorporated within the boundary material. For the passive wall, permeability data relative to oxygen have been collected from literature sources and also measured using specific oxygen transmission rate experiments. For the active walls, scavenger kinetic constants were estimated from data obtained using test bottles prepared with varying scavenger concentrations. Numerical predictions in both cases have been verified by comparison with data on gas concentration in water filled bottles maintained under controlled conditions for periods of up to 6 months.

Key words: Food packaging, PET bottles, Shelf-life, Passive wall, Active wall.

INTRODUCTION

The use of plastic, and specifically Poly(ethylene terephthalate) (PET), as bottle material for food storage is continuously increasing. Although PET and other plastic materials generally ensure good protection from environmental contamination, efforts are still being made to improve their barrier properties, especially in cases where particularly stringent requirements are imposed by the nature of a packaged food.

There are various ways to improve the barrier properties of plastic packaging

walls, which can generally be classified as providing either passive or active protection (Lange and Wyser, 2003). Passive protection consists in simply supplementing the original plastic material with a further component with superior barrier properties, so that the overall performance is improved. Active protection, on the other hand, implies the addition of a scavenging compound to the plastic matrix; this reacts with, and thus consumes, the permeating gas, thereby greatly reducing, for example, contact of a sensitive food product with oxygen (Vermeiren *et al.*, 1999).

Although the methodology presented here is quite general, in this study we restrict ourselves to the case of PET bottle with oxygen as the permeating gases. The overall amount of oxygen entering the bottle wall is the quantity needed to estimate shelf life, and this parameter is estimated by measuring oxygen concentration in the water filling the bottle.

MATERIALS AND METHODS

The Mathematical models

The system under consideration is very simple and it consists of a bottle, made up of a material which allows some gas permeation through its wall, filled to a certain point with liquid, with the remaining volume (headspace) occupied by gas. For the usual case of bottle thickness much smaller than bottle length, so that a 1-D model can be assumed, entering oxygen flux it is given by:

$$N_{O2} = -D_{O2,P}\left.\frac{\partial c_{O2,P}}{\partial x}\right|_{x=Z} \tag{1}$$

where $c_{O2,P}$ is the oxygen concentration in the wall of thickness Z.

In the most general case to be considered here, an immobile scavenger compound S can be dispersed homogeneously within the wall where it reacts irreversibly with oxygen. Mass balance equations for within the wall may be written (Yang *et al.*, 2001):

$$\frac{\partial c_{O2,P}}{\partial t} = D_{O2.P}\frac{\partial^2 c_{O2,P}}{\partial x^2} - kc_{O2,P}c_S \tag{2}$$

$$\frac{\partial c_S}{\partial t} = -\nu_s kc_{O2,P}c_S \tag{3}$$

The solution of these two partial differential equations, with the appropriate boundary and initial conditions, delivers the oxygen concentration profile in the packaging wall and hence, by means of eq. (1), the oxygen flux, N_{O2}, into the bottle.

Experimental procedures

The values of relevant PET parameters were supported by direct measurements of oxygen transmission rate carried out by using a MOCON OX-TRAN Model 2/21 permeability meter. Different types of bottles were prepared and tested in the present work, by stretch blow moulding of preforms using a commercial machine. These preforms consisted of either pure PET, or PET to which varying quantities of enhancing material had been added. Bottles used in this work contained a liquid volume of 500, 1000 and 1500 ml, with headspace volumes varying from 15 to 45 ml. Individual bottle surface area was estimated from the geometrical draw-

ing, whereas its average thickness was estimated from the knowledge of the total bottle weight and polymer density. Tests were carried out by first filling the bottle with a known amount of distilled water, which was purged with nitrogen and then sealed with a metal cap; the bottle was then left in a constant temperature (25 °C) and ambient pressure environment. Oxygen concentration in the water phase was measured at regular intervals using a non invasive technique (OXY-4TRACE supplied by PreSens, Regensburg, Germany).

RESULTS

The first case investigated was of a passive PET bottle, for which the knowledge of PET permeability is the only information necessary to carry out predictions. Overall, not a great deal of information is available regarding PET bottles, the exception being the work by Liu *et al.* (2004) which reports oxygen permeabilities in the range of 1.9 -2.5 10^{-19} $m^3m/\ m^2Pas$ for pure PET blown bottles. Measured permeability by direct OTR measurements was 2.23 10^{-19} $m^3m/\ m^2Pas$ for a pure PET sample cut from a bottle and 1.25 10^{-19} $m^3m/\ m^2Pas$ for a sample of PET with 5% low permeability material added. Nielsen relationship for the permeability of passive PET material

$$\frac{P}{P_{PET}} = \frac{1-\varepsilon}{1+\frac{\alpha\varepsilon}{2}} \tag{4}$$

was found to give a good prediction of experimental behaviour and utilized for the successive calculation. Good agreement between the change of oxygen concentration with time for bottles of PET containing different amounts of inert material – up to 6.71% by volume – and model predictions, Figure 1, was obtained.

PET bottles containing an active component to slow down the oxygen transmission rate were finally investigated. Figure 2 shows how the present approach, with

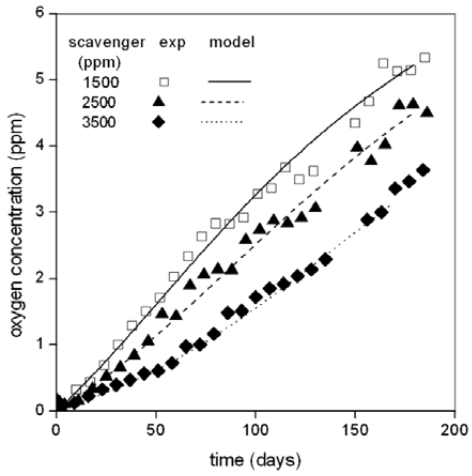

Figure 1 – Oxygen concentration in a 1500 ml bottle function of time. The effect of inert material addition to standard PET.

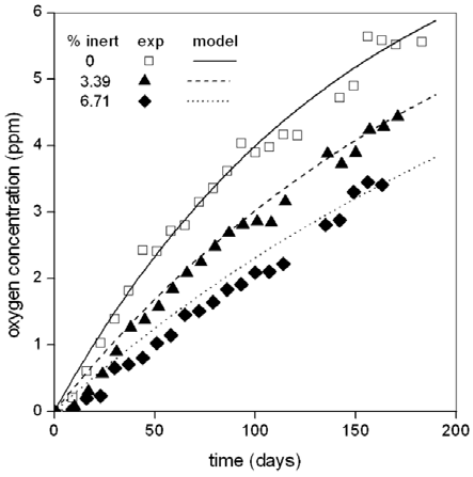

Figure 2 - Oxygen concentration in a 500 ml bottle function of time. The effect of scavenger material addition to standard PET.

the proper value of the kinetic constant, is quite capable of reproducing experimental behaviour over the whole range of time investigated, for every scavenger loading in the PET bottle wall. The obvious consequence is that we are now in a position to "design" the correct bottle which can satisfy costumer requirements, by utilising a specific scavenger material, characterized by its kinetic constant k, with the appropriate value of initial concentration.

CONCLUSIONS

The general approach presented here allows gas permeation rate through plastic walls, and therefore food shelf life, to be estimated. The procedure is quite general although has been applied here to the case of PET bottles with oxygen as permeating gas. The agreement with experimental data is very good when the correct parameters, such as PET permeability or scavenger kinetic constants, are utilized.

Acknowledgement

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HPLC PROFILE AND EVOLUTION DURING STORAGE OF TRIGLYCERIDES AND DIGLYCERIDES IN VIRGIN OLIVE OILS

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ABSTRACT

High-performance liquid chromatography has been used for determining the triglyceridice and diglyceridice composition of olive oils from main, minor and neglected Sicilians cultivars.

The HPLC analysis has allowed the identification and the quantification of 16 triglycerides. Among these, OOO, POO+SOL, OOL and POL, have shown a content higher than 84% of the total area of peaks in the chromatographic profile. Dioleine (OO) and oleopalmitine (OP) resulted the most representative diglycerides.

Analyses have been made in January 2007 and repeated after six months, submitting samples to a simulation of shelf life, to verify as the storage conditions may influence the hydrolitic processes of triglycerides, producing variations in the diglyceridic profile. The importance of study about the diglycerides is in fact to impute the correlation between the content in mono and diglycerides and the oxidative stability of oils.

The experimentation has allowed to verify meaningful decrements in oleopalmitine (OP) content, while opposite course have shown the dipalmitine (PP), and the stearilpalmitine (SP).

Principal component analysis (PCA), were applied to data of content of various triglycerides and diglycerides, to explore their capacity to discriminate varieties of olive oil, belonging to a Sicilian origin denomination.

That statistic analysis permitted moreover to differentiate significantly samples in the two different storage periods.

Key words: Diglycerides, olive oil, shelf life, triglycerides.

INTRODUCTION

Virgin olive oils consist predominantly of triacylglycerols (TAG) that generally follow a unique and typical pattern in the glycerol molecule being characteristic in the different edible vegetable oils. The advantage of using TAGprofile comparing to fatty acid (FA) profile is that the stereospecific distribution of FA on the glycerol molecule is genetically controlled and thus, the information of intact TAGs is usually higher (Aparicio&Aparicio-Ruı´z, 2000). The quantitative determination of partial glycerides (diacylglycerols) present in lipid materials in recent years has been the subject of numeros studies.

This is explained in part by the established correlation between the content of mono- and diglycerides and the oxidative stability of the oils. Based on the content of diglycerides and their fatty acid composition, it appear possible to develop analytical indices that will safeguard oils as well as indicate the state of a product freshness.

The aim of this work was: study triglyceride and diglyceride profile of virgin olive oils; verify as the storage conditions may influence the hydrolitic processes of triglycerides, producing variations in the diglyceridic profile; study the correlation between the content in tri and diglycerides and the oxidative stability of oils.

MATERIAL AND METHODS

Eight monovarietal Sicilian olive oil samples, were analyzed during the crop seasons 2006/2007. Samples were collected at Campo Carboj, Experimental Camp of Agricoltural and Forest Assessorship of Sicilian Region, situated in Sciacca (Agrigento).

Samples were divided in three different categories: main varieties *(Cerasuola, Nocellara del Belice, Tonda Iblea),* minor varieties *(Calatina, Crastu, Minuta),* neglected varieties *(Bottone di Gallo, Vaddarica).*

Triglyceride and diglyceride composition was determined according to Regulation EC 2568/91 (Annex VIII). A 10% solution of the samples to be analyzed was prepared by weighing 0.25 g of the samples in to a 5 ml graduated flask and making 2.5 ml with acetone. An HPLC system (Thermo Electron) equipped with a differential refractometer detector was employed, using a Ultra C18-ODS2 column (250 x 4.6 mm, 3 μm particle size; Resteck).

Setting were: column oven, 25 °C; elution solvent: acetone-acetonitrile (60:40) at a rate of 1.6 ml/min, for 17 min, from 1.6 to 2.3, for 63 min, 2.3 ml/min, for 40 min, and an injection volume of 10 μl of the sample as indicated above. It was assumeed that the sum of the areas of the peaks corresponding to the various TGs was equal to 100%, and the relative percentage of each TG was calculated.

Identification of triglycerides and diglycerides was made using standard (Supelco, USA).

Tocopherols content was determined according to Rovellini *et al.* (1997), an HPLC system Thermo-Electron Finnigan Surveyor equipped whit PDA; analysis conditions were as follows: Merck column ODS-2 (100x4.6 mm); T 25 °C; λ 292 nm; flow rate 1ml/min; volume injected 20 μl. α-Tocopherol (Fluka), was used as external standards for quantitative analysis.

RESULTS AND DISCUSSION

HPLC analysis of triacylglycerols permitted the identification and quantification of 16 triacylglycerols (TAGs). Among these OOO, POO+SOL, OOL and POL accounted for more than 84% of the total area of peaks in the chromatographic profile. The level of triolein (OOO) was remarkably high, ranging from 24,28 to 47,33%.

Trigliceride profile data's was recognized with Principal component analysis (PCA) independent mathematical models. PCA is a commonly used multivariate technique which acts unsupervised, and it helps us to find in what aspect a sample is different from another. The number of principal components has been decided by cross validation, and it is observed that two principal components are enough to explain the 62,2% of the data variance. The interpretation of the results of a principal component analysis is usually carried out by visualization of the component scores and loadings. In Fig. 2 the loading vectors for the two components are plotted and in Fig. 3 the score vectors for the two components are plotted. The used notation for triglycerides makes mention to the acids that they present in their structure, being O = oleic acid; P = palmitic acid; S = stearic acid; L = linoleic acid; Ln = linolenic acid; Ga = gadoleic acid.

This statistical method was able to discriminate succesfully cultivars examined. In particular, infact, *Calatina* and *Nocellara del Belice*, influenced especially by theirs OOO, SOO and GaOO contents, were differentiated from *Vaddarica, Minuta* and *Tonda iblea,* influenced instead by theirs OLLn, OLL, LLL and PLL contents.

Whereas *Bottone di gallo* influenced by theirs POO+SOL, SOP and OOLn contents discriminated compared to *Cerasuola* influenced in particular by theirs OOO, POLn and OOL contents.

Dioleine (OO) and oleopalmitine (OP) resulted the most representative diglycerides. But the distribution wasn't homogeneous between the cultivar under investigation. In fact, the main cultivars *Nocellara del Belice* and *Tonda Iblea* have shown a higher level of OO, respectively 53.81 and 52.48 %, compared to OP, 28.16 and 23.67%.

We also check the evolution of tri- and diglyceridic composition, examining the oils in January 2007 and analysis by after 6 months (subjecting the samples to a simulation of shelf life). The samples of oil have been, in fact, introduced in dark glass bottle and kept at room temperature and in the light, for 6 months.

About triglyceridic composition experimentation didn't show significant changes in glyceridic distribution.

In diglyceridic profile, instead, the experimentation has been possible to check decreases significant load to oleopalmitine (OP). The cv *Calatina* is decreased from 39.62 to 10.98%, the cv *Cerasuola* from 50.38 to 14.71% and the cv *Crastu* from 43.76 to 10.83%. Opposite trend showed instead the dipalmitine (PP). Always these cultivars have displayed respectively, the following changes: cv *Calatina*, from 7.37 to 34.21%; cv *Cerasuola*, from 9.76 to 40.11%; cv *Crastu* from 12.48 to 37.66%.

The stearilpalmitina (SP) also showed significant increases over the experimentation. In particular the cv *Calatina* went from an initial 1, 90 to a 6, 12%, the cv *Tonda Iblea* from 0, 61 to 5.72% and finally the cv *Vaddarica* 0, 77 to 7.60%.

The substantial variations which are registered to load component of digliceridic over 6 months of the trial, Tab.1, probably are attributable to the following factors. Since the glyceridic fraction is formed at 97-98% from triglycerides and that the diglycerides are not more than 2.8-3%, and that they are directly from hidrolytic processes about the triglycerides, a little significant change about triglycerides

Tab.1 Trigliceride and digliceride evolution

Cultivar	JANUARY		JUNE	
	Diglyc.	Triglyc.	Diglyc.	Triglyc.
Bottone di gallo	3,7	96,3	4,2	95,8
Calatina	2,6	97,4	3,2	96,8
Cerasuola	3,6	96,4	3,6	96,4
Crastu	2,5	97,5	3,1	96,9
Minuta	1,9	98,1	2,3	97,7
Nocellara del Belice	3,4	96,6	4,1	95,9
Tonda iblea	3,1	96,9	4,1	95,9
Vaddarica	3,2	96,8	2,6	97,4

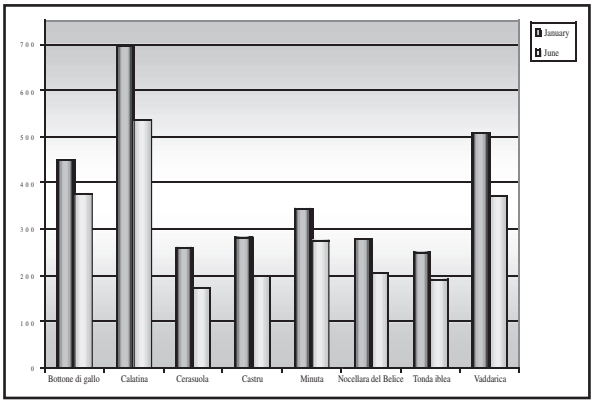

Fig.1 Tocopherols

induce a change at the expense of the digliceridic fraction much more important and significant.

The testing of samples submitted in shelf life simulation infact showed that, for nearly all the samples, a general increase in the level of diglycerides, occurred after the six months of testing.

This demonstrates to an increased process hydrolytic borne of triglycerides caused by prolonged exposure to light and the continuous changes thermal, which meant, as shown in Fig. 1, also a general decrease in the level of tocopherols and therefore of part of the assets antioxidant.

In conclusion we can say that the analysis of di-glycerides compared with that of the triglycerides can be taken as an index of quality of the oils and their state of conservation.

The unchanged level of diglycerides over time may be considered an index of preservation of an oil that has been put to the shelter from triggering factors hidrolytic processes which high temperature, continuous exposure to light and low heritage in antioxidants compounds.

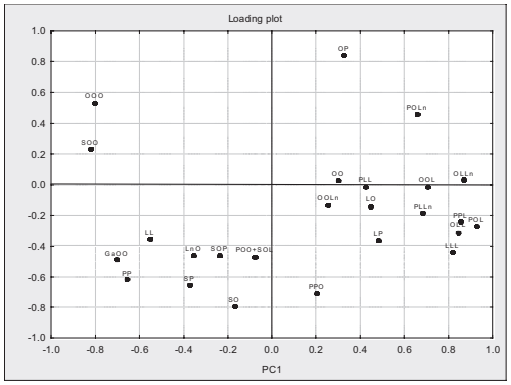

Fig. 2 Loading plot of tri-diglyceride compounds

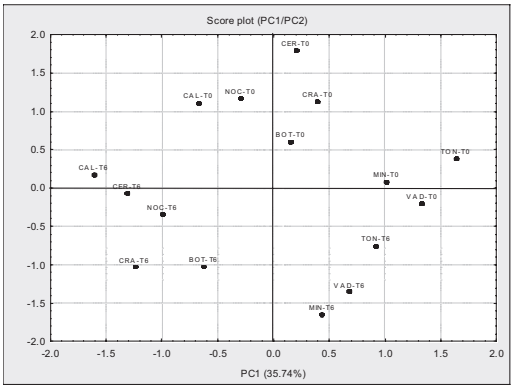

Fig. 3 Score plot of discriminant functions

Tab. 2 Triglycerides in January.

Cultivar	Bottone di gallo		Calatina		Cerasuola		Crastu		Minuta		Nocellara del Belice		Tonda iblea		Vaddarica	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LLL	0,11	0,02	0,06	0,02	0,16	0,01	0,08	0,03	0,37	0,02	0,09	0,02	0,43	0,02	0,49	0,07
OLLn	0,35	0,05	0,29	0,04	0,38	0,06	0,41	0,09	0,45	0,01	0,29	0,04	0,55	0,03	0,48	0,09
PLLn	0,07	0,11	0,08	0,11	0,08	0,01	0,11	0,00	0,15	0,00	0,10	0,01	0,21	0,06	0,19	0,09
OLL	1,10	0,15	0,91	0,11	2,41	0,58	1,77	0,02	4,26	0,13	1,74	0,01	4,23	0,21	5,27	0,55
OOLn	1,69	0,38	2,32	0,16	1,33	0,15	1,84	0,69	1,89	0,04	1,58	0,02	1,11	0,10	2,41	0,65
PLL	1,56	0,22	1,04	0,36	0,46	0,03	0,71	0,21	0,71	0,00	0,45	0,01	1,92	0,05	0,99	0,59
POLn	0,75	0,71	0,30	0,40	0,69	0,31	0,84	0,96	0,30	0,00	0,74	0,04	1,28	0,13	0,39	0,32
OOL	11,55	0,31	9,20	0,15	17,59	2,44	13,30	0,28	16,08	0,25	13,76	0,10	15,68	0,86	16,39	0,75
POL	8,74	0,84	4,35	0,70	8,64	3,03	10,51	4,89	11,64	0,09	7,16	0,05	12,01	0,09	12,00	0,41
PPL	1,28	0,56	0,97	0,65	1,84	1,88	1,96	1,85	2,34	0,02	0,82	0,01	2,79	1,54	2,04	0,04
OOO	31,66	0,01	45,69	2,32	40,08	2,35	34,14	3,25	27,11	0,49	37,29	0,07	26,33	0,37	24,44	0,23
POO+SOL	29,52	0,19	23,74	0,05	18,55	0,47	25,27	2,88	25,22	0,42	24,82	0,09	24,53	0,36	24,06	0,83
PPO	5,93	0,55	3,67	1,30	2,16	0,08	4,42	1,01	5,01	0,54	3,71	0,13	4,99	0,61	5,35	0,26
GaOO	0,39	0,09	0,85	0,17	0,51	0,08	0,27	0,15	0,33	0,11	0,47	0,10	0,22	0,07	0,43	0,37
SOO	3,84	0,97	5,00	0,92	4,51	0,07	3,35	0,01	2,87	0,03	5,27	0,24	2,73	0,25	3,16	0,88
SOP	1,46	0,35	1,52	0,65	0,61	0,87	1,01	0,13	1,27	0,02	1,71	0,14	0,97	0,03	1,91	0,32

Tab. 3 Triglycerides in June.

Cultivar	Bottone di gallo		Calatina		Cerasuola		Crastu		Minuta		Nocellara del Belice		Tonda iblea		Vaddarica	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LLL	0,17	0,17	0,05	0,00	0,12	0,03	0,08	0,06	0,36	0,06	0,12	0,00	0,55	0,06	0,40	0,01
OLLn	0,12	0,05	0,18	0,01	0,22	0,05	0,25	0,05	0,44	0,07	0,32	0,02	0,59	0,03	0,34	0,05
PLLn	0,00	0,00	0,03	0,00	0,06	0,02	0,05	0,00	0,29	0,16	0,11	0,01	0,21	0,01	0,05	0,07
OLL	1,23	0,18	0,69	0,01	2,12	0,07	1,33	0,05	3,64	0,21	1,54	0,07	4,10	0,03	4,09	0,36
OOLn	1,41	0,09	1,88	0,16	1,15	0,04	1,20	0,17	1,75	0,01	1,77	0,12	1,82	0,04	2,61	0,08
PLL	1,11	0,19	0,76	0,12	0,70	0,06	0,59	0,07	0,68	0,03	0,72	0,07	1,05	0,10	0,82	0,13
POLn	0,27	0,10	0,12	0,06	0,16	0,08	0,05	0,00	0,30	0,07	0,08	0,00	0,43	0,02	0,60	0,38
OOL	9,91	0,15	8,19	0,37	15,26	0,80	11,39	0,30	15,07	0,09	12,45	0,47	16,27	0,08	15,83	0,16
POL	7,71	0,42	3,56	0,08	6,14	0,44	6,54	0,66	11,23	0,08	6,70	0,14	11,84	0,25	12,18	0,32
PPL	2,04	0,30	0,55	0,17	0,83	0,54	0,73	1,04	2,34	0,05	0,92	0,17	1,76	0,34	2,57	0,11
OOO	30,51	0,12	46,51	0,93	41,21	2,08	36,37	0,09	26,42	0,39	35,51	0,20	26,02	0,40	23,92	0,70
POO+SOL	31,49	0,27	24,54	0,05	20,61	0,15	29,18	0,85	25,60	0,64	24,85	0,27	25,08	0,44	25,03	0,15
PPO	7,45	0,05	4,11	0,00	2,72	0,12	5,91	0,64	5,95	0,55	4,74	0,73	4,99	0,74	5,78	0,17
GaOO	0,75	0,20	1,00	0,20	0,70	0,24	1,31	0,22	0,96	0,40	1,34	0,72	0,46	0,25	0,87	0,85
SOO	3,68	0,62	6,51	0,28	6,38	0,57	3,95	0,01	3,12	0,71	6,60	1,23	3,61	0,20	3,41	0,04
SOP	2,16	0,17	1,32	0,48	1,62	1,08	1,07	1,51	1,85	0,77	2,24	0,40	1,24	0,25	1,48	0,18

Tab. 4 Diglycerides content in January.

Cultivar	Bottone di gallo		Calatina		Cerasuola		Crastu		Minuta		Nocellara del Belice		Tonda iblea		Vaddarica	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LnO	0,42	0,00	0,22	0,32	0,33	0,06	0,80	1,13	0,44	0,10	0,46	0,02	0,51	0,33	0,33	0,46
LL	0,02	0,03	0,21	0,30	0,00	0,00	0,12	0,18	0,36	0,14	0,46	0,12	0,31	0,01	0,07	0,10
LO	1,12	0,13	0,63	0,01	1,90	0,04	1,44	0,28	6,06	0,00	0,99	0,13	4,73	0,09	6,57	1,40
LP	2,58	0,12	3,88	0,12	2,09	0,04	2,06	0,08	2,89	0,20	1,78	0,16	1,59	0,00	2,45	0,75
OO	47,53	2,02	39,12	0,10	28,35	0,53	33,44	0,13	31,68	0,35	53,81	1,31	52,48	0,33	43,52	3,59
OP	30,15	0,72	39,62	0,84	50,38	0,12	43,76	1,27	36,08	0,08	28,16	0,59	23,67	0,31	29,40	5,12
PP	11,37	1,57	7,37	0,41	9,76	0,19	12,48	0,84	15,07	0,08	8,73	1,12	9,07	0,50	10,44	3,73
SO	5,65	0,84	7,03	0,43	5,07	0,16	4,71	0,90	6,10	0,36	3,20	0,11	7,03	0,25	6,46	1,86
SP	1,15	0,12	1,90	0,23	2,13	0,09	1,19	0,17	1,33	0,06	2,40	0,14	0,61	0,02	0,77	0,42

Tab. 5 Triglycerides in June.

Cultivar	Bottone di gallo		Calatina		Cerasuola		Crastu		Minuta		Nocellara del Belice		Tonda iblea		Vaddarica	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LnO	0,47	0,01	1,92	1,56	1,49	1,33	1,81	0,23	2,34	2,13	1,96	1,75	2,39	2,59	0,00	0,00
LL	0,73	0,23	0,50	0,27	3,23	0,21	1,82	0,12	1,15	0,02	1,16	0,39	0,70	0,01	0,00	0,00
LO	2,58	0,60	3,05	0,13	2,43	0,35	2,83	0,26	1,74	0,00	1,70	0,12	2,11	0,31	1,49	0,36
LP	0,70	0,02	0,62	0,12	0,89	0,19	1,02	0,35	5,92	0,11	0,51	0,04	4,30	0,68	7,82	1,05
OO	44,30	3,35	36,67	1,47	23,28	0,05	26,71	0,33	33,39	1,34	52,75	0,14	52,23	0,53	38,42	1,68
OP	7,92	0,04	10,98	1,13	14,71	2,16	10,83	0,74	12,03	0,55	7,95	2,13	10,68	2,21	9,00	0,49
PP	28,67	2,00	34,21	0,81	40,11	0,87	37,66	0,38	27,09	1,43	24,92	1,91	16,19	0,29	23,79	0,63
SO	10,12	1,97	5,93	0,69	8,39	0,25	12,77	0,71	10,83	0,14	6,58	2,02	5,69	0,26	11,86	1,09
SP	4,50	0,25	6,12	0,37	5,48	0,59	4,54	0,24	5,51	0,13	2,48	0,19	5,72	0,21	7,60	0,86

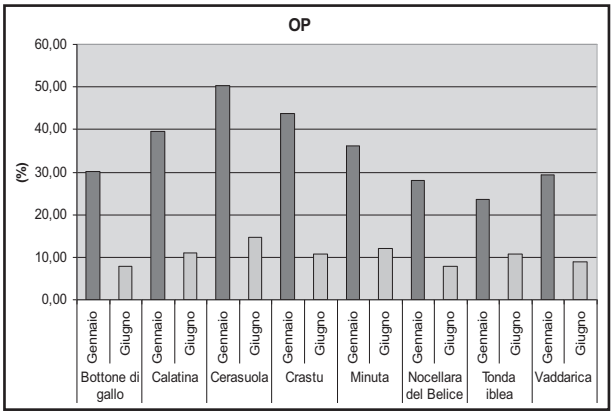

Fig. 4

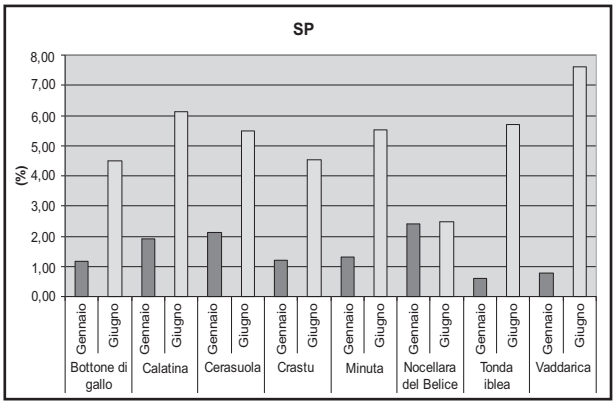

Fig. 5

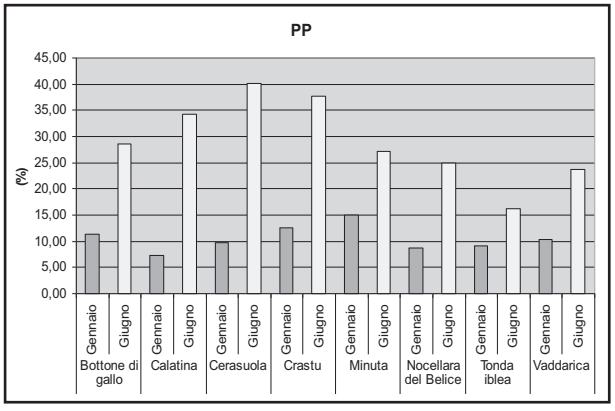

Fig. 6

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PREDICTIVE MODELING OF THE FRESHNESS OF MINCED BEEF MEAT STORED IN MAP AT DIFFERENT TEMPERATURES

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ABSTRACT

The aims of this work were: 1) to follow the freshness decay of packaged minced beef meat stored at different temperatures (4.3, 8.1 and 15.5°C) by applying both traditional methods (microbiological counts, TBA assay, headspace gas composition) and non-invasive methods (e-nose and color measurement); 2) to model the kinetics in order to obtain information about the maximum acceptability time as function of storage conditions. The minced beef, packaged with high gas barrier materials in MA was directly supplied by a manufacturer at the beginning of its commercial life and analysed at different times according to the storage temperature. Shelf life study revealed the ability of the traditional methods in describing the kinetic of freshness decay. The classical modeling of the experimental data and the comparison with microbiological or chemical thresholds allowed the setting, for each index, of a stability time exceeding which the meat was no longer acceptable. The quality decay of meat was also evaluated by inspecting the headspace fingerprint of the same set of samples by means of a commercial e-nose. A clear discrimination between "fresh", "old" and "very old" samples was obtained by using PCA and CA, determining at each temperature a specific range of stability time. Colour attributes were evaluated by using conventional colorimeter. All the stability times estimated for each index allowed the building of a generalized time-temperature tolerance chart, obtaining also a Q10 mean value. The results show that the Q10 values from the traditional methods (3.6-3.8 range) were accordingly overlapped with the Q10 estimated through e-nose and colour indexes (3.4 and 3.3 respectively).

Key words: freshness; e-nose; minced beef meat; shelf life; time-temperature charts.

INTRODUCTION

Consumers' concerns about the quality and safety of fresh meat are continuously increasing. Especially, reliable methods for assessing the freshness of meat would benefit consumers, the meat industry and the retailers. In the meat group and at this regard, minced meat is a very "critical" product. Colour changes (browning), rancidity progress, aroma profile decay and microbial spoilage affect the perceived quality of the meat: these trends can be slowed with chilling, modified atmosphere packaging, quality assurance and application of correct hygienic practices during the first stages of cutting, grinding and preparation. Freshness can be explored in its different sides, generally correlated: as safety assurance, as sensory qualities retention, as physical-chemical properties expression. Since microbial analysis is expensive and time consuming, alternative methods involving chemical changes have been proposed but, in the last years, non invasive and less time consuming approaches have been also assessed. In this work, a modelization of minced beef meat shelf life decay was tentatively approached on the basis of microbiological indices (TBC), chemical-physical tests (colour and headspace gas composition trends), rancidity evolution (TBA) and volatile profile (e-nose). The main goal was to estimate a stability time and its dependence from storage temperature in order to extrapolate useful information about the practices of commercialization and home storage of a very delicate and perishable fresh food.

MATERIALS AND METHODS

Minced beef meat packaged in PS trays (600 g), sealed with high gas barrier film and preserved in MA (30% CO_2 and 70% O_2) were taken at the beginning of their commercial life and stored at three different controlled temperatures (4.3, 8.1 and 15.5°C) for 3-12 days. At established storage times, different analyses were performed: chemical-physical tests (TBA with spectrophotometric method, heasdspace gas composition by gascromatography, hue by Lab colorimeter), microbiological assay (TBC according to ISO regulations) and e-nose measurements (PEN 2 model, WMA Airsense Analitics Inc., equipped with 10 MOS sensors, differently selective to volatile compounds).

The methods applied are according to standard procedures, well described in meat scientific literature (Nollett, 2007). For e-nose approach we referred to a method recently developed (Benedetti *et al.*, 2005). The all data were evaluated through simple mathematical models with a Tablecurve 2D software v. 4.0 (Jandel Scientific, San Rafael, CA, USA). The data obtained from the sensor array of the electronic nose were analysed by Principal Component Analysis (PCA) and Cluster Analysis (CA) using the XLSTAT v. 2007.2 (Addinsoft 1995-2007).

RESULTS AND DISCUSSION

TBA trend revealed a zero order kinetic (figure 1). For secondary oxidation products, no legal threshold exists, but from literature data limit of 1 mg malonaldehyde/kg meat has been suggested for sensory perceived rancidity (Watts, 1962). So, a time threshold at the three storage temperatures has been computed, as shown in figure.

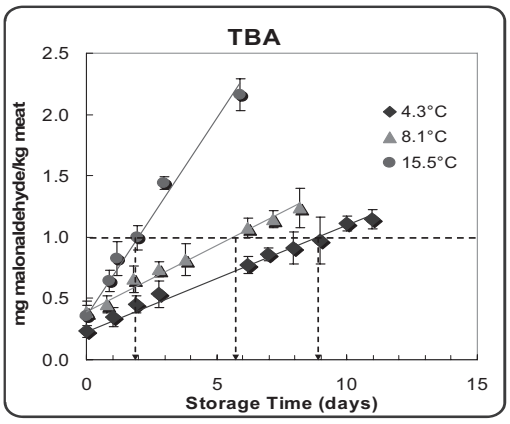

Fig. 1: Trend of TBA index (expressed as malonaldeyde) in MAP minced meat stored at different temperatures.

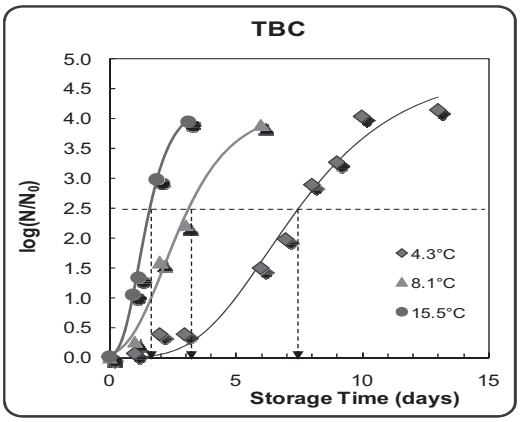

Fig. 2: Trend of Total Bacterial Count in MAP minced meat stored at different temperatures.

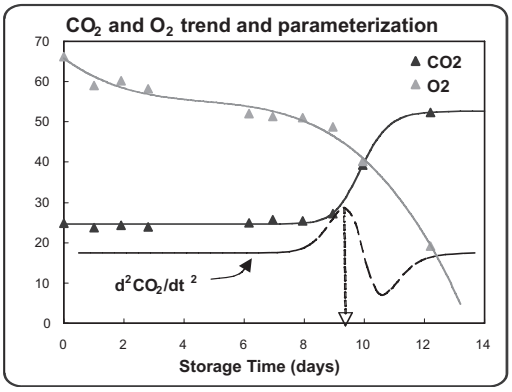

Fig. 3: CO_2 and O_2 trends in the headspace of MAP packaged minced meat stored at 4.3°C.

At a given temperature, the growth of microbial population follows three main steps, namely, lag phase, exponential phase and a stationary phase. This behavior can be satisfactorily described with the Gompertz function (Zwietering et al, 1991) as illustrated in figure 2 that shows the Total Bacterial Count kinetics at the three storage temperatures. The TBC remained for a short time at the starting level (104 CFU/g) and in some cases reached final values of 107-108 CFU/g. The kinetics model also allowed evaluation of an acceptability range time, namely the time necessary to reach the Total Bacterial Count value of 5x105 CFU/g as established by EU 2073/2005 Directive. For the parameterization purposes, this value has been set as 2.5 LogN/No, taking into account an average microbial population in fresh meat of 104 CFU/g. Total coliforms (not reported) were below the legal requirements in all the storage conditions.

The main effect of microbial growth is the oxygen consumption in the surrounding headspace and the CO_2 production. This phenomenon has been investigated by gaschromatography: results are shown in figure 3, with reference to the storage at the lowest temperature. A simple transition kinetic has been applied to experimental data in order to identify (after second derivative transformation) a threshold time, namely the time corresponding to the maximum of the second derivative of the function. Results for others storage conditions confirmed the overlapping with microbiological shelf life estimations.

Use of the e-nose combined with multivariate statistical elaboration (PCA and CA-Ward method, square Euclidian distance) was able to discriminate among the different samples, as shown in figure 4. The two first principal components represented 83% of the total variance and their biplot (loadings–sensors and scores–

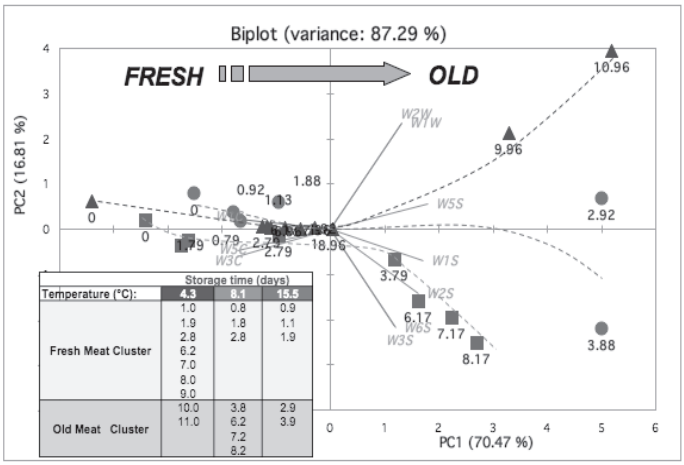

Fig. 4: Biplot of sensor responses and samples scores during storage of ground minced meat at different temperatures. Insert refer to results of Cluster Analysis.

samples, figure 4) allowed a separation of the samples according to the storage conditions (confirmed by cluster analysis, as shown in the figure 4 insert). Samples were distributed along PC1 and PC2 according to the storage time and storage temperature, respectively. In order to define a pseudo stability-time for freshness maintenance another statistical approach was performed, considering the trend of PC1 scores of samples vs. the storage time, so to extrapolate the time corresponding to the minimum of the second derivative of the transition function interpolating PC1 scores at each temperature.

Lab colorimetric evaluations showed an hue decrease (browning, computed as a-redness-/ b – yellowness- ratio) during storage, interpolated with a pseudo-sigmoidal function. As for headspace composition, this trend was parameterized by inspection of the second derivative, allowing the computation of a threshold time for each storage test (7.8, 2.6 and 1.3 days, respectively, increasing storage temperature).

All the considered indices are in good agreement for the shelf life parameterization, so a time-temperature tolerance chart has been prepared. Figure 5 shows the corresponding results and includes the Q_{10} average value (n-fold decrease of the shelf life for a 10 °C temperature increase, Labuza, 1982). On the basis of these results, freshness of minced meat packaged in MAP is maintained for about 8 days at 4°C (recommended storage temperature), 3-4 days at 8°C (usual temperature in household refrigerators) and 1 day at 20°C (room temperature).

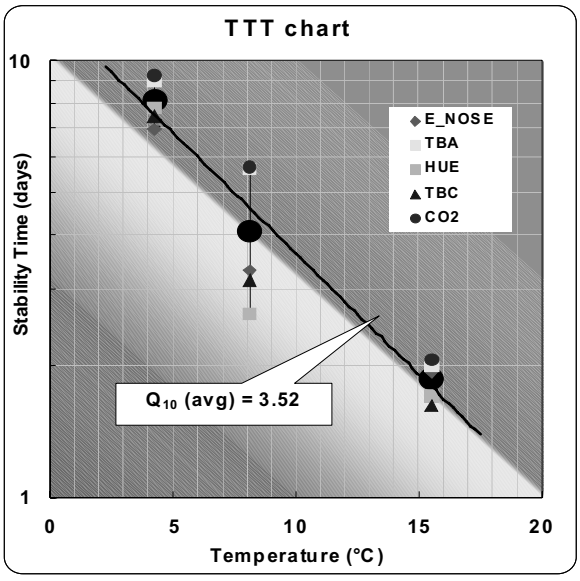

Fig. 5: Time Temperature Tolerance chart for shelf life of minced meat

CONCLUSIONS

Shelf life studies require in the practice a fast and pragmatic approach: when the safety is assured, the sensory quality decay can be

monitored and parameterized. In this perspective, as demonstrated in this work, the electronic nose can easily contribute to take into account the complex evolution of the aroma profile. Other classical analytical indices related to sensory decay (in the case of minced meat: colour, headspace composition, secondary lipid oxidation products) can support this pragmatic approach. The shelf life time ranges obtained by the e-nose, microbiological survey and other sensoristic or analytical techniques are substantially overlapped. Our results confirmed that fresh minced beef meat is a very perishable and delicate commodity and that storage temperature plays a critical role in quality maintenance.

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TURNING THE SENSORY ACCEPTABILITY LIMIT INTO AN ANALYTICAL ONE FOR SHELF-LIFE STUDIES

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ABSTRACT

The shelf-life of most foods is mainly determined by the changes of the sensory acceptability. Consequently, a consumer panel would be the most appropriate tool to determine their shelf-life. However, this approach is very expensive and difficult to perform routinely. The best working way should be the identification of an analytical index related to the sensorial acceptability. The aim of the present research was to turn the sensory limits, assessed by survival analysis of acceptability data obtained by a consumer panel, into analytical indexes of some selected shelf-stable products. To this purpose, the oxidation kinetics and the acceptance limits of some selected bakery products (biscuits, bread sticks and crackers) were evaluated during storage. Results indicate that peroxide value could be a representative index of the quality depletion of bakery products. In fact, its changes were found to be correlated to the sensory acceptance. The availability of this relation may allow easily predicting consumer response during food storage leading to reliable estimation of shelf-life.

Key words: Bakery products, consumer acceptance, oxidation, shelf-life.

INTRODUCTION

It is well known that oxidative reactions cause the formation of off-flavors accounting for the consumers' rejection of the product (Grosso and Resurrection, 2002). Consequently, a consumer panel would be the most appropriate tool to determine when the food reaches the end of its life by sniffing the product. This approach conflicts with the industrial needs because it is very expensive and difficult to perform routinely. More useful for routine shelf-life evaluation would be the identification of a chemical quality index, related to the sensorial acceptability, which could be

measured by a quick and simple analytical method. The identification of such relationship is a key step in developing a simple and ready to use shelf-life prediction model. From an industrial point of view, one of the widest used oxidation index is the peroxide value (PV) because its evaluation is very easy also with the laboratory equipments owned by small and medium companies. In addition, it should be considered that, even if hydroperoxides are not volatile, they are well known precursors of volatile secondary oxidation products which are sensory perceivable. For this reason, the possibility to correlate the peroxide value with the sensory acceptance limits appears interesting in order to find out methodologies for a rapid prediction of the product shelf-life by the quality assurance management.

On the basis of these observations, the aim of the present work was to turn the sensory limits, assessed by survival analysis of acceptability data obtained by a consumer panel, into analytical indexes of some selected bakery products. To these purposes biscuits, bread sticks and crackers were considered. The oxidation kinetics and the acceptance limits were evaluated during storage at 45 °C.

MATERIAL AND METHODS

Sample preparation

Fresh made commercial biscuits, bread sticks and crackers were purchased at an Italian factory just after their production. Original packages were stored in oven 45 ° C.

Analytical determination

Fat separation. Sample fat was obtained by solid-liquid extraction using diethyl ether– petroleum ether (Carlo Erba, Italy) mixtures (1:1 v/v). In particular, ground biscuits and solvent mixture in the ratio 1:2.5 w/v were stirred at room temperature for 1 h. After filtration through filter paper (Whatman n. 1), the fat was separated from the solvent by evaporation (Heidolf Instruments, mod. 4001, Germany).

Peroxide value analysis. The peroxide values (PV) of the fat samples were carried out according to AOAC (1995).

Sensory analysis. The end of shelf-life of biscuits stored at different temperatures was determined through sensory analysis by applying the survival analysis (Gomez *et al.*, 2001; Hough *et al.*, 2003, 2004). The samples were tested by a 70 member consumer panel, approximately 50 % males and 50 % females with ages ranging from 20 to 45 years. Panelists were asked to sniff the samples and answer the question: “Would you normally consume this product? Yes or No”. During the sensory session each panelist sniffs 6 samples of biscuits, crackers or bread sticks with different storage time. The CensorReg procedures from S-PLUS (Insightful Corporation, Seattle, USA, ver. 7) were used according to Hough *et al.* (2003, 2004).

Data analysis. The results are the average of at least three determinations and the coefficients of variation were less than 5 % for peroxide value determinations. Linear regression analysis by least squares regression was performed by Windows Excel and the goodness of fitting evaluated on the basis of statistical parameters of fitting.

RESULTS AND CONCLUSION

Fig. 1 shows the changes of the percentage of consumers rejecting the product

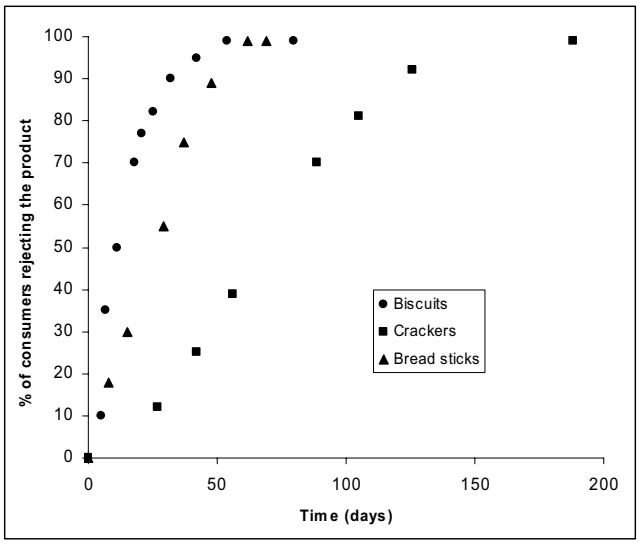

Figure 1. Percentage of consumers rejecting the products as a function of storage time at 45 °C.

as a function of storage at 45 °C of biscuits, crackers and bread sticks. As expected, during food life, the percentage of consumers rejecting the product progressively increased. In particular, the acceptance of biscuits decreases much faster than that of bread sticks and crackers. The latter appears the most stable products. The sensory acceptability data could allow a direct estimation of the product shelf-life which is exactly the time needed to reach a selected percentage of consumers rejecting the product. For instance, the industry could select as shelf-life limits the time so that 50 % of the consumers sniffing the product found it not acceptable. It must be pointed out that using this methodology it is possible to select for the same product different acceptability limits. In fact, these limits, although affected by food chemical and physical stability, can also depend on company policy and choices.

Although the approach of survival analysis is very effective and provides company-tailored assessment of product shelf-life, it is indeed hard to be performed routinely. For this reason, the identification of the relation between consumer acceptance and an appropriate chemical index would be extremely useful. To his purpose, the peroxide value changes were measured during the storage of bakery products (Fig.2). As expected, peroxide values increased during storage showing different kinetics depending on the bakery product considered. In particular, the development of oxidation resulted higher in biscuits than that in bread sticks and crackers.

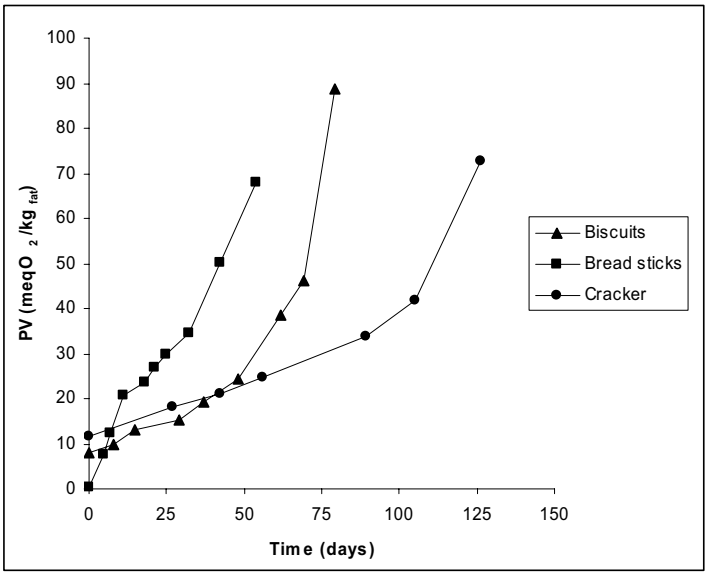

Figure 2. Changes in peroxide value of biscuits, crackers and bread sticks stored at 45 °C.

In order to identify the relation between PV and

Table 1. Linear regression parameters (slope, intercept, determination coefficient and p value) of percentage of consumers rejecting the product as a function of the corresponding peroxide value.

Product	Slope (%consumer rejecting /meqO2kg-1fat)	Intercept	p	R^2
Biscuits	2.67±0.14	1.45±0.96	<0.05	0.98
Crackers	2.88±0.22	-34.89±6.13	<0.05	0.97
Bread sticks	5.49±0.66	-30.18±8.11	<0.05	0.96

Table 2. Peroxide value corresponding to 50 % consumers rejecting the sample of biscuits, crackers and bread sticks.

Product	Peroxide value ($meqO_2kg^{-1}fat$)	95 % lower limit	95 % upper limit
Biscuits	14.8	11.3	19.4
Crackers	29.4	27.6	32.9
Bread sticks	12.4	11.4	14.2

acceptance, the peroxide value in correspondence of the time at which different percentages of the consumers reject the samples was calculated for each of the product considered and the regression analysis was performed. Table 1 shows the results obtained. The statistical analysis demonstrated the good correlation between PV and acceptance. The mathematical relationship obtained could be used to calculate the peroxide value limit of shelf-life corresponding to a selected % consumer rejection. For instance, considering that 50 % consumer rejection is a well accepted limit in shelf-life studies (Cardelli and Labuza, 2001), the PV limit of the bakery product considered can be obtained (Table 2). It is evident that, while the PV limit for biscuits and break sticks is not significant different and close to 12 meqO2kg-1fat, the peroxide value at which 50 % of consumer reject crackers is significantly higher (29 meqO2kg-1fat). This result indicates that there is not a unique PV limit for bakery product and such limit should be estimated for each product considered.

Results obtained highlights that the PV is a representative index of the quality depletion of bakery product during their life on the shelf. In fact, its changes were found to be linearly related to the consumer acceptability. Thus, knowing the relationship between PV and acceptability, it is possible to predict the product shelf-life in a rapidly and saving money way.

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APPLICATION OF BOOTSTRAP METHOD TO MODELLING OF MICROBIAL FOOD QUALITY CHANGE

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ABSTRACT

Bootstrap method, a computer-intensive statistical technique to estimate the distribution of a statistic was applied to deal with uncertainty and variability of the experimental data in stochastic prediction modelling of microbial quality change of chill-stored prepared foods. Three different bootstrapping methods for the curve-fitting to the microbial count data were compared in determining the parameters of Baranyi's growth model: nonlinear regression to static version function with resampling residuals onto the all the experimental microbial count data; static version regression onto mean counts at sampling times; dynamic version fitting of differential equations onto the bootstrapped mean counts. All the methods outputted almost same mean values of the parameters with the method of the dynamic form resulting in the largest, but suitable distribution of parameters with affordable confidence interval of the predicted microbial count.

Key words: Baranyi growth model, aerobic bacteria, nonlinear regression, confidence limit.

INTRODUCTION

Quantitative modelling of microbial growth is useful for shelf life determination of perishable foods labile to microbiological spoilage. The mathematical model describes the microbial growth with its parameters usually determined by curve fitting procedure to the experimental data. Stochastic models deal with the problem of uncertainty and variation of experimental data in the estimation of the microbial growth.

Bootstrap method is a computer-intensive statistical technique to estimate the distribution of a statistic for stochastic predicted modelling. This study aims to

compare different bootstrap methods in estimating the parameters of a primary model of microbial growth, particularly with emphasis on testing the validity of the method proposed by Lee *et al.* (2007). The effect of different methods on the confidence interval of predicted microbial growth was also investigated.

MATERIALS AND METHODS

Two experimental data set of aerobic bacterial growth on Korean style seasoned soybean sprouts and fan-fried seasoned meat patties at respective constant temperatures of 0 and 5°C were available in our laboratory for this research (Fig. 1).

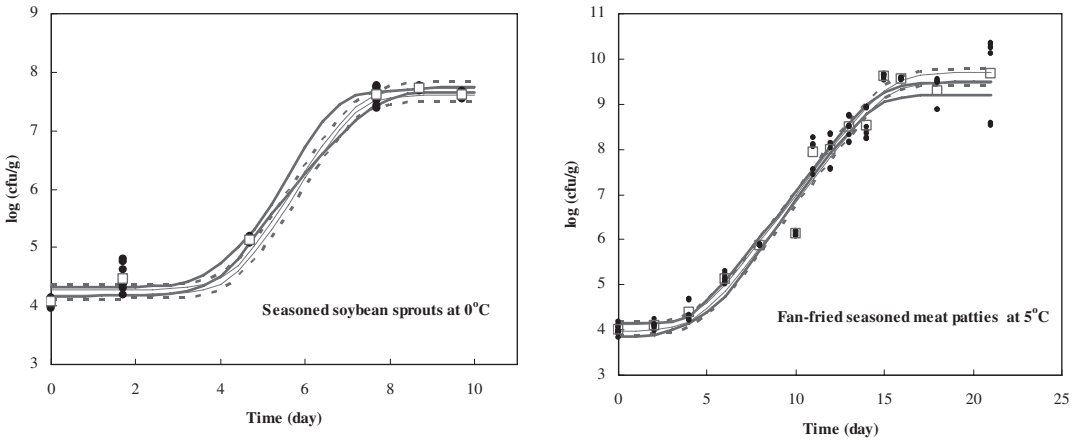

Fig. 1. Aerobic bacterial growth of the seasoned soybean sprouts and fan-fried seasoned meat patties stored at 0 and 10oC, respectively. Nine individual plate counts were measured for each experimental sampling time except for initial measurement with 15 replicates. •: individual plate count; □: mean count; —: 95% confidence limit based on static version (Eq. 1) with all the experimental data; ·····: 95% confidence limit based on static version (Eq. 1) with average experimental data; □: 95% confidence limit based on dynamic version (Eqs. 3 and 4).

First, model-based resampling of the residuals was applied to the regression of the microbial growth data according to the static version of the Baranyi and Roberts microbial growth model (1994):

$$\log N = \log N_o + \frac{\mu_{max}}{\ln 10} \cdot A - \frac{1}{\ln 10} \cdot \ln\left(1 + \frac{e^{\mu_{max} \cdot A} - 1}{10^{(\log N_{max} - \log N_o)}}\right) \quad (1)$$

where A is defined by $A = t + \frac{1}{\mu_{max}} \cdot \ln\left(\frac{e^{-\mu_{max} t} + 1/(e^{t_{lag}\mu_{max}} - 1)}{1 + 1/(e^{t_{lag}\mu_{max}} - 1)}\right)$, μmax is the maximum specific growth rate (1/day), N is the microbial count in cfu/g at time t, N_o and N_{max} are the initial and maximum cell densities in cfu/g, respectively, and t_{lag} is lag time (day).

Eq. 1 is a form reparameterizing q_o to t_{lag} from the original equation given by Baranyi and Roberts (1994) according to Eq. 2.

$$q_o = \frac{1}{e^{t_{lag}\,\mu_{max}} - 1} \tag{2}$$

where qo is the initial state for the physiological state of the cell population.

The bootstrapping procedure starts with curve fitting of the static version Eq. 1 to the experimental data based on the criteria of minimizing sum of error squares. The nonlinear regression subroutine Rnlin from IMSL Library (Visual Numerics, Houston, Texas, USA) was used to obtain a set of parameters (t_{lag}, log N_o, μ_{max}, and log N_{max}). The centred residuals from the best-fit microbial count estimate of regression curve were then resampled randomly with replacement and added to the best-fit value to form a new set of microbial count data vs. time, which was subsequently used again for another nonlinear regression with repetition of 1000 times The regression procedure was conducted separately for a set consisting of all the experimental data, and also for a mean microbial count data set.

As another way of bootstrapped curve-fitting, the dynamic version model of Baranyi and Roberts (1994) in differential equations of logarithmic form (Eqs. 3 and 4) was applied to estimating 1000 sets of the parameters (log q_o, log N_o, μ_{max}, and log N_{max}) following the method of Lee *et al.* (2007).

$$\frac{d(\log q)}{dt} = \frac{\mu_{max}}{\ln 10} \tag{3}$$

$$\frac{d(\log N)}{dt} = \frac{\mu_{max}}{\ln 10}\left(\frac{1}{1 + 10^{-\log q}}\right)\left(1 - 10^{(\log N - \log N_{max})}\right) \tag{4}$$

where q is the physiological state of the cell population at time t. Each experimental set consisting of mean microbial counts during storage was constructed by resamping individual plate counts and then supplied for the parameter estimation, which was done by minimising the sum of squares of errors between the experimental data and those simulated by solving differential Eqs. 3 and 4 for a guessed parameter set.

The parameter, t_{lag} of Eq. 1 obtained from static version regression was converted to qo following Eq. 2 to compare the different methods in the estimation.

RESULTS AND CONCLUSION

When the data in Fig. 1 were used for parameter estimations of static version Eq. 1 and dynamic version Eqs. 3 and 4, three methods applied produced nearly same mean values of the parameters (Table 1). Even with very slight difference among the methods, the mean parameter values were less different between two types of regressions to static Eq. 1 than between any of static equation regressions and dynamic differential equation fitting. Nonlinear regression to static Eq. 1 with all the plate count data resulted in the narrowest ranges of parameters relatively closer to those from adopting mean plate count on each storage time than those of fitting to the dynamic Eqs. 3 and 4 (Table 1).

From 1000 sets of microbial growth model parameters for each bootstrapping method, 1000 growth curves can be obtained by substituting them into Eq. 1 or Eqs. 3 and 4. The 95% confidence limit for any storage time was obtained from 2.5% and 97.5% percentiles of the estimated microbial counts (Fig. 1). Three methods

Table 1. Microbial growth model parameters determined by different bootstrapping methods for aerobic bacterial growth on two prepared foods

Method	Parameters of Baranyi and Roberts model (Eqs. 1-4)			
	log qo	log No	μmax	log Nmax
Seasoned soybean sprouts at 0°C				
Nonlinear regression to static Eq. 1 with all the experimental data	-4.870±0.043	4.239±0.009	2.645±0.112	7.668±0.010
Nonlinear regression to static Eq. 1 with averaged experimental data	-4.872±0.115	4.238±0.116	2.645±0.015	7.667±0.136
Fitting with dynamic differential Eqs. 3 and 4	-4.014±1.159	4.249±0.082	2.383±0.530	7.703±0.050
Fan-fried seasoned meat patties at 5°C				
Nonlinear regression to static Eq. 1 with all the experimental data	-2.233±0.030	4.050±0.063	1.176±0.014	9.593±0.103
Nonlinear regression to static Eq. 1 with averaged experimental data	-2.233±0.100	4.054±0.129	1.200±0.033	9.600±0.154
Fitting with dynamic differential Eqs. 3 and 4	-2.239±0.581	3.993±0.160	1.204±0.092	9.347±0.147

Value is given as mean ± 2 standard deviations.

applied generally gave almost identical microbial growth patterns and similar count estimates with comparable but a little different confidence bands. The two kinds of static version regression showed the similar steady confidence bands with a little narrower one for the case using all the experimental data points. The dynamic version gave the time variant confidence interval generally close to that of the static version regression with average count on each sampling time. The experimental and curve-fitting errors have been taken more appropriately into consideration by the dynamic version bootstrapping. The method is also noted to give confidence range similar to those of static version regressions as shown in Fig. 1.

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INFLUENCE OF STORAGE TIME ON THE COMPOSITION OF VOLATILE COMPOUNDS OF AIR DRIED, FROZEN AND FREEZE DRIED THYME AND ROSEMARY CULTIVATED IN SARDINIA

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ABSTRACT

This paper deals with the effect of different stabilizing techniques on the evolution of the volatiles in rosemary (*Rosmarinus officinalis* L) and thyme (*Thymus officinalis* L.) cultivated in Sardinia during nine months of storage. Fresh leaves were collected and soon divided in four batches, which were subjected to hydro distillation and GC-MS analysis, the first batch as fresh, the second one after drying in a laboratory pilot dryer, the third after freezing in a forced air freezer and the fourth after freeze drying in a laboratory freeze dryer.

All the samples were adequately packaged and stored. Samples for analysis were taken at 3 months intervals. The fresh, stabilised and stored plant material were hydro distilled for 4 hours using a Clevenger-type. The oils were analysed in duplicate by gas chromatography, using a flame ionization detector. Qualitative analysis was done by GC/Mass and mass units were monitored from 10 to 450 at 70 eV.

Results of the evolution of volatile compounds of the differently samples seem to evidence that the best way to stabilize the herbs is freezing.

Key words: *Rosmarinus officinalis* L.; *Thymus officinalis* L.; essential oils; stabilisation; storage.

INTRODUCTION

The shelf life of spices is traditionally extended by drying. Fresh herbs, due to their high water content, undergo micro-organism growth and adverse biochemical reactions. On the other hand drying may result in a lot of physical and chemical alterations. Air and oven-dehydration are the main methods used to stabilize spices. During oven drying, in general, losses of volatile compounds are directly dependent on the temperature and time used. Anyway, other unit operations that may be proposed are freezing and freeze-drying.

Thyme and rosemary grow both wild in the Mediterranean basin and they are very much appreciated for their aromatic, antimicrobial and antioxidant properties (Dorman and Deans, 2000; Nguyen *et al.*, 2000). The effect of mechanical air drying on the volatiles of *T. officinalis* and *R. officinalis* have been extensively reported (Blanco *et al.*, 2002; Fadel and El-Massry, 2000; Raghavan *et al.*, 1995; Venskutonis, 1997). However, we have no knowledge about changes during storage on volatile composition of air dried, freeze dried or frozen samples of these two species cultivated in Sardinia.

The aim of this paper is to verify the effects of the above cited stabilizing techniques on the evolution of the volatiles in rosemary (*Rosmarinus officinalis* L) and thyme (*Thymus officinalis* L.) cultivated in Sardinia during nine months in storage.

MATERIALS AND METHODS

Fresh leaves were collected and soon divided in four batches, which were subjected to hydro distillation and GC-MS analysis, the first batch as fresh, the second one after drying in a laboratory pilot dryer, the third after freezing in a forced air freezer and the fourth after freeze drying in a laboratory freeze dryer. Drying was done at 45°C and 1250 m^3/h for thyme and 38°C and 300 m^3/h for rosemary. Freeze drying was done with an Edwards Modulyo freeze dryer at a condenser temperature of -52°C, shelf temperature of 20°C and pressure below 10 Pa for 24 hours. The oven dried samples were packaged both under vacuum inside a polypropylene film and at atmospheric pressure inside polyethylene bags, while freeze dried herbs were packaged in an aluminate film under vacuum; both samples were stored in the dark at 20°C. Frozen herbs were packaged in polyethylene bags and stored in the dark at -18°C. Samples for analysis were taken at 3 months intervals up to nine months. The fresh, stabilised and stored plant material were hydro distilled for 4 hours using a Clevenger-type and the oils were stored at –20°C before analyses, which were carried out on two replicates of each sample by gas chromatography, using a flame ionization detector. Qualitative analysis was done by GC/Mass and mass units were monitored from 10 to 450 at 70 eV, as reported in a previous paper (Piga *et al.*, 2007).

RESULTS AND DISCUSSION

Rosemary essential oil yield showed the most significant reduction in freeze dried samples, while frozen herbs evidenced the least reduction. Results of GC/MS analyses of frozen and freeze dried samples showed that borneol, γ-terpinene and mirtenol had the major losses during storage, (myrtenol disappeared after 9 months in storage on freeze-dried samples). Dried samples evidenced a major decrease also in β-pinene, terpinolene, and methyl charvacrol.

Thyme essential oil yield showed the most significant reduction in air dried samples, while frozen herbs evidenced the least reduction. Results of GC/MS analyses of frozen and freeze dried samples showed that terpinel-4-ol, thymol methyl ether, carvacrol methyl ether and caryophyllene ether had the major losses during storage, (carvacrol methyl ether and caryophyllene ether disappeared after 9 months in storage). Dried samples evidenced a major decrease also in α-thuyene, octen-3-ol, myrcene, γ-terpinene and carvacrol.

Packaging material of dried herbs resulted in contrasting results.

From these preliminary results we can state that the best stabilising unit operation is freezing for both herbs.

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APPLICATION OF GAB OR OTHER MODELS FOR WATER SORPTION ISOTHERMS DETERMINATION OF TRADITIONAL BAKERY PRODUCTS

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ABSTRACT

The vapour sorption isotherms of five typical baked foods were determined at 20°C. In particular, the methodology proposed by Cost Projects 90 and 90 bis have been used. The latter procedure implies using a proper number of pieces of each product. The products have been closed in desiccators, in which nine different equilibrium relative (ERH) humidity have been created. The relative humidity inside desiccators have been checked daily until equilibrium, before placing samples. Moisture content of samples has been measured by a gravimetric method, while water activity has been determined by a dew-point hygrometer. All obtained data have been interpolated to fit the GAB equation or other equations and the mean relative percentage deviation modulus (E%) calculated.

Key words: bakery products; biscuits; cookies; shelf-life; sorption isotherms.

INTRODUCTION

The shelf-life of packaged foods is strictly dependent on product formulation and technology, on packaging characteristics, mostly as barrier to gases and light, and

on storage conditions. The shelf-life can be predicted both with the classic, but time and cost expensive, simulated approach, either by normal or accelerated testing, or by a more simple and flexible mathematical model approach, which takes in consideration all the variables related to product/packaging/environment system (Fava *et al.*, 2000). One of the key factors in setting up the mathematical model is the obtainment of food sorption isotherms.

The aim of this work was to obtain the sorption isotherms of five typical Sardinian bakery products, in order to use them in further mathematical modelling for shelf life evaluation.

MATERIALS AND METHODS

Five traditional bakery products ("tiriccas", "copulettas", "papassinos", biscuits and savoyards) were obtained by a local plant and soon transported at our lab, where they were immediately used for the determinations. The vapour sorption isotherms were determined at 20°C. In particular, the methodology proposed by Cost Projects 90 and 90 bis have been used (Wolf *et al.*, 1985). The latter procedure implies using a proper number of pieces of each product. The products have been closed in desiccators, in which nine different equilibrium relative (ERH) humidity have been created by using nine salts, ranging from 0.11 to 0.92 aw, and weight changes at different times have been measured until a constant value has been attained. The relative humidity inside desiccators has been checked daily until equilibrium with a Micropack Humidity Data Logger (Mesa Laboratories, Inc., Colorado USA), before placing samples. Moisture was evaluated in triplicate according to the AOAC method (AOAC International 2000), while water activity has been determined by a dew-point hygrometer (Aqualab Series 3, Decagon, Pullman USA). All obtained data have been interpolated to fit the GAB equation or other equations using TableCurve 2D software Version 5.01. To evaluate the goodness of fit of each model, the mean relative percentage deviation modulus (E%) was used. The E% is widely adopted throughout the literature, with a modulus value below 10% indicative of a good fit for practical purposes (Lomauro *et al.*, 1985).

RESULTS AND DISCUSSION

The Table 1 shows the estimated parameters of model coefficients and the corresponding mean relative percentage modulus, that describe the goodness of fit of the isotherms of the five bakery products. Moisture sorption isotherms of the five products clearly show a sigmoid (Type II) shape. Examination of the results indicate that the GAB and BET models best describes the experimental adsorption and desorption data for the bakery products considered throughout the entire range of water activity. In fact, the E(%) value was always below 10% when using BET and GAB models. Modelling of sorption data with BET and GAB equations allows the determination of monolayer moisture content values, mo, which ranged 0.0345-0.0542 g/g on dry basis and 0.0355-0.0599 on dry basis, respectively. The values are in the range found for other starchy based foods (Lomauro *et al.*, 1985; Palou et al., 1997; Kim *et al.*, 1998). It is noteworthy the higher mo value of tiriccas and copulettas, that differ from the other products, as they have a short crust.

Table 1 - Estimated values of coefficients and mean relative percentage deviation module obtained for sorption models applied to experimental adsorption data

Model	Constants	Products				
		Tiriccas	Papassinos	Biscuits	Savoyards	Copulettas
GAB	Mo	0.0599	0.0355	0.0413	0.0378	0.0570
	K	0.9770	1.0541	0.9766	1.0187	0.9955
	c	4.4936	2.7933	3.8600	3.0227	1.8191
	E(%)	1.96	2.91	5.52	1.76	8.61
BET	Mo	0.0534	0.0497	0.0345	0.0451	0.0542
	c	7.311	1.3695	8.977	1.7649	2.2873
	E(%)	2.21	4.78	3.34	5.41	6.38
Oswin	a	2.4071	3.021	3.430	4.5430	4.0387
	b	0.5544	-0.7032	-0.560	-0.1683	-0.030
	E(%)	15.23	22.67	36.66	42.78	37.81
Chen	a	0.1138	0.2218	0.110	-0.0958	-0.1687
	b	-1.0038	-1.8121	-1.450	-2.0091	-1.8029
	E(%)	19.84	32.18	44.86	51.10	47.23
Henderson	a	-0.7163	-0.8511	-0.7441	-0.4018	-0.2871
	b	2.2591	2.8051	3.1824	4.1594	3.6718
	E(%)	15.58	21.45	35.14	40.76	35.48

The data reported can be useful for food manufacturers to predict the shelf life of these products.

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SENSORY EVALUATION AND STATISTICAL ANALYSIS TO ASSESS THE CHANGES DURING STORAGE IN EXTRUDED CEREALS

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ABSTRACT

Sensory evaluation by a semi-trained panel and statistical analysis of data were carried out to evaluate the organoleptic properties of corn flakes during storage and the effect of adding ascorbic acid or tocopherols. The obtained results allowed to evaluate the effects of storage and of added antioxidants on odour and flavour of corn flakes, pointing out that tocopherols are more effective than ascorbic acid in preventing off-odours and off-flavours. Moreover, statistical analysis revealed to be a powerful tool allowing to approach sensory analysis even when semi-trained, small panels are involved.

Key words: Corn flakes; natural antioxidants; sensory analysis; statistical analysis; storage.

INTRODUCTION

Nowadays the quality control of foods is of major concern in food industry. An important aspect of this matter deals with the sensory properties of foods and their evolution during storage. Sensory analysis of food products allows to evaluate them using criteria that guide consumers in their choices. Thus, it is not completely replaceable by the assessment of chemical parameters.

On the other hand, a well-trained, professional panel represents a cost, that is not always affordable, especially in small industries that often carry out sensory evaluation of the products with semi-trained, not professional small panels. In

these cases, the main limits of sensory analysis, that regard its repeatability and objectivity, can jeopardize its efficacy. In order to overcome these limits, statistical tools applied to sensory analysis appear to be very useful (King *et al.* 1995; Bárcenas *et al.* 2000; Peron 2000; Ruiz-Capillas and Moral 2003).

Corn flakes are interested by lipid-related off-flavours that compromise their shelf-life (Paradiso *et al.* 2008). The behaviour of the organoleptic properties of corn flakes during storage has been studied in this investigation, by means of sensory evaluation, carried out by a semi-trained panel, and statistical analysis of data, in order to supply objective information about the effects of storage and of the employment of natural antioxidants.

MATERIALS AND METHODS

Corn flakes were produced using an industrial production line, as described in Paradiso *et al.* (2008). The flakes produced through the traditional technology (control, Co) were compared with flakes added during mixing with 0.02 % w/w of natural tocopherols (T) or 0.1 % of ascorbic acid (Aa). The flakes were packed in 500 g polypropylene bags, stored for one year and submitted to descriptive sensory analysis after 90, 180, 270, 360 days of storage. The descriptors adopted were the following: fresh product odour (FPO), fresh product flavour (FPF), pungent odour (PO), rancid odour (RO), stale odour (SO), rancid flavour (RF), stale flavour (SF), overall acceptability (OA). The intensity of every attribute was expressed on a 10-cm unstructured linear scale. A semi-trained panel, confident with the product, was formed. The number of panellists was 5, that is the lower limit suggested for panels (King *et al.* 1995). Panellists evaluated fresh and aged flakes to familiarize with flavours and with the vocabulary and the intensity range of the sensory attributes. Panellists received a tray containing samples, randomly coded, a glass of water and an evaluation form with a pencil. Statistical analyses (ANOVA, Tukey Test for multiple comparisons and PCA) were performed with the XLStat software (Addinsoft Inc., New York).

RESULTS AND DISCUSSION

The sensory scores attributed by the panelists were submitted to three-way ANOVA (*antioxidant, time and panellist*). Because of the long duration of the investigation, the second order interaction *panellist* × *time* was considered as a possible source of variance. The results are reported in Table 1. For all descriptors except one (SF) the model fit, shown by R^2, resulted significant at $p < 0.05$, so that the explanatory variables resulted significant in most cases. A significant effect of the time was observed in almost all descriptors, which showed, as expected, significant decreases in the freshness and in the overall acceptability and remarkable increases of all the oxidation-related off-flavours, with the exception of the rancid flavour, for which the Tukey Test did not reveal significant differences.

Panel test revealed also significant differences attributable to the antioxidants employed. In particular, it appreciated the effectiveness of tocopherols in preventing the onset of off-flavours, already reported in our previous work (Paradiso *et al.* 2008) on the basis of sensory and chemical data. Also the flakes added with ascorbic acid showed better sensory properties than those perceived in Co. Nevertheless, the

Table 1 - Results of three-way ANOVA and Tukey Test for multiple comparisons on the data of the sensory evaluation.

DESCRIPTORS[a]	R^2	TIME (days)[c]				ANTIOXIDANT[c,d]			PAN[e].	PAN. × TIME[e]
		90	180	270	360	Co	Aa	T		
FPO	0.75[b]	A	AB	BC	C	B	B	A		-
FPF	0.81	A	AB	A	B	B	AB	A		-
PO	0.78	C	B	B	A	A	AB	B		-
RO	0.83	B	A	AB	AB	A	B	C		
SO	0.69	B	B	B	A	A	B	B	-	
RF	0.57	-	-	-	-	A	B	B	-	-
SF	0.50	B	AB	AB	A	A	B	B	-	-
OA	0.66	A	AB	AB	B	B	B	A		-

[a] - FPO: fresh product odour, FPF: fresh product flavour, PO: pungent odour, RO: rancid odour, SO: stale odour, RF: rancid flavour, SF: stale flavour, OA, overall acceptability; b - bold character means significance at $p < 0.05$; c - different letters on each row within each variable mean significant difference at $p < 0.05$ (A > B > C);d - Co: control, Aa: flakes added with ascorbic acid, T: flakes added with tocopherols e - PAN.: panellists, the symbol ▲ means a significant effect at $p < 0.05$.

improving effect of ascorbic acid resulted less pronounced respect to tocopherols, being intermediate between Co and T. The latter, in fact, had for all descriptors a significant effect respect to control. On the other hand, Aa showed characteristics alternately similar to Co or T. Only RO clearly distinguished the three typologies. These differences could be appreciated disaggregating the variance sources of the raw data, even if the semi-trained panel could not warrant homogeneity, as pointed out by the significant effect, in some cases, of the explanatory variable *panellist*, and of the interaction *panellist* × *time*.

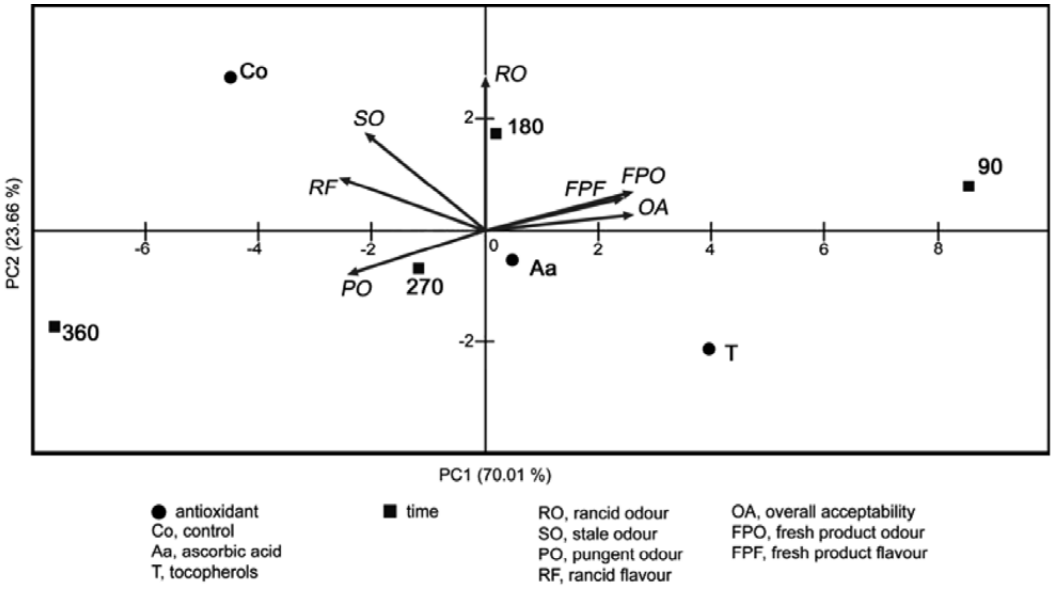

Figure 1 – Biplot of the principal components analysis.

A subsequent step was to apply multivariate analysis to improve the data interpretation. In order to include only the variance attributable to storage and antioxidants, principal components analysis (PCA) was performed on the parameters of the linear models obtained by three-way ANOVA. Only the parameters of *time* and *antioxidant* variables were taken, and the model of SF, that was not significant, was excluded.

Figure 1 reports the biplot of PCA. PC1 (70 % of variance explained) is positively correlated to freshness and acceptability and negatively correlated to SO, PO, and RF. PC2 (23 %) is closely correlated to RO and shows partial correlations with the other descriptors. Ageing is mainly described by PC1. After 180 days a peak in RO was observed: in the subsequent phases it was probably covered by a more intense PO, while the RF continued to increase. The effect of the use of antioxidants was mainly inverse to that of time. Nevertheless, considering PC2 it is possible to notice the different effects of the two explanatory variables. In fact, positive freshness and acceptability descriptors and PO seem to be particularly linked to ageing, while the other sensory properties are in larger amount influenced by antioxidants, being tocopherols, at the experimental conditions adopted, more effective than ascorbic acid.

In conclusion, it was possible to evaluate the effects of storage and of added antioxidants on odour and flavour of corn flakes, pointing out that tocopherols are more effective than ascorbic acid in preventing off-odours and off-flavours; moreover, statistical analysis revealed to be a powerful tool that could allow to approach sensory analysis even when semi-trained, small panels are involved.

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EVOLUTION OF THE VOLATILE COMPOUNDS IN VACUUM-PACKED RIPENED SAUSAGES DURING STORAGE

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ABSTRACT

An experimental investigation was carried out in order to appraise the evolution of volatile compounds in ripened sausages during vacuum storage, by SPME-GC/MS technique. The obtained results pointed out the influence of vacuum storage on the evolution of volatile compounds. In particular, the substances originated from carbohydrate fermentation showed an increase during time, while those derived from spices decreased. Volatile compounds derived from the oxidation of lipids, that are among the chiefly responsible of sensorial properties of meat products, were detected in low percentages. Furthermore, during storage they showed a significant increase starting from two months of storage.

Key words: ripened sausages; SPME-GC/MS; vacuum-storage; volatile compounds.

INTRODUCTION

Many factors influence the characteristic flavour of ripened sausages, such as ripening time and conditions (Viallon *et al.*, 1996; Sunesen *et al.*, 2001), the type of microbial starter (Montel *et al.*, 1998; Olesen *et al.*, 2000; Erkkilä *et al.*, 2001), the development of surface moulds (Bruna *et al.*, 2003; Sunesen *et al.*, 2003), additives and spices included in the meat dough formulation (Meynier *et al.*, 1999; Herranz *et al.*, 2005; Carrapiso, 2007).

After the production phase, to keep unaltered the qualitative characteristics until consume, appropriate packaging solutions are required. Among them, under vacuum packaging is commonly used to store the ripened sausages. The anaerobic conditions, however, can negatively affect the quality characteristics of the product. In fact, as underlined in a previous research by the same authors (Summo *et al.*,

2006), a 40-day vacuum storage of ripened sausages determined a worsening of their sensory properties.

The aim of the present paper was to evaluate, by SPME-GC/MS analysis, the evolution of the volatile compounds of ripened sausages during a 5-month vacuum storage.

MATERIAL AND METHODS

Twelve industrial sausages were sampled after ripening. Two samples (T0) were immediately analyzed, while those remaining were vacuum-packed with a polyethylene/polyester film (thickness 95 μ, permeability to oxygen < 15 cm^3 m^{-2} 24 h^{-1} at 20°C and 75% R.H., permeability to water < 1.5 g m^{-2} 24 h^{-1} at 23 °C and 85% R.H.). The vacuum-packed samples were stored at 4°C in the dark for 5 months, and analysed (two samples per storage time) after 1, 2, 3, 4, and 5 months of storage (T1-T5).

The determination of the volatile compounds was carried out by SPME-GC/MS, using a gas-cromatograph Agilent equipped by a mass spectrometer 5975C, a SPME fiber assembly 50/30 μm DVB/Carboxen/PDMS of Supelco, a Supelco capillary column SPB-624 30 m × 0.25 mm × 1.4 μm, under the following conditions: injection port temperature, 250°C; helium pressure, 30 kPa; oven temperatures, 40°C for 10 min then 5°C/min to 230°C and final isothermal for 10 min. Mass detector was set at the following conditions: detector voltage, 500V; interface temperature 250°C; source temperature 250°C; ionization energy 70 eV; emission 200 Å, scan range 30-270 amu. Peak identification was performed comparing retention times with those of the standards (purchased from Sigma-Aldrich) and by computer matching with the reference mass spectra of NIST and Wiley libraries. Data obtained were submitted to ANOVA and multiple comparison (Tukey test) by XLStat software.

RESULTS AND DISCUSSION

Figure 1 shows the evolution of the volatile compounds during vacuum storage of ripened sausages, grouped according to their origin (Viallon *et al.*, 1996; Sunesen *et al.*, 2001). Before being packed (T0), the sausages showed a head-space profile characterised by a high concentration of compounds derived from the added spices, as well as substances originated from carbohydrate fermentation. The oxidation compounds were detected at low concentrations (about 3%), while among those derived from acid esterification derived by microbial activity, the most abundant was ethyl acetate (data not shown).

During the whole storage period an evolution of the head-space was observed. In particular, the substances originated from carbohydrate fermentation showed an increase of their percent concentration, related to the activity of microflora in anaerobic conditions, reaching the maximum level at the fourth month of storage, with a value higher than 53%. Also the esters increased during storage, with the amount of 12% at the end of the considered period. Besides, a decrease of the level of compounds derived from spices added to the meat dough was observed. On the contrary, volatile compounds deriving from the oxidative phenomena raised their levels, with significant differences ($p < 0.05$) after the second month of storage.

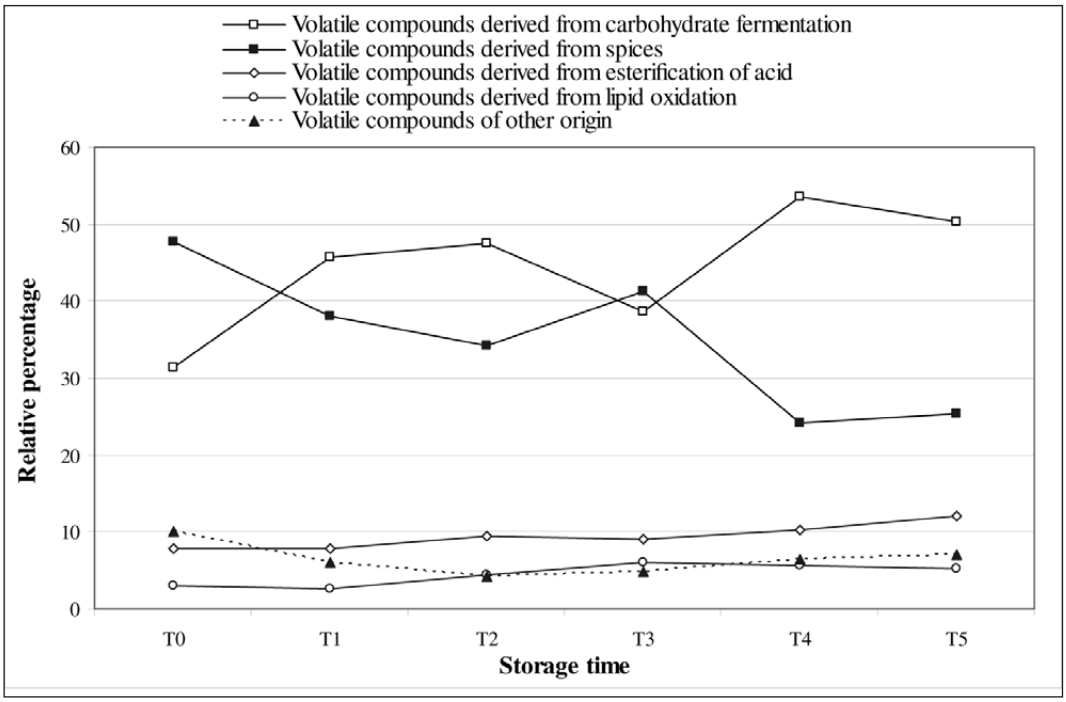

Figure 1. Evolution of the volatile compounds of ripened sausages during vacuum storage grouped according to their origin.

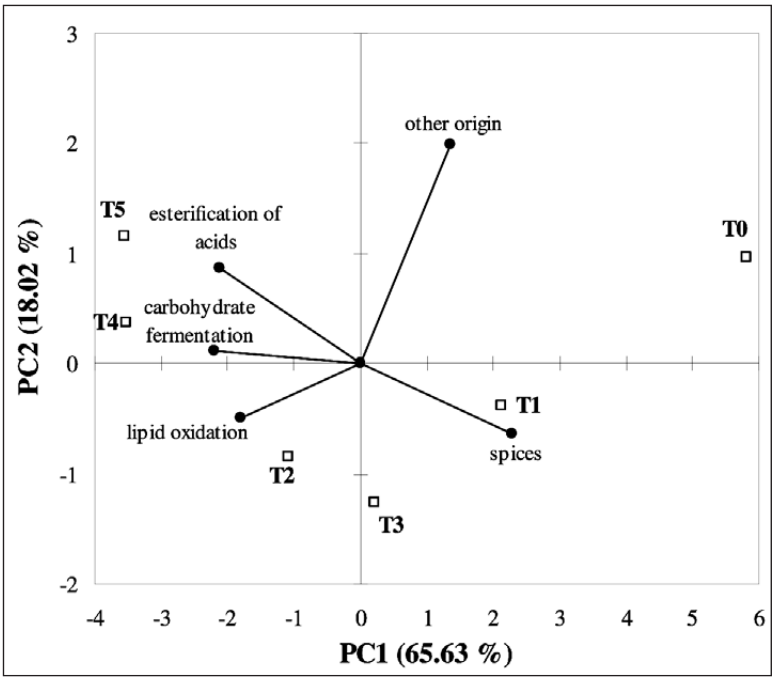

Figure 2. Principal component analysis of the volatile compounds in the ripened sausages during vacuum storage grouped according to their origin.

The latter compounds showed the highest concentration at the third month, with a value of 6.10%. Prolonging the storage time, the oxidation compounds showed a slight decrease not statistically significant. The evolution of the oxidative phenomena observed may be due to the oxygen present in the internal part of the sausages. It has to be remarked that the volatile compounds deriving from the oxidation of lipids, due to their low perception thresholds, are among the chiefly responsible of sensorial properties of meat products also at low concentrations (Montel *et al.*, 1998).

Figure 2 shows the results of the principal component analysis of the sausage samples as a function of volatile compounds, grouped basing on their origin. The first two principal components justified 83% of the observed variability. PC1, alone accounting for more than 65% of variability, resulted to be related to storage time. In particular, during the initial phase of ripening (T0 and T1) the sausages were characterized by high concentrations of volatile compounds derived from spices, while those stored for intermediate periods of time (T2 and T3) resulted to be characterized by the increase of volatile compounds due to lipid oxidation. Sausages stored under vacuum for prolonged periods of time (T4 and T5) were characterized by high levels of compounds derived from both carbohydrate fermentation and acid esterification.

In conclusion, the research evidenced the efficacy of SPME-GC/MS analysis in monitoring the evolution of volatile compounds during vacuum storage of ripened sausages.

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IMPORTANCE OF THE INFORMATION ABOUT THE SHELF LIFE OF FOOD FOR CONSUMERS

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ABSTRACT

The purpose of the research was to assess the importance of the information about the durability of a food product compared to other bits of information placed on the packaging or label of the product.

A questionnaire-based pilot survey was performed, on the basis of a unique questionnaire prepared by the authors for that purpose. The survey was related to the frequently purchased groups of products. The study focused on the factors determining the decision of the customers concerning the purchase, on the basis of the information available on the packaging. Then the hierarchy of importance of specific groups of information was determined, with particular attention being paid to the information about the durability of the product.

The presented research proved that comprehensive, reliable, legible and transparent information about the product is an essential element of the food security and of the decisions actually taken by the customers.

Key words: consumers' preferences, information function of packaging, quality of food product, shelf life of food.

INTRODUCTION

The trend to use the unit packaging of food products as a market message carrier is growing. The visual aspect of the packaging, by their labelling, becomes very important for the consumer and often determines the final purchase decision. The labelling shall mean any words, particulars, trade marks, brand name, pictorial matter or symbol relating to a foodstuff and placed on any packaging (AMPUERO *et al.*, 2005; LISIŃSKA-KUŚNIERZ *et al.* 2005).

The legal rules governing the labelling, with a general nature and applied horizontally to all the food products marketed in the EU are provided in Directive

2000/13/EC and Directive 2003/89/EC. The main objective of the legislation on food labelling are to (COMMISION STAFF WORKING DOCUMENT, 2008; DIRECTIVE 2000/13/EC; DIRECTIVE 2003/89/EC):
– enable consumers to make informed, safe, healthy and sustainable choices,
– provide consumers with relevant, useful and legitimately expected information,
– ensure the smooth functioning of the internal market.

The specific information provided on a compulsory basis during food product labelling includes the information about the shelf life a foodstuff. It is specified on the packaging as: the date of minimum durability lub use by" date. The date of minimum durability of a foodstuff shall be the date until which the foodstuff retains its specific properties when properly stored. The date shall be proceded by the words: "Best before..." when the date includes an indication of the day, or "Best before end..." in other cases. The words shall be accompanied by: either the date itself, or a reference to where the date is given on the labelling.

In the case of foodstuffs which, from the microbiological point of view, are highly perishable and are therefore likely after a short period to constitute an immediate danger to human health, the date of minimum durability shall be replaced by the "use by" date.

According to the research conducted in 2005 at the order of Burean Europeen des Unions de Consommateurs (BEUC), in such countries as Germany, Denmark, Spain, Hungary and Poland, the consumers, when purchasing food products pay a particular attention to the date of minimum durability or the 'best before' date (86% respondents), and are much less concerned with the brand (only 70%). According to the opinion of The European Commision Impact Assessment Board (IAB) of 2008, there are serious problems with the legibility of the information placed on the packaging (WWW.BEUC.EU, 2008; WWW.EC.EUROPA.EU, 2008).

Although the framework Directive 2000/13/EC requires that the mandatory requirements be easy to understand, market in a conspicuons place and in such a way as to be easily visible, clearly legible and indelible, there is widespread complaint that labels are neither legible or understandable. The most frequent complaint in particular is the size of the type face (DIRECTIVE 2000/13/EC).

SUBJECT AND METHODOLOGY OF THE RESEARCH

The purpose of the research was to identify the preferences of the consumers concerning the kind and form of the information which ought to be placed on the packaging of food products. The intent of the research was also to verify two hypotheses. The first assumed that the date of minimum durability is one of the most important bits of information placed on foodstuff packaging. The other alleged that such information is hardly visible and the consumers, in particular aged persons, have problems with locating it.

The research was based on a method combining a questionnaire with an interview. The respondents were selected according to the method of typical individuals selection. The survey included a group of so-called 'grey consumers', i.e. persons aged more than 45, who are not fully professionally active and have entitled to receive pension or retirement benefits. That group of consumers was chosen for this survey on purpose (ANTONIDES *et al.*, 1998; GROVER *et al.*, 2006; UNDERWOOD, 2003).

In Poland the group in question is growing, and according to a demographic forecast, in 2010 persons aged more than 45 will constitute almost 50% of the total population. The domestic publications provide no sufficient information as to the preferences of that group in respect of the labelling. Undertaking such research was even more important because of the fact that the international research proved that the perception of packaging by older consumers is different than with the other age groups.

The survey comprised 400 respondents living in Poland, in a large urban agglomeration and in the peripheries thereof.

The consumers' requirements with respect to information apply to any group of products, but particularly to those purchased routinely, therefore the survey was focused on the preferences related to the packaging of the so-called snack products. Products such as: chips, salt sticks, crackers, peanuts, bars etc. are known and purchased often, mostly because of their flavour value.

OUTCOME OF THE RESEARCH AND ITS ANALYSIS

To verify the preferences of grey consumers with respect to the labelling of snack food packaging, the researchers asked them firstly how often they bought such products. On the basis of the results obtained, we can state that such food is purchased by the grey consumers regardless of the age group and socio-economic factors (sex, place of residence, education, professional background and financial status) usually once a week (36%), or several times a week (23% respondents). 18 % respondents buy once a month or even more rarely, and every 15th examined consumer makes a purchase every day. It is thus evident that such products are quite often purchased and consumed in spite of the fact that could not constitute a key element of nutrition, because of health reasons.

In that group, salt sticks are the most frequently purchased product (57%), followed by the crackers (34%) and chocolate bars (31%). Besides the sticks, women buy chocolate bars and crackers more often, while men – peanuts and chips.

The research demonstrated that the three principal factors determining the consumers' decision about the purchase of the products in question are: price (76%), habit (69%) and 'best before' date (66%). Much less often the consumers base their decision on such factors as: promotion (29%), advertisement (26%) and various data on the packaging (25%).

Asked to rank the bits of information which they read on the packaging, the respondents recognised the date of minimum durability (74%), as the most important. The date was followed by: the product name /brand name/, business name (49%), calorific value (40%), the list of ingredients (38%), the net quantity (28%), caution e.g. allergenic ingredients (17%), any special storage conditions (10,5%), promotional marks (6%) and ecological marks (3%). The number of declarations concerning reading the 'best before' date is not dependent on the socio-economic factors. The only differentiation occurs with respect to the sex category: women declare to read such information more often (78%) than men (68%). The visibility of the 'best before' date is weak according to 68% respondents (font too small). For that reason, the respondents were asked to propose the area of the packaging where they wished to have such information. The majority of the respondents (61%) wished to have the date near the name of the product (that tendency becomes more marked in line with age). Also more than half (55%) of the respondents, in

particular those aged 45-50 (79%), accepted the date placed below the inscription „Best before". The consumers want that this bit of information – of key importance for them – stand out at once and be made distinct, so that they need not hesitate whether it is indeed the 'best before' date or the number of the lot (that was revealed by the interviews). Only every tenth respondent accepts the date in question being placed on the seam of the packaging, which is a customary practice among the manufacturers of the products. Among the elements of the packaging which according to the examined persons lead to a lack of legibility or to difficulties with deciphering the information placed on the packaging, the respondents emphasised the following: font size (70%), graphic elements (17%), packaging size (14%) and packaging structure (13%). Grey consumers were also asked whether the quantity of information placed on the packaging is adequate. As many as 70% respondents acknowledged it as adequate, this share increasing in line with the level of education. Only 7% declared that there was too much information (two times more men than women). The age group which estimated that the quantity of information was too large were the respondents aged 66-70 (as many as 23%). The research also demonstrated that the colour of the packaging, as background for all inscriptions, is important for older persons. More than 63% declared that they paid attention to the appearance of the packaging and to the colour. For women, the colour of the snack food packaging is more important than for men. As to the age categories, persons aged 51-59 pay the most attention to the external appearance, and the persons aged 66-70 – the least (only 41%).

CONCLUSION

The informative potential of the packaging is one of the material factors determining decisions about the purchase of a product. The information about the durability of the product placed on the packaging as the date of minimum durability is a very important piece of information for the consumers. Grey consumers believe that that aspect ranks as the third most important decision-making factor, after the price and the habit, during the purchase of a frequently bought food product.

The findings of the research confirmed that the consumers have many objections to the information conveyed by the packaging of the products available on the Polish market. A large fraction of the information is hardly visible and the consumers have difficulty in reading it. Among the bits of information which according to the grey consumers can be read only with difficulty (unclear, font too small) the 'best before' date ranked as the first. Recapitulating this analysis of the research conducted, we wish to point out that new forms of labelling and new plastic formats ought to be developed and designed, so as to identify the solutions best adapted to the expectations of older consumers. The plastic form of the packaging should be such as to attract the attention of the consumer and create a stimulus to purchase the product, without reducing the legibility and understandability of the information.

It is worth noting that IAB in Brussels is undertaking action in that respect, to be followed by legal acts aiming to minimise the less important information and ensure good visibility of those pieces of information which are important in the light of the consumer's interest protection (COMMISION STAFF WORKING DOCUMENT, 2008; WWW.DECISIA.FR, 2008).

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MODIFIED ATMOSPHERE PACKAGE DESIGN OF MINIMALLY PROCESSED BROCCOLI

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ABSTRACT

The aim of this work was to study the effect of the oxygen, carbon dioxide and temperature on the respiration rate of minimally processed broccoli in order to design a modified atmosphere package. The respiration rate of minimally processed broccoli were determined in a modified closed system at 5, 7, 15 and 20°C with 2, 5, 10, 15 and 21% of oxygen and nitrogen as balance. The respiration rate was also determined at 0, 10, and 20% of carbon dioxide in air at 15°C. The effect of temperature was also studied on product conditioned at 3%O_2 and 15% CO_2. Michealis and Menten and Arrhenius models were used to describe the effect of independent factors on product respiration rate. The maximum respiration rate estimated at reference temperature was 90±20 ml kg^{-1} h^{-1}, the Km value (the oxygen concentration at the average of the maximum respiration rate) was 1.65 ±0,04, and the Kmc value was 1.9 (R^2=0,99). Activation energy (Ea) of the respiration rate process was estimated at 81 kJ mol^{-1}. Results highlighted that the model is appropriate to design MAP for minimally processed broccoli. Nevertheless validation at more temperature must be performed. Moreover, the time to reach equilibrium inside the pack must be optimize to assure that the designed package can extend the product shelf life.

Key words: Modified atmosphere package, modelling, respiration rate; ready-to-eat broccoli.

INTRODUCTION

Modified atmosphere packaging (MAP) can potentially reduce the problems associated with processed fresh product, leading to significantly longer shelf-life. Nevertheless, improperly designed MAP systems may be ineffective or even shorten the shelf-life of fresh-cut products. Therefore, to ensure an optimal gas composition during product shelf life, an engineering approach has to be followed to model all the variables that play a critical role (Torrieri *et al.*, 2008). One of the most impor-

tant factor to package design is the respiration rate. Thus, the objectives of this work were: (i) to study the effect of the oxygen, carbon dioxide and temperature on the respiration rate of minimally processed broccoli; (ii) to verify the goodness of the Michealis and Menten and Arrhenius models in describing respiration rate under different O_2-CO_2 concentrations and different temperatures; (iii) to design a modified atmosphere package for the minimally processed broccoli.

MATERIALS AND METHODS

Broccoli (*Brassica Rapa var. Sylvestris*) were bought the day of the test by a local farmer, cleaned by using tap water and minimally processed before testing. The respiration rate (R) of the product were determined in a modified closed system at 5, 7, 15 and 20°C with 2, 5, 10, 15 and 21% of oxygen and nitrogen as balance and 0, 10, and 20% of carbon dioxide in air at 15°C. The effect of temperature was also studied at 3% of oxygen and 15% of carbon dioxide. The effect of oxygen, carbon dioxide and temperature was described by Michealis and Menten competitive model with the parameters depending by the temperature following an Arrhenius type relationship (eq.1) :

$$RO_2 = \frac{R_{\max_0} \exp\left(-\frac{Ea_1}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)\cdot yO_2}{k_{m_0}\exp\left(-\frac{Ea_2}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)\cdot K_{I_0}\exp\left(-\frac{Ea_3}{R}\left(1-\frac{yO_2}{K_I}\right)\right)+yO_2} \quad (1)$$

Model parameters were estimated by non linear regression analysis by means of last square methods.

The respiration rate (RO_2) at the equilibrium gas composition (3% O_2- 15% CO_2) and film permeability (KPO_2) were used to optimize the product weight to be packed to reach the optimal gas composition. Thus, product weight (M) was calculated by solving:

$$\frac{kP_{O_2}\cdot A}{X}\cdot\left(y_{O_2}^{out}-y_{O_2}^{eq}\right)=M\cdot R_{O_2} \quad (2)$$

To validate the model, the O_2 and CO_2 variation in the package head space were predicted solving the mass balance around the package by using as product input the above selected product weight and the Michealis and Menten and Arrhenius parameters. The dynamic exchange of O_2 and CO_2 inside the package was modeled with the symbolic language of dynamic systems by using SIMILE modeling environment (www.simulistics.com). The prediction was then compared with experimental data obtained by packaging the product and monitoring the head space composition (O_2-CO_2) during storage time at constant temperature.

RESULTS

Figure 1 shows O_2 consumption rates (RO_2) of minimally processed broccoli stored at 5, 7, 15, and 20°C as a function of the oxygen concentration (%) in the jar head

Table 1. Respiration rate of minimally processed broccoli at 3% O_2 and 15% CO_2 at 15°C

T	RO_2	RCO_2	RQ
5	15±6	24±10	1
7	28±6	22±7	0.8
15	55±10	40±11	0.7
20	99±9	72±10	0.7

Table 2.Parameters of MAP design

	Constant Tref=12.5°C)	Ea (kJ/mol)
Vmax	90	81
km	1.65	16
kmc	1.9	24

space. The oxygen consumption rate increases with O2 concentration and decreases with the temperature decreases. In figure 1 the solid lines represent the respiration rate as predicted by the Michaelis and Menten model.

The agreement with our experimental findings is very satisfactory (0.94<R2adj<0.99).

Temperature affects respiration rates much more than oxygen concentration: increasing the temperature from 5 to 20°C results, for samples stored in air, in a 87% change in RO_2, whereas the respiration rate decreases by 60% on lowering the O_2 concentration from 21% to 2%. Carbon dioxide has a significant effect on broccoli respiration rate. At 15°C by increasing carbon dioxide from 0% to 20% the respiration rate decrease by 30% (data not showed). To study the combined effect of oxygen and carbon dioxide, the respiration rate was also determined at 3% of oxygen and 15% of carbon dioxide (Table 1). Respiration rate increase as temperature increase by 85%. The effect of MAP is more evident at high temperature. At 20°C the respiration rate decreased due to MAP by 37%, whereas no effect were observed at 5°C. A slight decreases of RQ were observed as the temperature increased from 5°C to 20°C. Figure 2 shows the dependence of Michealis and Menten parameters from temperature. The activation energy (Ea) of the respiration rate process and of the inhibitions effect and the Michealis and Menten parameters at reference temperature are reported in table 2. The Arrhenius-type relationship was then included in the Michealis and Menten competitive model to allow for the variation of parameters with temperature. Figure 3 shows the change in O_2 and CO_2 concentrations versus time inside the permeable package containing minimally processed broccoli stored at 5°C. The figure also shows the gas composition changes as predicted by the model (eq.1). The product mass to be packed to reach an equilibrium gas composition of 3% of O_2 and 15% of CO_2 is 50g. The model predicts reasonable well experimental data, but equilibrium time must be optimized.

In conclusion, the respiration rate of minimally processed broccoli is well de-

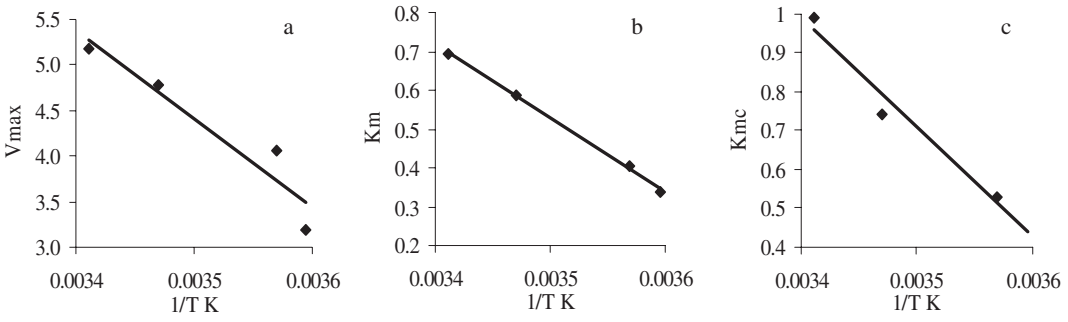

Figure 2. Effect of temperature on Vmax (a) km (b) and Kmc (c)

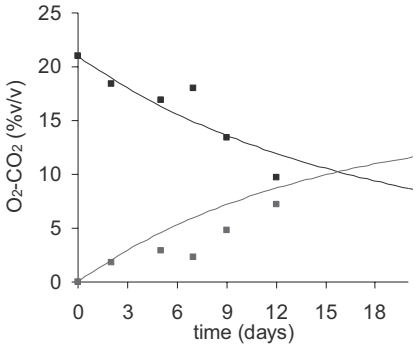

Figure 3. O_2 and CO_2 exchange in the headspace of broccoli MAP

scribed by a Michaelis-Menten competitive model combined with an Arrhenius-type equation. Preliminary results showed that the model is appropriate to design MAP for minimally processed broccoli, but validation at more temperature must be performed. The time to reach equilibrium inside the pack must be optimize to assure that the package extend the product shelf life.

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FOOD SHELF LIFE ISSUE IN THE ASPECT OF THE MANUFACTURER -MARKET-CONSUMER RELATIONSHIPS

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ABSTRACT

The increasingly higher demands and preferences of consumers, resulting foremost from economic development and the faster pace of life, as well as the need to meet consumer protection policy objectives and intensifying competition, have induced food producers to introduce high quality products to the market in attractive packaging that effectively ensures a long shelf-life.

In the economic terms, the issue of the food shelf life can also be considered from a narrower perspective, that is, in view of the incurred costs directly affecting the obtained economically justified durability of food, as well as from a wider perspective – taking into account all the market actors involved, which in a market economy environment becomes particularly important.

The purpose of the paper is to present the problem of food durability against a context of shaping the relationships of the kind manufacturer-market-consumer. The author have proposed a model of customers' expectations and experiences related to durability.

It has also been pointed out that such relationships are strongly connected with the degree of market success of the product and of the manufacturer, and with the implementation of the customer protection policy.

Key words: consumer protection policy, relationships of the manufacturer-market-consumer, shelf-life of food.

INTRODUCTION

The quality of food can be defined as the sum of those properties of a food article which determine its fitness for the satisfaction of the needs of a contemporary consumer. Those needs can be met if the product fulfils the requirements of the regulations of law in force, has the nutritious value desired by the consumer, is

compliant with the health requirements, has the expected technological value and the desired sensory properties throughout the period of shelf-life (COLES, 2005; TAUB *et al.*, 1998).

On the basis of the findings of research presented in trade publications, by Cardelli and Labuza (Cardelli *et al.*, 2003; Labuza, 2000), we can state that the properties of food most important for the consumer at the time of consumption include: external appearance, taste and aroma, which determine the sensory quality of a food product. The key factors affecting the sensory quality of the products include: level of original quality of the product, kind of packaging and the conditions of storage of the packaged product. In compliance with the description of those quality properties of food which influence the expectations and the perception of the consumer, proposed by Van Trijp and Steencamp (Van Trijp *et al.*, 1998) as well as Poulsen (Poulsen, 1996), the durability together with the product properties, health safety, and convenience of use, are classified as the internal quality properties of the product, while the properties of the production system, environment-related aspects and marketing constitute the external quality properties of food (Lisińska-Kuśnierz *et al.*, 2006; Luning *et al.*, 2002). Thus, one can affirm that the durability is one of the essential elements affecting the opinions of the consumer about the quality of the product and about the perception of the manufacturer on the market.

MODEL OF THE EXPECTATIONS AND EXPERIENCES OF THE CONSUMERS WITH RESPECT TO THE SHELF-LIFE OF FOOD

While analysing the issue of the durability against the background of developing relations of the type manufacturer-market-consumer, it is advisable to take into account the expectations and experience of the consumers with respect to the durability, because that aspect is closely related to the success achieved on the market by the product and its manufacturer, and to the realisation of the consumer protection policy (Lisińska-Kuśnierz *et al.*, 2000).

Considering the principles of the leading quality model developed by Van Trijp and Steenkamp (Van Trijp *et al.*, 1998) and modified by W.M. Jongen (Jongen, 1998), and taking into account the fact that the durability is one of the material quality properties of a food product, a model of the consumer expectations and experiences regarding the shelf-life of food was proposed in figure 1.

According to that model, the evaluation of the food durability by consumers occurs in two stages. At the moment when they make their choice and then purchase a food product, they express their expectations with respect to the durability of the packaged product. The proper durability is subjected to verification only during the consumption. The expectations with respect to the durability constitute an important factor in the behaviour of a consumer while making the choice among products, and the experience gained from the evaluation of the durability is indispensable while making a subsequent purchase, and acquiring the habit of selecting a given product and manufacturer.

The proposed model assumes that the evaluation of the expected durability may be affected by external and internal indicators. The external indicators are supplied principally in the information about the product durability placed on the packaging or on the label affixed to the product, the packaging system used and the construction form of the packaging, as well as the price of the product

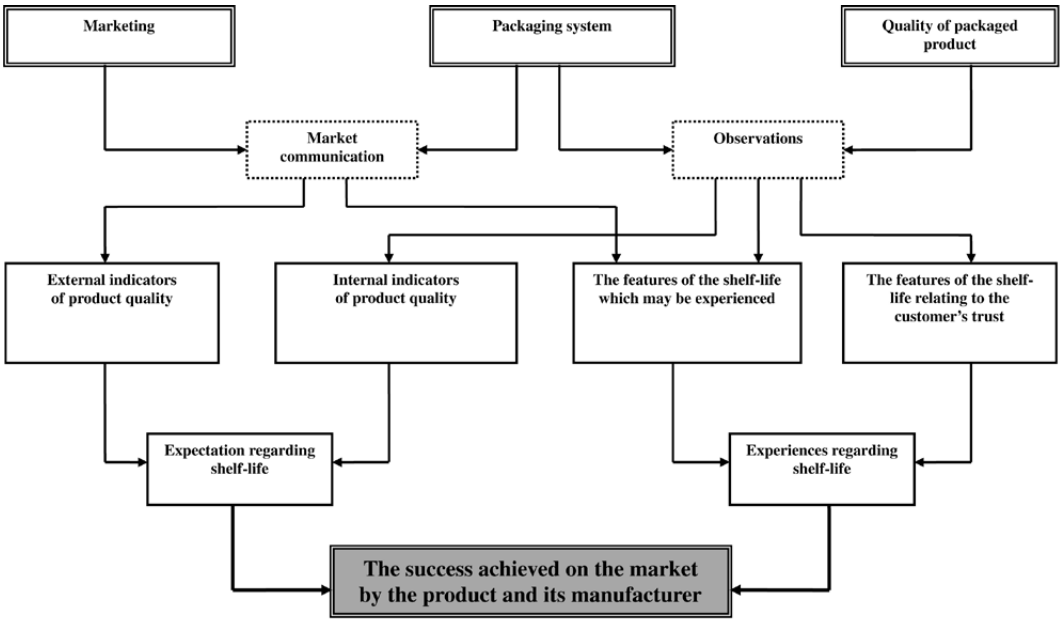

Fig. 1. A model of the consumer expectations and experiences regarding the shelf-life of food
Source: autor's work based on (JONGEN, 1998; LUNING *et al.*, 2002; VAN TRIJP *et al.*, 1998).

and the trade mark. The internal indicators relate to the product appearance, its colour or aroma.

On the basis of the research conducted by Steenkamp (Steenkamp, 1989) we can affirm that the proper experience of the durability is based on the combination of the perceived features related to the durability, where we distinguish trust and experience. The features of the durability which may be experienced are those to which the consumer can verify in the course of the consumption of a food product, for instance, flavour and aroma. The features relating to the customer's trust to the manufacturer, such as absence of additives or friendliness for the natural environment, cannot be verified by means of the personal experience of the consumers.

Alongside the observations of the customers themselves, the efficacy of the market communication is also a key element affecting both the expectations and the experience related to the durability. The process of the market communication is based mainly on the reliable information, broadcast with a specific purpose.

The emitter, operating on the supply side of the market, is concerned with sending a system of signals which would lead to a programmed response of the recipients for whom it was intended. The response to the information broadcast is a specific behaviour of the target recipient, providing a feedback information for the sender. The market communication is therefore based on the movement of at least two information flows, one from the sender to the recipient, and the other, constituting the result of the response to the broadcast message, from the recipient to the sender (Ucherek, 2006).

CONCLUSION

The essential purpose of the market messages sent is always the effort to draw the attention of the potential recipient (consumer), provoking his interest and activating his ability to memorise, liberating his need to possess, and then inciting him to take action in order to satisfy such need.

It is also important that the purchaser be convinced that his choice was appropriate and that he received full satisfaction as a result of that specific choice. If the consumers' expectations with respect to the durability are corroborated through their experience, then such situation constitutes a basis for market success achieved by the product and by the manufacturer, thanks to a reliably determined durability limit and to the efficient realisation of the marketing efforts in that respect.

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TEST OF THE SCOPE AND INTENSITY OF THE CHANGES OF PERMEABILITY OF OXYGEN THROUGH THE MODERN PACKAGING MATERIAL

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ABSTRACT

The purpose of the research undertaken was an analysis of the scope and dynamics of the changes in the barrier properties of the modern packaging materials used for packaging of peanuts, in the course of the storage of that product in varying microclimatic conditions. The object of the research was the laminated foil: (PETmet/LDPE).The determination of the oxygen permeability was made by means of OX-TRAN 100. The above analysis enables us to state that the use of the packaging materials with good barrier properties, in particular with respect to oxygen, and meeting the quality requirements provided in technical specifications, is a guarantee of a proper fulfilment of the protective function of the packaging which prevents the interference of external factors with the micro-climate inside the packaging, and as a result, reduces the risk of unfavourable changes in the packaged product.

Key words: barrier properties, packaging materials, transport of oxygen, varying microclimatic conditions.

INTRODUCTION

During storage and distribution, foods are exposed to a wide range of environmental conditions. Environmental factors such as temperature, humidity, oxygen and light can trigger several reaction mechanisms that may lead to food degradation. As a consequence of these mechanisms, foods may be to such an extend that they are either rejected by the consumer or they may become harmful to the person consuming them (Man *et al.*, 2000).

Among the technical properties, characterising the usefulness of the modern packaging material for the protection products quality, one should identify and analyse those properties which determine the scope of the changes occurring in the configuration: conditions environment – packaging material – crypto-climate in the packaging – changes in the product. The properties concerned include: barrier properties, in particular the oxygen- tightness. The transport of oxygen through the packaging materials, its mechanism and rapidity of the process depend on a multitude of elements, first of all on the chemical structure (Fishman *et al.*, 1996; Lisińska-Kuśnierz, *et al.*, 2005).

EXPERIMENTAL

The experimental material was the laminated foil PETmet/LDPE, with inter-layer print thick (thickness: 0,90 m^{-4}; basis weight: 83,90 g/m^2; the tensile load in length: 32N; the tensile load in breadth:35N; tensile strength in length: 26,5 MPa; tensile strength in breadth: 30 MPa; elongation in length: 50%; elongation in breadth: 46%, water vapor permeability: 1,40 g/m224h, oxygen permeability: 1,00 $cm^3/m224h0,1MPa$). That packaging material met the quality requirements determined in the Company Normalization Document (Company Normalization Document, 1995).

The aim of this work was to assessment the changes in the oxygen permeability (Pt) by packaging material. The determination of the oxygen permeability was made by means of OX-TRAN 100 (Research Procedure, 2004).

In view of the fact that one of the research objectives was to analyse the impact of the storage conditions on the scope and intensity of the changes of the above quality parameter of packaging materials, the tests were made in the course of such storage, in varying microclimatic conditions. The storage conditions applied in the research are as following (British Cellophane Limited, 1982; D/NI-04-04-01, 1997; PN-ISO 554, 1996; Lisinska-Kuśnierz, *et al.*, 2007).

Condition A: T=230C±2oC, RH= 50±5%, research cycle: 4 weeks, time of storage: 52 weeks;

Condition B: T=180C±1oC, RH= 75±2%, research cycle: 4 weeks, time of storage: 52 weeks;

Condition C: T=280C±1oC, RH= 75±2%, research cycle: 1 week, time of storage: 13 weeks;

Condition D: T=380C±1oC, RH= 85±2%, research cycle: 1 week, time of storage: 13 weeks.

In the case of the B, C and D scenarios, the storage tests were performed on the basis of the air conditioning chambers type KPW-1/4 (Quality Estimation of Packaging Materials and Packaging, 2005).

The statistical analysis of the obtained results was carried out using the appropriate modules of the Statistica 6,0 software. The average values, calculated on the basis of the results showing the changes of oxygen permeability (Pt) by analyzed material which are presented in table 1, were used to calculate the functions mapping the development of such changes during storage (Zelias *et al.*, 2004).

The formal models reflecting the dependence of the changes in the oxygen (Pt) permeability through the analysed laminate covered with a metallic layer, from the time of storage in varying micro-climatic conditions, were polynomial functions of the second and third degree, presented in table 2. For the oxygen permeability only

Table 1. The scope and intensity of the changes of the oxygen permeability (Pt) by packaging material PETmet/LDPE during storage in different storage conditions.

Kinds of storage conditions	Research cycle [week]*													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
The changes of the Pt during storage, $cm^3/m^224h0,1MPa$														
A	1,00	1,00	0,88	0,90	0,92	1,00	0,95	1,00	0,95	1,00	0,95	0,88	0,95	0,90
B	1,00	1,00	0,90	0,92	0,90	1,05	0,90	1,00	0,88	0,90	0,90	0,88	0,90	0,85
C	1,00	1,00	0,90	1,00	0,95	1,05	0,95	1,00	0,95	1,05	0,90	0,85	0,85	0,80
D	1,00	1,05	0,88	0,95	0,90	1,00	0,95	0,78	0,80	0,90	0,90	0,80	0,90	0,88
The indices of dynamics of the changes of the Pt during storage, %														
A	100	100	88,0	90,0	92,0	100	95,0	100	95,0	100	95,0	88,0	95,0	90,0
B	100	100	90,0	92,0	90,0	105,0	90,0	100	88,0	90,0	90,0	88,0	90,0	85,0
C	100	100	90,0	100	95,0	105,0	95,0	100,0	95,0	105,0	90,0	85,0	85,0	80,0
D	100	105,0	88,0	95,0	90,0	100	95,0	78,0	80,0	90,0	90,0	80,0	90,0	88,0
* Kinds of storage conditions: A, B - 4 weeks; C, D - 1 week														

Source: authors' work.

Table 2. Functions approximating the changes of the oxygen permeability (Pt) by packaging material PETmet/LDPE during storage in different storage conditions.

Kinds of storage conditions	Regression equation	Coefficient of determinatio n. R^2 (%)	Mean error of predicte se	Coefficient of variation Ve (%)
A	Pt = 0,9906 – 0,0388t + 0,0080t2 – 0,0004t3	61,10	0,04	4,22
B	Pt = 0,9832 – 0,0190t + 0,0009t2	69,44	0,0489	5,27
C	Pt = 0,9566 + 0,0204t - 0,0024t2	60,12	0,05	5,28
D	Pt = 0,9678 – 0,0272t + 0,0016t2	66,64	0,0624	6,88

Source: authors' work.

60-70% of the variability of that dependent variable is explained by the variability of the time of storage. However, the se and Ve values are so low (below 7%) as to enable us to state that the accuracy of the estimation is satisfactory.

The analysis of the models of functions illustrating the changes in the oxygen permeability in the course of storage presented in table 1 points to a similarity of the process mechanism regardless of the micro-climatic conditions of storage. The scope of the above changes remains limited. The indices of the dynamics of changes Pt in the course of storage calculated in relation to Pto in the case of normal conditions storage and standard conditions storage fall into the range of about 10% , while for accelerated conditions – about 15% Pto, as shown in table 1.

CONCLUSION

The effectiveness of the modern packaging system is determined principally by

the technical properties of the packaging material used. That material is a key factor in guaranteeing that the packaging fulfils the function of protecting the desired level of quality of the product – mainly thanks to the adequate barrier property such as the oxygen permeability by packaging material, air-tightness and mechanical resistance of the packaging. The properties of the packaging material, and their stability potential when exposed to external factors, determine the character and pace of the changes occurring in the product, by their influence on the packaging crypto-climate, which becomes particularly important in the case of packaged food products.

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OPTIMIZATION OF BLUEFIN TUNA FISHBURGER MAP: MIXTURE DESIGN AND TRIANGULAR SURFACE ANALYSIS

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ABSTRACT

This work is part of a project aimed at exploiting the edible part of bluefin tuna scrap by using them to produce fish burgers. The objective of the work was the optimization of bluefin tuna fishburger MAP by means of a mixture design and a triangular surface analysis. As dependent variable tuna fish burger colour (chromatic coordinate a*) was used. A linear constraints design was chosen and vertex and centroid points were selected (D optimal design). A quadratic model was selected as the most appropriate to fits the dependent variable response surfaces. The fat oxidation was also studied. Results of this preliminary work showed that, starting from tuna processing wastes, it can be realized a minimally processed seafood, as tuna fish burger. Mixture design and triangular surface analysis can be a good tools for gas composition optimization. High oxygen concentration and high level of carbon dioxide seems to prolong the product shelf life up to 9 days at 3°C.

Key words: tuna fish burger; waste re-use; modified atmosphere packaging; shelf life; mixture design and triangular surface analysis.

INTRODUCTION

This work is part of a project aimed at exploiting the edible part of bluefin tuna wastes for fish burger's production. The idea came from the recent positioning of sea cages for Mediterranean Bluefin Tuna (*Thunnus thynnus*) on-growing along

the Campania coasts. Actually, bluefin tunas are exclusively sold on the Japan markets and used as fresh product. Nevertheless, tuna processing leaves unused most parts of biomass (more than 50%). This parts are special wastes and so represent an additional cost (Art. 7 comma 3 del D.Lgs. 22/97). From the tuna head it can obtain about 30% of meat, that can be usable as ingredient for some peculiar processed seafoods. In Italy, the processed seafood's market is mainly represented by tuna in oil packed in glass jar or canned. Fresh fish burger could respond at the growing market of minimally processed food at high convenience level.

Nevertheless, fresh fish burger is a high perishable product and an appropriate MAP must be developed to extend product shelf life (Davis, 1999). Thus, the objective of this work was to optimize the gas mixture composition of fresh fish burgher MAP by using a mixture D-optimal design and triangular surface analysis. Because one of the major alteration process is the myoglobin oxydation and color is the most important quality index that affects consumer's preferences at purchasing time, color attribute was used as response variable during the optimization process.

Figure 1. Tuna fillet processing

Table 1. Experimental design

Samples	O_2	CO_2	N_2
1	20	0	80
2	20	0	80
3	60	20	20
4	60	20	20
5	60	40	0
6	60	40	0
7	60	40	0
8	40	60	0
9	60	0	40
10	60	0	40
11	20	40	40
12	20	40	40
13	20	40	40
14	20	20	60
15	40	30	30
16	40	30	30

MATERIALS AND METHODS

Bluefin Tunas (Thunnus thynnus) were ongrown during summer 2007 in a sea-cage near Procida island. Fillets were cut from the head of captured tunas and stocked at -20°C after bleeding in cold salt water. Frozen tuna samples were cut manually in small pieces (figure 1). 50 g of meat were placed between two permeable sheets of polymeric material and pressed gently to give the burger the required form. Fish burgers were modified atmosphere packaged (two per pack) in polyester tray by using a barrier film (Table 1).

Experimental design

To study the effect of oxygen, carbon dioxide and nitrogen on fish burger color during shelf life, a linear constraints design was chosen and vertex and centroid points were selected (D optimal design) (Table 1). A total of 16 samples were prepared and analyzed at different time (0, 1, 2, 5, 7, 9, 12 days) during storage at 3°C. The colorimetric parameter a* was used as response variable to optimize gas composition. Optimization was performed at 9 days of storage. The following quality indices were also monitored: (i) Gas composition (O_2-CO_2%); (ii) Weight loss; (iii) pH; (iv) MDA (nmol/g dw). The effect of time on *quality indices* was studied by ANOVA.

Figure 2. Tuna fish burger packed in MAP at different storage time: fresh sample (a); sample packed with 60% O2 and stored for 9 days (b); sample packed with (60%O_2 - 20%CO_2) and stored for 9 days.

RESULTS

Initial gas composition did not exchange during storage time ($p>0.05$). This results let suppose that tuna fish burger were low contaminated. Microbiological analysis have to be performed to confirm this hypothesis. ANOVA showed that storage time and gas composition have not effect on pH. The pH average value was 5.6 for all samples. All samples showed a weight loss during storage time. Samples stored in 60% O_2 - 40% CO_2 showed 0,37% (day 9) of weight loss whereas the maximum weight loss was observed for samples stored at 20%O_2-20%CO_2-60%N_2 (2,83% at day 9). Figure 2 shows the visual aspect of samples during storage time. As function of gas composition and time, the color changed from bright red (a) to a dark red (b) and, for same samples, to a yellow/red color (c). Figure 3 shows the response surface of colorimetric parameter a* at 9 days of storage modelled by using a quadratic polynomial model (a). Numerical optimization was performed and point prediction are reported in figure 4. From the triangular surface analysis, the best gas composition to optimize the colorimetric a* parameter after 9 days is 60% O_2 and 40% N_2. Results showed that triangular surface analysis can be a good tools for

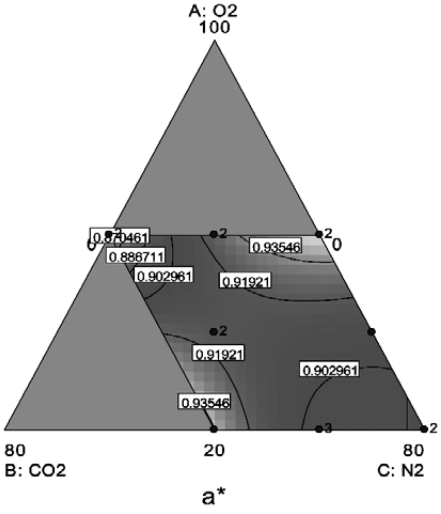

Figure 3. Triangular response surface of a* as function of gas composition

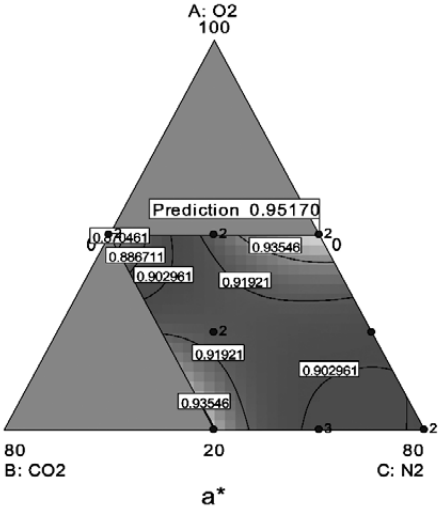

Figure 4. a* predicted by the model in order to optimize the value at 9 days.

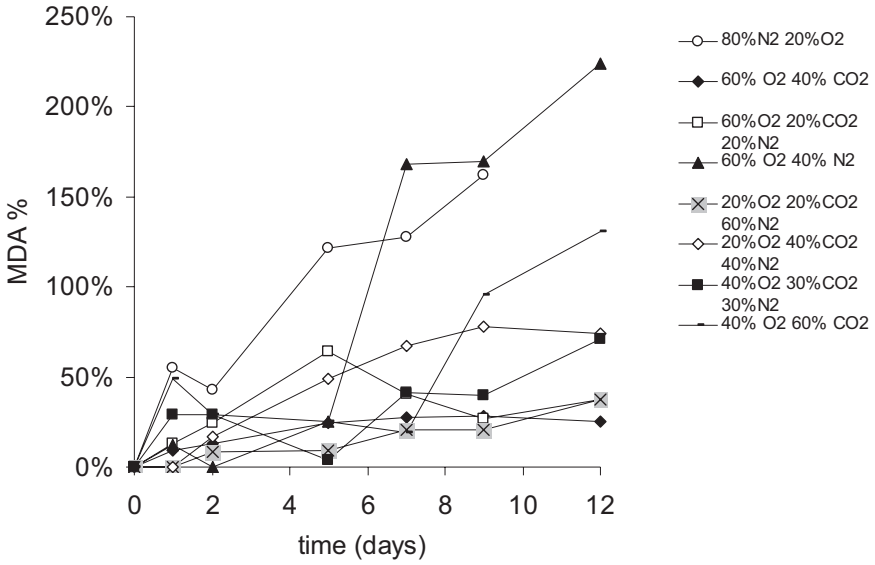

Figure 5: MDA vs. time of tuna fish burger packed in MAP and stored at 3°C.

gas composition optimization. Nevertheless, results are just preliminary to conclude that the gas composition identified is the optimal one to extend product shelf life. Figure 5 shows the oxidation results (MDA) as function of time of MAP samples. MDA results shows that the gas composition identified as the optimal one to optimize a* determined a fast oxidation of the sample, whereas the best atmosphere seems to be the one composed by 60% of oxygen and 40% of carbon dioxide.

In conclusion, the results of this preliminary work showed that, starting from tuna processing wastes, it can be realized a minimally processed seafood, as tuna fish burger.

Mixture design and triangular surface analysis can be a good tools for gas composition optimization. High oxygen concentration and high level of carbon dioxide seems to prolong the product shelf life up to 9 days at 3°C. Future works will be performed to optimize gas composition as function of all quality indices.

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ANALYSIS OF THE ESSENCE OF THE FOOD SHELF LIFE CONCEPT

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ABSTRACT

A generally acceptable definition of the food shelf life is yet to be developed. In the FAO/WHO documents, as well as in the UE legislation, the durability as such is not defined, instead, a concept of durability appears in one of the other definitions – of the possible manners of putting date labels on food products.

The purpose of these considerations is to present the diversity of the manners in which the concept of the food durability can be construed, and indicating the major factors which determine the durability of food. This is why different definitions of the food durability are quoted. On the basis of the analysis of the related trade publications it was found that the essence of the food durability concept, irrespective of the manner in which it is worded, is not more than the time span during which the product retains a determined level of quality, acceptable for the consumer, and depending from the nature of the packaged product.

Many factors affect the durability of food. Their aggregate influence has to be taken into account in any research aiming at determining such durability.

That is why this study focuses also on the mutual interconnections of the factors affecting the shelf life of a food product.

Key words: definition of the shelf life, factors affecting the shelf life of food, quality of food.

INTRODUCTION

Different definitions of the food durability concept can be found in the trade publications. For instance, the Institute of Food Technologists (IFT) defines "food durability as the period between the moment the product is manufactured and the moment when the same product is purchased in retail sale, during which time the product retains an acceptable level of quality with respect to nourishing value, taste, texture and appearance" (Robertson, 2000). An alternative definition is that "shelf life is the duration of that period between the packing of the and

its use, for which the quality of the product remains acceptable to the product user" (HINE, 1987).

According to M. Jaszewska and I. Fechner "the durability of a food product is a span of time from the manufacturing of such product until the moment when in determined conditions of storage the changes occurring in the product lead to the loss of specific properties, lower its physical-chemical or microbiological quality indicators below the limits set forth in the relevant standards, or lead to the loss of the consumers' acceptance for its properties" (Jaszewska, *et al.*, 2003).

On the basis of the above considerations, one can state that the essence of the food durability concept consists in the product potential to maintain a certain level of quality which is acceptable for the consumer, such level of quality being conditioned by the character of the packaged product. The measure of a food product durability is the period of time starting with the manufacturing of the integrated product, during which the product maintains a specific level of quality (Labuza, 2000; Ucherek *et al.*, 2005).

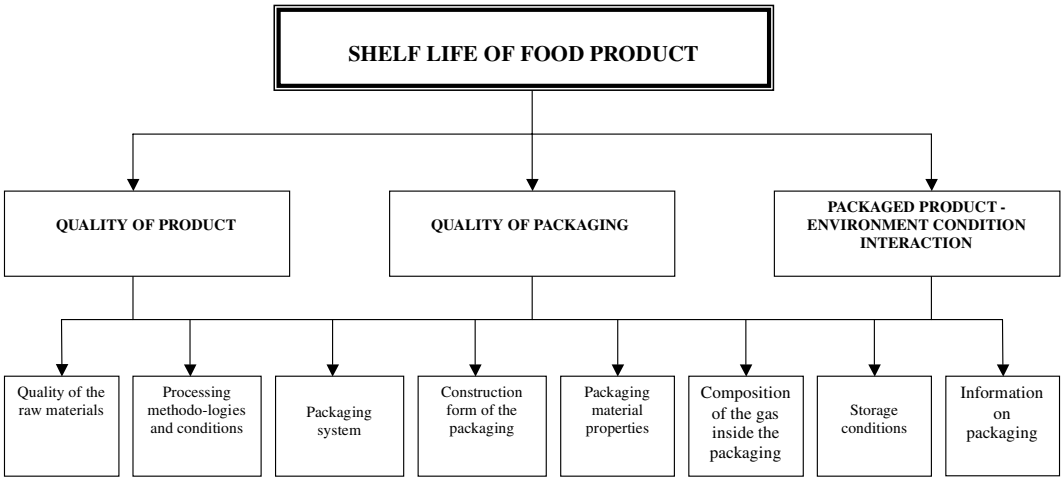
FACTORS DETERMINING THE SHELF-LIFE OF FOOD PRODUCTS

Many factors determine the durability of food. Their combined impact has to be taken into account in the research intended to help extend the durability of packaged products and forecast their period of durability. They are parts of a system which is composed of: a product with specific properties, packaged in a packaging unit with specific construction form, dimensions, barrier properties, mechanic resistance and other known quality parameters, a crypto-climate filling the space between the product and the packaging, and the external environment in which the packaged product is stored. The consequences of the complex interactions of all the factors operating on the product determine its durability (Lisińska-Kuśnierz, 1999).

The trade publications provide varying classifications of the above factors and the rank which the packaging occupies among them. That is due to the diversification of the functions and tasks fulfilled by the packaging. Thus, the role of the packaging in the protection of the durability of the packaged product is unquestionable. After a review of the trade publications, we can propose the following system of principal factors affecting the durability of foodstuffs (Brody, 2003; Lisińska-Kuśnierz, *et al.*, 2003; Robertson, 2000; Steel, 2004; Ucherek, 2007):

- quality of product,
- quality of packaging,
- packaged product-environment condition interaction.

The above key factors have to be considered on the basis of a comprehensive approach, combined with the analysis of the criteria elements applied in the assessment of their usefulness for a specific product. The most important criteria elements affecting the durability of the packaged product are: quality of the raw materials, processing methodologies and conditions, packaging system, construction form of the packaging, packaging material properties, composition of the gas mixture inside the packaging, storage conditions, and a reliable and transparent information about the storage conditions and product durability, placed directly on the packaging or on the label attached thereto. The interdependencies of the factors influencing the product durability with the individual criteria elements applied in the assessment of their usefulness have been presented on fig. 1 (Brody, 2003; Lisińska-Kuśnierz, *et al.*, 2003; Robertson, 2000; Steel, 2004; Ucherek, 2007).



Source: autor's work.

Fig. 1. The interdependencies of the factors influencing the product shelf-life with the individual criteria elements applied in the assessment of their usefulness

If a suitable level of food durability is to be guaranteed, not a single one of the above factors is to be omitted or treated lightly. Thus, in the research on the food products durability one ought to take into account the quality of the packaged product in combination with its internal and external environment.

CONCLUSION

A study of the trade publications led to the conclusion that there is no one unambiguous definition of the food durability concept, and the essence of that concept, irrespective of the definition proposed, can be reduced to the period during which the product maintains a determined level of quality, acceptable by the consumer, conditioned by the character of the integrated product.

It is also worth noting that the proposed system of key factors determining the durability of packaged products in connection with criteria elements used in their selection for a specific product should constitute a point of departure for any research of the food products durability.

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PROCESSING AND SHELF LIFE OPTIMIZATION OF OSMO-AIR-DRIED CRISPY APPLE RINGS

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ABSTRACT

New interest has recently arisen in the field of dehydrated apple products, used mainly as snack food. Crispness is the main quality parameter to be considered, as, for this kind of product, the consumer is more sensitive to differences in texture than in taste. In a previous study was evaluated the influence of different pre-treatments applied before air drying, as well as of the cultivar on the crispness of apple rings. The results obtained, using a specific chemiometric technique (Design of Experiment), evidenced that Golden Delicious is the most suitable apple cultivar among the ones considered and that a combined osmo-air drying process allowed dried apple rings to be obtained with limited structural collapse.

The aim of this work was to set a critical moisture content value above which osmo-air-dried crispy apple rings are no longer acceptable to the consumer. Furthermore, the feasibility of a correlation between the sensorial threshold and a crispness index value, obtained by a mechanical test, was evaluated.

Organic apple rings of cv Golden Delicious were dipped for 60 minutes in a concentrate sugar mixture (a_w=0.90) of fructose, sucrose and glucose in the same proportions as in the fresh apple, and then air dried at 80°C until constant weight was achieved. Water vapour sorption under various relative humidities was determined by monitoring moisture content over time. Apple rings not pre-treated were air-dried and monitored as a control. Crispness was assessed by means of both a bending-snapping test and a parallel sensory analysis.

Key words: adsorption isotherms, apple rings, crispness, osmo-air dehydration, texture.

INTRODUCTION

In the last few years functional products are of great interest to many research groups, due to an increasing demand for healthy, natural and tasty fruit-based innovative products. An example that could satisfy all these requirements are crispy dried apples, perceived as a healthy snack food. However, air drying of vegetable tissues involves the removal of large amounts of water and is characterized by extensive shrinkage and microstructural changes. Osmotic dehydration applied prior to air drying could improve final product quality, promoting great changes in tissue structure (Del Valle, Cuadros & Aguilera, 1998; Lewicki, 1998). In obtaining a long-lasting crispy product it should be considered that a low-moisture food is an heterogeneous system, consisting of many microregions with different compositions and concentrations, not necessarily in an equilibrium state (Aguilera & Stanley, 1999). So a change in microstructure could affect the consumer's quality perception, affected more by differences in texture than in taste, especially for snack food items, as demonstrated by Shewfelt (1999). Understanding water absorption in dried apples during shelf life is of utmost importance since it negatively affects final product quality, mainly in terms of crispness, which is the key parameter to be optimized and monitored during shelf life.

The aim of this work was to set a critical moisture content value for osmo-air-dried crispy apple rings, above which the product is no longer acceptable to the consumer. Moreover the possibility to correlate the sensorial threshold and a crispness index value was evaluated, in order to design, in a future work, a proper packaging able to extend the shelf life of such a kind of products.

MATERIALS AND METHODS

According to the results obtained in a preliminary study, osmo-air-dried apple rings were obtained as follows. Apples (cv Golden Delicious), were washed, dried, cored by a spoon soil auger (25.0 mm diameter), and mechanically cut (LT INOX, Kronen, Germany) into 5.0 mm thick rings. Then they were dipped for 60 minutes at 25°C in a concentrate sugar mixture (a_w=0.90) consisting of fructose, sucrose and glucose in the same proportions found in fresh apples. The solution was continuously recirculated through a peristaltic pump and the ratio fruit/solution was 1/3. Air drying was performed at 80°C, up to constant weight, using an alternate upward-downward air circulated drier, operating at an air speed of 1.5 ms^{-1}. Not pre-treated apple rings were dried as a control.

Water activity was measured by an electronic hygrometer (Aqua Lab. CX-2-Decagon Devices, Pullman, USA). Moisture content was determined according to Karl Fischer method after extraction in anhydrous methanol (ASTM D 6304-2004 a, 1-procedure A). Results are expressed as g H_2O/100 g solids.

Water vapour sorption isotherms were determined according to the procedures reported by Wolf *et al.*, (1985), and the principles described by Bell and Labuza (2000) . Dried apple rings were placed in sealed plastic boxes containing saturated salt solutions (LiCl anhydrous, CH_3COOK, $MgCl_2$ hexahydrate, K_2CO_3 anhydrous, $Mg(NO_3)_2$ hexahydrate) and stored in a climatic chamber at 25°C. The water activity (a_w) of these solutions was in the range of 0.11-0.52. Samples were weighed at regular intervals and the equilibrium was considered to be reached when the change in weight did not exceed 0.1% in 2 consecutive weightings at no less than 5-days

intervals. In this study equilibrium was achieved in three weeks. The same experiment was carried out using glycerol solutions at different concentrations in the same aw range. Apple rings stored in these boxes were used for sensory analysis.

Apple chips mechanical properties were determined using a bending-snapping test (Farris *et al.*, 2008). One apple disc at a time was placed on two supports; a third compressing bar was driven down at a speed of 10 mm/min, bending each specimen until it snapped. For sensory analysis a descriptive test was applied. Ten panelists expressed a judgment on crispness intensity on a free scale from 0 (low) to 10 (very high). Each session was repeated on two subsequent days. Results were elaborated with FIZZ, Software Solutions for Sensory Analysis and Consumer Test, Biosystemes, France.

RESULTS

The extremely high hygroscopicity of both air-dried and osmo-air-dried apple rings is evidenced by the adsorption isotherms in the range 0.11-0.52 a_w, as shown in Figure 1, where experimental data were interpolated by Lewicki equation (1998). The obtained equilibrium moisture content values dramatically increased with increasing water activity, leading to a steep slope of the curve.

As for sensory evaluation, there were significant differences in crispness scores between osmo air-dried and air-dried rings equilibrated at water activity below 0.24, osmo air-dried rings crispness being judged significantly higher. With the increase of the water activity values so the differences between the two samples diminished progressively.

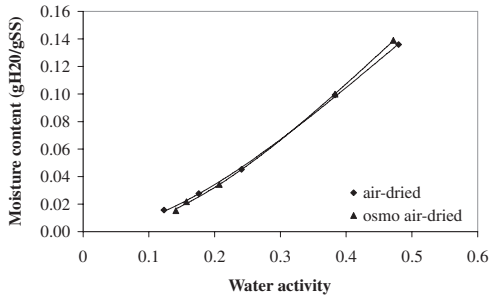

Figure 1. Experimental sorption isotherm curves in the aw range 0.11-0.52, for air-dried and osmo-air dried apple rings.

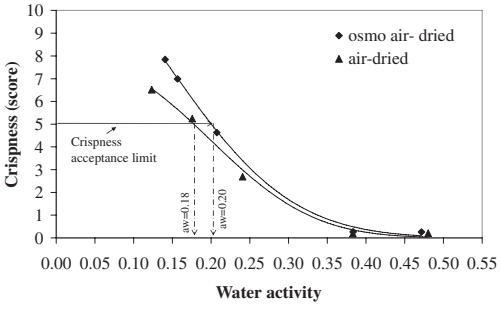

Figure 2. Effect of water activity of air-dried and osmo-air dried apple rings on crispness intensity estimated by sensory evaluation.

Comparing apple rings within the same group (osmo air-dried and air-dried rings) conditioned at different water activities, crispness was judged not significantly different for samples with aw below 0.18 (Figure 2), as stated by the analysis of variance. On the contrary, for higher aw crispness fell drastically and panelists assigned significantly lower scores. Considering score 5 as the threshold for product acceptability, a critical moisture content was set from the interpolation between sensory score and water activity by graphical method (Konopacka *et al.*, 2002), (Figure 2). For apple rings not pre-treated before air-drying the critical water activity was 0.18, while for osmo-air-dried samples it was 0.20. The critical moisture content value corresponding to the critical water activity, was graphically derived from the isotherm. It was equal to 2.9 g H_20/100g solids for the air dried apple rings and 3.2 g H_20/100g solids for the osmo air-dried.

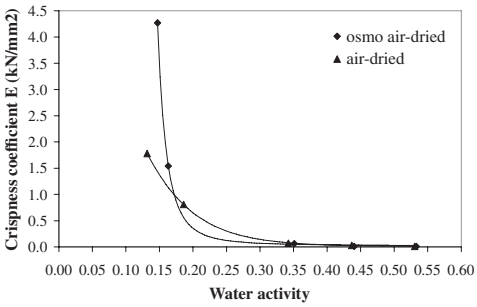

Figure 3. Effect of water activity of apple rings on crispness coefficient E.

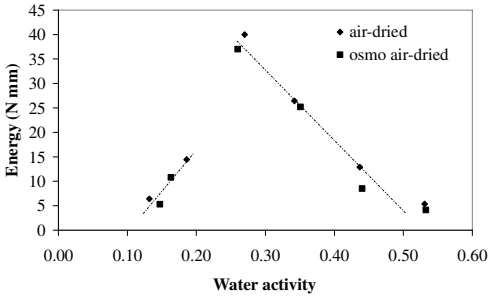

Figure 4. Energy to break-point as a function of water activity.

Results of bending-snapping test are shown in Figure 3. As for the sensory test, differences between air-dried and osmo-air-dried rings were assessed at water activity values lower than 0.20, when osmo air-dried apple rings had the highest crispness coefficient.

For both samples, crispness coefficient decreased progressively with the increase of water activity, reaching values close to zero above a_w 0.35.

Energy required for breaking the samples may be a good indicator of product crispness (Konopacka *et al.*, 2002), and it can be evaluated by the value of the area under the bending-snapping curves. By increasing water content the shape of the recorded curves changed from sharp and narrow to broad and flat (data not reported). This was due to the change of the food matrix from crispy and able to break into plastic. This change is described by the quick increase of the energy needed to break the chips for water activity higher than 0.20 (Figure 4), indicating that the critical water activity for apple rings is close to this value. The decrease of energy at high aw values, is due to the curve shape, which tends to flatten, with a long deformation (mm) but very low force values (N).

CONCLUSIONS

Results of both sensory and mechanical analyses evidenced that osmo-air-dried apple rings are crispier than the air-dried ones. This difference could be attributed to the different porosity of the specimens, linked to a better preservation of the apple tissue structure, which in turn can be ascribed to the partial concentration achieved during the osmotic pre-treatment. Sensory analysis allowed a critical moisture content to be set as crispness acceptance limit, being its value higher for osmo-air-dried apple rings. Furthermore, correlation was established between the sensorial acceptance threshold and the crispness coefficient E, above which the product is no longer acceptable to the consumers. Finally, osmotic dehydration before drying not only improved dried apple rings crispness, but also could allow a longer shelf life.

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SESSION II

"New Technologies for Shelf Life Extension"

Chairmen:

G. Barbosa-Canova (Washington St. Univ., USA)
D. Dainelli (Seal Air Corporation, Italy)

PACKAGING TECHNOLOGIES FOR FOOD QUALITY PRESERVATION - A REVIEW

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ABSTRACT

The food industry has since a few years started a move from simply manufacturing food & beverages to supplying nutrition and wellness. In the same time, a strong pressure on the packaging industry has arisen to reduce its global impact on the environment, which adds-up to the everlasting pressure on the economics of packaging.

This results in the situation that modern foods require always more protection against degradation factors such as oxygen, water vapour, light and microbiological issues, while using less packaging or packaging materials with lower intrinsic environmental impact.

This paper provides a review, which does not aim at being comprehensive, of the different approaches used for improving the overall performance of food packaging.

Key words: antimicrobial packaging, barrier, biopolymers, nanocomposites, oxygen scavengers.

INTRODUCTION

A strong move towards nutritional and healthy products can be observed in the food industry, through the addition of functional components, long-chain unsaturated fatty acids, and vitamins and through the reduction or suppression of suspicious and "non-natural" components such as salt, preservatives and other "E-numbered" ingredients. In parallel, the concept of "fresh-food" is also becoming increasingly important. These changes in the way of preparing food products have clearly increased the sensitivity of products to most degradation factors, such as oxygen, water vapour, light and microbiological degradation. In the same time, while the pressure on food packaging economics remains very strong, the general trends towards environmental performance improvement and sustainability has important consequences on the way packaging and packaging waste are looked at. Classical inorganic materials are nowadays often undesirable, and a move towards lighter

and permeable packages is made. With the increased sensitivity of foods mentioned before, simple packaging solutions are not adequate to guarantee food quality.

This paper aims at describing the main domains were developments are undertaken to improve packaging performance in terms of environment and product protection keeping in mind the paramount issue of economics. Three domains will be presented, namely barrier improvement, packaging materials based on renewable resources or biopolymers and active packaging.

Barrier improvement

The barrier solutions presently available on the market all have their drawbacks, e.g. cost, water-sensitivity, opacity or perceived environmental bad-will. This situation has stimulated the industry to provide new, more efficient barrier solutions which have been reviewed by Lange et al (2003) classifying innovation along five major lines: thin inorganic coatings; new barrier polymers; blends; organic barrier coatings; and nanocomposite materials. Since the publication of this paper, it is observed that the development of new barrier polymers and blends has not brought much new materials on the market. Organic coatings are not much of a subject anymore and vacuum deposited coating have been integrated into the field of nanocomposite materials, which is the field were the largest amount of publications can be found.

Nanocomposites

Historically, nanocomposite materials in the packaging field were mainly based on the addition of clay particles in polymers. The flake-shape of these particles once dispersed and oriented in the polymer matrix, increases the length of diffusion hence reducing the permeability. Theoretical calculations have shown that the addition of a few percent of nanoclay in polymers could yield barrier improvement factors (BIF) in the order of 100 to 1000 (Gusev et al, 2001). Current commercial products however have BIF's ranging up to 10, showing that although commercially available, the integration and exfoliation of clays still is in its infancy and is far from having reached its full potential. Many studies are still ongoing in this field. More recently, the field of nanocomposite has integrated vacuum coated materials, such as SiOx coated films or containers and metallised films, which are mature technologies for barrier improvement. These coatings are being produced since several decades but have been looked at as nanocomposites since 4 to 5 years only. BIF up to 100 are achieved with these technologies.

Biopolymers

In view of the environmental pressure on packaging materials, biopolymer is a very active field of research. Although the volume of this type of polymers for packaging applications still is very low, many niche applications have made their way to the market, although not always with an improvement of the environmental impact (Lundquist *et al.*, 2006). The material being by far the most used biopolymer is polylactic acid (PLA). Besides this, thermoplastic starches and polyhydroxyalcanoates (PHA) are completing the list of the main biopolymers available. In general, biopolymers have a fair barrier to oxygen but are sensitive to water and to water vapour permeation. Their application in food packaging is therefore quite limited at this point. Recent developments enabling the production of classical polymers from natural feedstock rather than petrol have been reported. One of the most promising routes followed is the production of Polyethylene (PE) form sugar cane. PE being both a fair barrier to moisture and a widely used sealing layer in multilayer

packaging films, it could not only contribute to the manufacturing of performing multilayer films, but also help to significantly reduce the environmental impact of packages, as the sealing layer represents in most cases the highest volume material in a multilayer structure.

Active Packaging

Active packaging can be defined as packaging that interacts chemically or biologically with its contents to extend shelf-life or modify the product during storage. They can act as remover (scavenger) of undesirable compounds such as oxygen, ethylene, aromas and water, or as part of the antimicrobial protection. Currently, the main applications and axes of research are oxygen scavenging and antimicrobial packaging.

Oxygen scavengers

Modified atmosphere packaging (MAP) is commonly used to extend the shelf-life of oxygen sensitive products. In some extreme cases, the level of residual oxygen achieved through MAP still is not sufficiently low to guarantee the quality of some products and other approaches have to be used, oxygen scavengers (OS), i.e. reducing compounds capable of fixing oxygen, being one of those. Historically, OS came in small sachets or labels that were directly introduced in the package. This system hardly was used in Europe due to normative limitations and consumer acceptance. Today's trend is to directly integrate the scavenger into a polymeric matrix. Although reducing the activity of the scavengers, this approach still facilitates the use of scavenger and its overall acceptance. OS materials include iron based compounds, low molecular weight (MW) hydrocarbons and some polymers. While the iron based OS can have detrimental effects on the mechanical properties and transparency of some matrix polymers, low MW hydrocarbon can generate degradation products inducing off-flavours to the product (Galdi *et al.*, 2007). Polymer based OS are therefore increasingly preferred.

Antimicrobial packaging

In order to follow the trend towards "fresher" foods and "clean labels", novel hurdles against microbiological spoilage of foods had to be developed, antimicrobial packaging being one of these. Antimicrobial packages can have the active component coated on the inner surface, combined with binders or even co-extruded with synthetic polymers. The bacteriocin nisin is the antimicrobial most commonly incorporated into films, but food grade acids and salts, chitosan, plant extracts, essential oils, enzymes and metallic nanoparticles (mainly silver) are also used (Joerger, 2007). In general, the colony forming units (CFU) reduction achieved by antimicrobial packaging remains fairly low, between 1.5 and 2.5 log10 reductions, making them adequate in the use as additional hurdle to bacterial growth. They could allow the reduction of another hurdle, such as heat treatment temperature, helping therefore improving food quality. The use of such films remains nevertheless relatively costly.

CONCLUSIONS

This short paper has reviewed some recent development in the packaging field that help extending the shelf-life of foods or improving their quality with respect to "fresher" foods, "clean labels", nutritional benefits and environmental impact

of packaging. Research trends in the field of barrier materials, biopolymers and active packages have been presented. Although no revolution has occurred in the last decade in the field of packaging, stepwise improvement and development of novel technologies will enable packaging to take an even bigger role in the quest for long-lasting fresh foods.

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DEVELOPMENT OF MODIFIED POLYMERIC SURFACES FOR THE ANALYSIS OF BACTERIAL ADHESION: INFLUENCE OF PHYSICOCHEMICAL PROPERTIES

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ABSTRACT

The surface of polypropylene (PP) was modified by the post irradiation grafting of different hydrophilic molecules. The polypropylene sheets were activated by electron beam radiation prior to the grafting reaction. Analyses were performed using X-ray photoelectron spectroscopy (XPS) to confirm the presence of grafted molecules on the surface of PP. The degree of grafting and the surface hydrophilicity of PP were determined using weighing and measuring the water contact angles. The zeta potential ζ and the isoelectric point (iep) were determined by the streaming potential method. Finally, Adhesion of *Listeria monocytogenes* on unmodified and modified PP surfaces was observed by Scanning Electron Microscopy.

Key words: biocontamination, polypropylene, radiation grafting, physico chemical properties.

INTRODUCTION

The microbiological quality of foods is currently a major challenge from both the economic and public health points of view. Indeed, the biocontamination of foods by pathogenic micro-organisms can cause food poisoning. In recent years, new infections have appeared while others have seen resurgence and cases of resistance have developed, not to mention new modes of transmission related to the use of novel processes. Current trends in consumption (i.e. more fresh products, more ready-to-eat meals), new technologies, the globalization of markets and the adaptability of micro-organisms are all factors which today require food safety control improvement. New techniques are being developed to reduce the risks of

contamination in finished products and thus ensure their hygienic quality. They will enable us to ensure that food contact surfaces (i.e. cutting tables, conveyors, containers, kitchen utensils as well as food packaging) do not serve as vehicles for transmission of pathogens. To reduce contamination risks in finished products and thus ensure their hygienic quality, companies currently have recourse to curative actions combined, or not, with preventive action. Up to now, curative actions have relied mainly on the use of disinfectants [Aarnisalo. K et al 2000]. Although effective, these compounds can be at the origin of toxicity or the appearance of bacterial resistance. As regards preventive approaches, they have usually been based solely on the choice of the material to be used (nature and finish of the surface or even topography). Recent works [Nonaka. T *et al.*, 1999 ; Yang. M.R *et al.*, 2002; Anjum. N *et al.*, 2006 ; Anjum. N, et al, 2008], nevertheless, lead us to envisage new strategies based on the development of new antimicrobial materials. As used herein, antimicrobial materials refer to material with biocide, bacteriostatic and or repellent activity.

1. Conception of polymeric materials with bioactive surface properties

To limit surface biocontamination, the approach we propose consists in modifying hydrophilicity and the zeta potential of the surface of plastic materials by radio grafting of hydrophilic monomers.

Our work concerned a plastic material having a dominant position in the agro food industry, namely polypropylene (PP). In order to get hydrophilic PP surfaces the grafting of acidic, basic or neutral monomers was carried out. The preferential monomers capable of acting as bacteriostatic or biocide agents are quaternary ammonium salts (QAS).

Subjected to an ionizing treatment (accelerated electrons) PP generates significant concentrations in peroxy radicals the lifespan of which is long enough for the material to be activated preliminary to the grafting.

After irradiation of the PP, the grafting step was performed in a closed reactor by immersion of the material in the monomer solution at a selected temperature (herein 70°C). The hydro peroxides break down and the radicals obtained bond covalently to the functionalized molecules

The degree of grafting was determinated in order to control the grafted amount of co monomer.

Surface properties of modified surfaces

The interesting observation in our grafting system is that the graft management on the PP surface is strongly governed by the composition of the reaction medium and surface grafting is accomplished under specific grafting parameters.

The grafting of DMA on a PP substrate resulted in an hydrophilic surface (θ = 30°), whatever the percentage of monomer in the reaction media and the degree of grafting. A hydrophilic surface was also obtained after grafting with AA, but only at high degrees (4%), and it is well known that such a high degree of grafting affects the physical structure of PP, notably its cristallinity. After the grafting of QAS, we obtained highly hydrophilic surfaces ($\theta \sim 30°$), but the grafting technique was quite difficult and reproducibility was limited. It was interesting to observe that the cografting of a highly reactive, hydrophilic monomer such as acrylic acid (AA) or dimethyl acrylamide (DMA), ensured "prehydrophilization" of the material which thus favored the grafting of QAS.

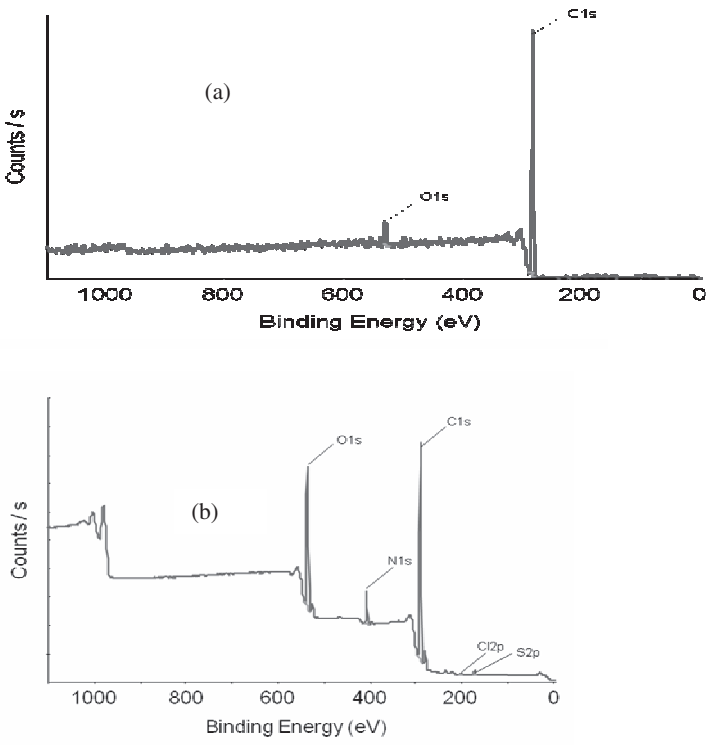


Figure 1: XPS spectra of (a) virgin PP and (b) PP grafted with comonomer mixture

Conventional X-ray photoelectron spectroscopy (XPS) analyses were performed to confirm the presence of QAS on the surface of PP grafted with comonomers (figure 1). XPS imaging results, shown in Figure 2, indicate that in our experimental system grafting takes place in a homogenous manner.

Zeta potential (ζ) measurements were also performed to determine the surface charge and the isoelectric point (iep). The zeta potentials of the grafted surface can vary over a broad range according to the choice of monomers. For the same surface hydrophilicity (θ ~ 30°) and depending on the nature of the monomers grafted it is thus possible to modulate the physicochemical characteristics which determine the phenomena governing adhesion and, probably, adapt them to a given application (table 1).

Adhesion of *Listeria monocytogenes* was observed on virgin and comonomer grafted PP by electron microscopy. The electron microscopic images illustrated that the extent of bacterial adhesion is higher in the case of virgin PP than on PP which is grafted with a comonomer mixture. The damage of the cells on the modified surfaces could be due to the graft molecules present on the modified surfaces (figure 3).

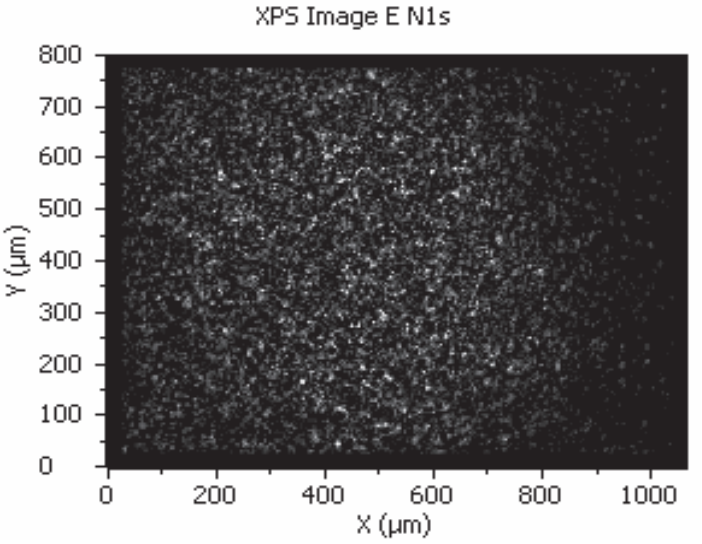
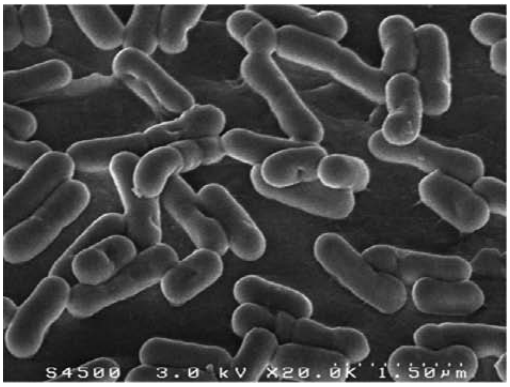
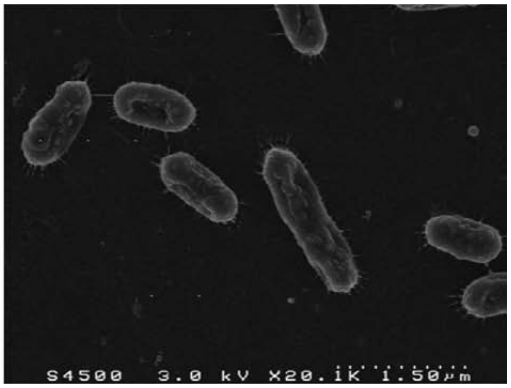


Figure 2 :XPS mapping of nitrogen



(a)



(b)

Figure 3 :Electron microscopic images of ungrafted and grafted PP showing adhesion of Listeria monocytogenes. (a) Virgin PP, (b) Grafted PP with a comonomer mixture AA/QAS.

Table 1 : Values of Zeta potential (ζ) at physiologic water pH and iep of virgin PP, PP grafted with monomers (AA, DMA, QAS) and comonomer mixtures

Sample	ζ (mV) at pH =5.5	iep
Virgin PP	-25	4.3
PP grafted with AA	-15	2.6
PP grafted with DMA	- 8	4.2
PP grafted with DMA/QAS	4	-
PP grafted with AA/QAS	7	5.8
PP grafted with QAS	30	7.9

CONCLUSION

The present work attempted to control the surface properties of polypropylene (PP) such as hydrophilicity, surface charge and isoelectric point (iep) by using neutral, cationic and anionic molecules and their mixture to avoid bacterial adhesion. To achieve this control, surface modification of PP sheets was done by radiation induced graft polymerization of monomers and comonomer mixtures. We show that depending on the functionality grafted and the conditions of grafting, it is possible to modulate the physicochemical parameters determining the phenomena that govern adhesion of a moderately hydrophilic bacterial strain such as *Listeria monocytogenes*.

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BIOBASED COATINGS AS A SOLUTION TO IMPROVE THE OVERALL PERFORMANCES OF PLASTIC FILMS

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ABSTRACT

The present research dealt with evaluating barrier, friction and optical properties of three different plastic films after deposition of a gelatin-based bio-coating. The composite films showed improved barrier properties against oxygen and UV radiation. Static and kinetic coefficients of friction were significantly decreased both in the film-to-film and in the film-to-metal tests, leading to a desirable value for many applications. However, bio-coated films showed lower optical performances in terms of transparency and haze. Not significant differences were observed as far as the water vapour permeability is concerned. The obtained data suggest that the lipid-protein coating tested in this study, in spite of its great potential for enhancing some characteristics of plastic packaging films, still exhibits negative aspects which necessitate further improvement.

INTRODUCTION

The application of very thin bio-layers (from few micron to few hundred nanometers) onto plastic substrates in the form of coatings is capturing an increasing interest in the recent few years. This is due not only to the opportunity to solve, at least partially, the waste disposal issue by introducing into the environment a reduced amount of petrol-based polymers, but also because of some potential advantages represented by the availability, low cost and biodegradability. These characteristics meet the consumers' trends, leading to a greater attention to both what is natural and environmental compatible. Furthermore, for industry, this provides innovative and economic solutions. For these reasons, biopolymers have a potential use as sustainable food packaging materials.

This paper examined the use of a lipid-protein matrix as a thin coating on three different plastic films widely used in the food packaging industry. In particular, pigskin gelatin was used as a protein to provide the barrier against oxygen and

the necessary strength to the biopolymeric layer. An acetylated monoglyceride was chosen as a lipid, in the attempt to provide both best water vapour barrier performances and friction properties of the final composite films. Finally, glycerol was used as a plasticizer to enhance the flexibility of the final structure, preventing coating cracking. The aim of the present work was to evaluate the effect of a lipid-protein bio-coating on some properties of plastic packaging films. To this purpose, barrier (oxygen and water vapour), friction (static and kinetic coefficients) and optical (haze, transparency and UV transmission) properties of three different plastic films (polypropylene, polyethylene terephthalate and low-density polyethylene) after the coating deposition were evaluated.

MATERIALS AND METHODS

Composite films (plastic substrates coated with the biodegradable thin layer) were prepared according to the procedure protected by International patent WO 2008/075396 A1. Both the neat plastic films and the composite ones (i.e. plastic web and coating) were tested using five independent replicates in regards to thickness, barrier and optical properties. Coefficients of friction were assessed using ten replicates.

Barrier properties (oxygen, water vapour) were determined by the use of permeabilimeters based on the nearly-isostatic methods. Optical properties (haze, transparency, UV-filter) were measured using an UV-Vis spectrophotometer coupled, when necessary, to an integrative sphere (150 mm). Friction properties (static and kinetic coefficients) were determined by means of a dynamometer. One-way ANOVA was used in order to check for differences between and within groups (each plastic film, coated and uncoated). The mean values, when appropriate, were separated by LSD's multiple range test at $p \leq 0.05$.

RESULTS AND CONCLUSIONS

Table 1 summarizes the results obtained in regard to the afore mentioned analyses on the three different bio-coatings/plastic films pairs (Fig. 1).

As can be seen, the addition of the biopolymeric layer led to a drastic decrease of the oxygen permeability, ranging from 73% for the OPP to 40% for the PET. The barrier effect of the coating is undoubtedly due to the presence of gelatin, which exhibits oxygen permeability roughly close to that of PVDC and EVOH films at 0% relative humidity. Only a small effect on the WVTR of the PET-coated film was measured ($\approx$ 15% decrease), whereas the presence of the bio-coating did not lead to any significant changes in the WV barrier characteristics of OPP and LDPE films. It could be explained considering that, at T = 23°C, OPP and LDPE themselves are excellent water vapour barriers, and the addition of a coating with a low fat-to-protein ratio is not enough to affect their initial performances. Transparency values (%) decreased 17% for OPP (90.46 – 75.04), 12% for PET (82.27 – 72.69) and 36% for LDPE (84.10 – 54.05). The deposition of the coating led to a consistent increase in haze for all the three plastic films. In particular, the haze index increased mainly for OPP (85.5%) followed by LDPE (78%) and PET (70%). The decrease in transparency of the plastic films after coating deposition is undoubtedly attributable to the lipid component. The area under each

Table 1. Thickness, Oxygen Transmission Rate (OTR), Water Vapour Transmission Rate (WVTR), Haze, Transparency and Coefficient of Friction of coated and uncoated plastic films

Film		Thickness (μm)	OTRa (cm^3m^{-2} day^{-1})	WVTRb (g m^{-2} day^{-1})	Haze (%)	Transparency (%T)	Coefficient of Friction (CoF)			
							Film-to-film		Film-to-metal	
							μk	μs	μk	μs
OPP	Uncoated	19.6 (± 1.41)	1715.67 (± 17.10)	1.09 (± 0.11)*	1.60 (± 0.04)	90.46 (± 0.25)	0.75 (± 0.05)	1.27 (± 0.22)	0.37 (± 0.04)	0.48 (± 0.04)*
	Coated	21.25 (± 1.5)	465.67 (± 47.35)	1.26 (± 0.04)*	10.97 (± 1.03)	75.04 (± 3.48)	0.17 (± 0.02)	0.52 (± 0.07)	0.21 (± 0.03)	0.47 (± 0.03)*
LDPE	Uncoated	47.5 (± 1.45)	2623.67 (± 47.50)	1.12 (± 0.19)*	5.71 (± 0.39)	84.10 (± 0.08)	0.64 (± 0.06)	0.18 (± 0.03)	0.86 (± 0.07)	0.46 (± 0.08)*
	Coated	49.1 (± 1.52)	1159.67 (± 52.60)	1.38 (± 0.01)*	26.13 (± 2.40)	54.05 (± 2.54)	0.29 (± 0.04)	0.20 (± 0.04)	0.51 (± 0.08)	0.46 (± 0.06)*
PET	Uncoated	12.3 (± 0.98)	112.33 (± 1.53)	15,78 (± 0.43)	3.14 (± 0.14)	82.27 (± 0.32)	0.42 (± 0.03)	0.54 (± 0.06)*	0.27 (± 0.02)	0.34 (± 0.02)
	Coated	13.85 (± 1.05)	67.10 (± 0.70)	13.53 (± 0.02)	10.49 (± 0.54)	72.69 (± 1.02)	0.22 (± 0.03)	0.58 (± 0.10)*	0.20 (± 0.02)	0.42 (± 0.03)

*Denote a not statistically significant difference between treatments within group (type of film) at $p \leq 0.05$ (or 95% confidence interval). Standard deviation is reported in brackets.
a 23± 0.5°C; 0% relative humidity on both sides of the sample.
b 23± 0.5°C; 90% relative humidity difference between wet and dry chambers of the instrument.

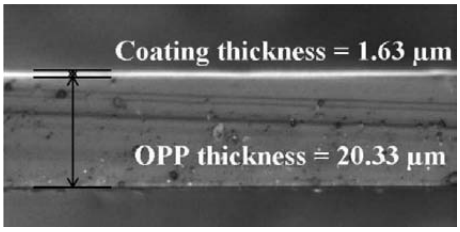

Fig. 1 OM cross-section of polypropylene coated film.

transmittance curve was also used to check for some differences in the UV transmission properties after the coating application. As reported in Fig. 2, the bio-coating led to an enhancement of the UV transmission properties for the OPP and LDPE films.

The major component involved in this 'blocking-UV' capacity is the protein. In particular, this UV-absorbing behaviour has to be attributed to the aromatic amino acids in the gelatin composition, like phenylalanine and tyrosine. In addition, further evidence was given by comparing the spectra obtained from films coated in accordance to the original formulation with those obtained from films coated in absence of the lipid component. As displayed by the Fig. 3, the transmittance spectra of OPP coated in the absence of the acetylated monoglyceride are downwards-shift-

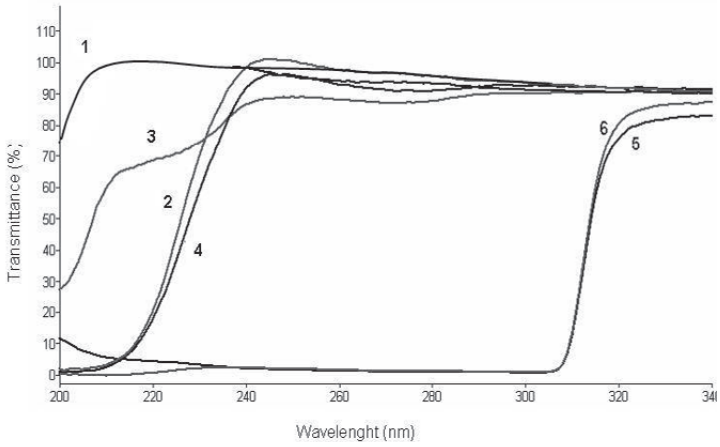

Fig. 2 UV light transmittance of uncoated (1) and coated (2) LDPE, uncoated (3) and coated (4) OPP, uncoated (5) and coated (6) PET.

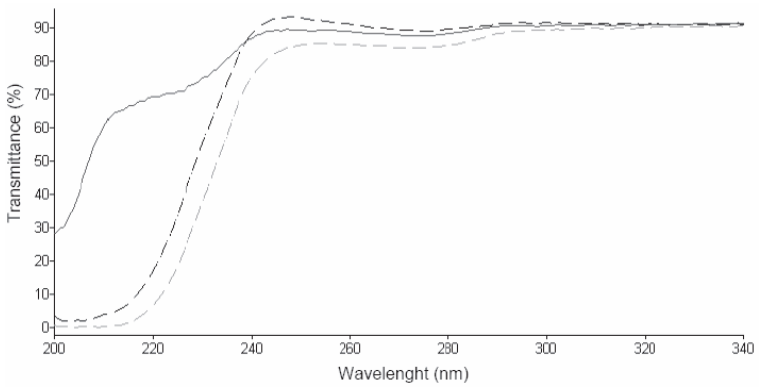

Fig. 3 UV light transmittance of uncoated OPP (straight¬¬¬¬¬¬¬), coated OPP (grey dotted) and coated OPP without the lipid component in the original formulation (black dotted).

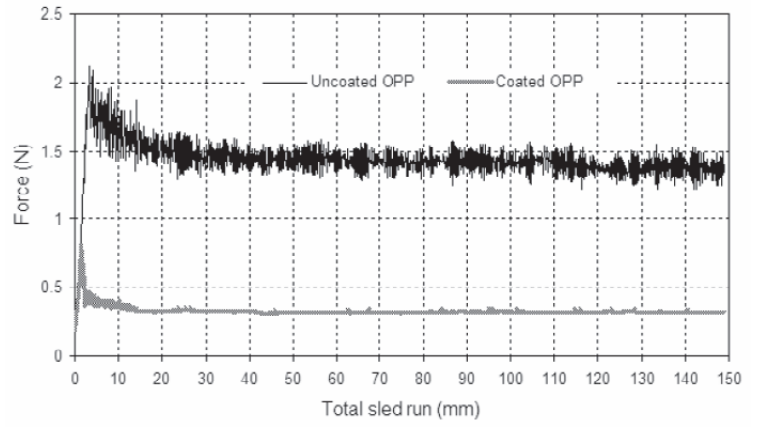

Fig. 4 Typical curves obtained for static and kinetic friction coefficients determination during the film-to-film test of uncoated and coated OPP.

ed with respect to those obtained with the lipid component in the formulation, confirming that gelatin is able to partially block the UV radiation, whereas the lipid drops dispersed in the coating reduce this effect.

Great differences may be observed after deposition of the thin coating on the plastic substrates (Fig. 4).

This is basically due to the specific friction characteristics of the coating, which are independent from its thickness and which could be affected by the type of substrate on which is spread on (e.g. the substrate's own smoothness) and by the coating uniformity, as shown by the results reported in Table 1. The overall positive effect arising from the deposition of the coating on plastic films is attributable to the lipid component, which acts as a true slip agent.

From the experimental results it can be concluded that the coating designed according to the proposed formulation does not seem at the moment the best way to promote the improvement of some important performances of plastic substrates. This is because the addition of a lipid component in the original formulation did not lead to any meaningful and significant improvement of the original characteristics, with the exception of the friction properties. The addition of the monoglyceride acetylated to the gelatin-based formulation indeed reduced some positive effects provided by the protein biopolymer, such as the optical properties (in terms of haze and transparency), the UV-absorbing capacities and likely the oxygen barrier property too. Although these negative effects, such composite layers may represent a potential way able to meet the increasing demand for more sustainable solutions, especially if some aspects will be improved on.

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GELATIN FILMS WITH HYDROPHOBIC PLASTICIZERS: MECHANICAL PROPERTIES, WATER VAPOR PERMEABILITY AND WATER SOLUBILITY

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ABSTRACT

The objective of this work was to develop gelatin based films using hydrophobic plasticizers: tributyl citrate or o-acetyltributyl citrate. A surface response methodology was used to evaluate the effect of the gelatin concentration, plasticizer concentration, lecithin concentration and plasticizer type on mechanical properties, solubility in water and water vapor permeability of films. The films were produced by casting technique. In general, the properties of the films based on gelatin and hydrophobic plasticizers were affected by plasticizers type and concentration.

Key words: edible films, citrate, physical properties, surface response.

INTRODUCTION

Poor water vapor barrier property is one of the main limitations for protein films applications. This characteristic is due to the hydrophilic character of proteins and plasticizers. The most common plasticizers used in biodegradable and/or edible films technology are the glycerol (Vanin *et al.*, 2005) and the sorbitol (Thomazine *et al.*, 2005). However, these plasticizers are highly hydrophilic and consequently, produce films high sensitive to the relative humidity conditions.

An alternative, not yet explored in the edible film technology, is the use of hydrophobic plasticizers, such as the citrates derivates (Heng *et al.*, 2003). The citrate esters, produced from the citric acid, are considered as non toxic (Labrecque *et al.*, 1997) and have great potential for application in the production of film based on proteins.

Thus, the objective of this work was to develop gelatin based films using hydrophobic plasticizers as the tributyl citrate and the o-acetyltributyl citrate. A surface response methodology (RSM) was used to evaluate the effect of the plasticizer type, plasticizer concentration, gelatin concentration and lecithin concentration on mechanical properties, water vapor permeability and solubility in water of films.

MATERIALS AND METHODS

An A type gelatin (Gelita South America), tributyl citrate (TB), o-acetiltributil citrate (AC) (Morflex, NC), lecithin (Caramuru, Brazil) and ethanol (Sinth) were used in this work without previous treatments.

The films were produced by casting (Vanin *et al.*, 2005; Thomazine *et al.*, 2005), with an average thickness of 0.080±0.008mm. Before the characterizations, the films were conditioned at 58% of relative humidity and 25°C, for 7 days.

The mechanical properties of films were evaluated by tensile test according Thomazine *et al.* (2005), and the solubility in water and the water vapor permeability were determined gravimetrically (Cuq *et al.*, 1997), always at 25°C. An experimental design (2^4) was used to study the effects of the gelatin concentration ($X_1=C_g$; -1=2 g/100g of FFS, +1=4g/100g of FFS), the plasticizer concentration ($X_2=C_p$; -1=25 g/100 g of gelatin, +1=50 g/100g of gelatin), the lecithin concentration ($X_3=C_L$; -1=40 g/100g of plasticizer, +1=60 g/100g of plasticizer) and the plasticizer type ($X_4=T_p$; -1=TB; +1=AC) on physical properties. The Statistical analyses were carried out using the Statistica program (Version 7.0).

RESULTS AND DISCUSSION

According the statistical analysis of the dependent variables, the linear model was statistically significant for mechanical properties and solubility in water. Thus, these models described with significant parameters were used to generate surface response graph, when the overall model was statistically significant ($p<0.05$) and predictive ($F_{calculated}>>F_{Listed}$).

The surfaces response allowed observe that the T_P and C_P influenced strongly the tensile strength (T) of the gelatin films, being that an increase on C_P provoked an important decrease on T (Figure 1a). These results were due to the plasticization

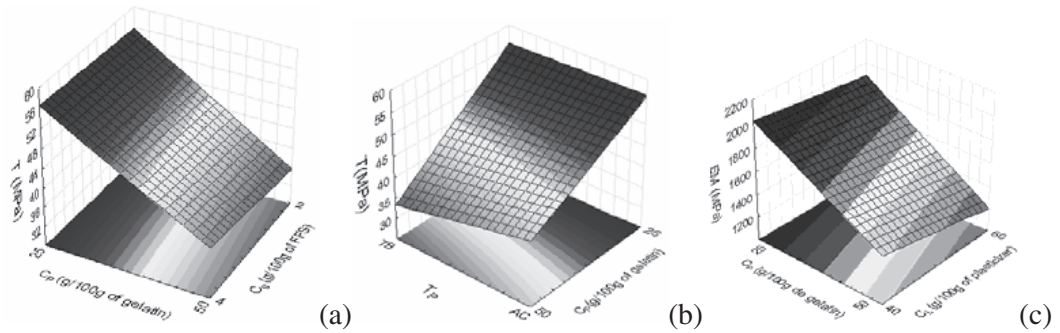

Figure 1: Surfaces response for the tensile strength (T) and elastic modulus (EM).

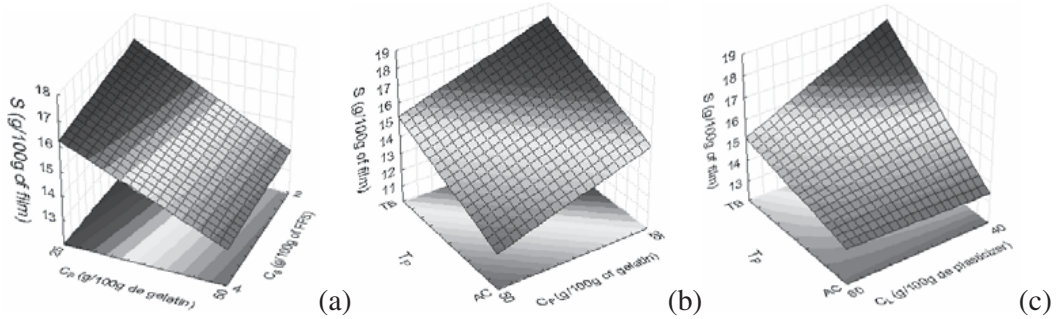

Figure 2: Surfaces response for the elongation at break (E).

of the protein matrix by the citrates similarly to the hydrophilic plasticizers. The T values of gelatin films with AC were higher than those of films with TB (Figure 1b). These results were similar to that observed for hydrophilic plasticizers (Vanin *et al.*, 2005, Cuq *et al.*, 1993). On another hand, the plasticizer effect was also observed on elastic modulus, with a subtly effect of the lecithin concentration (Figure 1c).

The elongation at break (E) was affected slightly by plasticizer type, plasticizer concentration and gelatin concentration, being the most important effect provoked by the lecithin concentration (Figure 2). The plasticizer TB provoked a slightly increasing (between 6 and 8%) on E which was not affected by AC plasticizer (Figure 2a). In overall, this behavior don't agree with that observed for films based on gelatin and lipids where the increase in the lipid concentration provoked an increase in E due to an increasing in the molecular mobility (Bertan *et al.*, 2005).

On another hand, an increase on the C_P caused a reduction of the solubility in water (S) of films (Figure 3), as expected, because the increase of C_P increased the hydrophobic character of the material. Moreover, the T_P affected differently S as function of the C_L. The AC plasticizer was not sensitive to C_L, but the reduction on CL (from 60 to 40%) increased S (from 15 to 18%) for films plasticized with TB. These films were less water soluble than other films based on gelatin (Carvalho and Grosso, 2004; Bertan *et al.*, 2005). Unfortunately, according the results of the analysis of variance, the water vapor permeability (WVP) data could not be represented by a linear model, and thus, a surface response graph cannot be plotted. The WVP of the films produced in this work varied between 3.4 and 5.9gmm/cm²hPa for films produces with TB, and between 4.6 and 5.7 gmm/cm2hPa for films with AC. The films with TB and AC have lower water vapor permeability than films based on gelatin/triacetine (Bertan *et al.*, 2005).

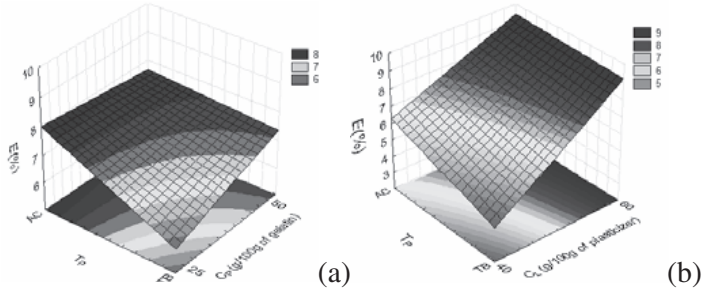

Figure 3: Surfaces response for the solubility in water (S).

CONCLUSION

The plasticizers studied were compatible with gelatin, producing flexible

films. In general manner, the properties of gelatin based films were significantly affected by the plasticizers concentration and type.

Acknowledgments: to FAPESP, for the financial support (06/00431-8).

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BARRIER PROPERTIES OF BIODEGRADABLE POLYESTERS TOWARDS AROMA COMPOUNDS

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ABSTRACT

The capability of two innovative packaging materials, poly(lactic acid), PLA, and poly(hydroxybutyrate), PHB to stabilise the aroma formulation of foodstuff during storage were investigated. Isotherms and solubility coefficients of ethyl acetate (EA) into PLA and PHB were determined using a microgravimetric method. The structural change of the PHB and PLA (with various degrees of crystallinity) films and their rheological behaviour before and after aroma sorption were respectively investigated by differential scanning calorimetry (DSC) and by dynamic mechanical analysis (DMTA). The gas barrier properties were measured through oxygen and helium permeation tests. For PLA, the degree of crystallinity seems to have no effect on gas barrier properties. PHB provides better helium and oxygen barrier properties than PLA. The solubility coefficients of EA are dependent on the film processing and on the activity of the aroma compound, a behaviour largely observed for semi-crystalline polymers. The mass uptakes of EA were the smallest in PHB and the PLA sample which is the most crystalline. It however seems that the high crystalline PHB performs better than PLA at the highest aroma compound activity.

Key words: PHB, PLA, aroma, barrier properties, sorption.

INTRODUCTION

The use of suitable packaging by the food industry has become a topic of great interest because of its potential to maintain quality throughout the shelf life of food products. Nevertheless, during storage transfer of compounds from food, such as

lipids or aroma compounds, can interact with the packaging causing modification and deterioration of gas barrier properties [Dury-Brun *et al.*, 2007]. Driven by environmental concerns, new polymers based on renewable resources are coming onto the market. One of the most prominent polymers among these materials is poly(lactic acid) (PLA) which is approved for food contact. The optimization of the PLA process in the field of food packaging is important, in particular as concerns the degree of crystallinity as it has the main influence on barrier properties. The aim of this work is to assess the thermal properties of PLA films with different degrees of crystallinity on gas and aroma barrier properties (oxygen, helium, ethyl acetate). Films were obtained by flat die extrusion and modified by compression-molding to modulate their crystallinity. The data were compared to an industrial sample (PLA Biophan) and to PHB.

MATERIALS AND METHODS

Two PLA materials were studied: PLA Biophan film and PLA Biomer L9000. PLA Biophan 121 (Treofan) is provided directly in film form. Its thickness is 30 μm. PLA Biomer L9000, 100% in L conformation, and PHB (Copersucar) were provided in pellet form. Ethyl acetate (EA) was provided by Sigma (purity 99.5 %).

Preparation of PLA films. Extruded PLA Biomer film (120 μm thickness) was prepared by flat die extrusion at 210°C. The roll temperature and roll speed were respectively fixed at 25°C and about 10m/min. In order to obtain an amorphous PLA film, the previously extruded film was compression-molded at 200°C and 3.4 bars for five minutes then quenched in water at room temperature. As PLA can recrystallise during heating, the amorphous films were maintained at different temperatures and durations under compression. The samples were then quenched in water to obtain various degrees of crystallinity (Recrystallised PLA)

Preparation of PHB films. PHB was first purified by solubilization under reflux with choloroform and reprecipitation with ethanol. The PHB powder was then compression-molded at 200°C and 3.4 bars for five minutes to obtain the films (250 μm thickness).

Differential scanning calorimetry (DSC) Two types of equipment were used: (i) a Pyris 1 (Perkin Elmer) to study the crystallinity of the samples (melting enthalpy of a 100% crystalline PLA = 93 J/g, [Solarski *et al.* 2005], enthalpy of a 100% crystalline PHB = 146 J/g [Inoue and Yoshie. 1992]). The tests were performed at 10°C/min from 0 to 200°C. (ii) a modulated DSC QSC 100 (TA Instruments) to study the glass transition. The heating scans were performed under sinusoidal temperature modulation with a heating rate of 2.5°C, a period of 40 s and a modulation of ± 0.265°C between 10 and 190°C. The tests were performed under nitrogen atmosphere in hermetic aluminium pans.

Oxygen and Helium permeability. The oxygen transmission rate was monitored at 23°C and 0% RH with a Systech 8001 apparatus. The helium transmission rate was measured at 23°C and 0% RH using a specific analyser (Cnam) and based on ISO 15105-2:2003.

Sorption isotherm method. The sorption isotherm of ethyl acetate (EA) was measured at 25°C and 0% RH using an electronic microbalance, IGA-002 (Hiden, Warrington (UK) with a sensitivity of 0.2 μg; sample weight: 30-40 mg).

Table 1: Gas permeability of PLA and PHB samples. n=2

Material	Helium transmission rate x1018 m3.m/(m2.s.Pa)	Oxygen transmission rate x1018 m3.m/(m2.s.Pa)	Crystallinity degree (%)
Extruded PLA	92	n.d*	2
Amorphous PLA	103	2.5	6
PLA Biophan	62	2.2	19
Recrystallized PLA	106	2.8	39
PHB	14	1.0	59

Dynamic mechanical analysis (DMTA). Measurements were carried out by a Tritec 2000 DMA from Triton technology at a frequency of 1 Hz. The polymer samples were heated from -50°C to 200°C at 2°C.min-1.

RESULTS AND CONCLUSIONS

Gas barrier properties. Helium and oxygen transmission values, respectively named HeTR and OTR, are given in Table 1. Results are compared with measurements performed on conventional packaging materials, polyethylene terephthalate (PET, HeTR = 10 10^{-18} m^3.m/(m^2.s.Pa) and OTR = 0.23 10^{-18} m^3.m/(m^2.s.Pa)) and polystyrene (PS, HeTR= 207 10^{-18} m^3.m/(m^2.s.Pa) and OTR= 19.5 10^{-18} m^3.m/(m^2.s.Pa). Compared to the conventional packaging polymers, PLA is intermediate between PET and PS, which are respectively classified as medium and low barrier material. The degree of crystallinity seems to have no effect on gas barrier properties. The gas barrier properties of PHB are better than those of PLA and closer to the value obtained for PET.

Plasticizing effect of aroma compounds on thermal and thermomechanical behaviours

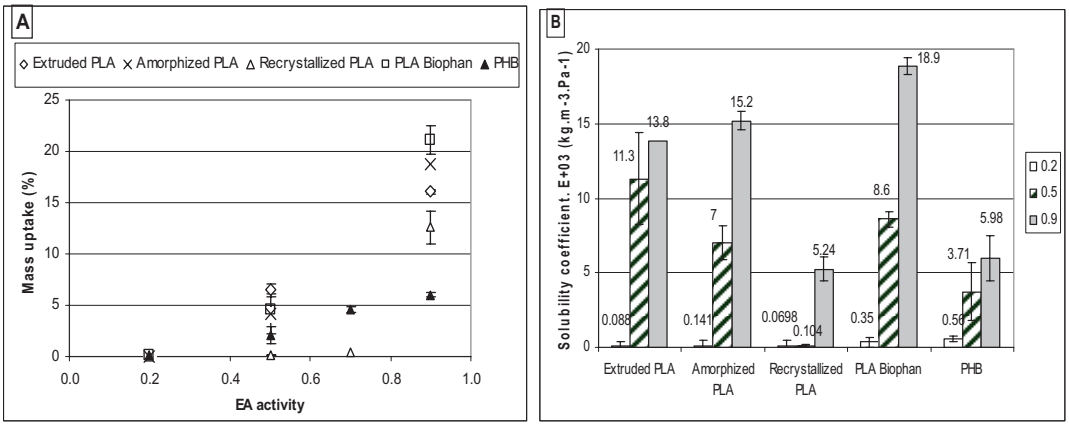
Measurement of the glass transition temperature (Tg) of the PLA samples was carried out before and after contact with ethyl acetate (EA) at 0.5 activity for 3 days in a hermetic vessel (Table 2). T_g was measured during the second heating. A net decrease of the Tg was observed due to the EA sorption. We can therefore conclude that the aroma compound has a plasticizing effect on PLA. This effect was further confirmed by DMTA (Table 2) after EA sorption. However on PHB film, plasticizing effect of EA was low even not noticeable.

Aroma sorption. The kinetics of sorption made it possible to calculate the solubility coefficients (S) of EA in PLA and PHB films in relation to the activity (p/p0) of EA (0.2, 0.5 and 0.9). For the four PLA, stationary states were reached only at 0.2 activity.

Table 2: Glass Transition (Tg) of PLA films before and after contact with ethyl acetate: analysis by DSC (n=3) and DMTA.

Material	DSC		DMTA	
	Tg before EA sorption (°C)	Tg after EA sorption (°C)	Tg before EA sorption (°C)	Tg after EA sorption (°C)
Extruded PLA	59.2 (+/-0.5)	38.5 (+/-0.5)	62.5	52.9
Amorphized PLA	58.0 (+/-1)	38.5 (+/-1)	63.8	49.9
PLA Biophan	54.7 (+/-1)	39.7 (+/-1)	67.5	55.1
Recrystallized PLA	59.1 (+/-1.6)	37.8 (+/-0.5)	69.6	51.9
PHB	-	-	15.4	12.2

At higher activity, kinetics of sorption revealed changes in the polymer morphology



Figures 1: A) Mass uptake (%) versus ethyl acetate activity B) : Solubility coefficients of ethyl acetate in the four PLA and PHB versus EA activity. n=3

throughout the sorption of EA. Mass uptake (figure 1A) and S (figure 1B) of EA into PLA and PHB were calculated and compared after 20 hours of contact. Mass uptake increases with the increase of EA activity whatever the polymer. Recrystallized PLA and PHB however showed the lowest mass uptake at the highest EA activity. This behavior can be related to their higher degrees of crystallinity.

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DEVELOPMENT AND APPLICATION OF AN ACTIVE PACKAGE TO INCREASE THE SHELF-LIFE OF "CALANDA PEACH"

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ABSTRACTS

Two different active packaging solutions were studied to protect and extend the shelf life of two varieties of Origin Denomination (D.O.) Calanda Peach(Prunus persica Sieb and Zucc), named JESCA and CALANTE. The packaging used was a active PET containing cinnamon extract or alternatively oregano as active agents. Storage at room temperature and at 0-0.5ºC was studied, and several parameters such as water loss, taste, aroma, sweetness, texture, color and firmness and a global acceptability evaluation were monitored and tested. The results obtained showed a shelf life of 12 days in perfect conditions and up to 20 days with a reduction of 20% of the fruits. The results are shown and discussed.

Key words: Active packaging, Calanda Peach, PET, Ripeness, Shelf-life.

INTRODUCTION

The active packing is that contributes to characteristics and properties to increase the shelf life of the product. Several active materials have been proposed in the last years but only some of them are nowadays commercially available(A. Rodríguez *et al.* 2007). The Calanda peach, one special variety but very sensitive to damage in which the added value is very high and consequently the presence of an active packaging contributes to conservation and benefit the product.

Nowadays, the shelf-life of Calanda Peach is short, from 3 to 5 days at 20 - 23ºC approximately, which limits its marketing to a small geographical area. The aim of this study is focused on extending as much as possible the shelf-life, using a new active polyethylene terephthalate (PET) packaging developed in our laboratory, with macro perforations with the application of active agents from the cinnamon and

oregano essential oils(C. Nerín *et al.* 2008), considered as natural food additives in the packaging material.

Significant variations in the process of the ripeness of the fruit, resulted in an increase of the shelf-life to a period between 17 and 20 days. The changes of color, ºbrix, weight and visual conditions were rigorously monitored. The results obtained are shown and discussed.

MATERIALS AND METHODS

The varieties of peach Calante and Jesca are two clones included in the D.O. Calanda Peachwas studiedas being the most representative. Two different formulations of active were used, based on the cinnamon and oregano essential oils. Additionally, two different types of active packaging were used: A)Tray of rigid PET, 18cmx18cmx7cm, with 24 perforations/dm^2 (perforations of 10mmx4mm) with a PET cap; B)Tray of PET rigid with adhesive active label of 10x10cm on the internal side of the PET cap, using also in this case two different active agents.JESCA variety was studied with both the active label and the active PET, whereas Calante was only studied in the presence of active PET. At last, peaches were stored at two temperatures, at room temperature, between 20 and 25ºC, and at low temperature, between 0 and 0.5 ºC.

Several variables were studied to evaluate the physicochemical characteristics, organoleptic behaviour and the chemical performance of both the packaging and the peaches with time, from time ceroto 20 days. The Color was measuredwith a Konica Minolta CR 400 ChromaMeter, using the illuminant C and the observer position 2. Also Brix Index was studied with Digital Refractometer MTD 045nD. The pulp of the peaches was crushed and homogenized for the measurement.*The water losswas* gravimetrically controlled versus time.The sensory characteristics by means of panels of tasting of 6 trained people, which evaluated *the external aspect, the firmness, the taste, the sweetness, the juiciness and the acceptability*. Every parameter was evaluated from 0 for very bad to 10 for excellent grade.

For the statistical analysis SPSS 13.0v.was used by means of comparisons of the stockings, ANOVA to establish significant differences, Pearson's coefficients of correlation, Linear univariant Models, with reliable levels of the 95%.

RESULTS AND DISCUSSION

From the first evaluations (12 days), can be seen the control is seriously damaged while the active packaging remains in good conditions, the same situation was found after 20 days in which the control was completely damaged whereas the active packaging protected the peaches for so long period. However, after 30 days remain to 0-0.5 °C, all peaches were seriously damaged the inside, from the area close up the seed towards the external area and the skin causing the darkening of the pulp. The effect was only visible when cutting and opening the peach, as the external appearance was excellent. This behaviour can be attributed to the cold temperature and has been described by other authors. Then, the cold damage was higher than the benefit from the active packaging and these tests were rejected. The rest of the study was focused on the tests at room temperature.

The results obtained with Calante peaches showed a slightly difference between

the control and the active packaging and consequently the shelf life was shorter than the expected at room temperature. This behaviour was attributed to the low effective concentration of active agents in the PET trays compared to that obtained using the active labels. As in this case the active PET was a prototype, further tests would be necessary to check this point and to improve as well the performance of the active PET.

Firmness and water loss were also evaluated versus time. It can be emphasized that the differences between the active and the control tests were also very high in Jesca tests. This means that the active agents protect the peaches on the ripening as well, maintaining not only the appearance but the firmness and color, all of them physical characteristics important for marketing purposes.

The *acceptability* was selected as representative parameter of the global sensorial quality of the peaches, since it turns out to be related and affected in a positive way by the improvement of the external aspect, the firmness, the taste, the sweetness and the juiciness, and in a negative way for the loss of water and the time. The fruits in the presence of the cinnamon essential oil reflect a longer shelf-life, comparatively to the reference fruits, that's to say, without active agents. In the same way, it was found that, with the concentrations of essential oils used in the formulation of the active compounds, the panel did not appreciate any change of taste or odor in the peaches, what is very important in terms of marketing and real application.

To consider a sample within the conditions of acceptable shelf life, a minimal value of 6 in the acceptability was established. It can be seen that up to 7 days, the values for the three cases were very similar while in the day 12, values lifted more belong to the signs in the presence of essential oils with a considerable difference in favor of the cinnamon. In addition, although in day 20 the three cases rank below the optimal value, the samples with essential oil look superior than the control.

ANOVA's analysis evidenced that there are no significant variations in the change of color nor ºbrix due to the use of active compounds, being the significant differences caused by time. In all cases it is noticed that the fruits with active packaging loose less water than in the case of the control, being the cinnamon what reflects less loss throughout the study.

CONCLUSIONS

The activities accomplished in this work allow us to confirm that the container with the formulation based on essential cinnamon oil like active agent, influence in a positive way the conservation of the peaches at room temperature. Acceptability increases of 6 days of conservation in the absence of active packaging, to 12 days in excellent conditions with the active container and 20 days with acceptable conditions for consumption.Deepening in the fact that the loss of water seems to be affected by the presence of active compounds.

ACKNOWLEDGEMENTS

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SHELF-LIFE STUDIES OF SLICED COOKED HAM PACKAGED IN POLYLACTIC ACID (PLA) TRAYS WITH OXYGEN SCAVENGERS

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ABSTRACT

The novel trend in food packaging is the extension of the shelf-life by means of mild technologies. This trend faces the challenge to offer "fresh like" foods products with reasonable shelf-lives and high global quality level. The reduction of food durability, however, determines a high impact of plastic packages on the urban waste management, because of their short life cycle. A possible solution to this problem could be the use of biodegradable/compostable packaging materials, obtained from annually renewable crops. Poly lactic acid (PLA) is one of the most promising compostable materials. In this work, the refrigerated shelf-life of sliced cooked ham packaged in PLA with Active device and different initial atmosphere modifications was evaluated.

Key words: active packaging, cooked-ham, MAP, oxygen scavengers, PLA, shelf-life.

INTRODUCTION

Modified Atmosphere Packaging is the answer to the increasing demand for fresh products that has required the industry to develop new and improved methods for maintaining food quality and extending shelf life. Typically longer shelf life for cooked ham is obtained with high level of CO_2 and absence of O_2 to avoid oxidation processes.

At present, one of the trends in food packaging is the use of bio-polymeric materials that can be considered an alternative to traditional plastics obtained from petrochemical industry. Nevertheless, bio-based materials are permeable to gases, for this reason the use of an oxygen scavenger can be a promising approach for those foods sensitive to oxidations. This work focused on the suit-

ability of expanded PLA tray lidded with PLA film for sliced cooked ham Modified Atmosphere Packaging.

MATERIALS AND METHODS

Sampling: Around 100g of sliced cooked ham were packaged under modified atmosphere in an automatic packaging machine, operating according to the vacuum compensation procedure. Cooked ham samples were packaged in Polylactic acid (PLA) trays laminated with a PLA film and lidded with a PLA film (thickness 30μm).

Three different PLA packaging solutions were analyzed:

A. MAP (70% N_2:30%CO_2).
B. Active Packaging - AP - (O_2 scavenger combined with a CO_2 emitter);
C. Active Packaging - AP - (O_2 scavenger combined with a CO_2 emitter) and MAP (100% N_2).

All samples were stored at 6-8°C and analyzed in triplicates at fixed intervals of time.

Microbial analysis: Total mesophilic and Lactobacillus spp. counts were controlled on the packaged samples, by means incubating 1 mL of known dilutions (from –2 to –8) of homogenised ham (10 g in sterile NaCl 1% solution) in PCA and MRS broth respectively. After incubation (4 days at 25°C), the microbial counts were performed and the results expressed as UFC/g.

pH: The surface pH of packaged ham was measured by means a pHmeter, equipped with a flat probe.

Gaseous atmosphere analysis: The gaseous atmosphere evolution inside the packages was monitored by analysing a known amount of headspace atmosphere (50-100 μL), withdrawn by a gastight syringe, through a silicon septum glued onto the package surface. The GC analysis was performed on a GC-TDC instrument, equipped with a CTRI column (Alletech Italia, Milan).

Colour evolution: ham colour evolution was measured by means a colorimeter MINOLTA Chromameter mod CR 400 and the results expressed as CIE L*,a*,b* parameters.

Weight loss: The weight changes of the packaged ham were controlled by weighing the entire packages on a laboratory technical balance.

RESULTS AND DISCUSSION

Atmosphere evolution

It is clear evident that, despite the low barrier offered by PLA, the active device is able to control the oxygen amount (samples C). The residual oxygen is also low in the packages which contain initially air (samples B). Instead, in the packages with only MAP (samples A), the O_2 percentage reaches a value around 10% after 16 days of storage, due to the natural permeability of the bio-polymer.

In figure 2, the CO_2 evolution inside the different packaging options is reported. The Active Packaging coupled with 100% N_2 (samples C) shows a CO_2 increase up to 7%, meanwhile in samples B the carbon dioxide percentage reaches a value of 10% in 16 days, may be for a more pronounced microbial growth due to the presence of air inside packages at time zero. As it could expected, traditional MAP

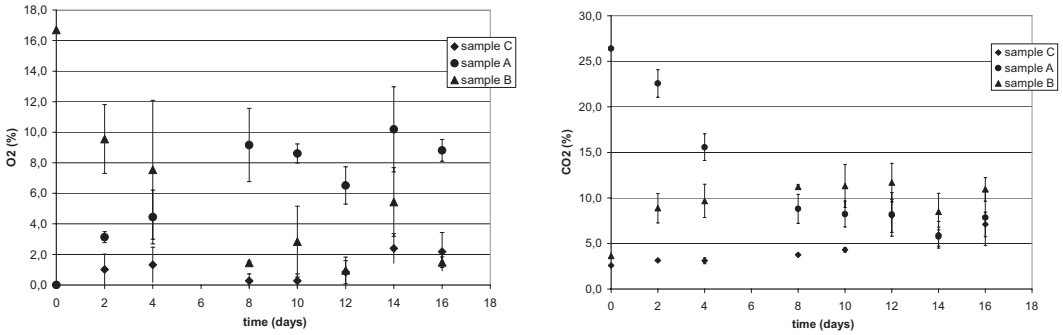

Fig.1-2: O_2 and CO_2 evolution of the samples A, B and C during storage

sample (A) shows a CO_2 decrease, which reaches a value of 9% around the 8th days of storage.

Microbial Analysis

The total mesophilic count is almost completely represented by Lactobacillus species which can inhibit the presence of others alterative microganisms. The Lag Phase of the microbial growth starts between days 8-10 in all the thesis, as shown in Fig.3. Sample C (AP with 100% N_2) shows the highest value at 12 days, it is evident that the starting high O_2 level supports the microbial growth. Very interesting is the result of the traditional MAP sample (A) with no active packaging: the evolution of the atmosphere due to the low barrier properties of PLA may causes a faster growth of the microrganisms; instead the Log phase in MAP samples is reached at the same time of the Active Packaging, in which the O_2 scavenger is present. Furthermore, the highest exponential growth is reached 2 days later then the Active Packaging with 100% Nitrogen (C). Sample B has an intermediate behaviour.

Colour

Cooked-ham packaged in air with the O_2 scavenger shows a significant changing in the colour after 8 days, as expected the presence of O_2 from time zero has negative effects on the aspect of the product. Very interesting is the result of sample C (AP with 100% N_2) and A (traditional MAP) in which the variation of the "L" parameter is not significant till 14 and 16 days respectively. Best result in samples C in which the "a" value decrease only from day 14, which means a changing toward green/grey.

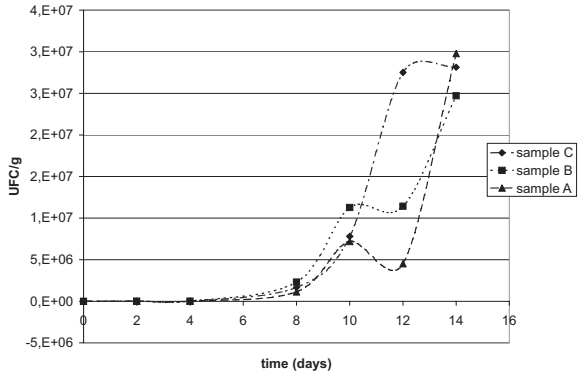

Fig.3: Microbial growth in samples A, B and C during storage.

Beside the L,a,b values, pictures of the products are taken during storage, and even if sample A is more stable during all the tested

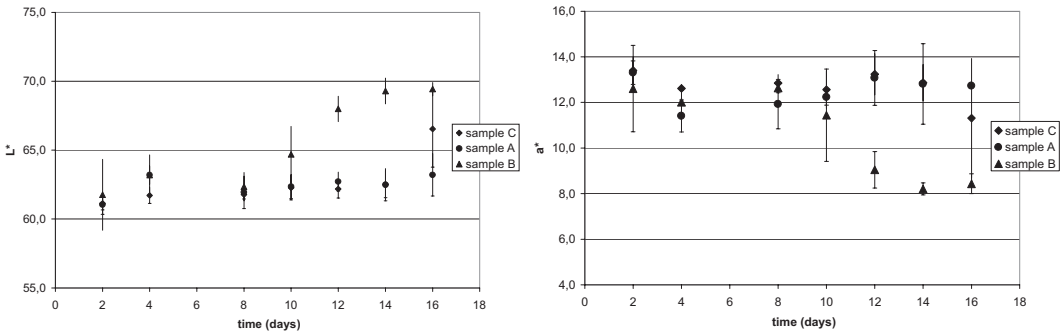

Fig. 4-5: Colour parameter L* and a* evolution during storage

period, the preferred solution is the AP combined with 100% N_2 in which the "a" value is constantly higher.

Weight loss

The highest value at the end of the shelf-life is 3% weight loss, which is acceptable for a very high humid product like cooked-ham. No significant difference between the three thesis.

CONCLUSIONS

PLA is a valid alternative to tradition polymers in the packaging of sliced take-away cooked-ham. The use of an O_2 absorber combined with a CO_2 emitter prolong the shelf-life up to 10 days at the challenge temperate of 6-8°C. The shelf-life is higher (14 days) when the Active Packaging is combined to MAP at very low O_2 content. The starting absence of O_2 guarantees the best colour of the product. MAP packaging without any Active Packaging surprisingly showed very good results, considering the high permeability to gases; colour and microbial count are comparable to the Active Packaging solutions. As usual the very high hygienic quality of the product and the control of the packaging process are fundamental for the good result of a packaging system.

OXYGEN CONSUMPTION DETERMINATION BY INNOVATIVE FLUORESCENT PROBE

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ABSTRACT

Oxidation of lipids in food is a general term for a complex process that results in generation of off-flavours and reduction in nutritional value. In order to track the main steps of lipid oxidation in food, total hydroperoxide value (POV) and volatile compounds are measured. Hydroperoxides are generally recognized as primary lipid oxidation products and volatile compound as secondary lipid oxidation products. Volatile compounds generate from the hydroperoxide degradation and are responsible of taste and flavour deterioration. Unfortunately, current available analytical methods to measure lipid oxidation are time consuming and they do not preserve the food system and the packaging.

In this study we present a new, sensitive, rapid and easy method to track lipid oxidation during storage preserving packaging and food system.

In order to correlate POV to oxygen consumption, food products containing long-chain polyunsaturated fatty acids were stored at 8°C for 1 month. Samples were analysed at the beginning of the storage and after 4, 7, 14, 21, 25 and 28 days, for POV by tritometric method. Oxygen consumption was determined by continuously monitoring the oxygen partial pressure in the package using a fluorescent probe.

Results showed a good correlation between POV and oxygen consumption, demonstrating the possibility of using the fluorescent probe to track lipid oxidation during storage preserving food matrix and packaging.

Key words: hydroperoxide value, long-chain polyunsaturated fatty acids, oxidation, oxygen consumption.

INTRODUCTION

One of the main trends in modern food industry is the one aiming at supplying nutrition and wellness rather than food products. In this sense, many food products are being complemented with nutritional ingredients, such as vitamins or long-chain polyunsaturated fatty acids (LC-PUFA).

The addition of such components increases the sensitivity of foods to oxidation, and a good understanding of the parameters influencing quality preservation is required to supply high quality foods with retained nutritional value at the end of their shelf life.

Classical method for the determination and characterisation of lipid oxidation, i.e. measurement of hydroperoxide and volatile formation, are time consuming and destructive methods, in the sense that one sample can not be measured over time.

The need for a simpler method allowing the monitoring of lipid oxidation as a function of time and storage conditions is therefore strong. The study presented in this paper proposes such a method based on oxygen consumption determination.

Fluorescent oxygen sensors enabling the non-destructive measurement of oxygen partial pressure in packaging headspace (Wolbeis *et al.* 1991 & 1998;) are used to monitor oxygen partial pressure evolution over time as well as packaging permeability (Lundquist *et al.* 2004, Wyser *et al.* 2006), permitting the determination of oxygen consumption by the product. This oxygen consumption is than compared to the formation of hydroperoxide as determined by classical methods. A correlation between those values would demonstrate enable monitoring of lipid oxidation during storage tests.

MATERIALS AND METHODS

Product

Yoghurt drinks packed in 100 mL polyethylene (HDPE) bottles and fortified by LC-PUFA were used for the study.

Determination of peroxide value

The peroxide value was determined by a titrometric method based on AOCS recommended practice Cd 8b-90: Peroxide value, Acetic acid - isooctane method.

Determination of package permeability

The oxygen permeability of the HDPE bottles has been determined by measuring the evolution of oxygen partial pressure inside bottles filled with water, containing a small amount of silver nitrate to avoid bacterial growth, as described in Wyser *et al.* (2006).

A Fibox 3-Trace oxygen analyser (Presens, Germany) was used for these measurements.

The measurements were performed in the same climatic conditions than the storage tests.

Determination of headspace oxygen partial pressure evolution

The method used for the monitoring of the oxygen partial pressure in the headspace was identical to the one used for the determination of the permeability, the bottles containing the tested products rather than water. The total amount of oxygen in the package was calculated by determining the volume of the headspace present in the package and by making the hypothesis that the solubility of oxygen in the product was equal to its solubility in water, and that product and headspace were at equilibrium at all times.

Storage

In order to correlate POV to oxygen consumption, packed product was stored at 8°C for 1 month (shelf life of the product). Samples were analysed at the beginning of the storage and after 4, 7, 14, 21, 25 and 28 days, for POV by tritometric method.

RESULTS & CONCLUSIONS

Figure 1 shows the evolution of the oxygen partial pressure in the headspace of a sample stored at 8°C over a period of 28 days.

The permeability of the bottles were determined to be 0.125 mL/pack/day at 8°C and considering an oxygen partial pressure difference of 212 mbar between the atmosphere and the headspace.

From these results the oxygen consumed by the product can be determined as the sum of the reduction of oxygen measured in the headspace and the oxygen entering the packaging, i.e. the oxygen permeation rate considering the actual partial pressure difference between atmosphere and package.

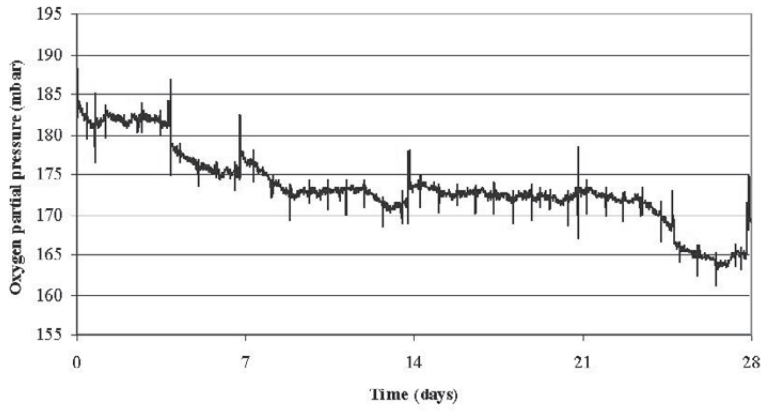

Figure 1: Oxygen partial pressure measured in packages containing LC-PUFA enriched yoghurt drinks during 8°C storage.

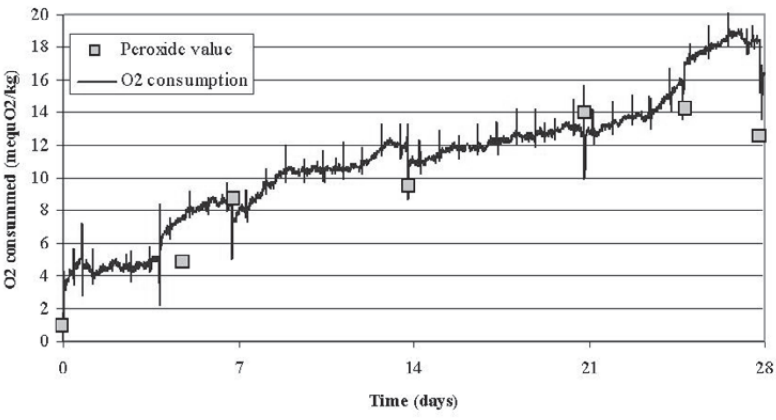

Figure 2: Oxygen consumption (continuous line) and peroxide values (grey squares) measured on LC-PUFA enriched yoghurt drinks during 8°C storage.

Figure 2 shows the results of this calculation, in mequ O_2/kg (continuous line) together with the measured peroxide values (grey squares).

An excellent correlation between both values can be found over most of the test duration.

At the end of the test, a slight deviation can be observed between the results of the two methods, probably due to the degradation of peroxides into volatiles. A determination of the

volatile in the headspace through head-space solid phase microextraction gas chromatography coupled to mass spectrometry (HS-SPME-GC/MS) could not confirm this hypothesis. It is however well known that the volatiles formed by the degradation of hydroperoxide are very prompt to permeate through polyethylene, which might explain the fact that a rapid increase in volatile concentration could not be observed.

This good correlation between POV and oxygen consumption, demonstrates the possibility of using the fluorescent probe oxygen monitoring to track lipid oxidation during storage, simplifying the experimental work and preserving food matrix and packaging allowing a continuous monitoring a product quality over an entire storage test.

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INHIBITION OF PATHOGENIC BACTERIA INOCULATED ON RAW CHICKEN BY AQUEOUS CHLORINE DIOXIDE TREATMENT

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ABSTRACT

Inactivation of *Escherichia coli* O157:H7, *Salmonella typhimurium, Listeria monocytogenes* and *Campylobacter jejuni* was evaluated on inoculated chicken by aqueous chlorine dioxide (ClO_2) treatment. Chicken samples were inoculated with 6-9 log CFU/mL of *E. coli* O157:H7, *S. typhimurium, L. monocytogenes,* and *C. jejuni,* respectively. Chicken samples were then treated with 0,50, and 100 ppm of ClO_2 solution and stored at 4±1□.

Aqueous ClO_2 treatment decreased the populations of the pathogenic bacteria on chicken breast and chicken leg. In particular, 100 ppm ClO_2 treatment on chicken breast and chicken leg reduced the populations of *E. coli* O157:H7, *S. typhimurium, L. monocytogenes,* and *C. jejuni* by 1.00-1.27, 1.37-1.44, 0.61-1.93, and 0.99-1.21 log CFU/g, respectively. Aqueous ClO_2 treatment on the growth of the bacteria was continuously in effect during storage, resulting in decrease of populations in *E. coli* O157:H7, *S. typhimurium, L. monocytogenes,* and *C. jejuni.*

The pH and volatile basic nitrogen (VBN) in chicken breast and chicken leg decreased with increasing ClO_2 concentration. Thiobarbituric acid reactive substance values of the chicken breast and chicken leg increased during storage, regardless of aqueous ClO_2 concentration.

Sensory evaluations results represented that the quality of the chicken breast and chicken leg treated with aqueous ClO_2 during storage was better than that of the control. These results indicate that aqueous ClO_2 treatment can be useful in improving the microbial safety of chicken breast and chicken leg during storage.

Key words: chicken, aqueous chlorine dioxide, pathogenic bacteria, quality, storage.

INTRODUCTION

Chicken is a very popular food due to low fat content and high nutritional value, yet it is highly perishable and always has the possibility of contamination of pathogens during processing and storage. Major bacterial contamination on chicken includes *Escherichia coli, Salmonella spp., Listeria monocytogenes,* and *Campylobacter* (Anang *et al.*, 2007)

To achieve bacterial decontamination, aqueous chlorine dioxide has been used as a food preservation method. Chlorine dioxide is a strong oxidizing agent and has a broad antimicrobial effectiveness (Wu and Kim, 2007). Regarding the effectiveness of chlorine dioxide in reducing the number of bacteria present in poultry-processing water, chlorine dioxide is more effective than chlorine. Therefore, in this study, we examined the effect of aqueous chlorine dioxide treatment to inactivate pathogenic bacteria, pH, VBN, lipid oxidation, and sensory evaluation of chicken breast and chicken leg during storage.

MATERIALS AND METHODS

Aqueous ClO_2 was prepared using a chlorine dioxide generating system as described previously (Youm et al, 2005). Samples were treated by dipping in 0,50, and 100 ppm aqueous chlorine dioxide solution for 10 min, respectively. Samples were then individually packaged in polyethylene terephthalate containers and stored at 4±1C. After ClO_2 treatment, samples (5 g) were removed using a sterile scalpel. Samples were then homogenized using a Stomacher for 3 min, filtered through a sterile cheese cloth, and diluted with 0.1% peptone water for microbial count. Serial dilutions were performed in triplicate on each selective agar plate. *E. coli* O157:H7 counts were determined by plating appropriately diluted samples onto Choromogenic E. coli/coliform medium. *S. typhimurium* were plated onto Salmonella Chromogenic agar base. All plates were incubated at 37C for 24 h. *L. monocytogenes* counts were determined by plating appropriately diluted samples onto Listeria selective agar base. Plates were incubated at 37C for 48 h. For *C. jejuni*, they were plated onto Brucella agar having 10% sheep blood under microaerophilic atmosphere, and plates were incubated at 42C for 24 h. Each microbial count was the mean of three determinations. Microbial counts were expressed as log CFU/g.

Measurement of volatile basic nitrogen (VBN) was determined according to the micro-diffusion method. Lipid oxidation was determined according to the method of Ahn and others (1998). Thiobarbituric acid-reactive substance (TBARS) values were expressed as mg malondialdehyde (MDA)/kg of sample. Samples were analyzed for their color, odor, and overall acceptability by 8 trained panelists. Sensory qualities of samples were evaluated using five point scoring method. Sensory scores were 5, very good; 4, good; 3, fair; 2, poor; and 1, very poor.

RESULTS

Aqueous ClO_2 treatment significantly decreased the populations of *E. coli* O157:H7, *S. typhimurium, L. monocytogenes,* and *C. jejuni* on chicken breast and chicken leg, compared to the control. In particular, 100 ppm ClO_2 treatment

Table 1. Effect of aqueous ClO_2 treatment on the growth of the pathogenic bacteria in chicken during storage

Pathogenic bacteria	ClO_2 (ppm)	Chicken breast Storage time (day)			Chicken leg Storage time (day)		
		0	2	4	0	2	4
E. coli O157:H7	0	5.92	7.26	6.27	5.69	7.50	7.09
	50	5.06	7.32	5.69	4.55	7.16	6.76
	100	4.92	6.84	4.60	4.42	6.80	6.66
S. typhimurium	0	5.53	5.64	5.65	5.28	5.32	5.47
	50	4.84	4.86	5.29	4.95	5.04	5.09
	100	4.16	4.57	4.95	3.84	4.20	4.51
L. monocytogenes	0	6.38	6.70	6.53	7.05	7.41	7.34
	50	6.10	6.45	6.33	6.29	7.09	6.31
	100	5.77	6.37	6.20	5.12	6.08	5.82
C. jejuni	0	7.94	8.12	7.28	8.08	8.60	7.61
	50	7.06	7.66	7.05	7.92	8.07	7.21
	100	6.73	7.57	6.52	7.09	7.31	6.41

(Unit: Log CFU/g)

on chicken breast and chicken leg reduced the populations of E. coli O157:H7, S. typhimurium, L. monocytogenes, and C. jejuni by 1.00-1.27, 1.37-1.44, 0.61-1.93, and 0.99-1.21 log CFU/g, respectively (Table 1).

The pH of the chicken breast and chicken leg decreased with increasing ClO_2 concentration. The results suggest that the pH of the chicken breast and chicken leg increases by formation of ammonia during storage. The VBN value is one of the indicators for deterioration. VBN values of the chicken breast and chicken leg increased during storage, and ClO_2 treatment reduced VBN values during storage. TBARS values of chicken breast and chicken leg increased during storage. However, there was no significant change in TBARS values among treatments. Sensory evaluation results showed that the quality of the chicken breast and chicken leg treated with aqueous ClO_2 during storage was better than that of the control.

CONCLUSION

The results clearly suggest that aqueous chlorine dioxide treatment can decrease the growth of pathogenic bacteria on chicken products without impairing qualities. Therefore, 100 ppm ClO_2 treatment can be used as a sanitizing agent instead of sodium hypochlorite during slaughtering process of chicken to improve microbial safety.

ACKNOWLEDGEMENT

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EFFECTS OF EUGENOL, *TRANS*-2-HEXENAL AND MODIFIED ATMOSPHERE ON THE MICROBIOLOGICAL SHELF LIFE ON FRESH-CUT APPLES

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ABSTRACT

The application of natural antimicrobial compounds to improve food quality has been increasing, based on the consumer's preference for foods without chemical additives. Eugenol and *trans*-2-hexenal have been known as having antimicrobial activity. Eugenol, used as a flavouring agent in cosmetics and food products, is active against pathogenic bacteria, fungi and virus. *Trans*-2-hexenal is a component of the aroma of fruit and vegetables. The results obtained in this work showed their antimicrobial activity, alone and as mixture, on mesophilic bacteria of Granny Smith apples.

Key words: fresh-cut apples, eugenol, *trans*-2-hexenal, shelf life, modified atmosphere.

INTRODUCTION

Marketing of fresh-cut, packaged and ready-to-eat products has increased rapidly due to increased consumer demand for fresh convenient foods. Minimally processed apples have a shorter shelf-life than their whole counterparts, because of higher susceptibility to microbial spoilage, increased respiration rate and ethylene production, which is stimulated by wounding of the tissue. Cell rupture from slicing of fruit is responsible for releasing intracellular products, such as enzymes, which can have a negative impact on the quality of cut apples (Olivas *et al.*, 2007). In recent years, the interest in the possible use of natural compounds, to prevent bacterial and fungal growth extending the shelf-life and to ensure the safety of

foods, has notably increased (Lanciotti *et al.*, 2003). Plants and plant products are a source of natural compounds; the ability of plant volatiles to inhibit decay microorganisms is one of the reason for interest in them as components of biological means for prolonging the shelf-life of minimally processed fruits and vegetables. In particular eugenol and *trans*-2-hexenal have been known as having antimicrobial activity. The aim of this work was to evaluate the effect of different concentrations of eugenol and *trans*-2-hexenal (alone and as mixture) on mesophilic cell load of apples, in relation to packaging atmosphere and storage temperature.

MATERIALS AND METHODS

Granny Smith apples were hand washed with drinkable water having, according to Italian law, 0.2 mg/L free chlorine. Fruits with no external injury were selected. Then, they were peeled and sliced with sharp knifes. The slices were pretreated, for 15 minutes with a solution containing 0.2% (w/w) of citric acid and 1.0% (w/w) of ascorbic acid and then packaged in low permeability bags (Tecnovac, San Paolo D'Argon, Bergamo, Italy)] by means of S100-Tecnovac equipment. The bags were 170mm×250mm long with properties specified by the manufacturer as follows: the film permeabilities to oxygen and carbon dioxide were 3.26×10^{-19}mol m m^{-2} s^{-1} Pa^{-1} and 9.23×10^{-19} mol m m^{-2} s^{-1} Pa^{-1} respectively and water vapour transmission rate of 1.62×10^{-10} kg m^{-2} $s^{-}1$. All of the samples were packaged in air (control atmosphere) and in a modified atmosphere (M.A.: 65% N_2, 30% CO_2, 5% O_2). The samples differed for the use of modified atmosphere and the amounts of *trans*-2-hexenal and eugenol. The concentration used calculated taking into account the total bags volume were as follows: 100 and 200 ppm both for trans-2-hexenal and eugenol. When used in mixture the concentrations were 50 + 50 ppm and 100 + 100 ppm for *trans*-2-hexenal and eugenol, respectively. The appropriate concentrations of *trans*-2-hexenal and eugenol or their mixture were introduced in the atmosphere, before packaging, by means of 9 mm soaked diameter filter disk (Antibiotica-Testblattchen. Shleicher and Shull, Dassel, Germany) put in a Durham tube. Samples were stored at 4 and 15 °C. As controls, samples were prepared at the same conditions, without volatile molecules, and stored at the same temperatures. Aliquots of 20 g of the different samples were diluted with saline solution (180 ml) and homogenized with Lab blender 400-Stomacher (Medical, Steward, London, U.K.). The appropriate decimal serial dilutions were plated onto Plate Count Agar (PCA, Oxoid, Milan, Italy) to enumerate mesophilic cell load, after the incubation at 30 °C for 48 h. Microbiological data are the average of at least two repetitions and are accompanied by standard deviation. The variability coefficients, expressed as the percentage ratio between the standard deviation and the mean value, were less than 7%.

RESULTS AND CONCLUSIONS

The antimicrobial properties of eugenol and *trans*-2-hexenal against apple mesophilic bacteria were investigated. The table 1a shows the evolution of microbial cell load (Log CFU/g) of mesophilic bacteria of Granny Smith apples packaged without and with antimicrobial compounds at different concentrations, in modified atmosphere (M.A.) and stored at 4 °C. The addition of eugenol and *trans*-2-hexenal

Table 1a: Cell load (Log CFU g^{-1}) accompanied by standard deviation of mesophilic bacteria of Granny Smith apples packaged in modified atmosphere and stored at 4 °C with and without antimicrobial compounds.

Time (days)	Log CFU g^{-1}						
	control	Trans-2-hexenal (ppm)		Eugenol (ppm)		Trans-2-hexenal + Eugenol (ppm)	
		100	200	100	200	50+50	100+100
0	4.26 ± 0.6	4.26 ± 0.6	4.26± 0.6	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6
2	3.30 ± 0.1	3.30 ± 0.1	3.30 ± 0.1	3.25 ± 0.2	4.48 ± 0.5	3.48 ±0.4	3.00 ± 0.3
4	3.38 ± 0.2	3.28 ± 0.2	3.28 ± 0.2	3.08 ± 0.3	3.36 ± 0.4	3.30 ± 0.3	- [a]
7	3.08 ± 0.1	3.60 ± 0.4	2.78 ± 0.1	3.08 ± 0.3	3.87 ± 0.4	3.11 ± 0.3	2.90 ± 0.2
9	3.32 ± 0.3	2.84 ± 0.3	2.90 ± 0.2	2.95 ± 0.3	2.78 ± 0.2	3.84 ± 0.4	3.34 ± 0.5
11	3.30 ± 0.5	2.00 ± 0.4	-	2.00 ± 0.2	2.78 ± 0.2	3.20 ± 0.3	2.30 ± 0.3
17	3.30 ± 0.4	2.00 ± 0.2	2.00 ± 0.1	2.30 ± 0.2	-	2.00 ± 0.2	-

[a] cell number < 10^2 CFU g^{-1}

Table 2: Cell load (Log CFU g^{-1}) accompanied by standard deviation of mesophilic bacteria of Granny Smith apples packaged in modified (a) and in control atmosphere (b) at 15 °C with and without antimicrobial compounds.

(2a)

Time (days)	Log CFU g^{-1}						
	control	Trans-2-hexenal (ppm)		Eugenol (ppm)		Trans-2-hexenal + Eugenol (ppm)	
		100	200	100	200	50+50	100+100
0	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6
1	3.38 ± 0.4	3.67 ± 0.4	3.80 ± 0.3	3.18 ± 0.2	3.54 ± 0.2	2.71 ± 0.1	3.21 ± 0.1
2	5.89 ± 0.5	5.96 ± 0.5	5.48 ± 0.5	5.85 ± 0.5	5.38 ± 0.4	5.04 ± 0.4	5.72 ± 0.6
4	6.15 ± 0.5	5.36 ± 0.5	5.60 ± 0.5	5.48 ± 0.6	6.25 ± 0.4	6.15 ± 0.1	6.20 ± 0.2
7	6.99 ± 0.6	5.38 ± 0.6	6.34 ± 0.5	6.18 ± 0.3	6.15 ± 0.2	6.42 ± 0.5	6.04 ± 0.1
9	7.58 ± 0.5	6.30 ± 0.5	6.54 ± 0.4	6.34 ± 0.0	6.35 ± 0.2	6.00 ± 0.1	6.30 ± 0.4
11	7.60 ± 0.7	6.30 ± 0.5	6.90 ± 0.5	5.98 ± 0.2	6.00 ± 0.3	6.60 ± 0.4	6.65 ± 0.0
17	7.70 ± 0.7	6.90 ± 0.6	6.50 ± 0.4	6.90 ± 0.6	6.90 ± 0.7	6.80 ± 0.1	6.50 ± 0.2

(2b)

Time (days)	Log CFU g^{-1}						
	control	Trans-2-hexenal (ppm)		Eugenol (ppm)		Trans-2-hexenal + Eugenol (ppm)	
		100	200	100	200	50+50	100+100
0	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6	4.26 ± 0.6
1	5.14 ± 0.5	4.79 ± 0.1	3.96 ± 0.0	5.48 ± 0.2	3.32 ± 0.3	3.18 ± 0.0	5.70 ± 0.4
2	5.48 ± 0.3	6.68 ± 0.2	6.48 ± 0.1	6.30 ± 0.0	5.54 ± 0.2	6.18 ± 0.5	6.78 ± 0.5
4	6.00 ± 0.6	6.30 ± 0.1	6.40 ± 0.3	6.54 ± 0.1	6.18 ± 0.0	5.96 ± 0.4	5.60 ± 0.1
7	6.18 ± 0.5	6.37 ± 0.4	5.90 ± 0.5	6.33 ± 0.5	6.88 ± 0.2	6.15 ± 0.3	6.25 ± 0.4
9	6.86 ± 0.4	6.30 ± 0.2	6.61 ± 0.3	6.57 ± 0.4	6.48 ± 0.6	6.30 ± 0.2	6.20 ± 0.2
11	7.00 ± 0.7	6.83 ± 0.0	6.50 ± 0.0	6.78 ± 0.2	6.78 ± 0.2	6.73 ± 0.6	6.30 ± 0.5
17	7.80 ± 0.6	6.60 ± 0.3	6.50 ± 0.1	6.90 ± 0.3	7.20 ± 0.7	7.10 ± 0.5	6.60 ± 0.0

influenced mesophilic cell load when the storage was prolonged. In fact, no antimicrobial activity was observed on mesophilic viability loss within 9 days; otherwise, after 11 days, eugenol and *trans*-2-hexenal showed a strong effect on mesophilic population. At the end of the experimental time, the maximum viability loss on the samples, added with 200 ppm of eugenol and with 100 + 100 ppm of eugenol and *trans*-2-hexenal, respectively, was observed. Due to the variability of the samples packaged in control atmosphere, no hypothesis was formulated on antimicrobial activity (data not shown). The samples packaged in M.A. and stored at 15 °C were more influenced by antimicrobial compounds than others; in fact the antimicrobial activity of the *trans*-2-hexenal, was observed within short storage time as a lower mesophilic cell load was recovered (5.36-5.60 Log CFU g^{-1} for the samples in the presence of 100 and 200 ppm, respectively and 6,15 Log CFU g^{-1} for the samples without *trans*-2-hexenal) after four days (tab. 2a). Similar results were obtained for the samples packaged in presence of the eugenol (alone and as synergic effect with *trans*-2-hexenal) (tab. 2a). In control atmosphere, the antimicrobial activity of both molecular compounds was effective after 9 days (tab. 2b). The results obtained provided evidence for the inhibitory effects of *trans*-2-hexenal and eugenol as gaseous compounds against mesophilic bacteria of apples slices. Further investigations are necessary to ensure the safety of minimally processed apples also in relation to apple variety, film package permeability and atmosphere conditions.

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APPLICATION OF MODIFIED ATMOSPHERE AND MASTERPACK PACKAGING TO IMPROVE THE CONSERVATION OF PORK MEAT UNDER REFRIGERATION

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ABSTRACT

Steaks of *longissimus dorsi* were packaged on expanded polystyrene trays covered with PVC films. Trays were placed in a masterpack (MP) containing three gas combinations: A) 75%O_2+25%CO_2, B) 50%O_2+50%CO_2, C) 100%CO_2, and stored under refrigeration (2.0±1.0°C) for 22 days. At 1st, 8th, 15th and 22nd storage days, 4 MP per treatment were removed from the refrigerated chamber, in order to submit samples to physical, chemical and microbial evaluations. It was found the development of *psychrotrophic aerobic* and *Pseudomonas*, from the 15th day storage, regardless the MA used. There was a significant treatment effect for some of the considered parameters, such as pH ($P<0.05$), L* ($P<0.07$), a* ($P<0.07$) and b* ($P<0.01$). There was a significative interaction ($P<0.01$) for TBARS values. The results for this experiment allow to conclude that the use of MA provides a shelf life extension for portioned pork meat up to 15 days storage.

Key words: color, gas mixture, TBARS, shelf life.

INTRODUCTION

In meat products, the loss of quality is mainly due to microbial growth, discoloration, lipid oxidation and surface dehydration (Sarantópoulos *et al.*, 2002). The gaseous atmosphere surrounding the meat is one of the most important factors affecting the color in the processes of storage and distribution, particularly when

associated with the presence of microorganisms, temperature and pH (Lanari *et al.*, 1995).

The modified atmosphere packaging can be used to extend the shelf life of great variety of foods, among them, fresh meats (Luno *et al.*, 2000; Cayuela *et al.*, 2004). In this sense, the aim of this work was to study the effect of the modified atmosphere system associated with master packaging on pork meat quality under refrigerated storage.

MATERIALS AND METHODS

Pork steaks, taken of the *Longissimus dorsi* (LD) muscle from 16 carcasses, were obtained of a local slaughterhouse. These steaks were placed on expanded polystyrene trays (3 steaks per tray) and covered with poly(vinyl chloride) films. The trays were placed in a secondary masterpack (78.5 x 48.5 cm, 0.35m^2, Cryovac) with high gas barrier property, six trays per masterpack (MP), containing three gas (O_2:CO_2) combinations: 75:25 (A); 50:50 (B); and 0:100 (C). After sealing, the atmosphere composition inside the MP was checked using a Dansensor gas analyzer (CheckPoint O_2/CO_2). No significant variation on the mixture was found during the storage.

The MPs were stored in a refrigerated chamber (2.0±1.0°C) up to 22 days. At 1st, 8th, 15th and 22nd storage days, 4 MP packaging with different gas combinations were removed from the refrigerated chamber, in order to submit samples to physical, chemical and microbial evaluations as follows:

Microbiological analysis were performed in raw material for *Salmonella, Total Coliforms, S. Aureus and Pseudomonads*, and in packed meat, in each storage time, for *psychrotrophic aerobic* and *Pseudomonads*, according to Silva *et al.* (2001).

- The pH of meat was measured with a portable pHmeter, with a glass electrode.
- The surface color of meats was analyzed using a colorimeter (MiniScan XE model, Hunterlab), using the CIELab scale (L*, a*, b*).
- The method of Vyncke *et al.* (1970) was used to determine the 2-thiobarbituric acid reactive substances (TBARS) in meat.
- A completely randomized in a factorial 3 x 4 design [3 gas combinations (A, B and C) and 4 storage times (1, 8, 15 and 22 days)], with 4 replicates for each gas combination x storage time of analysis, was used in this study. The statistical analyses of results were carried out using the GLM subroutine of SAS (2000).

RESULTS

The results of microbiological analysis in the raw meat did not show the presence of any of tested microorganisms. But, the statistical analysis showed interaction between the gas combinations and storage time ($P<0.05$) for aerobic *psychrotrophic* counts and effect of the storage time ($P<0.01$) for *Pseudomonads* counts (Table 1), although these counts had been low. For both bacteria, it was observed an important growth after the 15th day (Table 1).

Additionally, according to the results of statistical analysis, the pH of meat was affected by the interaction between gas combinations and storage time ($P<0.01$). Differences were observed only between values for 8th and 15th days, when de pH values from gas combination C (0:100) were slightly higher.

The values of TBARS increased during the storage, but the treatment C was

Table 1. Results of Aerobic *Psychrotrophic* and *Pseudomonads* analysis[a] of stored meat.

Storage time (days)	Aerobic *Psychrotrophic*			*Pseudomonads*
	$75\%O_2 : 25\%CO_2$	$50\%O_2 : 25\%CO_2$	$100\%CO_2$	
1	1.00 (0.42)	1.00 (0.42)	1.00 (0.42)	1.00 (0.23)
8	1.00 (0.42)	1.00 (0.42)	1.00 (0.42)	1.00 (0.23)
15	1.00 (0.42)	1.00 (0.42)	1.00 (0.42)	1.00 (0.23)
22	2.31 (0.42)	4.43 (0.42)	4.70 (0.42)	3.26 (0.23)

[a] $\log_{10}$ CFU/g of meat (standard error).

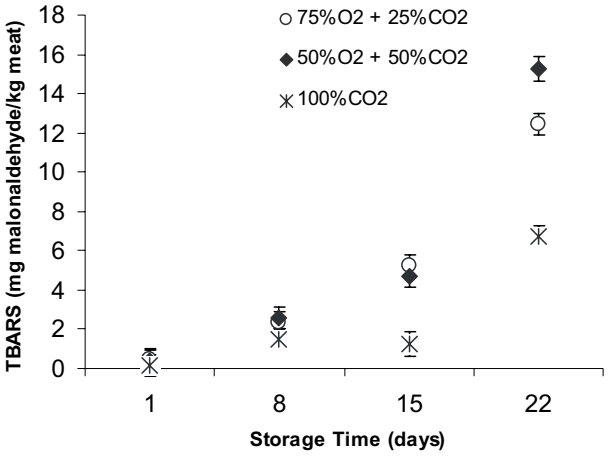

Figure1. Results of TBARS analysis (mg malonaldehyde/ kg meat) of packaged meat.

more efficient against lipid oxidation than the treatments A and B (Figure 1). There was a relationship between concentration of O_2 and TBARS values (Martinez *et al.*, 2005).

In terms of meat color, the parameters L* and a* were affected by gas combinations ($P<0.05$) and storage time ($P<0.01$), while parameter b* was affected only by gas combinations ($P<0.01$). The L* values for gas combination B were higher (62.9) than A and C (61.5). On the other hand, L* values increased during storage time, to 59.3 on 1st day to 63.6 on 22nd day. Sørheim *et al.* (1997) also observed that the values of L * increased until the 7th day, stabilizing until the end of the experiment (21 days). The a* values of meat packed with the gas combination A were higher (8.9), but considering the storage time, the results decreased from the 1st (8.9) to the 22nd (6.2) day. The gas combinations A and B have higher values of b* (~16.8) than C (15.9).

CONCLUSION

The results in this experiment allow to conclude that the use of MA allow a shelf life extension for pork steaks up to 15 days storage.

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EFFECTIVENESS OF ACTIVE AND MODIFIED ATMOSPHERE PACKAGING ON THE SHELF-LIFE EXTENSION OF A CHEESE TART

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ABSTRACT

The shelf life extension by MAP and AP of a typical cheese tart was studied. Baked tarts were packaged inside barrier to gas trays and wrapped with a barrier to gas and water film. Four batches were prepared: 1) Control; 2) MAP with different N_2/CO_2 ratios (70/30 and 20/80); 3) Trays with an iron oxide-based oxygen absorber. Tarts were stored at 20°C and sampled for analysis at 0, 7, 14, 27, 35 and 48 days. Determinations included microbiological analyses (total bacterial count, moulds, yeast and staphylococci), chemical-physical parameters (pH, water activity and dry matter), gas changes (CO_2, O_2 and N_2) inside MAP and AP trays, texture evolution and sensory analysis at our laboratories.

AP allowed a shelf life of 48 days, MAP shelf lives were of 14 and 34 days for 70/30 and (20/80), respectively, while control tarts spoiled after only 7 days.

Key words: active packaging; modified atmosphere packaging; pastry products; shelf life.

INTRODUCTION

Ambient cakes are intermediate to high moisture bakery products, as they have about 20% moisture and water activity (a_w) ranging from 0.65 to 0.95 (Smith and Simpson, 1995; Jones, 2000). The main causes affecting their shelf-life are first of all microbial spoilage, mostly by moulds, and secondly staling (Smith *et al.*, 2004).

The reduction of microbial spoilage of bakery products is preferably obtained by control of post baking contamination, mainly by using modified atmosphere (MAP) or active packaging (AP) (Smith and Simpson, 1995; Guynot *et al.*, 2003a; Guynot *et al.*, 2003b).

Extensive studies have been done on the effect of MAP and AP on the shelf life of different bakery products (Smith *et al.*, 1988; Ooraikul, 1991; Smith and Simpson, 1995; Guynot *et al.*, 2004), but, at our knowledge there are no reports on tarts with a cheese filling.

The aim of the present study was to verify the effects of MAP or AP packaging on extending the shelf life of a cheese tart.

MATERIALS AND METHODS

Cheese tarts were prepared following a traditional local recipe. Short pastry was prepared and pastry circles of 12 cm in diameter were obtained. The filling was obtained by mixing the different ingredients, mainly fresh grated ewe's cheese. An adequate amount of filling was layered in the center of the short crust circle, that was subsequently shaped to give a 8 cm in diameter tart. The tarts were baked at 180°C for 15 minutes in a rotor oven, cooled and packaged inside barrier to gas trays (two for each tray) and wrapped with a barrier to gas and water film. Four batches were prepared, the 1st being the control, the 2nd and the 3rd (MAP) by using different N_2/CO_2 ratios (70/30 and 20/80) and the 4th (AP) by placing a sachet of a iron oxide-based oxygen absorber inside trays. Tarts were stored at 20°C and sampled for analysis at 0, 7, 14, 27, 34 and 48 days. A ten-gram sample was homogenized in 90 mL of sterile water, and serial dilution was performed before plating. Total bacterial count, staphylococci, moulds and yeast were detected on appropriate media (CFU/g). Dry matter (dm), water activity (a_w) and pH were measured both on homogenized short pastry and filling. Texture was determined with a texture analyser (TA-XT2, Stable Microsystems, Surrey, UK) with a 50 kg load cell. Textural determinations were made in three tarts per each lot by using a blade set with knife edge for a cut test (HDP/BS), and a 5mm diameter cylinder probe (P/5), for a puncture test. Two indexes were used for both tests: a) maximum rupture force (as g); b) area under the curve (as g · mm) up to the maximum rupture force. The gas composition of at least three packages per each thesis were sampled and analyzed using a Combi Check 9800-1 gas analyzer (PBI-Dansensor, Denmark). Sensory analysis involved asking thirty-two untrained consumers to evaluate the overall acceptance of the samples by using an hedonic scale from 1 to 7 (1, terrible; 4, acceptable; 7, excellent) for colour, olfactory intensity, taste and consistency

RESULTS AND DISCUSSION

The O_2 concentration inside MAP and AP packages was close to 0% at the start of the experiment and increased only on MAP, which did not exceed 0.40%. The product has an a_w value higher than 0.9, thus is very susceptible to mould growth (Guynot *et al.*, 2003b). Control tarts evidenced mycelia after seven days of storage, while inhibition of mould growth was dependent on CO_2 concentration inside packages (Table 1). In fact, tarts spoiled after 14 and 34 days in 70/30 and

Table 1 – Total bacterial count (PCA)[x], yeast and mould (GYPD) and staphylococci (BP) growth (as CFU/g) during storage of a cheese tart packaged with MAP or AP.

Microbial media	Packaging	Storage time (days)					
		0	7	14	27	34	48
PCA	Control	$1.4x10^2$	$2.2x10^5$	-[w]	-	-	-
	70/30[y]	$1.4x10^2$	$9.8x10^3$	$2.1x10^4$	-	-	-
	20/80	$1.4x10^2$	$1.1x10^4$	$1.2x10^4$	$5.9x10^5$	$1.2x10^4$	$2.5x10^5$
	Absorber	$1.4x10^2$	<10	<10	<10	<10	<10
GYPD	Control	<10	$4.2x10^2$	-	-	-	-
	70/30	<10	<10	<10	-	-	-
	20/80	<10	<10	<10	<10	<10	$7.0x10^3$
	Absorber	<10	<10	<10	<10	<10	<10
BP	Control	<10	<10	-	-	-	-
	70/30	<10	<10	<10	-	-	-
	20/80	<10	<10	<10	<10	<10	<10
	Absorber	<10	<10	<10	<10	<10	<10

[x]PCA, Plate count agar; GYPD, Glucose yeast peptone dextrose agar; BP, Baird Parker Agar.
[y]70/30 = 70% N_2 and 30% CO_2; 20/80 = 20% N_2 and 80% CO_2.
[w]Sampling has been stopped due to visible mould growth on tarts.

20/80 MAP packages, respectively, while the use of oxygen absorber prevented mould growth up to the end of storage. The number of total viable cells increased in tarts inside MAP packages up to 10^5 CFU, while no colonies were found on AP packaged samples. Staphylococci were not detected. Tarts evidenced a strong hardening of control samples after 7 days in storage, MAP tarts hardened after 14 days (only in the external part in 70/30 samples), while tarts with absorber did not show significant changes in texture at the end of the 48 days in storage (data not shown). Sensory analysis gave values over the acceptability threshold for all the storage period and no significant differences were detected among samples (data not shown).

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USE OF CELL-FREE EXTRACT BY BIFIDOBACTERIA TO ENHANCE THE VITALITY OF *LACTOBACILLUS* SPP.

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ABSTRACT

Fermented foods are an effective system of interaction between bacteria and humans. Some of bacteria present in foods are able to colonize human gastrointestinal tract and this is the interpretation key of complex connection "*feeding-healthy*". This study was designed to investigate a new approach to develop the survival and vitality of probiotic strain normally used in manufacture of fermented milks. The bioactivity of cell-free extracts purified by bifidobacteria strains was studied in order to evaluate their influence on growth and survival of *Lactobacillus* spp. The results obtained showed a positive effect of bifidobacteria cell-free extracts.

Key words: bioactivity, cell-free extracts, probiotics, stability time.

INTRODUCTION

The term "probiotic" derives form Greek "*pro-bìos*" (for life) and, frequently, it is used to characterize the microorganisms which perform beneficial effects on health and life of human beings and animals. Fermented foods are an effective system of interaction between bacteria and humans. Bacteria present in foods are able to colonize human gastrointestinal tract and this is the interpretation key of complex connection "*feeding-healthy*". Since time began, fermented milks are considered probiotic foods; the possible therapeutical role of bacterial cultures used has made this category of products one among the first applicative areas of "*nutraceutic*". An essential value both on quality and shelf-life of a probiotic food is given by the therapeutical-healty valence, related to a definite minimum number of viable bacteria into the finished product at its deadline. The normative UNI 10538/93 sets a generic cell load not less than 10^7 CFU/g of probiotic food. So, to enhance probiotic populations growth and survival is a central concern in modern food industry. This study was designed to investigate a new approach to develop the survival

and vitality of probiotic strains normally used in manufacture of fermented milks. The bioactivity of cell-free extracts purified by dairy probiotic bifidobacteria was studied; in order to evaluate their influence on growth and survival of probiotic *Lactobacillus plantarum* strains.

MATERIALS AND METHODS

B. animalis DSM 10140, *B. subtile* DSM 20096 and *B. breve* DSM 20213, were examined throughout this study for the bioactivity of their cell-free extracts towards lactobacilli. The lactobacilli strains studied under the influence of *Bifidobacterium* spp. cell-free extracts were the following: *L. plantarum* c1 (Lc1), *L. plantarum* c83 (Lc83) and *L. plantarum* c99 (Lc99) (Campaniello *et al.*, 2005). *Bifidobacterium* spp. were grown in MRS broth (Oxoid, Milan, Italy) added with cysteine (0,05 w/v) (cMRS) (Sigma-Aldrich, Milan, Italy) (MRS), at 37 °C for 48 h. The cells were harvested from the broth by centrifugation at 5000 rpm at 5 °C, for 15 min and then washed twice with sterile saline solution (NaCl 9 g/l). The supernatant was filtered through a 0,45 μm Millipore filter (Whatman, Dassel, Germany) and the filtrate represented the cell-free hydrosoluble fraction (CFE) (Kaneko *et al.*, 1994). Frozen cultures of *L. plantarum* strains were thawed and pre-cultured in MRS broth (Oxoid, Milan, Italy) (MRS). Serial diluitions of cell suspensions were carried out to get the desired inoculum level (10^2 cfu ml^{-1}). The assays were performed in 250-ml Erlenmeyers, containing 100 ml of MRS broth. The cell-free filtrate from bifidobacteria, was added as a medium ingredient (1% v/v). The samples were inoculated with lactobacilli initial inocula (10^2 cfu/ml) and for each assay pure cultures of the microbial targets, without cell-free filtrate, were used as controls. The samples were incubated at 37 °C in anaerobic condition and the viable count of bifidobacteria was periodically evaluated on MRS agar, incubated at 30°C for 48h. The cell loads recorded for each microbial group were modelled according to the Gompertz equation modified by Zwietering *et al.* (1990); a negative Gompertz equation was used to model the cell load data of each microbial group in death phase. The Probiotic Stability Time (PST), defined as the time, over that the cell load preserve a value upper than 10^7 cfu/ml (hours), and the Probiotic Stability Time Index (PSTI), defined as percentage increase of PST, were calculated.

RESULTS AND CONCLUSIONS

The cell-free extracts influenced significantly all kinetic parameters both in growth and death phases of microbial targets. A stimulant effect on growth of all tested lactobacilli was registered, but Lc83 was the most susceptible among the tested microorganism under cell-free extract action (figure 1a). In fact, a significant ($P<0.001$) lowering of the lag phase in the samples was registered (4.22 hours, 3.99 hours, 4.93 hours and 11.94 hours for Lc83 under *B. animalis* CFE, *B. subtile* CFE, *B. breve* CFE, and control, respectively). Also a significant ($P<0.001$) increase of maximum increase of bacterial load attained at stationary phase was recorded. Lc83 was the most responsive strain to the action of cell-free extract by *B. breve*, in fact, a per cent increase of bacterial load attained at stationary phase equal to 13,84% was recorded (8.23% and 6.94% for Lc83 under *B. animalis* CFE and *B. subtile*, respectively). A slowing of death phase for all samples, in particular for Lc83

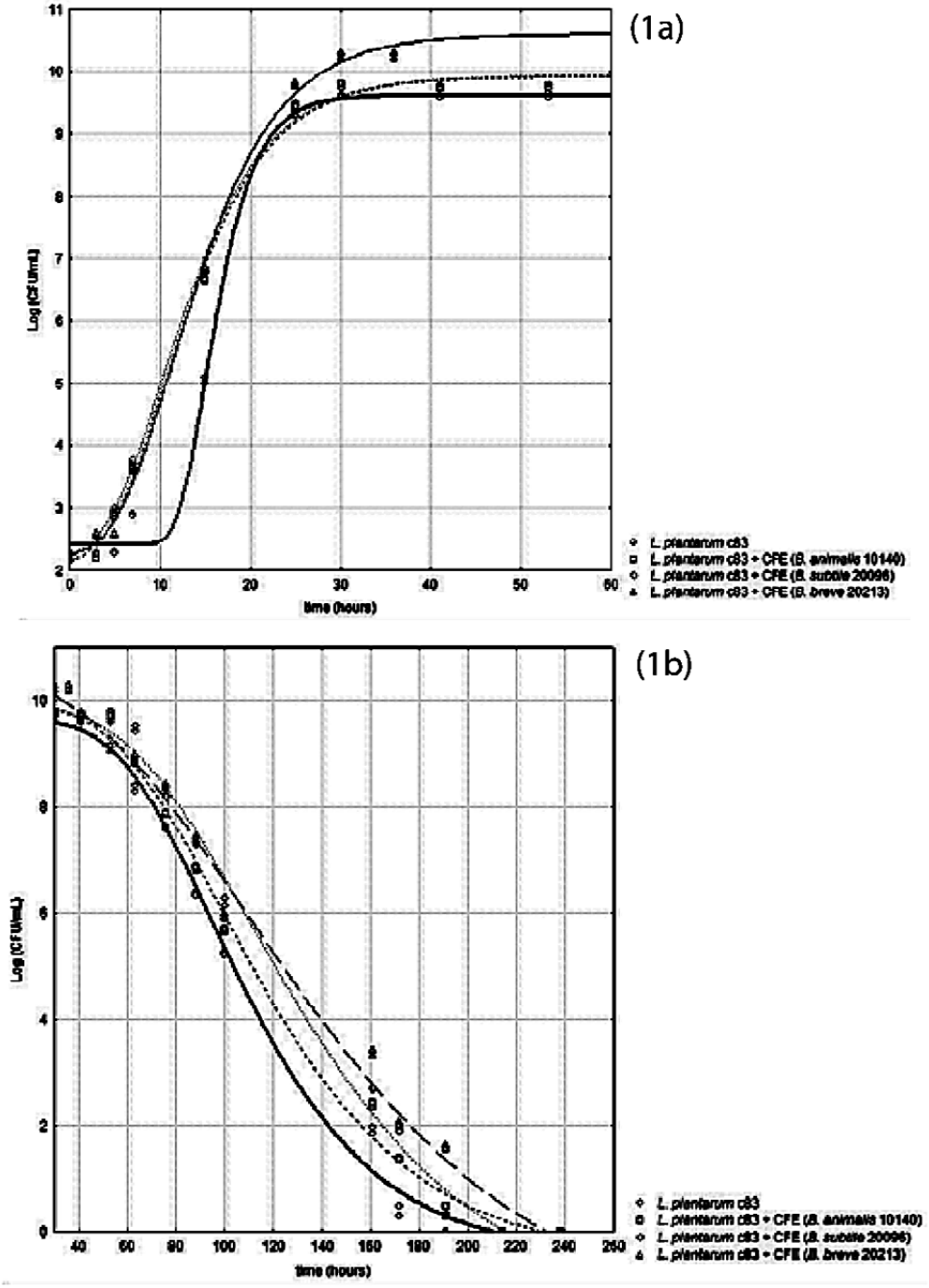

Figure 1: Evolution of *L. plantarum* strains in growth (a) and in death (b) phase with and without cell-free extracts obtained by *Bifidobacterium* spp. The curves represent the best fit of the model to the experimental data.

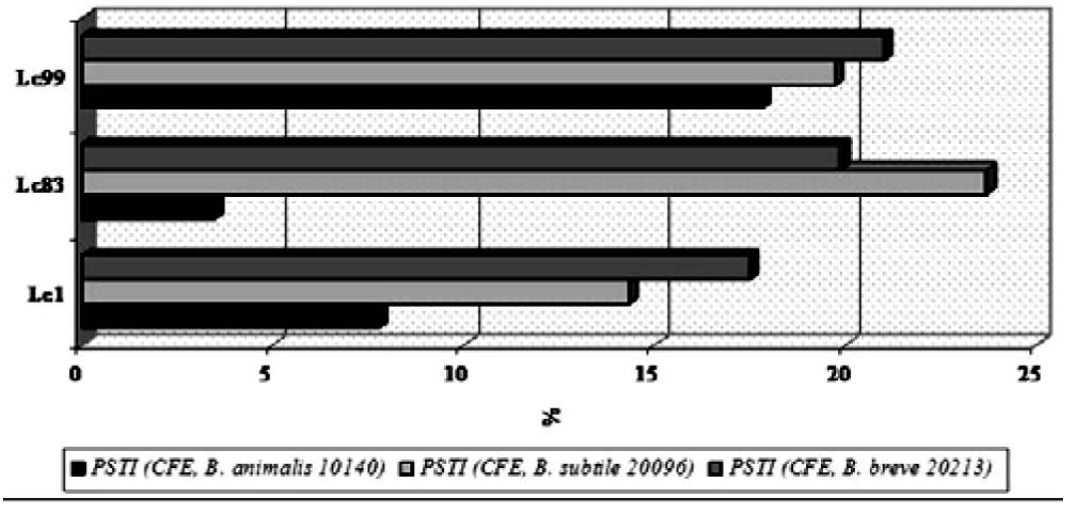

Figure 2: Percentage increase values of Probiotic Stability Time (PSTI) of *L. plantarum* strains grown under cell-free extracts obtained by *Bifidobacterium* spp., added as an ingredient in the growth medium.

under *B. breve* CFE action was recorded (Figura 1b). The Probiotic Stability Time (PST) hold an important role to determine the probiotic potentiality of *L. plantarum* strains and the possible prebiotic activity of bifidobacteria cell-free extracts. In order to quantitatively evaluate and to represent the obtained results, a Probiotic Stability Time Index was also calculated. Positive values of this standardized index were recorded for all sample (Figure 2). The highest values of PSTI was obtained for Lc83 under *B. subtile* CFE (23.70%), whereas the smallest value of PSTI was registered for Lc83 under *B. breve* CFE (3.50%). This result showed the great susceptibility of Lc83 to the cell-free extracts action. In general, the increase of Probiotic Stability Time was very high, in fact, values upper than 14.35% (Lc1 under *B. subtile* CFE) were recorded. Only for Lc1 under *B. breve* CFE, a low value was recorded (7.77%). The results obtained in our experimental conditions strengthen the hypothesis of prebiotic activity of these bifidobacteria cell-free extracts.

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GELATIN FILMS WITH LYCOPENE: MECHANICAL, WATER VAPOR BARRIER, COLOR, AND LIGHT BARRIER PROPERTIES

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ABSTRACT

The addition of lycopene in gelatin based films, more than providing a characteristic color to this material, may also improve its UV barrier property and also imply in the production of an active film because lycopene is a strong antioxidant agent. The aim of this work was to produce edible films based on gelatin containing several concentrations of lycopene to verify the effect of this pigment on some physical properties of these films. The lycopene concentration did not affect the mechanical properties nor the water vapor permeability of films, but affected strongly the films color parameters and the UV and visible light barrier properties. Thus, it can be concluded that lycopene is a potential additive for increasing UV-visible light barrier and color appearance of gelatin-based films without modifying the others physical properties of the films.

INTRODUCTION

Gelatin is a protein largely used in the pharmaceutical and food industry, produced in large scale at prices relatively low, and due to its functional properties it has been explored in the production of edible and/or biodegradable films (Sobral *et al.*, 2001; Carvalho and Grosso, 2004; Thomazine *et al.*, 2005). In overall, gelatin-based films present an effective gas barrier property at low and intermediary relative humidity, and have good mechanical properties in comparison with other films based on others biopolymers.

Moreover, gelatin has been also used for the production of active films, as support for active principles, as antimicrobial or antioxidant agents, for example (Gómez-Guillén *et al.*, 2007). But, the use of pigments to improve the color of films is not usual in the edible film production (Corat *et al.*, 2007). Normally, gelatin-based films are almost transparent and colorless, as a consequence of the blend of this biopolymer with plasticizers, which are normally, colorless low molecular components.

Thus, the incorporation of natural pigments, such as lycopene, in gelatin based films, additionally to furnish a characteristic color to this material, may also improve some film characteristics such as the UV barrier property. Lycopene is a natural red pigment considered as one of the major carotenoid in human diet. This pigment has a strong antioxidant character; with a singlet-oxygen-quenching ability (Weisburger, 2002). Thus, the use of this pigment will also imply in the production of an active film.

The aim of this work was to produce edible films based on gelatin containing several concentrations of lycopene and to verify the effect of this pigment on some physical properties of these films.

MATERIALS AND METHODS

An A type gelatin (Gelita South America), sorbitol (Synth) and lycopene (maltodextrine encapsulated, BASF) were used without previous treatments.

The films were prepared by casting technique (Sobral *et al.*, 2001), with the following compositions: 2 g of gelatin/100 g of film forming solution; 25g of sorbitol/100 g gelatin and 0.2; 0.4; 0.6; 0.8; and 1.0g lycopene/100g of gelatin. The films, with an averaged thickness of 0.008±0.008mm, were conditioned at 58% of relative humidity and 25°C, for 7 days, prior to characterizations.

The mechanical properties of films were evaluated by puncture tests with a TA.XT2i texturometer (Sobral *et al.*, 2001) and the water vapor permeability was determined gravimetrically (Cuq *et al.*, 1997) at 25°C. The color parameters (a*, b*, L*) were determined using a colorimeter MiniScan XE HunterLab (Sobral *et al.*, 2001) and the ultraviolet and visible light barrier properties of the films were measured (280 to 800 nm) using a UV spectrophotometer Libra S22 Biochrom (Corat *et al.*, 2007). All results were submitted to statistical analyses using the Statistica program (Version 7.0).

RESULTS AND DISCUSSION

Despite of some statistically differences observed, the effect of the lycopene concentrations on the puncture force (PF) or puncture deformation (PD) was not clear (Table 1). These properties did not increase nor decreased as a consequence of the increase of the pigment concentration. Conversely, according to the statistical analyses, the lycopene concentration did not affect the WVP of the films (Table 1). In a general manner, the values of these properties were comparable to the values of others gelatin films plasticized with glycerol and/or sorbitol (Sobral *et al.*, 2001, Thomazine *et al.*, 2005, Vanin *et al.*, 2001).

The color parameters of the films were strongly affected by the lycopene concentration (Table 1). The increase of the lycopene concentration turned the films less slight colored, reducing its whiteness ($L^* = -16.8C_L + 93.6$, $R^2 = 0.965$), but increasing the red ($a^* = 19.6C_L - 4.0$, $R^2 = 0.955$) and yellow ($b^* = 23.1C_L - 1.7$, $R^2 = 0.920$) character of the films. The color of lycopene is due to the extension of its system of conjugated carbon-carbon double bonds with 11 conjugated double bonds (Britton, 1995). However, the color intensity of a product will depend on the pigment concentration.

The results of the light barrier properties tests are presented in Table 2, as light

Table 1. Puncture force (PF) and deformation (PD), water vapor permeability (WVP) and color parameters (L*, a*, b*) of gelatin-based films containing lycopene.

C_L	PF (N)	PD (%)	WVP 10^{-8}	L*	a*	b*
0	30.0 ± 1.9^{a,b,c}	2.4 ± 0.2^{a}	4.1 ± 0.3^{a}	89.9 ± 0.0^{a}	-0.9 ± 0.00^{a,c}	2.6 ± 0.1^{a}
0.2	30.7 ± 1.8^{a,b}	1.9 ± 0.1^{b}	4.1 ± 0.2^{a}	86.0 ± 1.0^{b}	2.2 ± 0.2^{a}	8.6 ± 0.3^{b,c}
0.4	32.9 ± 1.1^{a}	2.6 ± 0.4^{a}	3.3 ± 0.3^{b}	84.3 ± 2.1^{b,c}	7.7 ± 2.7^{b}	14.7 ± 3.0^{c}
0.6	28.4 ± 2.5^{b,c}	2.7 ± 0.3^{a}	4.4 ± 0.4^{a}	81.8 ± 0.4^{c}	7.8 ± 0.5^{b}	16.3 ± 1.2^{c}
0.8	33.3 ± 2.6^{a}	2.7 ± 0.2^{a}	4.7 ± 0.6^{a}	76.9 ± 1.3^{d}	14.7 ± 2.0^{c}	23.0 ± 2.8^{a}
1.0	26.4 ± 2.0^{c}	2.3 ± 0.1^{a}	4.8 ± 0.3^{a}	73.6 ± 2.1^{e}	18.4 ± 2.9^{c}	28.0 ± 3.5^{a}

Note: C_L = lycopene concentration (g/100g of gelatin); Different letters represent significant differences ($p<0.05$) between averages obtained through the Duncan test.

transmittance. To be considered as a high light barrier, the film should present very low transmittance values in this analysis. Thus, the films based on gelatin containing lycopene can be considered as a high barrier to the UV light (200-280 nm), but also the increase in the pigment concentration still allowed producing a film practically impermeable to UV (T→0%) when C_L=1 g lycopen/100 g gelatin (Table 2).

In overall, the pigment concentration affected the light barrier property of films. But, the reduction in transmittance of some visible light, such as green light (λ=350nm), red light (λ=400nm) and violet light (λ=500nm) were remarkable (Table 2), differently from the behavior observed within films based on gelatin and colored using chlorophyll (Corat *et al.*, 2007). The highly conjugated double bonds of lycopene are also responsible for the high light absorption of this molecule (Britton, 1995), and consequently, by its light barrier character.

Table 2: Light transmittance (T) of gelatin-based films containing lycopene.

	T (%)					
λ (nm)	CL (g/100g of gelatin)					
	0.0	0.2	0.4	0.6	0.8	1.0
200	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
280	4.1±0.8	3.4±0.9	2.3±1.3	1.6±0.4	1.5±0.1	0.7±0.3
350	69.8±4.9	53.0±9.8	37.3±8.3	18.3±4.2	13.1±2.0	5.6±1.2
400	75.6±4.7	64.3±7.2	50.6±5.8	31.9±5.8	25.6±3.7	13.8±2.4
500	79.4±3.6	74.5±3.8	66.5±8.9	53.5±3.7	49.3±2.4	36.7±1.3
600	80.7±3.1	81.3±0.8	80.1±0.7	77.2±1.4	76.9±1.7	72.2±5.4
700	81.6±2.8	82.6±0.7	82.4±0.6	81.1±2.0	81.4±2.1	78.8±6.2
800	81.8±2.7	82.7±0.7	82.6±0.6	81.6±1.7	81.7±1.9	79.5±5.6

Note: C_L = lycopene concentration (g/100g of gelatin); λ= wavelengths. Different letters represent significant differences ($p< 0.05$) between averages obtained through the Duncan test.

CONCLUSION

Lycopene is a potential additive for increasing UV-visible light barrier and color appearance of gelatin-based films without modifying the others physical properties of the films.

ACKNOWLEDGMENTS

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DEVELOPMENT OF MODIFIED ATMOSPHERE AND HUMIDITY PACKAGING (MAHP) FOR FRESH MUSHROOMS

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ABSTRACT

Mushrooms have short shelf life and are very sensitive to humidity levels. Micro-perforated PVC film is normally used for packaging of mushrooms but high humidity levels causes condensation inside the package. Possible solution to control humidity would be to use desiccants in conjunction with modified atmosphere packaging. The objectives of the present study were to optimize the number of micro-perforations for achieving optimum levels of O_2 and CO_2; and to test the effectiveness of moisture absorber and scavenger for controlling relative humidity and CO2, respectively inside the package. A mixed type of moisture absorber containing bentonite, sorbitol, and $CaCl_2$ in the proportion of 0.55, 0.25 and 0.2 g per g, respectively was used in different amounts (0, 5, 10 and 15 g). The packages were covered with a PVC polymeric film, perforated with 2, 4, 6 and 8 holes of diameter 0.25 mm stored at 10°C for 5 days. The optimum number of holes was found to be 6 at 10°C yielding 5-6% O_2 concentration and 9-10% CO_2 concentration. Packages containing 5g of desiccant were better than those without moisture absorber and those containing 10 and 15 g of mixed desiccant. Mushrooms packed with 5 g of desiccant, 4 g of calcium hydroxide and perforated with 6 holes were still usable and could still be presented on the market and be sold.

Key words: Fresh produce, desiccant, micro-perforation, packaging, scavenger.

INTRODUCTION

Mushrooms (*Agaricus bisporus* L.) are one of the most perishable products; usually their shelf life is 1-3 days at ambient temperature. Mushrooms are conventionally packed in plastic punnets and over-wrapped with perforated PVC film and stored under refrigeration temperature. But high humidity levels, created due

to the high transpiration rate of mushrooms and poor water vapor permeability of the film, causes condensation inside the package as clearly seen underneath the film of the punnets being sold in the supermarket (Mahajan *et al.*, 2008a). A possible solution to control humidity would be to use desiccant in conjunction with MAP. The objectives of the present study were to optimize the number of micro-perforations and scavenger for achieving optimum levels of O_2 and CO_2; and to test the effectiveness of desiccant for controlling relative humidity inside the package.

MATERIALS AND METHODS

Button mushrooms grown at Renaniree Mushrooms, Macroom, Ireland were harvested and transported to the laboratory in chilled conditions. A mixed type of desiccant developed by Mahajan *et al.* (2008b) was used containing bentonite, sorbitol, and $CaCl_2$ in the proportion of 0.55, 0.25 and 0.2 g per g, respectively. Each package containing 250 grams of whole mushrooms was placed with a desiccant bag and covered with a PVC polymeric film, perforated with holes using a needle of diameter 0.25 mm. The factors and their levels are shown in the table. Calcium hydroxide was used as a CO_2 scavenger. After five days in the controlled temperature room at 10°C, the quality parameters such as mushroom weight loss, color and sensory parameters (9-point hedonic scale) were evaluated. Gas analysis was also performed during the storage period.

Factors	Levels
Number of holes :-	2, 4, 6, 8
Desiccant, g :-	0, 5, 10, 15
CO_2 scavenger, g :-	0, 2, 4

Gas analyser

Colorimeter

Package design:

A user friendly software (PACK-in-MAP) was used for designing MAP for mushrooms (Mahajan *et al.,* 2007). In the PACK-in-MAP software, the type of product, storage conditions, amount of product to be packed, and size and geometry of the package was defined. The software then selected the optimum range of O_2 and CO_2 and calculated the respiration rate according to the model reported by Cliffe-Byrnes *et al.* (2007) for that product. Based on this information, the software then selected the best possible film, calculated the film area and number of holes required to achieve the desired gas composition. Finally, the software simulated the O_2 and CO_2 changes over storage time and displayed the information in a graphical format.

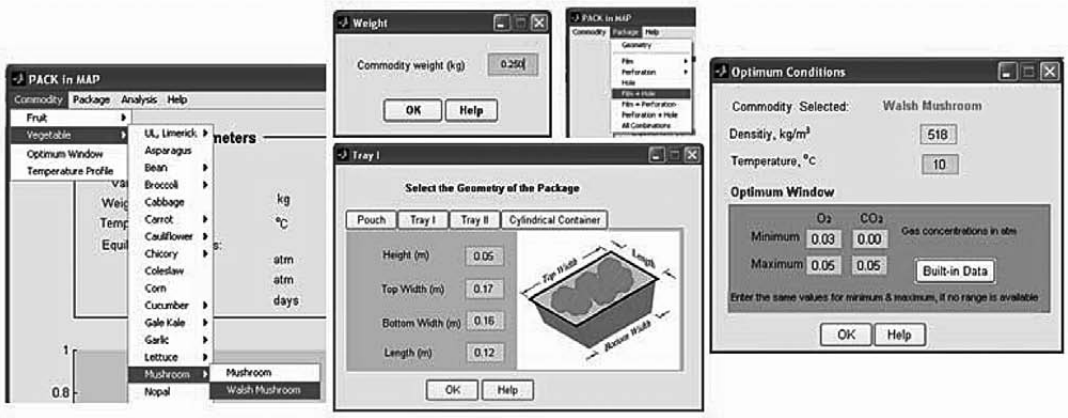

Figure 1. Screenshots of PACK-in-MAP software

RESULTS

Package validation:

The package with 2 holes yielded a very low O_2 concentration creating anoxia inside the packages whereas the package with 8 holes had 8% O_2 concentration which was above the optimum O_2 requirement for mushrooms. It was 2.5 and 5% when the packages were perforated with 4 and 6 holes, respectively. There was no significant difference of the number of holes on the CO_2 concentration, the average being 10.9 % after 25 h. The steady state gas composition was achieved within 25 h of storage in all the packages. The closest result to the target gas composition of 5% of O_2 was obtained with 6 holes however, it was not possible to obtain CO_2 within the target range, and therefore calcium hydroxide was used to scavenge

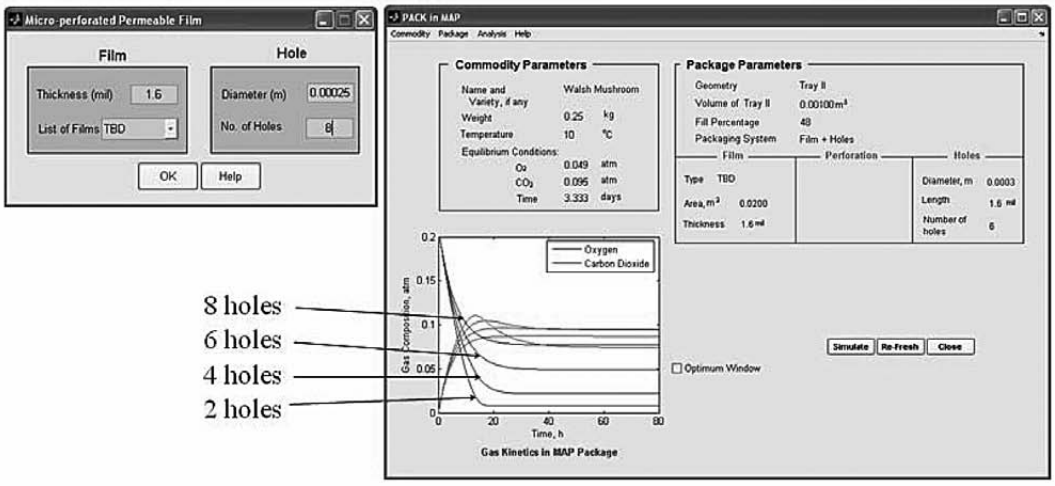

Figure 2. Screenshots of PACK-in-MAP software showing MAP design for mushrooms

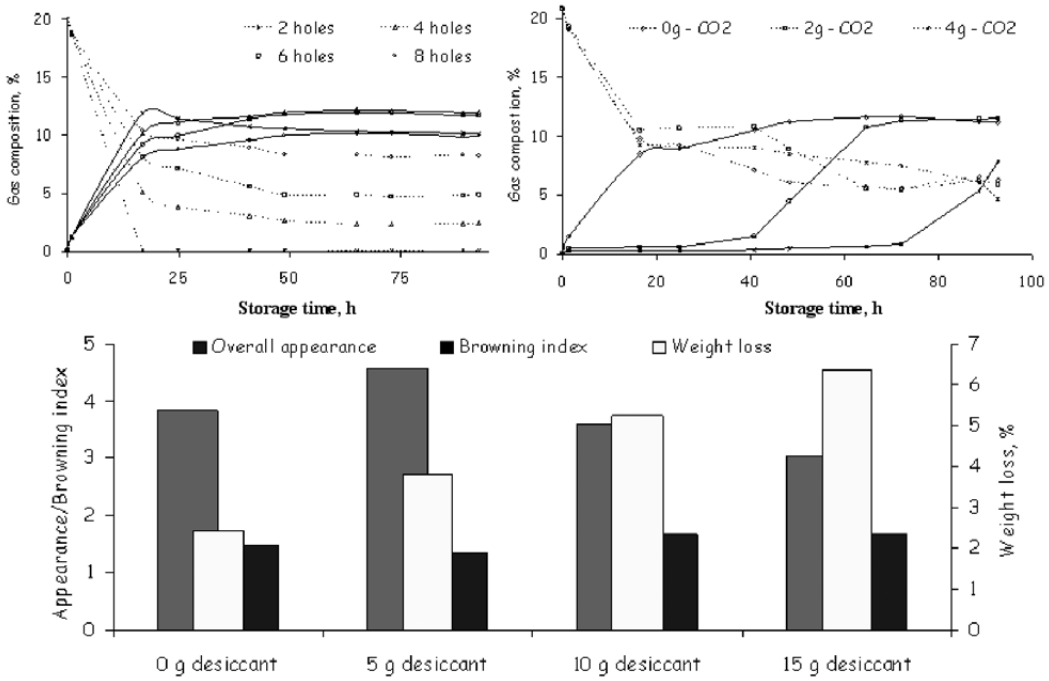

Figure 3. Experimental data showing changes in gas composition and quality of mushrooms during storage at 12°C

excess CO_2 from the package. The simulated gas composition by PACK-in-MAP software was in agreement with those obtained experimentally.

In packages containing 2 and 4 g of calcium hydroxide, the CO_2 concentration stayed at almost 0% up to 40 and 70 hours, respectively, and then it increased to 11% and 8% at the end of 95 h of storage. Therefore, the use of 4 g of calcium hydroxide and 6 holes allowed the package gas composition to closely approach the target level. The weight loss of mushrooms was found to be 2.2, 3.6, 5.2 and 6.1% after 5 days of storage with 0, 5, 10 and 15g of desiccant, respectively. It has been reported that the weight loss of more than 5% is considered bad for the quality of the mushroom as well for the economic aspect (Mahajan *et al.*, 2008). After five days, the overall quality was better in packages containing 5g of desiccant than in control package. With the higher amount of desiccant, the appearance was worst, getting closer to the limit of usability. The normalized browning index was actually better with 5 g of desiccant than that for samples with 0, 10 and 15 g of desiccant. Therefore, it was concluded that the optimal parameters for mushroom packaging were 5 g of desiccant, 6 micro-perforations of diameter 0.25 mm and 4 g of calcium hydroxide as a CO_2 scavenger.

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NEW PROCESSING TO EXTEND THE SHELF LIFE OF SLICED APPLES

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ABSTRACT

Edible food wraps made from fruit and vegetable ingredients could appeal to food makers looking for nutritious, colourful and eye-catching films for convenience foods, so they are becoming a new "attention point" for researchers.

The food industry has been taking reaping the rewards of the increase in consumer demand for convenient and minimally processed product like fresh-cut apples. This ready-to-eat product is a low-calorie snack that could be useful for school-canteen as for the more widespread snacks machines, encouraging children and adults to boost their fruit consumption.

Different studied were made to extend the shelf life of minimally processed fruits; our aim was to find an useful, simple to use and not chemical dipping solution for fresh-cut apples. We used natural thickeners as carragenin, xanthan and others just used for the same purpose as suggested by others authors.

The apples *Golden Delicious* were purchased from a commercial market. They were washed, cut and the slices were dipped in an anti-browning solution. Samples were treated with different edible coatings and packed in plastic trays with covers and stored at 4 °C. Colour, weight loss, texture and respiration rate were analysed.

The general appearance of the apple's slice, in terms of browning and the presence of off-odour were followed, during the storage, by a panel test of three person comparing the treated samples with a control, so the end of shelf life was evaluated.

Results showed the importance of the good application practice of an edible coating as the thickness and the drying method of the layer of the edible coating. The variation of initial colour (ΔE) was related with the uniformity of the layer of the coating.

Key words: anti-browning, edible coating, polysaccharides, shelf life.

INTRODUCTION

Edible coatings and films can provide an alternative for extending the post har-

vest life of fresh fruits and vegetables. Traditionally, films and coatings have been used to reduce water loss, but new film materials and edible coatings formulated with a wider range of permeability characteristics facilitate achieving a "modified atmosphere" effect in fresh fruits.

Edible films and coatings are generally produced using biological materials such as proteins, lipids and polysaccharides. Films made of polysaccharides or proteins usually have suitable mechanical and gas barrier properties but show high permeability to moisture and poor water vapor barrier properties. In contrast, films composed of lipids (waxes or other lipids) exhibit good water vapor barrier properties but show poor mechanical resistance and high oxygen permeability (Tanada-Palmu and Grosso, 2005). Coatings were formed directly on the surface of the fruit, while other films were previously formed and then used to pack fruit.

Different studied were made to extend the shelf life of minimally processed fruits, among these the use of antioxidant likes sulphites to reduce the browning (Pizzocaro F. *et al.*, 1993). Several packaging solutions considering the effects of variable contents of CO_2 and O_2 on the metabolism of the fruit, as modified atmosphere packaging (Beaudry R. M., 2000; Mathooko F. M., 1996) were also studied, as the activities of argon and other gases that showed variable beneficial effects (Mostardini F., Piergiovanni L., 2002).

The aim of this research was to find an useful, simple to use and not chemical dipping solution for fresh cut apples.

MATERIALS AND METHODS

Apples *Golden Delicious* were purchased from a market. They were washed, cut and the slices were dipped in an anti-browning solution as reported by Pizzocaro *et al.*, (1993). Samples were treated with different edible coatings (natural thickeners) and packed in plastic trays with covers and stored at 4 °C. The different coating

Fig.1 Untreated apple's slices

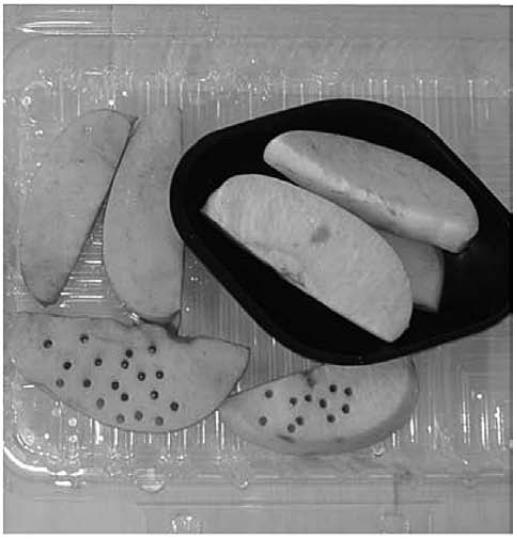

Fig.2 Coated apple's slices

formulations were prepared with aqueous or ethanol based solutions according to literature. Coatings were formed directly on the surface of the fruit by dipping the slices into the formulations and drying. Slices of apple were dipped in water, than dried and stored like the others and considered as control. Colour, weight loss, texture and respiration rate were analysed (data not showed).

During the storage, the general appearance of the apple's slice, in terms of browning and the presence of off-odour were followed by a panel test of three person comparing the treated samples with the control (Lu Z. *et al.*, 2005).

RESULTS AND CONCLUSIONS

Sensory evaluation showed that coatings maintained the visual quality of the apple's slices during the storage time, however, the appearance of some slices were unacceptable for the dissimilar layer of coatings. Results showed the importance of the good application practice for the thickness and for the drying method of the layer of the edible coating. The humidity of treated sample was always lower than untreated (control) so an useful reduction of the weight loss was achieved. The variation of initial colour (ΔE) was related with the uniformity of the layer of the coating while the texture was not strictly related.

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BIOBASED LAYERS AS A CONTROLLED RELEASE PACKAGING SYSTEM

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ABSTRACT

The interest towards new packaging solutions has increased in the recent years. The possibility to combine plastic films and biodegradable layers in the form of thin coatings represents only one of the most recent novelty in the field. In the present work, the use of such layers for controlled release packaging purposes is described.

INTRODUCTION

Plastic films are the most practical, economical and useful solution for packaging purposes since they took place in the every day life. However, the possibility to replace them, partially or totally, with new materials has increased in the last decades, especially in the attempt to solve the waste disposal issue. Early research addressed the obtainment of standing-alone edible/biodegradable films, with encouraging results. Recently, due to the difficulty in the development of biofilms with properties similar to those of synthetic films, increasing attention is being paid in the design of composite structures, i.e. obtained by coupling a synthetic substrate to a thinner layer of biodegradable origin. In this way it is possible to accomplish several goals, such as the improvement of specific properties like barrier, optical and mechanical. In the present study, biodegradable layers have been fabricated as a suitable tool for the release of active compounds. In particular, our research focused on the influence of the physical structure of these layers in controlling the above mentioned release. This study was hence aimed at demonstrating as the release of active compounds can be controlled, among other factors, by compositional

effects which lead to different structures in terms of morphology and localization of the active substances.

MATERIALS AND METHODS

A polyion-complex hydrogel from gelatin and low-methoxyl pectin as degradable biomaterial was prepared by heat-mixing pigskin gelatin (10% wt), citrus pectin (2% wt) and glycerol (5% wt) at 80°C for 1 h. Two additional solutions were prepared starting from the previous one by the addition, respectively, of a cross-linker (glutaraldehyde, 0.3% wt) and nanoclays (montmorillonite, 1% wt). As a reference, a 10% wt gelatin solution added of glycerol (5% wt) as a plasticizer was taken into consideration.

From each of these four solutions, films of 100 ± 2.5 μm were obtained by casting, using polycarbonate templates and then drying in a vacuum oven at 60°C x 24 h. Films were then kept at room temperature for further 24 h.

Films were then analyzed in terms of swelling and mechanical properties (both large deformation test by a dynamometer and dynamic mechanical analysis). Subsequently, morphological information was gained by microscope measurements, using E-SEM as well as the AFM technique.

Finally, the influence of the different morphology on the release properties of films was assessed by a release study, in which the release in 95% ethanol of an active compound (tocopherols, 3000 ppm) previously incorporated in the biodegradable film (pieces of 2 x 2 cm) was monitored over 20 days at a temperature of 40°C and under agitation (100 rpm).

RESULTS AND CONCLUSIONS

The four types of biodegradable film showed fairly different characteristics due to the specific starting molecules used for preparing the original hydrogels. As far as the mechanical properties are concerned, a different behavior has been detected in the large deformation (tensile) test. As it can be observed from Fig. 1, only gelatin films showed a stress-strain curve typical for the common synthetic materials, i.e. with an obvious first steep part (elastic zone).

In the other cases, the curve showed a typical plastic behavior. This is due to the disruption of the so called 'junction zones' (i.e. microcrystallites) which normally take place in gelatin-based matrices as a consequence of the well-known coil-helix transition phenomenon. At the same time, it can be noticed the improved elongation after pectin addition and both tensile strength and elongation improvement after cross-

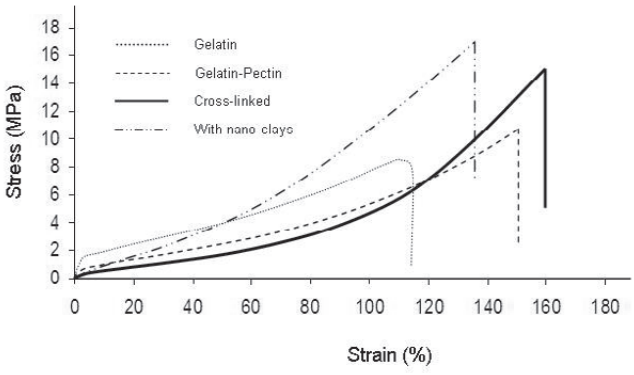

Fig.1 Mean curves of the tensile test of the four different type of bio-films used in the present work.

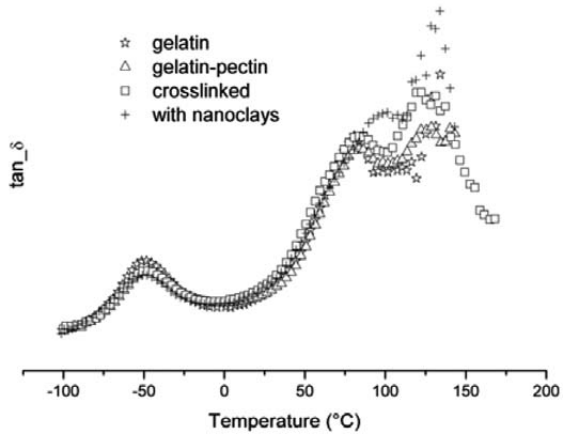

Fig. 2 DMA traces of the four different type of bio-films used in the present work.

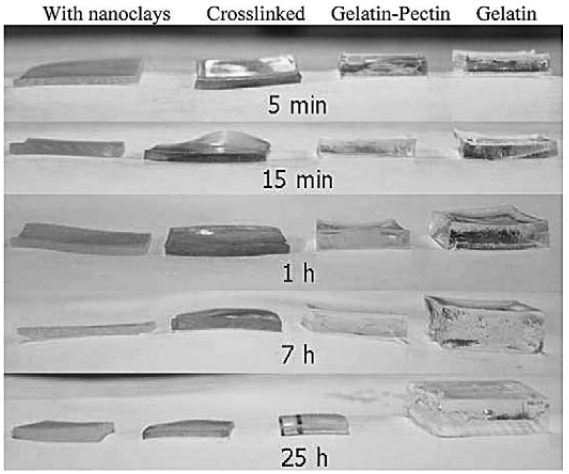

Fig. 3 Swelling behaviour of the different type of bio-films used in the present work after 5 times of immersion in distilled water (room temperature, 100 rpm agitation)

linking. The addition of nanoclays, contrary to what it was expected, led to only a slight tensile strength increase.

The DMA analysis showed only an important increase in the thermal stability of samples added with nanoclays, as it can be seen from the shifting of the peak at ~80°C to ~110°C (Fig. 2).

The swelling test evidenced a completely different resistance to water by the four types of bio-films (Fig.3). Although gelatin films swelled of a great extent compared to the other ones for a same time of immersion in distilled water, the addition of a limited amount of pectin (2% wt) dramatically influenced this property, thanks to the electrostatic interactions between the positively charged gelatin and the negatively charged pectin, which reduced the ability of water molecules of interacting with the charged sites of either biomacromolecules.

The crosslinking of gelatin chains further increased the resistance to water, whereas no evident changes were observed when nanoclays were incorporated into the hydrogel.

The cross sectional images collected through the environmental scanning electron microscope (E-SEM) and those gathered from the atomic force microscope (AFM) at the surface of the films allowed achieving a deeper understanding on the morphology changes due to the different starting formulations (Fig. 4 and Fig. 5).

In particular, gelatin films had evident discontinuous domains due to the junction of different molecules which recovered almost completely the original triple-helix conformation within a renaturation process. The addition of pectin led to the disappearing of such domains, as evidenced by the greater uniformity and homogeneity of the obtained films. Crosslinking produced the formation of a tight web due to the formation of new short and long range covalent bonds mediated by the glutaraldehyde molecules. Finally, E-SEM pictures revealed as the incorporation of nanoclays did not yield the exfoliation of the inorganic platelets, which on the contrary lumped together, forming aggregates and thus producing two distinct phases.

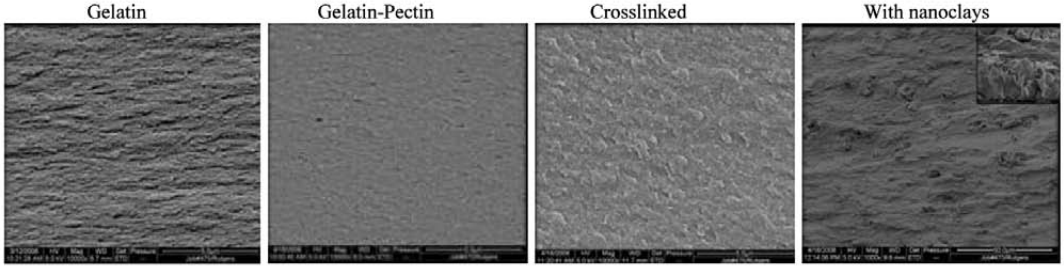

Fig. 4 E-SEM images of the different type of bio-films used in the present work (10000x magnification)

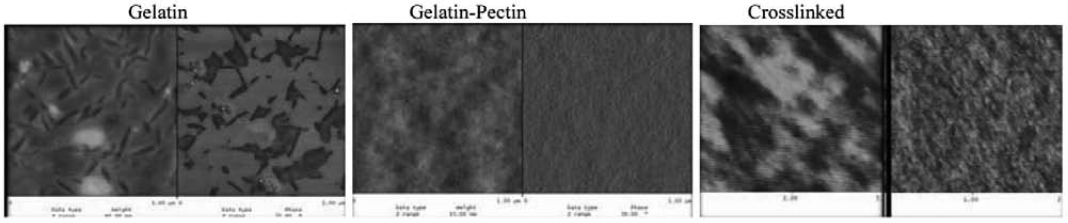

Fig. 5 AFM images of gelatin, gelatin-pectin and crosslinked gelatin-pectin films in *'height'* and *'phase'* modes

The results of the release study clearly demonstrated the influence of the morphology on the release property of a given bio-layer (Fig. 6). In particular, gelatin films released faster the total amount of the loaded tocopherols. Adding pectin slightly reduced the release rate over time. Crosslinking led to meaningful differences, exploitable in practice. Nanoclays acted similarly to the crosslinker, however releasing a higher % of the active compound.

In conclusion, release of active compounds can be studied, controlled, and designed also considering compositional effects, which play a fundamental role in the development of different structures in terms of morphology and localization of active compound. Biopolymers appear as a promising tool for achieving this task, probably more easily than synthetic polymers due to their pronounced versatility.

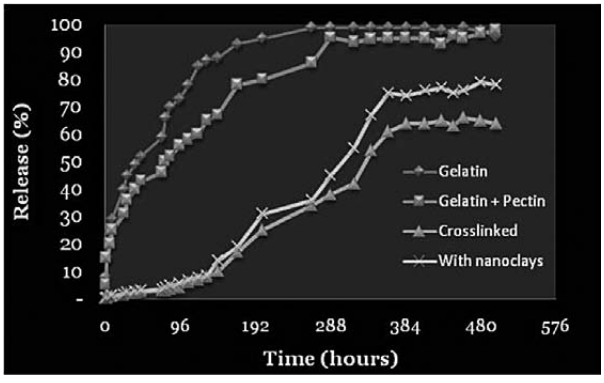

Fig. 6 Release curve of tocopherols from the four different type of bio-films used in this study.

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RELEASE KINETICS AND ANTIOXIDANT ACTIVITY OF TWO NATURAL COMPOUNDS FROM POLYMER FILMS INTO DIFFERENT FOOD SIMULANTS

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ABSTRACT

Promising active packaging materials are those ones containing antioxidant compounds which are initially incorporated into the polymer film and are then released into the packaged food product. The release is aimed to provide a sufficient concentration of antioxidant in order to prevent lipid oxidation and rancidity of the foodstuff and increase nutritive value on food.

In this work α-tocopherol and thymol were chosen as food grade antioxidant compounds and incorporated into a biodegradable polymer film (PCL) by means of a two step-process (mixing and extrusion). The obtained active materials were studied in order to study the release kinetics and the antioxidant activity into a fat food simulants. Release kinetics of the actives compounds from the different films were monitored at 25°C by using spectrophotometric and/or chromatographic methods.

The incorporation of α-tocopherol and thymol into polyolefin film (LDPE) has also been investigated for comparative purposes.The determination of the antioxidant capacity of active packaging into the food stimulant media is fundamental for an adequate selection of the most appropriate packaging for each foodstuff. To this aim tests were performed in order to assess the effectiveness of the released α-tocopherol and thymol in preventing the oxidation of linoleic acid in the chosen food simunats.

Key words: biodegradable polymer, Extrusion, linoleic acid, lipid oxidation, Thymol, α-tocopherol.

INTRODUCTION

Active packaging provides additional functions enabling the package to interact with the food to improve food quality, safety and convenience. Several food products such as fruit juice, milk, sauce, fish are perishable by nature and require protection from oxidation during their preparation, storage and distribution to give them desired shelf-life. Many preservatives, such ascorbic acid, various plant extracts, silver-substituted zeolite, lysozymes, and chlorine dioxide, have been successfully incorporated in packaging materials to confer antimicrobial activity in food packaging (Appendini & Hotchkiss, 2002). Other active compounds, such as antioxidants, can be incorporated into or coated onto food packaging materials to control the oxidation of fatty components and pigments. Chan Ho Lee e*t al.* (2004) have assessed the validity of antioxidant activity of α-tocopherol and nisin on coated paper. Alpha-Tocopherol is a natural antioxidant usually added during the manufacturing of infant formulas in order to improve vitamin E supply and/or to prevent lipid oxidation (N. Rodrigo at al. 2002). Alpha-tocopherol release from LDPE has been already investigated by Heirlings *et al.* (2004) and Siro *et al.* (2006). In this work the effect of a different matrix, a biodegradable polymer such as polycaprolactone, on the release and the use of a different natural antioxidant, such as thymol, has been investigated.

MATERIALS AND METHODS

Two different polymeric matrixes (LDPE and PCL) and two different natural substances (α tocopherol, thymol) were used to obtain active antioxidant films. The polyolefinic film LDPE Riblene® was purchased from Grinplast s.r.l (Italy) while the biodegradable polyester PCL CAPA® 6800 was purchased from Solvay (UK). Vitamin E is one of the most effective natural antioxidants that protect e.g. polyunsaturated fatty acids in foods from oxidation during storage. Alpha-Tocopherol was purchased by (Sigma-Aldrich, Italy). Thymol is a constituent of oil of thyme, a naturally occurring mixture of compounds in the plant Thymus vulgaris (thyme). It is important for its antimicrobial and antioxidant properties. In this study it was purchased by (Sigma-Aldrich, Italy).

Film preparation

The active films were prepared through a three-step process. 50g of polymer, thymol (α-tocopherol) (3% w/w) were fed into a mixer (HAAKE Rheocord 9000). Rotors' speed of rotation was set equal to 20 rpm and mixing time was equal to 6 min. The mixed matter was afterwards collected, pressed using a COLLIN P300P press and then pellettized. A twin co-rotating screw extruder (Thermoprism) was used (L/D=24). The temperature profile from the feeding zone to the die was different for each polymer (80-110°C PCL, 100-150°C PE). The extruded film was pressed with a calender into a thin film (300 micron)

Antioxidant release

Release profile of alpha-tocopherol and tymol from the two investigated polymer matrix (ldpe and pcl) into food simulant was studied. as food stimulant, 95% ethanol was used, as normally used olive oil has a relatively high original alpha-tocopherol content.

The prepared active films (2 g) were put into a container and brought in contact with 150 ml of food simulant at ambient temperature, under moderate stirring. The active compounds release kinetics were evaluated by monitoring the antioxidant concentration in the surrounding solution until an equilibrium value was reached. each data shown is the average value of three replicas. Quantitative determination of alpha-tocopherol and thymol into 95% ethanol solution were made by using spectrophotometric and chromatographic methods. The calibration curve was constructed for peak area/intensty against alpha-tocopherol and thymol concentration of standard solutions from 100 to 600 ppm, with 3 replicate samples for each active compound concentration.

Antioxidant activity of the release α- Tocopherol and Thymol

Antioxidant activity of both tocopherol and thymol released from PCL and LDPE films into ethanol was tested in a model system following the method proposed by Pryor *et al.*(1993). The model system consists of micelles of sodium dodecyl sulfate (SDS) mixed with linoleic acid. ABAP (2,2.-azobis-2-amidinopropane dihydrocloride) is used as the oxidation initiator. Fifty µl of tocopherol (thymol) released in ethanol (asintotic value) were added to the linoleic acid emulsion. The absorption of light by the linoleic emulsion was measured using a spectrophotometer at 232 nm (A232). This absorbance is related to the amount of conjugated diene hydroperoxide of linoleic acid formed during oxidation and, thus, provides a measure of the oxidation extent.

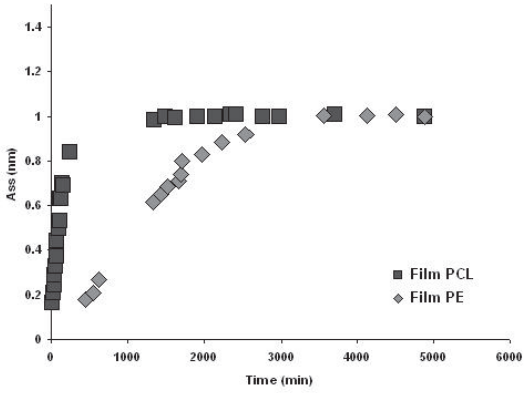

Fig.1. Release kinetics of Alpha-tocopherol from LDPE and PCL matrices in 95% EtOH.

RESULTS AND CONCLUSIONS

In the present study the release processes of tocopherol and thymol from the PCL and LDPE matrices was followed by the determination of the amounts of both antioxidant compounds migrated into the food simulant at 25°C as a function of time. Tocopherol release kinetics from both LDPE and PCL are reported in Figure 1. The obtained data show that the reached asynthotic value is almost the same from both matrices.

Tymol release data are reported in Figure 2, it can be noticed that the amount of thymol released from PCL is higher than that released from LDPE. This is probably due to the fact that less

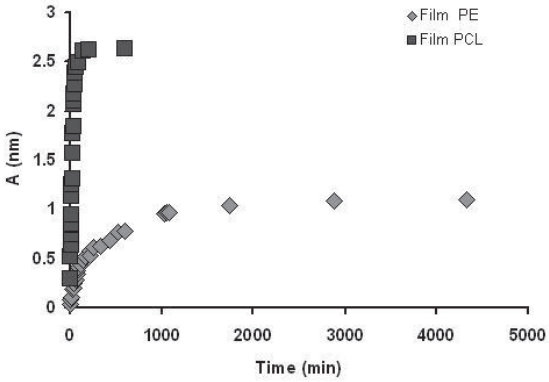

Fig.2. Release kinetics of Thymol from LDPE and PCL matrices in 95% EtOH.

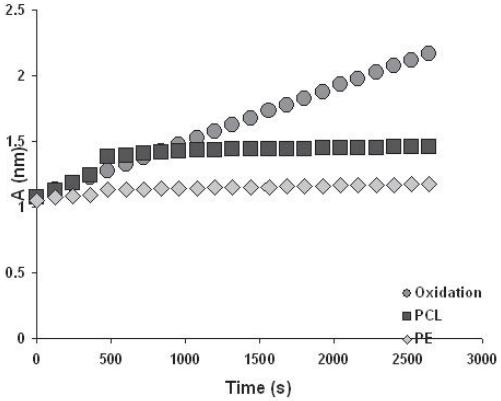

Fig.3. Antioxiadant activity of Alpha-tocopherol released from LDPE and PCL matrices in 95% EtOH.

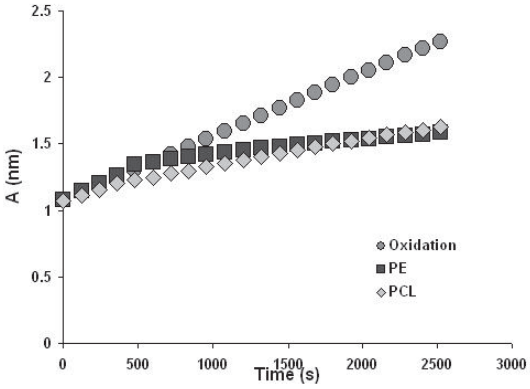

Fig.4. Antioxiadant activity of Thymol released from LDPE and PCL matrices in 95% EtOH.

amount of antioxidant is lost during the extrusion process because of its oxidative degradation. As both antioxidant are fat soluble, fast migration from LDPE and PCL was expected. The only difference can be ascribed to the time needed to reach the equilibrium value, being 25h from LDPE and 60h from PCL for tocopherol and 2h from PCL and 60h from LDPE for thymol. This is an important information for real food packaging applications: the initial high initial rate of AO release, in fact, could inhibit the initiation step of oxidation that takes place at the early stage of the storage.

Figures 3 and 4 show that the addition of the released antioxidants to the model solution (4 minutes after the beginning of the oxidation) causes an immediate change in the slope of the curve, due to their capacity to inhibit the oxidation of the unsatured fatty acid. The obtained results show that both tocopherol and thymol released from both PCL and LDPE films are effective in reducing the oxidation of linoleic acid.

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LYSOZYME IMMOBILIZATION ON CELLULOSE-BASED MATERIALS AS A TOOL FOR FOOD PROTECTION

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ABSTRACT

In our study, we addressed binding and controlled release of lysozyme from paper prepared from different components, and in the presence/absence of carboxymethyl cellulose (CMC). The stability of lysozyme binding to various agents (chaotropes, detergents, and salts) was also assessed. Incorporation of CMC in papers significantly increased the amount of bound lysozyme and the stability of the binding to repeated washing with buffer or with buffered chaotropes, but decreased the stability of binding towards anionic detergents. As expected, high salt concentrations resulted in complete removal of the protein.

Key words: carboxymethyl cellulose, lysozyme binding and controlled release, binding stability.

INTRODUCTION

Increasing consumer demand for safer foods and longer product shelf life calls for the development of novel food-processing and packaging strategies. In this frame, biodegradable protein- and polysaccharide-based films might act not only as packaging materials, but also as carriers for a number of food additives, including antimicrobial proteins.

Several proteins can be used as active agents in food packaging in view of their selective antimicrobial activity. They act through various mechanisms: by enzymatic action (as best exemplified by lysozyme), by altering the permeability of bacterial cell membranes (as nisin-like proteins do), or by sequestering micronutrients es-

sential for bacterial growth (as do iron-sequestering proteins such as lactoferrin). They can be grafted or immobilized at the surface of the material and act by contact effect, taking advantage of the fact that most – if not all – these proteins have a positive charge at neutral pH.

Previous work has shown that lysozyme binding to various "plain" papers relates to the materials and processes used for paper production. Release of lysozyme bound to "plain" paper materials by various agents acting on a given type of chemical interactions (e.g.: NaCl, electrostatic; urea, H-bonds, SDS, hydrophobic and electrostatic) has shown that different types of interaction may occur. Binding and release of lysozyme from papers having different composition and/or prepared with various production processes were studied in order to achieve a specific bio-based functional material suitable for controlled release of antimicrobial proteins.

MATERIALS AND METHODS

Materials

Commercial bleached hardwood and softwood kraft pulps were used. Sodium carboxymethylcellulose (CMC – WALOCEL CRT 60000 PPA07, substitution degree 0.7-0.8) was from Wolff Cellulosic. Purified lysozyme was provided by Sigma.

Table 1 Paper composition

Sheet	Low Fiber (%)	Long Fiber (%)	CMC (%)
A	20	80	-
B	20	80	2,5
C	80	20	-
D	80	20	2,5
E	50	50	1,25

Sheet forming and testing

Paper sheets were prepared with a Rapid Köthen System HAAGE BBS type, according to the standard ISO 5269-2:2004, starting with the composition given in Table 1.

The sheets were wet pressed, and dried under restraint either for 7 min at 95°C or at room temperature (21°C). A custom process for the production of paper in the presence of amounts of CMC compatible with the mechanical properties of paper itself was set up.

The topochemistry of CMC attachment was investigated by using conductometric titration to determine the total charge of the support. Physical properties, such as tensile index, density, and tensile energy absorption (TEA) were determined according to ISO 1924-2:1994 with an INSTRON 1122 dynamometer. The wettability of paper sheets was determined by using the Dataphysical Contact Angle System OCA. Uptake and release of bound lysozyme was assessed spectrophotometrically, by using published extinction coefficients.

RESULTS AND DISCUSSION

Binding of lysozyme to paper prepared with different ratios of short/long celulose fibers, and in the presence/absence of an anionic cellulose derivative (carboxymethyl cellulose, CMC) was studied. The amount of carboxylic groups (Table 2) titrated on individual paper samples indicated that it is possible to increase and modulate the surface charge density of paper by incorporating CMC, and that incorpora-

Table 2. Titratable acidity in papers of different composition

Sheet	Low Fiber (%)	Long Fiber (%)	CMC (%)	COOH (meqkg^{-1})
A	20	80	-	39
B	20	80	2,5	132
C	80	20	-	56
D	80	20	2,5	146
E	50	50	1,25	100

Table 3. Physical properties of paper dried at room temperature and at 95°C.

Sheet	Process	ADS (%)	Density (kgm^{-3})	TEA (KJkg^{-1})	Contact Angle (° after 200 s)
D_1	Hot dry (95°C)	53.50	868	2 ± 0.3	65±6
D_2	Soft dry (21°C)	68.89	632	1.5 ± 0.15	<10

at room temperature (D2). In particular in D2, both the density and the TEA index were lower, whereas the wettability was higher, as shown in Table 3. The lysozyme-binding capacity was higher in D2 than in D1.

The stability of lysozyme binding to the various papers towards solutions of various agents (chaotropes, detergents, and salts) was also assessed in order to define the nature of the interaction between the protein and the various types of paper. Presence of CMC greatly increased the stability of the binding to repeated washing with buffer at different pH or with buffered non-ionic chaotropes (8 M urea), but decreased the stability of binding towards anionic detergents (1% sodium dodecyl sulphate). As expected for a mainly ionic interaction at neutral pH between the positively charged lysozyme and the negatively charged matrix, high salt (NaCl) concentrations resulted in almost complete removal of the adsorbed protein. It has been verified that immobilization of lysozyme on CMC affects only marginally the structural and functional properties of lysozyme. A proper balance between ionic and non ionic interactions (as determined by CMC tion of CMC in paper increased significantly the amount of bound lysozyme (Figure 1). CMC incorporation resulted in a 3-fold increase in the amount of bound protein when using short-fiber paper (from 20% to 60% of the added lysozyme) and in a 2-fold increase when using long-fiber paper (from 30% to 65% of the added lysozyme).

The production process is also important for the final properties of each paper. Sheets that were wet pressed and dried under restraint for 7 min at 95°C (D1) were different from sheets dried

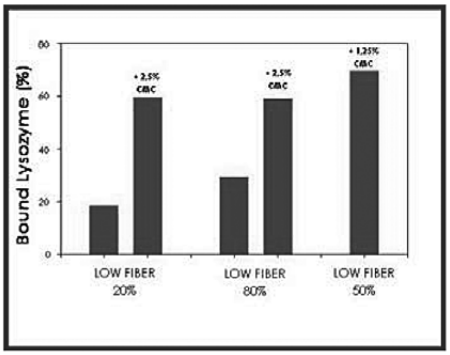

Fig. 1. Binding of lysozyme to various paper samples

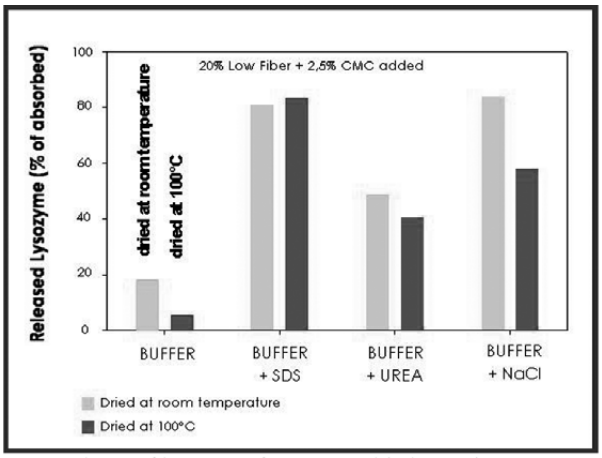

Fig. 2. Release of lysozyme from paper dried at various temperature.

addition and by the type of fiber) allows to optimize lysozyme binding and release to custom-prepared paper.

The relevance of the different types of interactions among lysozyme and custom-made paper is evident also after drying the protein-loaded support at 100°C. As shown in Figure 2, drying an antimicrobial-loaded custom-made paper at high temperature impairs only marginally the release of the active protein.

CONCLUSIONS

Antimicrobial proteins may be efficiently absorbed onto and released from paper-based products. Chemical forces other than electrostatic interactions are relevant to binding and release of positively charged antimicrobial proteins. Chemical and physical properties of the paper supports may be modified by changing the composition and the production process, thus allowing to modulate absorption/desorption of the antimicrobials, while retaining the performance of the packaging material. These cellulose-based packaging materials could be useful for extending the shelf life of fresh or wet foods.

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CHARACTERIZATION OF CHITOSAN-BASED ACTIVE NANOCOMPOSITE FILMS

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ABSTRACT

Biopolymers are widely studied in the field of food packaging in order to improve food quality while reducing waste disposal; however, their use has been restricted due to water sensitivity and poor mechanical properties, especially on moist environments. Recently nanocomposites materials obtained by adding nanosized clay mineral to natural and/or synthetic organic polymers have attracted the attention of several researchers. Clay particles, properly dispersed in the polymer matrix, even at a low loading level (1-5 wt%), greatly improve mechanical and barrier properties of the obtained nanocomposites material. In this work different chitosan-based nanocomposites films were prepared using the sonication and solvent casting techniques by incorporating different amounts of unmodified MMT (Na-MMT). The thermal behaviour and mass transport properties were characterized using X-ray diffraction, DSC, TGA and water and oxygen permeabilimeter. Obtained results show that a certain degree of intercalation is obtained, thus indicating that chitosan chain penetrates into the galleries of the clay. Barrier properties of chitosan films were considerably affected, in particular nanocomposites water and oxygen permeability considerably decreased, depending on different amount of loaded nanofiller.

Key words: Biopolymers, nanocomposites, antimicrobial, active packaging.

INTRODUCTION

Plastic material are generally no biodegradable causing many environmental problems associated with their disposal. In order to overcome these problems, in the last years natural polymers have received a great attention. But their applications in everyday life has been limited due to their poor properties, i.e. water sensitivity and relatively low stiffness and strength (Rhim *et al.*, 2006). A possible strategy to improve their performance and to impart them better physical and structural

properties is the incorporation of nanosized clay minerals such as layered silicate dispersed at nanometric level into the macromolecular matrix. Because of their high aspect ratios and high surface area, the clay particles, if properly dispersed in the polimer matrix at a loading level of 1-5%wt impart unique combinations of physical and chemical properties that make these nanocomposites attractive for making films and coatings for a variety of industrial applications (Alexandre and Dubois, 2000). Although numerous research work on polymer-clay nanocomposites have been performed, the matrices of these nanocomposites have mainly been synthetic polymer. The literature available for natural biopolymer-based nanocomposite materials is limited (Sinha Ray and Bousmina, 2005). Aim of this work is to develop and to study a biodegradable "active" nanocomposites material for application in food packaging having antimicrobial properties, using chitosan as biopolymer and nanoscale layered silicates such as MMT as filler.

MATERIAL AND METHODS

Materials

Chitosan, a polysaccharide derived from chitin, 9-(1,4)- linked 2-deoxy-2-amino-Dglucopyranose units having inherent antimicrobial properties was purchased by Sigma Aldrich. A commercial montmorillonite was used as filler: Cloisite-Na+ was kindly supplied by Sud Chemie, and used as received. It is a natural sodium montmorillonite whose particle size range between 2-13 μm. Glacial acetic acid (HAc) obtained from Aldrich Chemicals was used as solvent for chitosan. Glycerol by Fluka was used as plasticizer.

Preparation of chitosan/MMT-Na film

A chitosan aqueous solution of 2% wt was prepared by dissolving 2 g di chitosan powder in 100 ml of acetic acid solution (1%v/v) while stirring and heating for 20 min at 90°C using a hot plate. After the temperature was diminuited at 60°C, glycerol (25% wt based on chitosan)) was added and stirred for 20 min at 60°C until it was completely dispersed. Nanoclay solutions were prepared by dispersing two different clay compositions (5% and 10%wt based on chitosan) into 100 ml of acetic acid solution (1%v/v) and vigorously stirring for 1 hour. Afterwards, the montmorillonite solution was slowly added into the chitosan solution. The mixtures were stirred for 1 hour and then sonicated for 30 min at 25°C in a bath-type ultrasound sonicator. The solution was cast into cleaned and levelled glass plates and dried at ambient conditions (T = 27 °C and UR% 50-55%) for three days until the solvent was completely evaporated. The cast film was dried overnight in a vacum oven at 25°C.

Methods

X-ray diffraction tests were performed with a Philips X-ray generator and a Philips diffractometer. The X-ray beam was nickel-filtered Cu-Kα radiation with a wavelength of 1.54 Å. Diffraction intensity data were collected in $2\theta = 5° - 60°$ range; for each 2θ value the intensity determination was repeated six times; hence, each diffraction pattern consists of six different sets of data.

Thermal analysis was performed using a TGA 2950 (TA Instruments) by increasing the temperature from 25°C to 600°C at the heating rate of 20°C/min. Degradation temperatures were calculated as the inflection point of the curve.

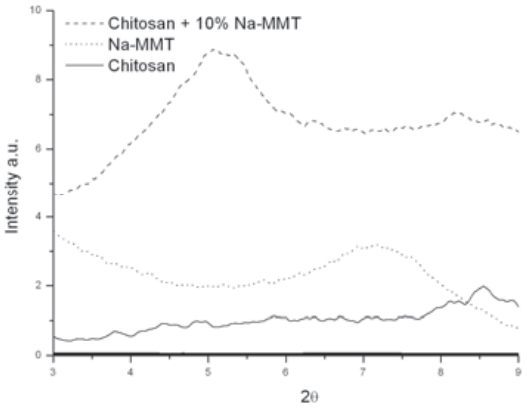

Fig.1. X-Ray diffractograms for neat chitosan film, Na-MMt powder and nanocomposite chitosan film.

Main thermal transitions of the samples were assessed by a differential scanning calorimeter DSC 2920 (TA Instruments) cooled with the RCS cooling system.

Permeability measurements were performed with a Permatran Mocon® (Model W 3/31) and OxTran Mocon® (Model ML 2/20); samples with a surface area of 5 cm2 were tested at 25°C. Water permeation tests were conducted by keeping the relative humidity at the downstream side of the film equal to zero and at the upstream side of the film equal to 35%. Oxygen permeation tests were performed in dry conditions.

RESULTS AND CONCLUSION

In nanocomposite materials, when the polymer chains spread into the tunnels of clay, the distance between the individual layers, called d-spacing, increases with respect to its intrinsic value. Results obtained from X-Ray analysis (Fig.1) show that the peak of MMT was shifted from 7° to 5° after its incorporation into the polymer, thus indicating the obtainment of an intercalated nanocomposite structure (the inorganic layers remain parallel with each other).

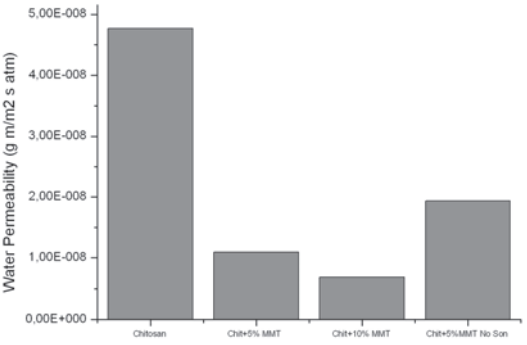

Fig.2. Water permeability of chitosan and nanocomposite films. T=25°C. aw upstream=0.35

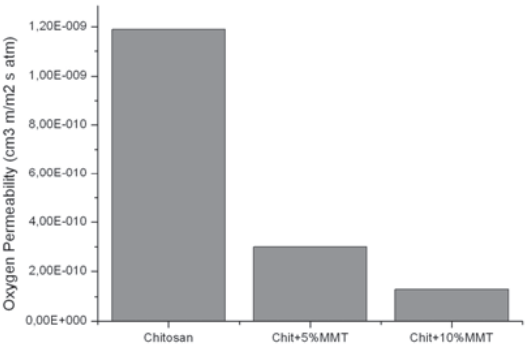

Fig.3. Oxygen permeability of chitosan and nanocomposite films. T=25°C. Dry conditions.

Thermo-gravimetric analysis (data not shown) indicates that nanocomposite chitosan film decompose at higher temperature than neat chitosan film, thus indicating that the presence of nanoclay increases the oxidative degradation. DSC spectra of chitosan (data not shown) show an endothermic peak most likely due to the evaporation of water vapor contained in the chitosan film. The exothermic peak, instead, may be attributed to the decomposition of chitosan. No effect of MMT presence can be observed. Permeability measurement show a relevant decrease of both water and oxygen permeability even at low (5%w/w) MMT content. The presence of higher amount of the clay (up to

10% w/w) show an higher barrier effect on the obtained intercalated nanocomposite. As shown in figures 2 and 3, the increase of barrier properties of the nanocomposite film is due to both the presence of the clay which increases the small molecular weight compound diffusive path and to the effect of sonication technique.

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BIO-MICROCAPSULES PRODUCTION FOR A CONTROLLED RELEASE OF NATURAL ANTIMICROBIALS FROM PACKAGING MATERIALS

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ABSTRACT

In this work, bio-microcapsules of chitosan have been developed by a membrane process using both polymeric and ceramic membranes. This technique permitted the formation of monodispersed bio-polymer droplets which were then cross linked with a natural additive adapted for this polymer structure that enhanced the water resistance of chitosan. Furthermore, two different types of natural antimicrobial were included in the capsules making the loading both during their production and after the droplet formation. The most effective entrapping method was then selected considering the amount of natural antimicrobial incorporated. The antimicrobial activity of the microcapsules was assayed by turbidimetric methods against bacteria and yeasts selected as dangerous and common microorganisms, which may be present in fresh food. Results are reported about the relationship between the amount of natural active substances released by microcapsules and the different degree of crosslinking, as well as the effects of the technological variables investigated on the kinetics of antimicrobial release.

Key words: Bio-capsules formation, membrane technique, antimicrobial release.

INTRODUCTION

The water resistance modulation of biodegradable packaging materials is of primary importance because it allows the use of the packaging material both in

contact with fresh foods and as active packaging system. In fact, the release of active compounds at rates suitable for specific sensitive foods very often implies the moisturization and the modification of the matrix where the active substances are incorporated into.

In this perspective, micro-encapsulation is a promising technology for protecting the natural active substances from the stresses and damages that can occur during the package manufacturing, for improving the capsules distribution in film, for preventing or minimising the loss of efficacy and for modulating the water resistance of the biodegradable materials. Thanks to these effects and according to their structure, the microcapsules could better control the release of the active substances and promote the interaction of the film with the active substances carrier (Rosca *et al.*, 2007). In this work, bio-microcapsules of chitosan (Srinivasa *et al.*, 2007) have been developed by a membrane process using a polymeric film. This technique permitted the formation of monodispersed bio-polymer droplets which were then cross linked with a natural additive adapted for this polymer structure that enhanced the water resistance of chitosan. Antimicrobial activity of microcapsules was assayed either in solid and liquid cultures of microrganisms selected as dangerous and which may be present in fresh food.

MATERIALS AND METHODS

Capsules preparation

The polymer, chitosan (medium MW, Sigma Aldrich), with (up to 1 wt.%) and without active agents was dissolved in a solution water/acetic acid at room temperature. The solution was magnetically stirred for at least 1 day to allow the polymer to be completely dissolved. Microcapsules have been prepared following two different procedures: a) "syringe method" b) "membrane method":

a) Used to prove the feasibility of making capsules. Capsules have been prepared by adding the polymer solution in a syringe and pressing it until the droplets were formed. The droplet enters in contact with a liquid mixture made of water/NaOH/ crosslinker and the active compound, purified and semi-purified (phase 3).

b) The polymer solution (phase 1) was add to the feed tank and pressed through the mono-pore film of polyethylene (PE) which has a pore diameter of 300 mm. The droplets formed (phase 2) went in contact with the (phase 3) (Figoli *et al.*, 2007). Capsules were then left in this mixture for 24 and 48 h and then recovered using a filter paper. Capsules were left to evaporate overnight at room temperature and set in an oven under vacuum for 24h to completely remove the solvent.

Microrganisms and culture conditions

Several microbial strains were tested, in particular: Aspergillus niger, B. cereus, B. subilis, C. utilis, Enterococcus faecalis, E. coli, L. monocytogenes, Penicillium chrysogenum, Pseudomonas putida, Rhizopus oryzae, Rhodotorula rubra, Staphilococcus aureus, S. cerevisiae. They all belong to the internal collection of the Section of Industrial Microbiology of University of Milan (MIM). Yeast and filamentous fungi were grown on MEA (Malt Extract Agar) medium, of the following composition (g l-1): malt extract 20, soybean peptone 2, agar 15, glucose 20, pH 5.8, sterilisation at 118 °C for 20 min. Bacterial strains were grown on TSA (Tryptic Soy Agar, Difco) medium. Cultures were maintained as frozen stocks at - 20 °C in appropriate liquid medium in presence of 10 % glycerol (w/v), and propagated twice before use in experiments.

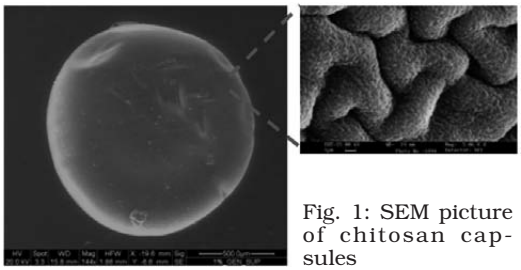

Fig. 1: SEM picture of chitosan capsules

Antimicrobial activity assay

Antimicrobial activity against indicator strains was tested either in solid and liquid cultures. As regards the first condition, 0.1 ml of a microbial suspension was added in 5 ml warm culture medium present in a Petri dish. After solidification, 20 mg of the natural antimicrobial was added, and then covered with other 5 ml culture medium. Samples were incubated at 30°C for 24 h. Antimicrobial activity was evidenced as halo of growth inhibition around the added antimicrobial. As regards assay carried out in liquid cultures, 100 ml Erlenmeyer flasks containing 10 ml TSB or MEB (media without agar) sterile medium, were inoculated with 0.1 ml of the microbial suspension, and 20 mg of the antimicrobial compound was suddenly added. A negative control sample was also set-up, without the antimicrobial. Flasks were incubated at 30 °C on an alternative shaker (200 rpm) and samples taken at appropriate intervals. Cultures were analysed for absorbance (OD 600 nm), while cell count (cells/ml) was performed employing a haemocytometer Bürker apparatus (Atanassova *et al.*, 2003).

RESULTS AND DISCUSSION

Chitosan microcapsules containing different concentration of active compounds have been prepared following the procedure above reported. A Scanning Electron Microscopy of the surface chitosan capsule, without active compound, is shown in Figure 1. The capsules present a spherical shape and a roughness surface. The capsules have an average diameter of 900 mm. Capsules containing active compound in the concentration up to 1% have been also prepared. Chitosan capsules without any active compound are usually white/transparent while the ones with the active compound resulted brown in colour as shown in figure 2. This was a clear evidence that the active compound was loaded in the microcapsules.

Antimicrobial activity against indicator strains was tested either in solid and liquid cultures. The results are summarised in table 1. In particular, the different types of active agents (purified and semi-purified) were compared as well as the effect of the cross-linker glutaraldehyde and the "additive" crosslinker .

The semipurified natural antimicrobial evidenced higher activity than the corresponding purified sample. Chitosan

Table 1. Antimicrobial activity of the different types of chitosan microcapsules prepared

	BIOPOLYMER			CHITOSAN, 2%	CHITOSAN, 2%	CHITOSAN, 2%	CHITOSAN, 2%
	ANTIMICROBIAL	SEMI-PURIFIED	PURIFIED			SEMI-PURIFIED, 1%	SEMI-PURIFIED, 1
	CROSS-LINKER			ADDITIVE	GLUTARALDEHYDE, 1%	ADDITIVE	ADDITIVE
	CROSS-LINKING TIME					24 h	48 h
M	*Saccaromyces cerevisiae*	++	-	-	-	-	+
I	*Candida utilis*	-	-	-	-	-	-
C	*Rhodotorula rubra*	++	-	-	-	-	+
R	*Enterococcus faecalis*	+	-	-	-	-	-
O	*Staphylococcus aureus*	++	+	-	-	-	+
R	*Bacillus subtilis*	++	+	-	-	-	+
G	*Bacillus cereus*	++	-	-	+/-	-	+
A	*Listeria monocytogenes*	++	+/-	-	-	-	+
N	*Pseudomonas putida*	-	-	-	-	-	-
I	*Escherichia coli*	-	-	-	-	-	-
S	*Rhizopus oryzae*	++	-	-	-	+/-	+
M	*Aspegillus niger*	++	-	-	+/-	+/-	+
S	*Penicillium chrysogenum*	++	+/-	-	-	-	+/-

crosslinked bio-microcapsules were more active after 48 h of reticulation than after 24 h. In particular, Bacillus, Listeria and the yeast strains were well inhibited by the contact active biocapsules.

CONCLUSIONS

The formation of mono-dispersed chitosan capsules, with and without active compounds, has been successfully carried out both using the “syringe” and the membrane technique.

Even if in some cases a certain degree of activity reduction was evidenced, bio-microcapsules proved their efficacy against bacteria and yeast. Furthermore, the antimicrobial activity tested in solid or liquid cultures proved an efficient procedure to evaluate bio- macrocapsule antimicrobial activity.

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INCORPORATION OF SAKACIN A INTO EDIBLE FILMS TO CONTROL *LISTERIA MONOCYTOGENES* IN READY-TO-EAT FOODS

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ABSTRACT

Bacteriocins have the potential to be used in bio preservation. The aim of this study was to evaluate the effectiveness of sakacin A against representative isolates of *Listeria monocytogenes* from food borne outbreaks; to study the activity of sakacin A in ready-to-eat (RTE) foods; and to identify a suitable biopolymer for coating. The results obtained demonstrated the possible use of the bacteriocin as antimicrobial agent in packaging applications.

Key words: active packaging, *L. monocytogenes*, RTE foods, sakacin A.

INTRODUCTION

Food active packaging represents an innovative way to inhibit and control microbial growth in foods while maintaining quality and safety (Cutter, 2006). RTE foods have been implicated in food borne outbreaks associated with *Listeria monocytogenes*.

In particular, the psychotropic and ubiquitous nature of this food borne pathogen makes its control difficult.

The aim of this study was to evaluate the effectiveness of sakacin A, a bacteriocin produced by *Lactobacillus sakei* DSMZ 6333, against representative isolates of *L. monocytogenes* (epidemic clones, Chen et al, 2007); to study the activity of sakacin A and to identify a suitable biopolymer to deliver the bacteriocin onto RTE food systems.

MATERIAL AND METHODS

Sakacin A activity against epidemic clones of Listeria monocytogenes *and its fate in food systems*

A modified well diffusion method (Schillinger and Lucke, 1989) was used to verify the antimicrobial activity of sakacin A against 9 strains representative epidemic clones of *L. monocytogenes* (Table 1). The most resistant strain and the most sensitive strain were used in the following experiments to represent least and worst case scenarios. To determine if some components in food products interfere with sakacin activity, food slurries of cheddar cheese, smoked salmon and turkey breast were combined with sakacin solution, held at 4°C and assayed for antimicrobial activity (Rose et al, 1999). Sakacin direct addition to turkey breast surface was also evaluated since loss of bacteriocin activity can occur. Foods were UV treated, cut in sections and contaminated. Treated samples were inoculated with sakacin solutions, control samples with sterile water. The slices were held at 4°C for 2 weeks aerobically and analyzed for *L. monocytogenes* population (Nguyen et al, 2008).

Selection of suitable biopolymers, films preparation and antimicrobial activity of the sakacin coating

Alginate films were made using the protocol developed by Cutter and Siragusa (1997), corn zein films by Janes et al (2002) and pullulan films by manufacturer's instructions (Hayashibara & Company). Antimicrobial activity of edible films was qualitatively determined using the plate overlay assay (Cutter, 1999).

RESULTS AND CONCLUSION

Sakacin A demonstrated antimicrobial activity against all the analyzed epidemic clones in plate overlay assays. Results are reported in Table 1. No interactions between sakacin and food components occurred when bacteriocin solutions were combined with foods. Sakacin A in direct contact with contaminated turkey breasts was effective. Remaining populations of *L. monocytogenes* are reported in Figure 1: 2 log reductions for J1-123 and 3 logs for R2-764 were observed after 14 day at 4°C. Pullulan films demonstrated the best characteristics: transparent, colorless,

TABLE 1. *L. monocytogenes* strains representative of the 4 epidemic clones analyzed in this study and their sensitivity to sakacin activity, measured as diameter of inhibition halos.

Strain	Outbreak	EC *L. monocytogenes*	Diameter of inhibition
J1-110	1985 California soft cheese outbreak	ECI	12mm
J1-123	1983–1987 Switzerland soft cheese outbreak	ECI	11mm
J1-120	1981 Canada coleslaw outbreak	ECI	12 mm
R2-764	2002 U.S. turkey deli outbreak	ECII	18 mm
N1-227	1998 U.S. hot-dog associated infection	ECII	16 mm
J1-101	1989 U.S. hot dog outbreak	ECIII	15 mm
R2-603	2000 U.S. turkey deli outbreak	ECIII	17 mm
J1-220	1979 Boston vegetable outbreak	ECIV	15 mm
J1-116	1989 United Kingdom Pâté outbreak	ECIV	13 mm

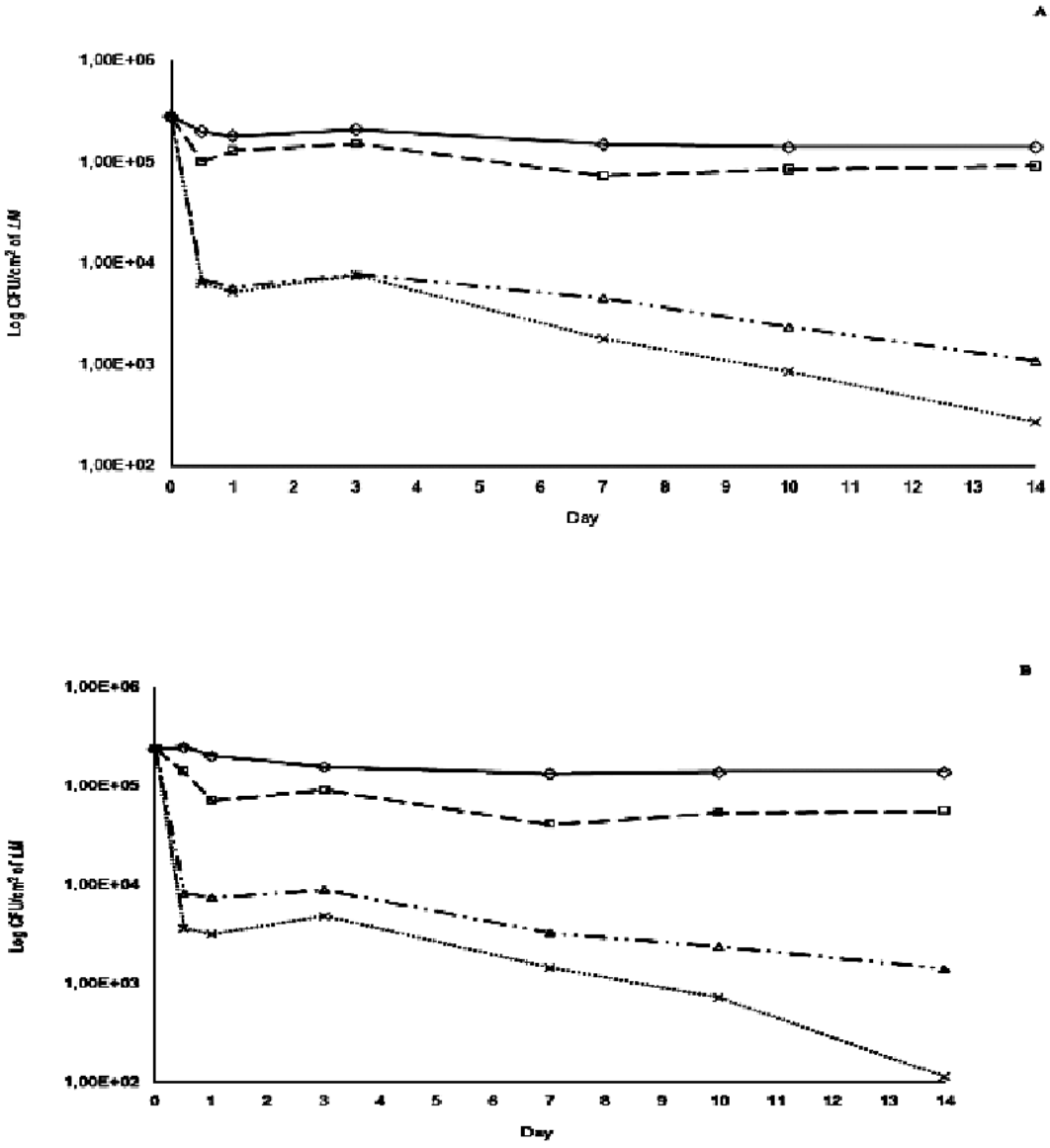

Figure 1. Remaining populations of *L. monocytogenes* J1-123 (A) and R2-764 (B) viable count observed in contaminated turkey breast samples at 4°C, treated by direct contact with sakacin solutions: control (◇),10 mg/ml (□), 20 mg/ml (Δ) and 40 mg/ml (×).

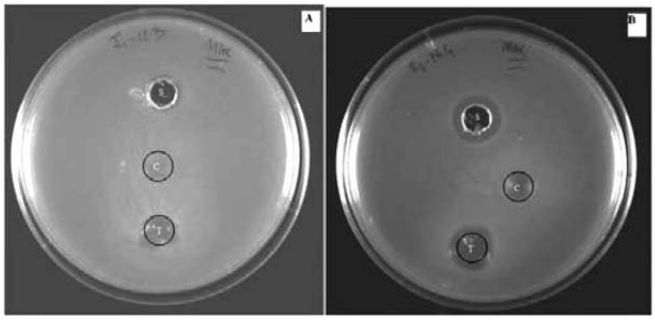

Figure 2. Qualitative antimicrobial activity evaluation of pullulan sakacin A activated films against *L. monocytogenes* J1-123 (A) and R2-764 (B). S: sakacin solution at 20 mg/ml; C: control film without sakacin; T: treated film with sakacin (20 mg/ml).

thick and the highest antimicrobial activity against the epidemic clones. Clear zones of inhibition were found around sakacin A-coated films (Figure 2). Sakacin A-coated pullulan films appear to inhibit the growth of *L. monocytogenes* in plate overlay assays. Additional experiments will investigate kinetic release of sakacin from the biopolymer and activity against RTE foods inoculated with pathogen.

Acknowledgments: Thanks to Dr. Knabel (Food Science Dept, Penn State) for providing the isolates used in this study.

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EVALUATION OF FLEXIBLE PACKAGING STRUCTURES FOR HIGH PRESSURE STERILIZATION

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ABSTRACT

In this study has been analysed the effect of high pressure sterilization (90-115 °C, 5000-8000 atm) and Pasteurization (35-40 °C, 5000-8000 atm) on flexible polymeric structures for food packaging. These are the conditions generally used at industrial level to treat packaged food. Effects on single materials as well as on multilayer structures have been examined in terms of morphological/structural, high pressure PVT, mechanical, calorimetric as well as in terms of mass transport (gases and water vapour sorption and permeation) properties, in order to assess the effect of this technology on the features of flexible packaging structures.

Both non-biodegradable and biodegradable commercial polymer films were analysed as well as non-biodegradable multilayer structures, including structures containing metallized layers or aluminium foil layers. The relevant effects are reported, including

delamination which occurred in multilayer structures containing metallized layers.

Key words: gas permeability, pressure, packaging film.

INTRODUCTION

High Pressure Processing (HPP) is a method of food processing where packaged food is subjected to elevated pressures with or without the addition of heat, to achieve microbial inactivation without compromising consumer-desired qualities (Le-Bail *et al.*, 2006). HPP treatments inactivates most vegetative bacteria, retaining food quality, maintaining natural freshness, and extending microbiological shelf life.

In a typical HPP process, the product is packaged in a flexible container (usually a pouch or plastic bottle) and is loaded into a high pressure chamber filled with a pressure-transmitting (hydraulic) fluid. The hydraulic fluid (normally water) in the chamber is pressurized with a pump, and this pressure is transmitted through the package into the food itself.

Here we report on the results of the first part of the research activity where test were performed on single packaging films. The second part of the research, which is now starting, will be focused on the treatment of packaged food.

MATERIALS AND METHODS

The following commercial packaging films used for the tests, are treated at pressure of 8000 bar and temperature at 35-40°C (pasteurization).

Biodegradable commercial film: PCL, PLA and commercial film LLDPE (Simaplast), PET, Aluminum foil.

The following commercial packaging films used for the tests, are treated at pressure of 8000 bar and temperature at 90-115°C (sterilization).

Monolayer samples: LLDPE, PET.

Multilayer samples (*ici*men$_{due}$): PET/ [cop LLDPE –Octene], PETmet/ [LLDPE], PET/PA/luminium foil/LLDPE.

Permeability tests were performed in a gas membrane-gas configuration (dry conditions) (Del Nobile *et al.*, 1999). The technique used was based on the detection of the pressure increase at the downstream side of the polymer film pressurized at the upstream side. The permeability was computed from the slope of the linear steady-state part of the curve, representing the permeated gas volume as a function of time. The tests were performed at an upstream pressure of 1 atmophere.

For vapour water permeability measure it is used an automatic instrument of Multiperm Extrasolution for permeability analyzer.

The thermal analysis was made using a DSC Q1000 TA Instruments, while the sorption Analyzer using the Q5000 SA TA Instruments.

RESULTS AND CONCLUSIONS

Thermal analysis of all the investigated samples don't show any difference between treated high pressure films and untreated films.

All the investigated samples and structures, with the exception of multilayer structures including aluminum foil or metallized layers, do not display significant changes of gas permeability (CO_2 and O_2) after HPP, showing only a small increase of permeability was observed as compared to untreated samples (see, for example, figure 1 for the case of LLDPE)

However, multilayer structures containing aluminum foil show a decrease of gas permeability (see figure 2) and an increase of barrier properties after HPP: this effect could be related to the presence of possible microdefects (pin-holes) (Del Nobile *et al.*, 1999; Murray) in the untreated metal layer which can be 'closed' as a consequence of the action of high imposed pressure, determining an effective decrease of permeability. Moreover, the bilayer laminated structure made by metallized PET and LLDPE shows, after HPP Sterilization and Pasteurization a dramatic mechanical damage of the film consisting in a delamination. This effect is possibly

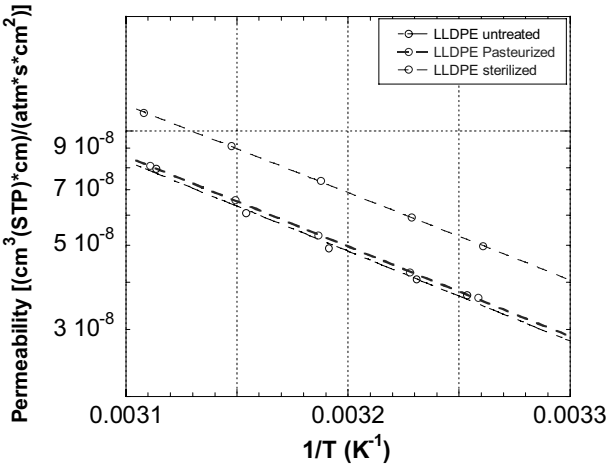

Figure 1

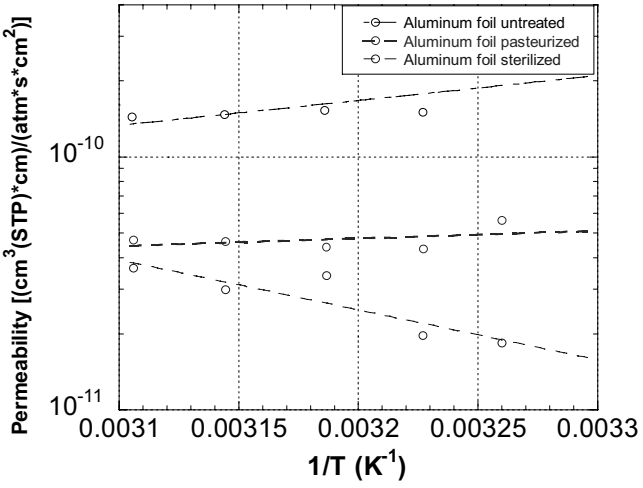

Figure 2

due to the difference in compressibilities of the polymer and metal layers, which, under high pressures, promote the development of interlaminar shear stresses which, in turn, promote the delamination.

Water vapour sorption displayed some differences between treated and untreated samples, with particular reference to LLDPE Simaplast

In conclusion, the result suggest that flexible packaging structures can withstand the conditions imposed by HPP as indicated also by other studies (Caner *et al.*, 2004), with exception of multilayer metallized structures. Tests are in progress to verify this conclusion for the case of packaged food. Moreover, different types of adhesive and optimized metallization procedures are under investigation to make metallized multilayer structures suitable for HPP applications.

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EFFECT OF MAP ON PHYSICO-CHEMICAL CHARACTERISTICS OF SHELL HEN EGGS

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ABSTRACT

The aim of this study was to compare some physico-chemical properties (weight loss, pH, Haugh unit, gel hardenss and foam drainage) of non packed eggs and eggs packed in high barrier plastic pouches with three atmospheres (air, 100% N_2, 100% CO_2) during 28 days of storage at 25°C. MAP permitted a strong reduction of the weight loss from the product. While the greatest quality decline was observed for the control, eggs packed in CO_2 maintained the initial values of Haugh unit during storage and the albumen pH was about 2 points lower than the control. Gel hardness showed a strict correlation with raw albumen pH and CO_2 MAP caused the formation of a soft and puffy coagulum. In terms of liquid drainage, foam stability decreased during storage for all samples but eggs packed in 100% CO_2 showed a more stable foam compared with fresh eggs until the fourth day. These findings suggest that the exposition of fresh eggs to CO_2 enriched atmospheres could be apply as a natural method to modulate albumen pH and indirectly the rheological properties of coagulated albumen and foam.

Key words*:* Foaming, Heat coagulation, Modified atmosphere packaging, Shelf life, Shell eggs, Storage.

INTRODUCTION

Eggs are an inexpensive source of high quality proteins and other nutrients. However, they are highly perishable and can rapidly lose their quality. The lost of egg quality attributes promoted by aging happens because eggs are breathable material and they allow moisture and CO_2 to permeate through the shell (Caner, 2005). In EU, except for few countries, for fresh egg preservation, stabilizing treatments such as those traditionally used in USA by the food industry for sanitation (washing with

aqueous solution of chemical agents) and microbes growth inhibition (refrigeration) are not allowed. The use of modified atmosphere packaging (MAP) could be a useful alternative to the traditional hurdles commonly used for food stabilization and it agrees with EU restrictions for fresh eggs preservation. From a technological point of view, egg may be considered a multifunctional ingredient since it can perform several functions by its coagulating, foaming, emulsifying, colouring, flavouring, anticrystallizing and nutritional properties. Products of egg albumen are important food commodities because they are used in a vast number of different food formulations (Liang and Kristinsson, 2005). To our knowledge, little data is available regarding the influence of MAP on the shelf-life of packed whole eggs, both in terms of quality maintenance of the product during storage and of the technological properties of egg constituents. The aim of this study was to compare some physico-chemical properties of non packed eggs with eggs packed in high barrier plastic pouches with three different atmospheres (air, 100% N_2, 100% CO_2) during 28 days of storage at 25°C.

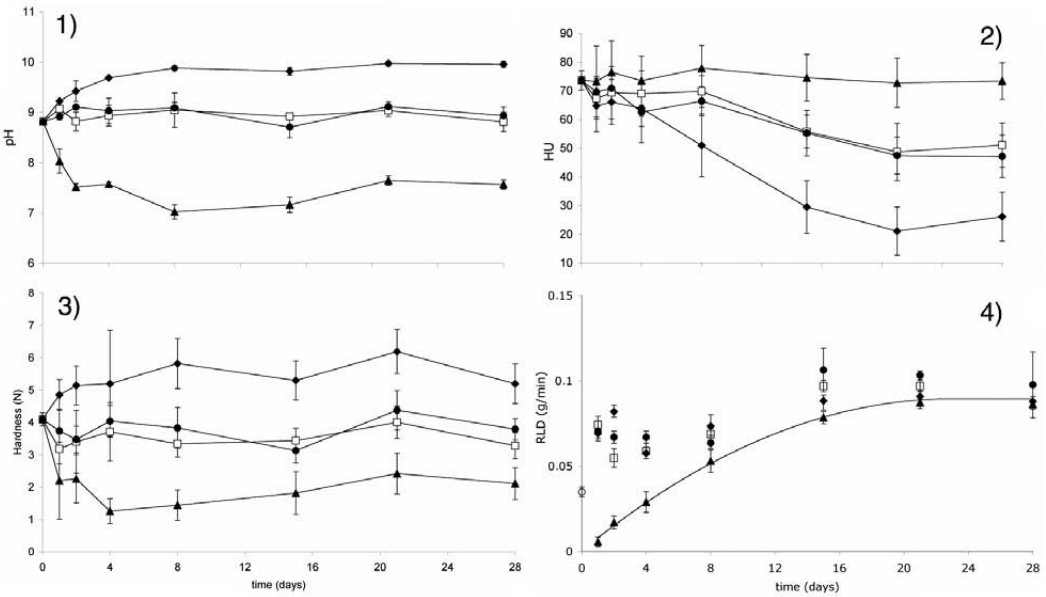
MATERIALS AND METHODS

1170 eggs (average weight of 68.05 ± 4.23 g) were obtained from one flock of Hyline brown hens of about 32 weeks age. Clean eggs laid the day before were placed on 140 plastic supports and 105 filled supports were packed in 105 high barrier multilayer (PE-PA-PE) pouches (20 x 30 cm, 105 μm thick) (Reber snc - Reggio Emilia - Italy). The samples were packed using a quaternary gas mixer and a gas-flushing welding machine. The following samples were prepared: Control (not packed); Air (packed in air); N_2 (packed in 100% N_2); CO_2 (packed in 100% CO_2). Albumen of fresh eggs (zero time) and of egg samples were analysed after 1, 2, 4, 8, 15, 21 and 28 days of storage at 25 ± 2°C. Weight loss (%) of eggs was calculated by subtracting the final weight from the initial weight and then dividing by the initial weight. The pH of egg white was measured using a pH-meter. The Haugh unit (HU) was determined at 25°C according to Cabeza *et al.* (2005). This index proposed by Haugh (1937) is yet extensively used to define interior egg quality, assaying the degree of egg freshness and it is a function of thick albumen height and egg weight. To a decrease of albumen quality corresponds a HU reduction. The hardness (N) of coagulated albumen was determined with a similar method of that of HammershØj *et al.* (2002). After heat coagulation at 90 ± 2°C for 30 min, cylindrical samples of coagulated albumen were taken using a core borer and than subjected to texture profile analysis (TPA) using a texture analyser mod. HD500 (Stable Micro Systems – Surrey - UK) equipped with a 50-kg load cell and a cylindrical probe mod. P/25. Test speed was 0.5 mm s^{-1} and the samples were compressed twice to 50% of the original thickness (5 mm). The foam drainage of the albumen was determined with a similar method of that of Lechevalier *et al.* (2005). Foam was obtained at room temperature by whipping 100 g of gently mixed albumen with a stand mixer at the maximum speed for 2 min. A glass cylinder was filled with a fraction of the obtained foam and placed on a funnel inserted in a graduated cylinder. The liquid amount drained into the cylinder was weighted every 10 min during 60 min of analysis.

RESULTS AND DISCUSSION

The weight loss of eggs during storage is mainly caused by evaporation of water

and loss of CO_2 (Caner, 2005). Eggs packaging permitted a strong reduction of the weight loss during storage, from the 6 to the 0.5% (data not showed). Albumen pH (Fig. 1) of Control sample showed an increasing trend from the beginning to the end of storage caused by CO_2 loss trough the shell. Samples packed in air and in 100% N_2 evidenced quite constant values of albumen pH during storage, while the use of 100% CO_2 was responsible of a fast and marked pH decrease as a consequence of CO_2 solubilization in the albumen. The average HU value of the fresh eggs (Fig. 2) was about 74 that corresponds to the one of a fresh, good quality egg (Caner, 2005). This value rapidly decreased for the Control sample, in agreement with previous investigation about quality modification of fresh eggs during storage (Jones *et al.*, 2005). All packed samples, in particular those in CO_2, better preserved eggs in terms of HU compared with the Control. The hardness (Fig. 3) of coagulated albumen showed a strict correlation with raw albumen pH. The values of hardness for Control sample showed an increasing trend during the first height day of storage, maintaining the highest hardness levels until the end of the experiment. CO_2 sample showed a fast and marked pH decrease reaching 7.03 ± 0.14 after height days of storage as a consequence of CO_2 solubilization in the albumen. This phenomenon was caused by the formation of a soft and puffy coagulum, due to the expansion of CO_2 during heating as detected from a visual examination of the heat coagulated albumen, while Control, Air and N_2 samples had a typical compact structure. In order to have a more exhaustive parameter bound to foam stability, the slope of the straight line obtained by linear regression of the liquid drainage data for fresh egg and the different samples was taken as Rate of Liquid Drainage (RLD) during the whole storage period (Fig. 4). All the samples showed an increasing trend of RLD during storage, as the stability of the foam became lower and lower with the passing of storage time. Compared



Figs. 1-2-3-4 - pH, HU, hardness (N) and rate of liquid drainage (RLD) values of shell egg samples during 28 days of storage at 25°C. Control (♦): not packed; Air (□): packed in air; N_2 (●): packed in 100% N_2; CO_2 (▲): packed in 100% CO_2.

with fresh eggs, Air, Control and N_2 samples showed lower foam stability (higher value of RLD) from the beginning of storage. In general, these samples had similar values of RLD that showed a strong increase from the height to the sixteenth day of storage. The foam stability of CO_2 sample albumen was higher than that of the fresh eggs, but only until the fourth day of storage, reaching values similar to those of the other samples from the eighth day.

CONCLUSIONS

Eggs packaging permitted a strong reduction of the product weight loss during storage and the limitation of HU decrease and pH increase. The greatest decline of interior egg quality was observed for the not packed sample (highest weight loss, pH increase and HU decrease). The substitution of the air in the package headspace with 100% N_2 did not promote any benefit to the product. Eggs packed in CO_2 maintained the initial values of HU for the whole storage period. Our results suggest that the exposition of fresh eggs to CO_2 enriched atmospheres could be apply as a natural method to decrease the pH of egg albumen and to indirectly change the rheological properties of heat coagulated albumen and foam. From an industrial point of view, the application of this finding could permit the substitution of more artificial, complicated, and expensive process commonly applied to change the albumen pH. In the optic of fresh shell eggs commercialization, MAP eggs could represent a tailored innovative food product, with specific characteristics bound to its destination (e.g. shell eggs special for foaming).

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PHOTOCATALYTIC DEGRADATION OF PROTEIN ALLERGENS BY TITANIUM DIOXIDE INCLUDED IN PLASTIC FILMS. PRELIMINARY EXPERIMENTAL RESULTS

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ABSTRACT

The effects of UV irradiated titanium dioxide on some structural features of egg white albumin, an anti-nutritional factor and a common food allergen that shares a number of structural features with other protein food allergens, were addressed. Formation of covalently bound aggregates (stabilized by di-sulfide or di-tyrosine bounds) is one of the possible effects induced by photo oxidation. The treatment-dependent formation of soluble protein aggregates was evaluated through Static and Dynamic Light Scattering (allowing to assess the presence of aggregates), and by SDS-PAGE (that makes evident the amount and the size of aggregates stabilized by disulfide bridges).

Formation of aggregates occurred upon exposure to UV light with all the preparations of titanium dioxide used in this study. The amount of aggregates was dependent on the titanium dioxide preparation, but the size of these aggregates, as detected by DLS, was not. Static Light Scattering results indicate that aggregation stems for the oxidation of aromatic aminoacids, that lead to the formation of dityrosine bonds. Exposure to UV light in the presence of titanium dioxide also induced the exposure of otherwise buried -SH residues that may be involved in the formation/exchange of disulfide bonds, usually representing the main interaction stabilizing protein aggregates.

INTRODUCTION

The relevance of food allergy and food intolerance is increasing, and this calls for the development of novel food-processing and packaging strategies that could meet also the consumer demand for safer foods. It should be noted than several foods prepared from allergen-free ingredients may undergo accidental contamination, or may be the substrate for the growth of allergen-producing organisms (from bacteria to molds and mites). The allergen proteins produced by any of these species are then difficult if not impossible to remove from the food. Thus, simple and safe preventive procedures are required, that - ideally - may be activated when the chances of contamination are the highest.

Several alloforms of titanium dioxide are capable of eliciting photochemical reactions when exposed to near-UV light, through a variety of mechanisms. These properties have been exploited, among others, for environmental remediation and for preventing urban or industrial pollution. Titanium dioxide is a widely used pigment that is very suitable for the preparation of coatings, safe for humans, and therefore represents a promising candidate for the preparation of photo-activable packaging.

MATERIALS AND METHODS

Titanium dioxide (anatase crystals) was obtained from two different sources: TiO_2 Hombikat UV–100, and TiO_2 Velox KO–320 (indicated here as "TiO_2 A" and "TiO_2 B", respectively). Egg white albumin, an anti-nutritional factor and a common food allergen that shares a number of structural features with other protein food allergens, was from SIGMA. A vinyl copolymer water emulsion (Caglificio Clerici) was used for thin films production by laboratory casting.

RESULTS AND DISCUSSION

TiO_2 inclusion in plastic film

The inclusion of TiO_2 in plastic films for food contact is a hard task. Problems that have to be solved are many, including: uniform dispersion of TiO_2; finding conditions ensuring tight anchoring of the nanopowder to the plastic matrix but making it possible the contact with allergens; minimising of the

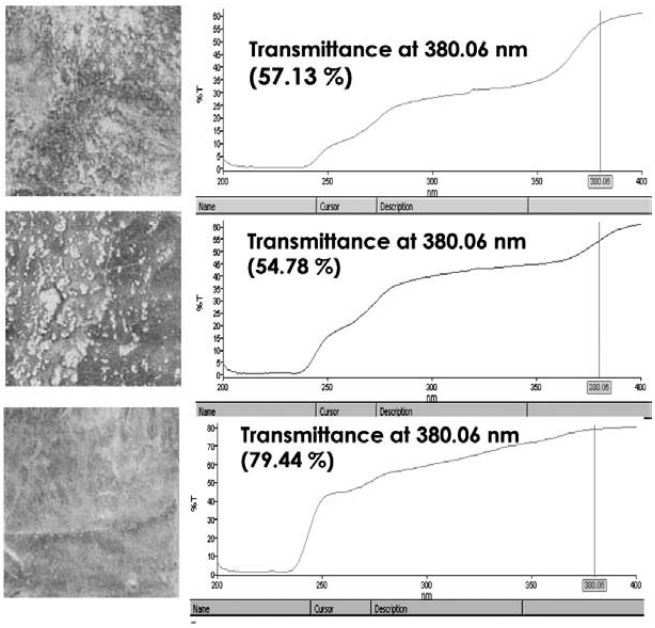

Figure 1. The UV spectra of the different films manufactured. At 380.06 nm, the wavelength of Ti2 activation, transmittance is reduced accordin to the different technques of incusionusd

aggregation phenomena of TiO_2 particles; the transmission of effective UV wavelengths in adequate intensities. Preliminary experiments indicated that the hydration of particles by a very long stirring in water and the mode of addition of TiO_2 to the polymer during casting are most critical, deeply changing the transmittance properties of the included films. In Fig. 1 the UV spectra of the different films are reported. The transmittance at 380.06 nm, the wavelength of TiO_2 activation, is about 84% in the pure plastic film and differently reduced according to the different techniques of inclusion used.

Increasing amounts of TiO_2 were added to buffered solutions of chicken egg ovalbumin (10 mg/ml in phosphate buffer, pH 7), and the resulting suspensions were exposed to the light from various types of fluorescent lamps for different times. In a second set of experiments, three films with TiO_2 included by means of different techniques were used in place of the suspended solid form.

SDS-PAGE and exposed thiols

SDS-PAGE performed under non-reducing conditions indicated that polymeric species are already present in the not irradiated protein, but their amount increase after exposure to UV light. In these experimental conditions it is not possible discriminate between

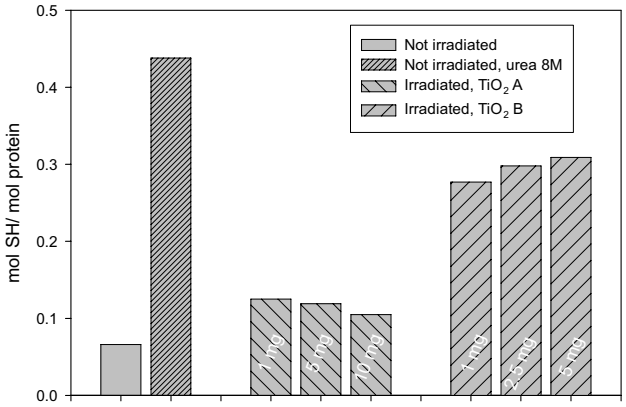

Figure 2. Accessible thiols in native and urea-denatured ovalbumin, and in ovalbumin following UV irradiation in the presence of various amount of different alloforms of TiO2.

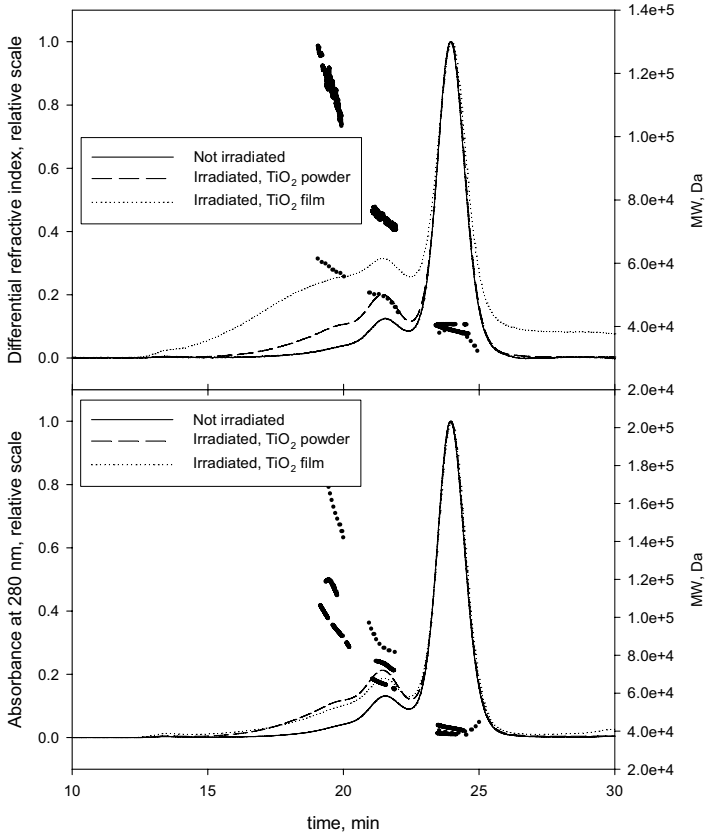

Figure 3. SEC-HPLC/LS profiles for variously treated ovalbumin samples. Top: refractive index detector; bottom, UV absorbance detector. Thick lines give the average MW for each protein peak.

di-sulfide or di-tyrosine bounds, and samples that were UV-irradiated in the absence or in the presence of TiO_2 do not show significant differences. However, the number of exposed thiols (figure 2), increases upon treatment, showing modifications that are "dose dependent" on the amount of titanium dioxide used. Is interesting to note that different TiO_2 preparations gave different effects. When TiO_2 was incorporated in the film, the decrease in transmittance impaired the photocatalytic effect.

SE-HPLC/light scattering

Size Exclusion Chromatography is more sensible than SDS-PAGE in measuring the amount of aggregates, and Light Scattering can calculate the absolute MW (thick line in the figures) of aggregates by comparing the intensity of the scattering with the concentration of the protein. The observed deviation among the two signals (Figure 3) indicates that photo-oxidation involved tyrosine residues, that modify the extinction coefficient of the protein. Moreover, the differential refractive index detector highlights deviation of the baseline for the sample treated in the presence of the vinyl acetate copolymer, probably due to photo-degradation of the film.

CONCLUSIONS

It is possible to identify "dose dependent" effects, suggesting a catalytic action of TiO_2 in protein modification, at least when analyzing the effects on thiol exposure. Studies currently being carried on are aimed at an improving our understanding of the role of titanium dioxide, also in relationship to the intensity and wavelength of the UV light inducing the structural modifications observed in the model allergen protein used in these studies described. Extension of our approaches to other food protein allergens and use of immunochemical techniques to verify any change of immunoreactivity ensuing from structural modifications are in our plans as well.

SESSION III

"Shelf Life Testing"

Chairmen:

F. Mencarelli (University of Tuscia, Italy)
K.L. Yam (Rutgers University, USA)

DIFFUSE REFLECTANCE UV-VIS SPECTROSCOPY IN FOOD MONITORING

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ABSTRACT

In food analysis, UV-Vis spectroscopy in the transmission mode is widely used for the evaluation of molecular species like pigments, proteins, fats, etc. Usually, a complex, destructive procedure for the extraction and separation of each component to be measured is required. The use of non-destructive UV-Vis spectroscopy is presented, performed in the Diffuse Reflectance mode (DRS-UV-Vis). The proposed method, being non-destructive, allows following the natural evolution of food during conservation, and results show that the technique can be used on two different classes of food, Golden Delicious apples and beef meat (thigh), both studied without any preliminary treatment.

Key words: DR-UV-Vis spectroscopy, food monitoring, fruit ripening, pigments, shelf-life.

INTRODUCTION

The quality of food, as perceived by consumers, is the complex result of many chemical or physical transformations occurring during both production and shelf-life. To ensure highest quality, careful controls are to be employed in the whole food supply chain, by using more sensitive and precise methods than those available at the present and, not secondarily, by increasing the number of controls along the chain. Unfortunately the number of measurements currently carried out is less than desirable. The main problem is that such measurements are usually based on destructive methods, the species to be measured being isolated through extraction processes (Wrolstad R. E. *et al.* 2005). These operations require well-equipped laboratories, skilled operators and are time-consuming, so constituting a serious

limitation. Especially for the big distribution platforms, choice of rapid, reliable and non destructive analytical tools for indicators of quality and ripeness is therefore of paramount importance.

Recently, methods based on spectroscopies in ranges UV-Vis and NIR have become available. UV-Vis spectroscopy yields specific absorption bands of pigments, the concentration and transformation of which are related to external characteristics (colour) and ripeness/freshness of food. Usually, measurement of pigments requires their extraction (Antolovich M. *et al.* 2000), so that artefacts may be obtained. Recently, also the non-destructive UV-Vis measurement in Diffuse Reflectance has been employed to evaluate pigments content of food (Merzlyak M. N. *et al.* 2003). The aim of the present work is to understand if this method can be useful to follow food pigment transformation during food natural ripening / degradation to determine fruits ripen level and meat freshness. Two different systems were considered: Golden Delicious apples and beef meat (thigh).

MATERIALS AND METHODS

Fruit considered was apples Golden Delicious from the same cultivar, kept two months in storage room under controlled atmosphere before the analysis. Each apples were divided into four quadrants, to take into account different sun exposures of each single fruit; in each quadrant a zone in the equatorial region was selected where measurements were performed. During the whole period of experimentation, apples were kept in air at r. t. Natural transformations occurring with senescence (51 days) were followed by DRS-UV-Vis. Meat was from beef thigh, purchased in a common butcher shop. It was cut, kept at r.t. and continuously analyzed for 3 days by DRS-UV-Vis. Both fruits and meat samples were measured without any preliminary preparation.

UV-Vis measurements were carried out in Diffuse Reflectance (DR) mode. A UV-Vis double beam spectrophotometer Varian Cary 500 was used, equipped with an integrating sphere. For background subtraction, the reference was Spectralon®. Spectra were collected in 200-800 nm region with a resolution of 2 nm. Spectra have been manipulated to calculate the concentration of absorbing species by the Kubelka-Munk function, F(R).

RESULTS AND CONCLUSION

Figure 1A compares spectra of an unripe Golden Delicious apple (dashed line) and of a ripe one (solid line). Bands visible in the UV-Vis range, from 200 to 800 nm, are the signatures of chlorophyll and carotenoids (Merzlyak M. N. *et al.* 2003). The former is one of the best indicators for the ripening level because of its concentration in peel is maximum in unripe fruits and decreases with time, reaching a minimum constant value at the right level of ripeness.

The characteristic bands of chlorophylls are centred at 678 nm, attributed to chlorophyll *a*, with a shoulder at 650 nm, due to chlorophyll *b*, and at 434 nm, attributed to the Soret band of chlorophyll *a*, an absorption due to the heme group (see circles in figure 1A). The bands at 480 nm, 447 and 422 nm are due to carotenoids, principally xanthophylls, responsible of the photo-protective and anti-oxidant agents (see asterisks in figure 1A). Bands at 447 and 422 nm are not

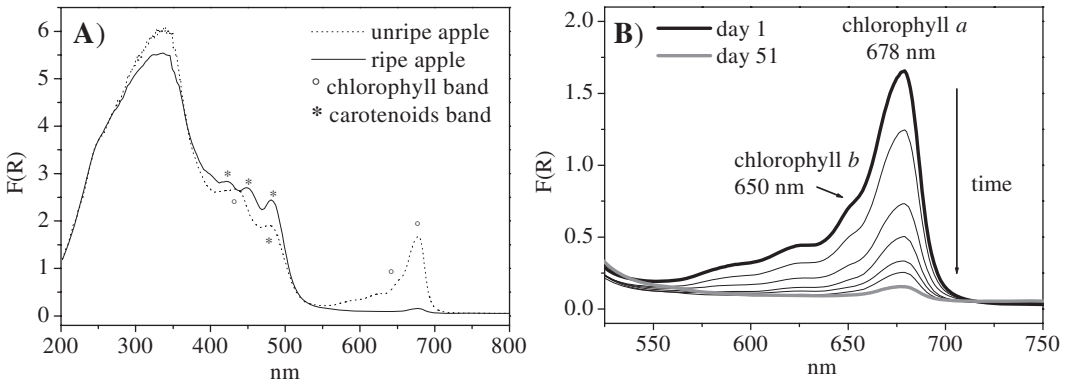

Fig 1. A) UV-Vis spectra of an unripe (dashed curve) and a ripe (solid curve) Golden Delicious apples collected in diffuse reflectance mode. B) Time evolution of UV-Vis spectra of apples stored at r.t. for 51 days. Day 1 corresponds to the day of delivery.

visible in the spectrum of unripe fruit because overlapped by chlorophyll Soret band at 434 nm.

The changes in the spectra can be given a quantitative description; figure 1B) depicts the set of spectra obtained as a function of time for a single specimen stored at room temperature and ambient atmosphere and analyzed for a period of 51 days (band of chlorophyll). Progressive ripening process, implying the degradation of chlorophyll, is witnessed by the bands at 434, 650 and 678 nm, quickly decreasing in intensities during the firsts day, then at a lower rate when approaching the 51th day; meanwhile the set of overlapping bands assigned to carotenoids (350-500 nm) progressively increase. Spectra confirm that chlorophyll content is linked to ripening level and the band intensity at 678 nm, directly linked to chlorophyll concentration by Kubelka-Munk equation, may be chosen as a marker of ripening progress.

About meat, the main pigment present in muscles is myoglobin, a globular heme-derived protein which transports O_2 inside muscle fibers. Myoglobin and derivatives may link different species, as O_2, CO, NO, whose presence depends on chemical

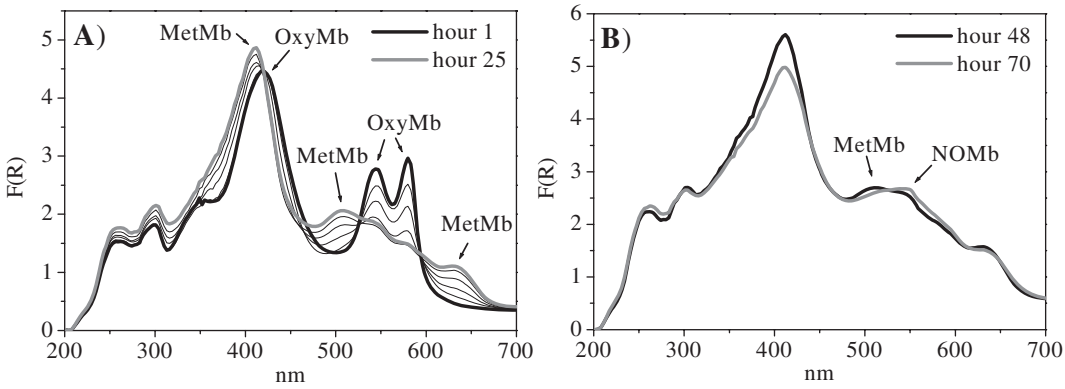

Fig 2. Meat analysis of beef thigh during 70 hours.

background (bacteria, atmosphere composition). Each myoglobin derivative has a characteristic spectrum that influences meat color (Gill C. O. 2003,). Fresh meat is characterized by the presence of oxymyoglobin (OxyMb in figure 2, myoglobin with O_2), whose main peaks are at 419, 545 and 580 nm. In aged meat, after 25 h, it was possible to identify metmyoglobin (MetMb), due to oxydation of heme ring Fe^{2+} to Fe^{3+}, bands centered at 410, 508 and 628 nm, and after 70 h nitric oxide myoglobin (NOMb), myoglobin carrying NO coming from proteins decomposition, whose typical peak is at 547 nm. As for apples, also in this case it is possible to identify a "marker" of meat freshness, as the presence of NOMb witnesses the degradation of meat proteins.

In conclusion, DR-UV-Vis spectroscopy results to be a fast and not destructive method to evaluate the state of food investigating pigments composition. Being not-destructive, it is possible to follow natural evolution of food degradation process, allowing the identification of specific markers directly linked to food freshness (as chlorophyll for fruits and myoglobine state in meat). It is so proposed that DRS-UV-Vis spectroscopy may be a powerful method to follow the evolution of important indicators monitoring the shelf-life of several classes of food.

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SHELF-LIFE AND QUALITY ASPECTS OF CHILLED COOKED ARTICHOCKS (*CYNARA CARDUNCULUS* SUBSP. *SCOLYMUS*)

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ABSTRACT

Chilled-cooked foods can be defined as perishable foods which have undergone a mild heat or pasteurisation process and which, to extend the time during which they remain wholesome, are intended to be kept in the temperature range of 0-4° C. Products include pre-prepared meals, pasta, rice, vegetable, soups and sauces. In this work the suitability and shelf-life of different Artichockes (*Cynara cardunculus* subsp. *Scolymus*) cultivars (*Violetto di Provenza*, *Violetto di Sicilia*, *Madrigal* e *Harmony)* to chilled cooked production was tested to set up a technological process and to formulate a new commercial item.

There were individuated the best solutions of time and temperature of treatment to preserve sensory quality and microbiological safety. There were selected some enzymatic markers to describe the main product alterations: Polyphenoloxidase (PPO, E.C. 1.14.18.1), Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) related to browning, and Pectinmethylesterase (PME, EC 3.1.1.11), Polygalacturonase (PG, EC 3.2.1.15) related to softening.

There was selected the most suitable cultivar on the basis of the previous biological (enzymatic) and chemical-sensorial markers (PPO, PAL; PME,; PG,) analyzed on raw plant material before processing and on processed vegetable during shelf-life.

Key words: cook-chill vegetable, artichokes, Polyphenoloxidase, Pectinmethylesterase, Polygalacturonase, Phenilalaninammoniolyase.

INTRODUCTION

Chilled-cooked foods can be defined as perishable foods which have been half

cooked or have undergone a mild heat or pasteurisation process and which, to extend the time during which they remain wholesome, are intended to be kept in the temperature range of 0-4°C. Products include pre-prepared meals, pasta, rice, soups and sauces.

The products may be assembled after separate cooking of individual components, chilling and then packing in the final container. Alternatively, the components may be cooked individually, packaged, sealed and chilled in the final container. A third possibility is for meal components to be vacuum packed, and then to receive a pasteurisation process which gives the potential for a longer shelf life under chill storage conditions because of the reduced risk of post-process contamination (the sous-vide process). Consumption of cook-chill foods from the manufactures should be within 5 days maximum, although there is no legal limit for that. The Advantages of Cook-chill foods are:

Labour savings – Cost savings –Higher quality meals .

Artichoke (*Cynara scolymus L*) is a vegetable cultivated in the Mediterranean area. The central part of the flower bud is the edible portion of the plant known as a globe artichoke and is widely consumed. This has considerable protein and mineral contents, a low percentage of lipids, and a high proportion of dietary fiber [Gon˜I *et al.*, 2005], polyphenolic substances, and fructan-oligosaccharides. Dietary polyphenols are currently attracting much interest because of their antioxidative, anti-inflammatory, and anticarcinogenic effects [Yang *et al.*, 2001]. Globe artichoke has been described as an important source of natural phenolic antioxidants such as hydroxycinnamic acids and flavones.

In this study the suitability and shelf-life of different Artichockes (*Cynara cardunculus* subsp. *Scolymus*) cultivars (*Violetto di Provenza*, *Violetto di Sicilia*, *Madrigal* e *Harmony)* to chilled cocked production was tested to set up a technological process and to formulate a new commercial item. There were analyzed the main quality alterations such as browning, softening loss of nutritional value and microbial spoilage. In this research we supposed that the latter could be related to the activities of endogenous enzymes: Poliphenoloxidase (PPO), Phenylalanine ammonia-lyase (PAL) related to browning; Pectinmethylesterase (PME, EC 3.1.1.11), Polygalacturonase (PG, EC 3.2.1.15) related to softening.

MATERIALS AND METHODS

Samples preparation

Samples (*Cynara cardunculus* subsp. *Scolymus*, *Violetto di Provenza*, *Violetto di Sicilia*, *Madrigal* e *Harmony cv*) were harvest in the experimental fields of DACPA (Scienze Agronomiche, Agrochimiche e Delle Produzioni Animali), in Ramacca countryside (Catania, Italy), transported to laboratory in air-conditioned vehicles and processed immediately in the laboratory. The heads were gently washed with chlorinated drinking water (200 ppm chlorine) and excess water was removed with a manual salad spinner, afterwards, external leaves were removed and the top was sliced off. Partially processed samples were packaged under vacuum in plastic boxes pasteurized at different temperatures and times. Finally chilled cooked artichoke were chilled stored at 4°C for up 10 days.

Enzymes extraction and assay

PME extraction and assay was carried out according to Hagerman A.E *et al.* [1986];

PPO extraction and assay was carried out according to Espin [1996];
PAL extraction and assay was carried out according to Key and Salveit, [1986];
PG extraction and assay was carried out according to Themmen, *et al.* [1982].

Sensorial analysis

Total Visual quality was scored on a scale from 1 to 9; 1=unusable, 3=fair (useable but not saleable), 5=fair (limit of marketability), 7=good, 9=excellent, fresh appearance. Intermediate numbers were assigned where appropriate. One visual quality score was given to an entire sample.

Off-flavour production was scored on a scale from 1 to 9; 1= total absence 9= very definite presence. Intermediate numbers were assigned where appropriate [López-Gálvez G**.** et al, 1997]

Physical-chemical analysis

Total Polyphenols**:** total polyphenols analysis was carried out according to Waterman and Mole al., 1994;

Browning index**:** browning index was carried out according to Ferrer *et al.*, [2005];

Antioxidant power**:** antioxidant power was carried out according Brand-William [1995];

Texture measurement: texture was determinated by using a texture analyzer;

Sugar content: sugar content was carried out according to McCollum *et al.*, [1988].

Soluble fiber content: soluble fiber content was carried out according to Khanum *et al.*, [2000].

Protein content: protein content was carried out according to Bradford method.

Chlorophyll: Chlorophyll content was carried out according to [Mackinney, G *et al.*, 1941].

Microbiological analysis

Microbiological analysis (PCA**)** was carried out according to Oxoid manual

RESULT AND DISCUSSION

PROCESS SETUP

Time and temperature selection

There were tested to different processing:

100 °C for 30 minutes

Preliminary blanching (80 °C for 5 minutes) and 100 °C for 30 minutes.

Samples treated at 100 °C for 30 minutes, without blanching showed at To time, good sensory quality, total enzymes inactivation (PPO, PAL, PME, PG) and a good spoilage reduction (99%), but the bags bulged during chilled storage due to CO_2 production that came from survival of thermophilic spore-forming bacteria.

Sample processed by preliminary blanching showed at To time good sensory quality, total enzyme inactivation (PPO, PAL, PME, PG) good spoilage reduction (99%) and no bulging during chilled-storage, although heat treatment decreased nutritional value of vegetable.

Table.1 Effect of process on protein denaturation, chlorophyll and polyphenols variation

	violetto di provenza	*violetto di sicilia*	*harmony*	*madrigal*
protein decrease %	62,51	47,92	43,12	25,26
virtual chorophill increase %	50,85	103,45	91,24	165,36
polyphenols increase %	52,56	78,09	131,73	100,93

Effect of heat treatment on nutritional quality.

Table 1 showed the effect of process "preliminary blanching (80 °C for 5 minutes) and 100 °C for 30 minutes" on protein denaturations and chlorophyll and polyphenols variations. Heat treatment strongly decreased protein content particularly in *Violetto di Provenza* (62.51%) and *Violetto di Sicilia cultivar* (47.92%). Heat treatment converted chlorophyll of artichokes in pheophytin, this alterations determinated a virtual chlorophyll increase (Tab. 1). Degradation of chlorophylls has been studied in literature since their bright green colour of vegetable is usually more pleasing to the consumer than the olive–brown colour of pheophytins [Schwartz and Lorenzo, 1991] Chemically, the colour change is due to the conversion of chlorophyll *a* and *b* to pheophytin *a* and *b* [Jones *et al.*, 1977 and Canjura *et al.*, 1991]. It is known that hydrogen ions can transform chlorophylls into their corresponding pheophytins by replacing the magnesium (Mg) atom in the porphyrin ring.]. In fact, the most common mechanism of chlorophyll degradation seems to be acid-catalysed transformation pheophytin.

Heat treatment increased virtually polyphenol content thanks to its effect on cell wall softening that made easy their extraction particulary in Harmony and Madrigal cultivar (Tab. 1).

Analysis On Raw Cultivars

Sensorial analysis

Different cultivars showed a similar quality and total absence of off-flavors.

Chemical-physical analysis

Raw cultivars were physically-chemically characterized as described in Tab. 2. *Violetto di Provenza* and *Violetto di Sicilia* were the richest in polyphenolic, sugar and fiber content, while chlorophyll and protein content were quite similar among cultivars; *Violetto di Provenza* and *Violetto di Sicilia* cultivar showed the higher cut resistance.

Enzymatic analysis

Row cultivars were enzymatically characterized as described in Figure 1.

Table 2 physical-chemical characterization of artichoke cultivars.

	violetto di sicilia	*violetto di provenza*	*harmony*	*madrigal*
fiber (%/ fresh weigh)	15,83±1	18,12±1	10,81±1	8,75±1
cut resistance (N)	2,1175±	2,01±	1,23375±	1,08625±
sugar mg/g	2,19±0,11	2,04±0,11	0,91±0,11	1,04±0,11
protein mg/g	4,18±0,38	3,96±0,38	4,35±0,38	4,55±0,38
total polyphenol mg/g	5,04±1,18	4,70±1,18	3,26 ±1,18	3,39 ±1,18
chlorophyll mg/100g	1,56 ±0.51	1,97 ±0.51	2,36 ±0.51	1,85 ±0.51

Harmony and *Madrigal* cultivars had the higher PAL, PPO and PG enzymatic activity, while PME activity was quite similar among cultivars. These results suggested that *Violetto di Provenza* and *Violetto di Sicilia*, thanks to the lower enzymatic activities, could be more suitable to the chilled cooked production.

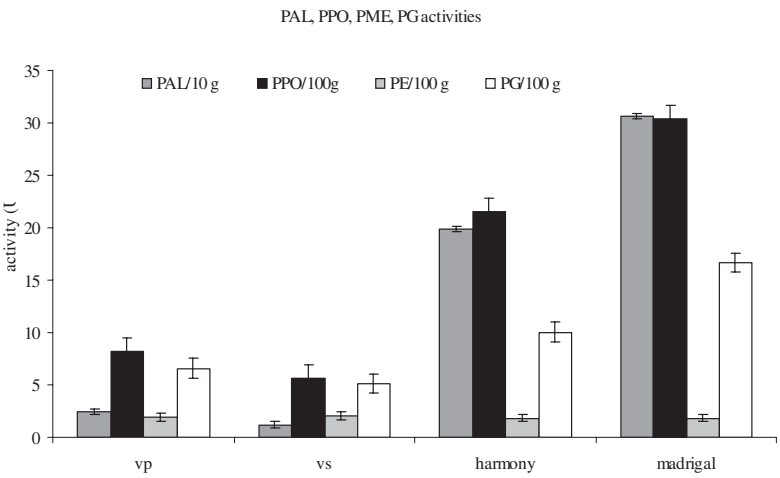

Figure 1

Analysis On Processed Cultivars: Chilled Coocked Artichocks During Shelf-Life.

Microbiological analysis

Each sample had a low spoilage, in particular *Violetto di Provenza* cultivar had the lowest spoilage (≤ 10 UFC/10 g tissue) during shelf-life.

Sensorial analysis

During chilled-storage of chilled coocked artichocks**,** total quality decreased and off-flavour increased in each samples, in particular *Madrigal* had the stronger and quicker softening and tissue degradations, while Harmony had the stronger and quicker off-flavour development (fig. 2).

Physical-chemical analysis

Browning index: Each cultivar didn't show browning during chilled-storage, thanks to the combination of enzyme thermal inactivation and vacuum process of processed samples (fig. 3).

Cut Resistance Cut-resistance decreased in each cultivar during chilled-storage of processed samples, particularly in *Harmony* and *Madrigal* (fig. 3).

Total protein: Cultivars didn't show protein degradation during chilled-storage of processed samples except for *Madrigal* (fig. 4).

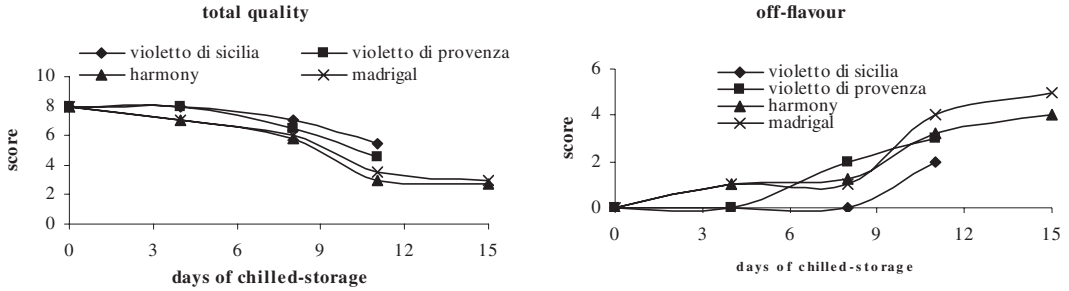

Figure 2

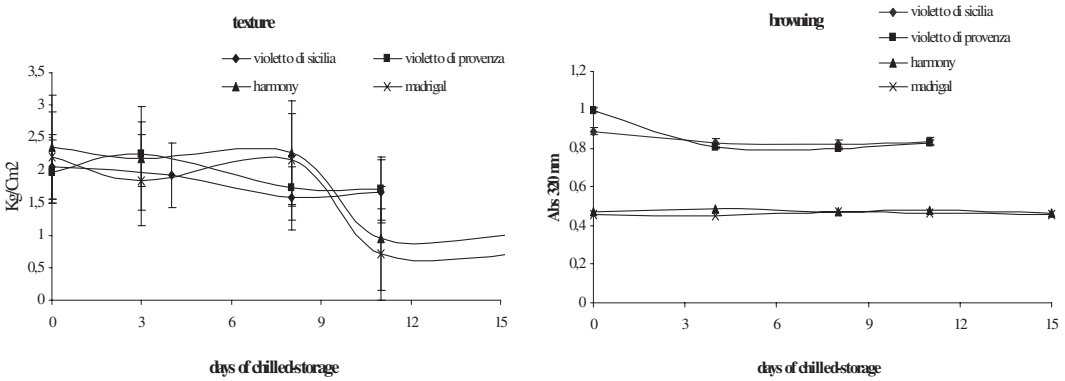

Figure 3

Chlorophyll: Cultivars didn't show protein degradation during chilled-storage of processed samples except for *Harmony* (fig. 4).

Sugar: Cultivars didn't show sugar degradation during chilled-storage of processed samples (fig. 4).

Antioxidant power: Antioxidant power decreased in each cultivar during chilled-storage of processed samples, particularly in *Violetto di Sicilia* and *Violetto di Provenza*. (fig. 4).

Polyphenols: total polyphenol decreased in *Harmony* and *Madrigal* cultivars during chilled-storage of processed samples, while in *Violetto di Sicilia* and *Violetto di Provenza* didn't show strong variations (fig. 4).

Enzymatic analysis: PAL, PME, PG, PPO

PAL, PME, PG, PPO All the enzyme selected and studied in the raw vegetable, PAL, PME, PG, PPO, were inactivated by the previous process, for this reason no activities were found in the processed chilled cooked artichokes during shelf-life.

CONCLUSIONS

"Preliminary blanching (80 °C for 5 minutes) and 100 °C for 30 minutes" treatment was individuated as the best solutions of time and temperature to preserve sensory quality and microbiological safety of chilled coocked artichocks.

Violetto di Provenza and *Violetto di Sicilia* were individuated as most suitable cultivar to processing on the basis of the enzymatic and chemical-sensorial markers analyzed on raw plant material before processing and on processed vegetable during shelf-life.

Heat treatment didn't affect strongly chemical and nutritional value of artichokes except for protein denaturation.

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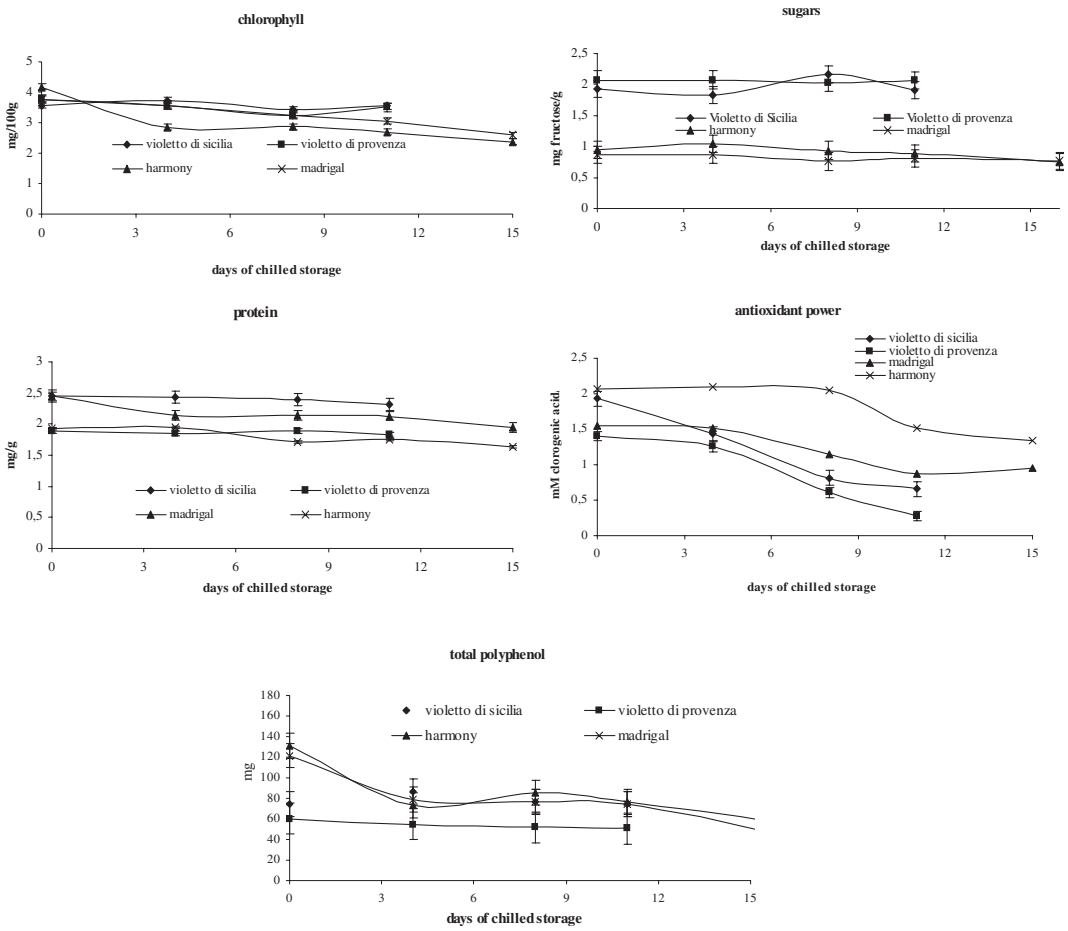

Figure 4

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RAPID DETERMINATION OF STYRENE AND ETHYLBENZENE IN PACKAGED YOGHURT

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ABSTRACT

In this study, a headspace solid phase microextraction coupled with fast high resolution capillary gas chromatography (HS-SPME/fastGC) method has been developed for the rapid determination of styrene and ethylbenzene in yoghurt. In order to optimize the analitycal procedure the effects of various parameters on the extraction and separation efficiency, such as sample volume/headspace volume ratio, sample heating temperature and time extraction, column efficiency, speed enhancement factor etc., were studied. Styrene and other volatile compounds have been quantified using the standard addition method. The method has been applied to commercial yoghurt samples at the production time and during their shelf-life.

Key words: ethylbenzene, fast-GC, migration, shelf-life, styrene.

INTRODUCTION

Styrene is one of the most important industrial chemicals produced mainly by catalytic dehydrogenation of ethylbenzene and utilized principally for production of polystyrene and styrene copolymers. Polystyrene is used for packaging fresh meat, cheese, fresh fruits, vegetables, cookies and delicatessen foods in its expanded form, whereas high impact polystyrene is used for fresh dairy products, such as yoghurt, and tea and coffee vending cups (Nerìn *et al.*, 1998). The migration of styrene monomer, which may affect the quality of products stored inside, varies with the physical and chemical characteristics of the polymer and of the food, namely the concentration of the residual styrene monomer in the polymer, the diffusion coefficient, the diffusion distance, the storage time and the duration of the migration process (Tang *et al.*, 2000; Vandenburg and Gramshaw, 1997; Tawfik and Huyghebeart, 1998). From literature data the levels of styrene in food

packed range from 5 ppb to 30 ppb (Tang *et al.*, 2000; Heikes *et al.*, 1995). The highest styrene ingestion risk is correlated to its key metabolite, the styrene 7, 8-oxide, since various in vitro and in vivo studies revealed it do be carcinogenic in experimental animals. Since traces of residual ethylbenzene might be also present in polystyrene, it cannot be excluded that during food contact ethylbenzene may migrate into food from polystyrene packaging material. From literature data the levels of ethylbenzene in food packed range from 6 ppb to 39 ppb (Tang *et al.*, 2000). In this study, a headspace solid phase microextraction coupled with fast high resolution capillary gas chromatography (HS-SPME/fastGC) method has been developed for the rapid determination of styrene and ethylbenzene in yoghurt. The determination has been carried out on yoghurt samples at the production time and during the shelf-life.

MATERIALS AND METHODS

Sampling

Twenty-four commercial yoghurt samples were purchased from a local dairy industry. Yoghurt was prepared with pasteurized whole cow milk and lactic acid cultures (*S. thermophilus* and *L. bulgaricus*) and it was packaged in polystyrene containers closed with an aluminium foil lid. Eight yoghurt jars from the same batch were collected three times during a production month and transferred to our laboratory under refrigerated conditions (+ 4 °C). The yoghurt samples were analysed immediately upon receipt and during the storage at 4 °C till the expiry date fixed in 40 days after production. Each sample was analysed in duplicate.

SPME procedures

The volatile components were extracted by HS-SPME. SPME was performed with a commercially available fibre using a DVB/CAR/PDMS fibre, 50/30 μm film thickness (Supelco, Bellefonte, PA, USA). Using a 7 mL vial, 2 g about exactly weighted of each sample was dissolved in 1.8 mL of saturated NaCl aqueous solution keeping the vial at 40 °C; equilibrium time, 6 min; extraction time, 3 min. After sampling, the SPME fibre was introduced onto the fast-GC injector at 260 °C, for 3 min.

Fast-GC Analysis

A Fast-GC Dani Master was used to analyse the sample headspace components. Injector temperature, 260 °C; injection mode, splitless; column, DN-Wax, 10 m, 0.1mm, 0.1 μm (Dani Instruments, SPA, Milan, Italy); oven temperature, 45 °C, to 160 °C at a rate of 40 °C/min, and to 240 °C at a rate of 30 °C/min; carrier gas, helium at constant pressure of 10 psi; detector FID; detector temperature 270 °C.

Quantitative analysis

Styrene and ethylbenzene were quantified in all yoghurt samples using the method of standard additions. Standard (Sigma-Aldrich s.r.l., Milan, Italy) solutions were added to multiple aliquots of a glass packaged yoghurt sample. The sample alone was analysed, too. The samples were analysed as previously described. Compounds were quantified based on calibration curves (R^2= 0,9981 for styrene, R^2= 0,9973 for ethylbenzene) generated by plotting the detector response versus the amount spiked of each standard. Each sample measurement was repeated two times.

Statistical Analysis

Statgraphic plus software, 5.1 version was used to perform the statistical analysis of the data obtained. ANOVA and Duncan test were applied to the data to determine the presence of significant differences (P-value < 0.05) during the refrigeration.

RESULTS

Fast GC using narrow-bore columns achieves the desired resolution of compounds of a complex mixture in the shortest possible time; SPME is an innovative solvent-free extraction procedure that is compatible with high-speed separation, leading to the reduction of both the SPME equilibration and analyte separation time. In order to optimize the analitycal method the effects of various parameters on the extraction and separation efficiency, such as sample volume/headspace volume ratio, sample heating temperature and time extraction, column efficiency, speed enhancement factor etc., were studied. The repeatability of the developed method was determined by analyzing three different samples of the same yoghurt under identical experimental conditions; the coefficient of variation (CV %) for the three replicates of the same sample was inferior to 10 % for all the identified compounds. A large number of volatile compounds have been detected. Among them both the key aroma compounds of yoghurt, and aromatic compounds (styrene, ethylbenzene) released from packaging material were identified. A SPME-fastGC volatile profile of a yoghurt sample is reported in Figure 1.

Styrene and ethylbenzene content was monitored during storage at 4 °C till the expiry date (Table 1). Styrene was present in trace at production time, then its level increased up to 23 days of refrigeration reaching 15.91 ppb and finally the amount decreased to 7.03 ppb at the end of the shelf-life. Ethybenzene were detected in all the analysed samples but in very low amount (inferior to 1 ppb) except for yoghurt sample at 23rd day of storage that showed a level of 1.02 ppb.

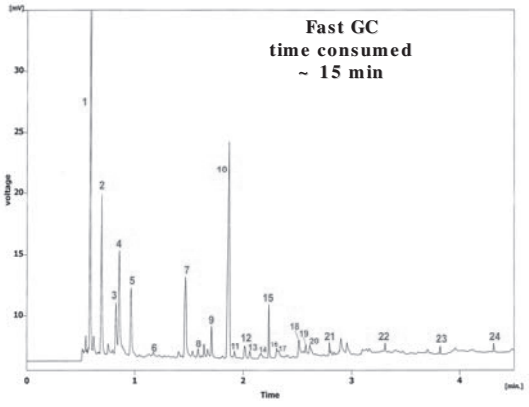

Fig. 1 - SPME-fastGC volatile profile of a yoghurt sample at day 1
1) acetaldheyde 2) diacetyl 3) acetone 4) 2-butanone 5) 2,3-pentanedione 6) ethylbenzene 7)2-eptanone 8) limonene 9) styrene 10) acetoin 11) 2-octanone 12) 6-methyl-5-hepten-2-one 13) 2-hydroxy-3-pentanone 14) 2-nonanone 15) acetic acid 16) 2-ethyl-1-hexanol 17)1-octanol 18) 2-undecanone 19) butanoic acid 20) ethyl decanoate 21) acetophenone 22) hexanoic acid 23) octanoic acid 24) decanoic acid

Table 1 - Average content (ppb) of styrene and etylbenzene in samples analysed during the storage at 4°C

Days of refrigeration	Styrene	Ethylbenzene
1	tr a[1]	tr a
10	3,17b	tr a
14	4,13b	tr a
18	9,96d	tr a
23	15,91e	1, 02b
35	9,01cd	tr a
38	11,03d	tr a
42	7,03c	tr a

[1]Different letters (a, b, c, d, e) indicate significant differences between values; tr < 1 ppb

The proposed HS-SPME/fastGC procedure is appropriate for determination of styrene and ethylbenzene migrated into food packed; it is rapid and sensitive enough for migration control. The method showed a good repeatability in terms of peak areas and retention times, a peak resolution comparable with that of conventional GC and a great reduction of analytical times. The time consumed for analysis, including sampling, SPME and Fast-GC analysis, was about 15 minutes. Using the method of standard additions, styrene and ethylbenzene have been quantified and statistical treatment of the quantitative results enabled to study the variation of their content during the shelf-life as result of migration from packaging material. These advantages have been demonstrated for a food matrix characterised by a low amount of volatiles, such as the yoghurt.

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VOLATILE TRANSFER BETWEEN SOLID FOOD AND PACKAGING

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ABSTRACT

The objective of this work was to better understand volatile molecule (oxygen, water vapour and especially aroma compounds) transfer in and through cellulosic (treated paper) and thermoplastic (bi-oriented polypropylene, biOPP) packaging films by taking into account their interaction with a flavoured (ethyl hexanoate, cis-3-hexenol and benzaldehyde) sponge cake during storage in controlled conditions. An integrated and multidisciplinary approach was used to study the system aroma compound-food matrix-packaging. The sensory quality of the sponge cake after storage in the packaging films was firstly studied by an olfactometry technique. The results obtained were then explained by determining: i) the aroma compound retention in the sponge cake after storage in the packaging films; ii) the permeability of the packaging films to volatile molecules and their aroma compound solubility and diffusivity, by using sorption and permeation methods in presence or not of the food matrix; iii) the microstructure, wettability and surface tension of the packaging films.

From the sensory study, the sponge cake odour quality was lower if the treated paper rather than the biOPP film was used in accelerated ageing conditions. This difference was explained by the modification of the aroma compound retention in the sponge cake after storage, due to aroma compound transfer through both packaging films. Whatever the volatile molecule (oxygen, water vapour and aroma compounds), the treated paper was more permeable than the biOPP. The aroma compound permeability difference between both films was mainly due to their diffusivity difference. Although being higher in the biOPP film than in the treated paper, the aroma compound solubility in both packaging films was on the same range of magnitude. The solubility values were related to the hydrophobic surface of both packaging films and the different diffusivity values were related to their microstructure, due to their different nature, composition and treatment.

Key words: Affinity, Microstructure, Packaging, Permeability, Storage, Volatile molecule.

INTRODUCTION

Food shelf life is highly dependent on mass transfers occurring in food / packaging systems, particularly volatile molecule transfers such as oxygen, water vapour and aroma compounds. The packaging permeability property is an important parameter and depends on several factors linked to the composition and structure of the packaging, the physico-chemical properties of the volatile molecule and the conditions of the external environment (temperature and relative humidity (RH)). If numerous literature can be found on that subject for plastic films, only few information are available for packaging films based on paper (Chalier *et al.*, 2007 ; Schuman *et al.*, 2004), although they offer the advantage of being recyclable and / or biodegradable. Particularly, flavour transfer through packaging can lead to modifications of the organoleptic quality of food (Ducruet *et al.*, 2001). Liquid models closed to real food or aqueous solutions containing some of the food ingredients were mostly considered for such studies (Van Willige *et al.*, 2000). Aroma vapour transfer through packaging if released from a solid food matrix was little studied until now to the authors' knowledge and was one of the goals of the French project CANAL ARLE.

In that context, the objective of this work was to better understand the transfer of small volatile molecules, particularly aroma compounds, through one cellulosic film compared to one thermoplastic film placed in indirect contact with a flavoured solid food matrix during storage in controlled conditions. For that purpose, the sensory quality of the flavoured solid food matrix after storage in the packaging films was firstly studied. The results obtained were then explained by determining the transfer properties (permeability, solubility and diffusivity coefficients) of the packaging films to volatile molecules as well as by investigating the structural and surface properties of the packaging films.

MATERIALS AND METHODS

Packaging films : A coextruded biaxially oriented polypropylene film (biOPP) whose thickness and density were respectively 38.3 μm and 0.95 was used as a reference in this study. The treated paper is a paper which was firstly impregnated with an acrylic-based latex 1; secondly supercalendered on both sides; thirdly coated with the same weight of an acrylic-based latex 2 on both sides. Such coating provides this paper with a water vapour barrier and a dense surface. The thickness and density of the treated paper were respectively 36.5 μm and 1.14.

Sponge cake : A sponge cake was chosen as model of intermediate moisture food and only the crumb was considered in the whole study.

Aroma compounds : Flavouring of the sponge cake dough was carried out by using 3 aroma compounds (ethyl hexanoate, cis-3-hexenol and benzaldehyde) which are very often found in flavour formulas.

Methods: The readers will refer to the published articles of the authors for more details concerning the different methods used in this work (Dury-Brun *et al.*, 2008a,b).

RESULTS AND CONCLUSIONS

The sponge cake flavoured with the 3 aroma compounds was stored for 7 days at 36°C and 78 % RH gradient in a glass packaging, the plastic film and the treated

paper. After this time, its headspace was extracted by solid-phase microextraction (SPME) then analysed by direct-gas chromatography-olfactometry by trained assessors who rated the odour similarity of this extract with one obtained from the glass packaging stored under the same conditions. The higher the score, the higher was the odour similarity between the tested and the glass packaging extracts. It was checked that odour similarity was the highest with the glass packaging. Odour similarity was significantly lower for the treated-paper than for the biOPP which odour similarity was significantly lower than for the glass packaging. These differences in odour quality of the sponge cake after storage in the different packaging films were further explained by determining the concentration of the aroma compounds in the sponge cake after storage in the glass or tested packaging. The flavour balance in the sponge cake was modified by use of the biOPP as packaging because relative amounts of aroma compounds were changed compared to the glass packaging. After storage in the treated paper, the sponge cake flavour profile was totally modified and aroma compounds were almost entirely lost. To further understand these results, the permeability property of both packaging films was studied for the 3 aroma compounds and for water vapour. Whatever the volatile molecule, permeability was higher through the treated paper than through the biOPP. The same conclusion was found with oxygen. The permeability coefficient was not significantly different between the aroma compounds studied for both packaging films. The influence of the packaging film on the transfer property was thus more important than the nature and physico-chemical properties of the aroma compounds used in this study. To better explain the permeability difference between both films, the solubility and diffusivity of ethyl hexanoate was determined at 25°C and 0 % RH, for 0.5 ethyl hexanoate vapour activity. Ethyl hexanoate solubility coefficient was on the same range of magnitude for both films, respectively 0.530 kg. m^{-3}. Pa^{-1} for the biOPP and 0.259 kg. m^{-3}. Pa^{-1} for the treated paper. This can be explained by their surface affinity for this hydrophobic molecule which was close from the measurement of their contact angle (respectively 15° and 18° for the biOPP and the treated paper). Nevertheless, ethyl hexanoate solubility coefficient was significantly higher in the biOPP than in the treated paper because biOPP was entirely hydrophobic whereas only surfaces of the treated paper were hydrophobic due to the latex-based coating. Ethyl hexanoate diffusivity coefficient was significantly higher through the treated paper (2372×10^{-16} m^2. s^{-1}) than through the biOPP (125×10^{-16} m^2. s^{-1}). Such diffusivity difference between both packaging films was previously found for water vapour and was related to structural differences between packaging films. Conversely to biOPP which is a dense material, the core of the treated paper was still partially porous although it was compressed by a supercalendering treatment. The transfer behaviour of both packaging films would thus be mainly controlled by their diffusivity property linked to their density / porosity.

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APPLICATION OF BIO-BASED POLYMERS FOR FRESH MEAT PACKAGING

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ABSTRACT

This research is focused on the application of biopolymers for fresh meat packaging, as a potential replacement to the commercial PVC film wrapped on expanded polystyrene (PS) tray system. For each experimental thesis, beef steaks were packed using an expanded polylactic acid (PLA) tray, having a biodegradable pad and either wrapped with a PVC film, or heat-sealed with three different biopolymeric films: PLA, Mater-Bi 1 and Mater-Bi 2. As control, meat samples were packed in PS trays with a polyolefinic pad wrapped with a PVC film, hence replicating a commercially available packaging system for fresh meat. All the samples were stored at 4 °C for 8 days and the meat quality was assessed throughout the whole storage period by using physical, chemical and microbiological indexes (pH, colour, lipid oxidation, weight loss, psychrophilic bacteria, *Pseudomonas spp., Brochothrix thermosphacta*). The experimental data indicated that no significant differences exist among the various packaging systems investigated.

Key words: biopolymers; fresh beef meat; packaging; Podolian cattle; shelf-life.

INTRODUCTION

Bio-based polymers or biopolymers are obtained from renewable resources, and being both compostable and biodegradable, their utilisation in food packaging may represent a good technological opportunity for reducing the amount of plastic wastes, with positive consequences on the environment (Alves *et al.*, 2006; Cutter, 2006). In particular, biopolymers for food packaging have, in general, a

relatively high permeability to O_2 and H_2O; therefore, their utilization is of interest mainly for those foodstuffs, such as meat, having few gas and water vapour barrier requirements, with a shelf-life limited to a few days of storage (Avella *et al.*, 2005).

Packaging of fresh beef meat can be realised in several ways: under vacuum, under air, in very permeable packages, usually made using expanded polystyrene (PS) trays wrapped with PVC film, allowing proper O_2 intake for right colour retention, or under an O_2 enriched atmosphere in barrier packaging materials. Interesting results have been recently also reported regarding the use of biopolymeric materials for meat packaging in combination with the use of PS trays or protective atmosphere (Cannarsi *et al.*, 2005; Cornini *et al.*, 2005).

In this work, packaging of fresh beef meat using an expanded biopolymeric tray made of polylactic acid (PLA) and heat-sealed with 3 different biopolymeric films, was investigated as a potential replacement for commercial packaging systems based on expanded PS tray over-wrapped with PVC.

MATERIALS AND METHODS

Meat was obtained from six different Podolian young bulls, all fed with the same diet, and slaughtered at 18 months of age, with a mean weight of 444 ± 21 kg. Dressed carcasses were divided into two sides and chilled for 24 h at 4°C. Afterwards, the *Longissimus lumborum* muscle was taken from the left side of each animal, and all the removed sections were vacuum-packaged and aged at 4°C until 8 days post-mortem.

The muscles were then cut and for each thesis two steaks of similar size (100 g, thickness 1 cm) were packed using an expanded PLA tray (23 cm x 14.5 cm x 3.5 cm) having a biodegradable pad (Coopbox Europe S.p.a, Italy), and either overwrapped with a PVC film, or heat-sealed with three different biopolymeric films: PLA (Treophan, Germany), Mater-Bi 1, a monolayer film, and Mater-Bi 2, a coextruded tri-layer film having better mechanical performances. Both Mater-Bi films (Novamont, Italy) were compostable and based on polyesters partially derived from renewable sources (Tab. 1). As control, meat samples were packed using PS trays with a polyolefinic pad (Coopbox Europe), wrapped with a PVC film, hence replicating a commercially available packaging system for fresh meat.

All samples were stored at 4°C for 8 days and the meat quality was assessed throughout the whole storage period by using physical, chemical and microbiological indexes. Colour was measured on the meat surface using a Minolta Chroma meter CR-300 with a D 65 illuminant, using CIELAB L*, a*, b* and Hue angle values. The oxidative deterioration of lipids was assessed according to Buege and Aust (1978) and expressed as mg malondialdehyde (MDA)/kg meat, while metmyoglobin content, expressed as percentage of total myoglobin content, was calculated according to Krzywicki (1979). Weight loss was determined gravimetrically and expressed as percentage of the original weight, while pH was measured using a pH meter equipped with a probe for solids. Microbiological quality of the samples was monitored by assessing the levels of psychrophilic bacteria, *Pseudomonas* spp. and *Brochothrix thermosphacta.* Statistical analysis of the data (ANOVA, LSD test, Correlation analysis) was performed by using the statistical package SAS (ver. 9.1., 2005).

RESULTS AND DISCUSSION

All the parameters considered for meat quality evaluation resulted influenced only by storage time (Tab. 2) and not by the packaging system of meat (Tab. 3). In particular, the pH value increased significantly over the storage period ($P < 0.001$) and according to Nychas *et al.* (1998) this trend is probably due to an increase in the production of proteases by psycrotrophic bacteria. However, in the present study no significant correlation between pH value and level of psychrophilic bacteria was recorded (Tab. 4).

Consumers relate the bright red colour of meat with freshness, and this parameter, besides depending on oxymyoglobin levels, is also affected by breed and rearing system. In particular, Podolian meat is naturally characterized by a darker colour, due to the extensive rearing system and to the utilization of pasture as the unique feeding resource (Napolitano and Girolami, 2001). In comparison with the data reported in literature for other fresh beef breeds, all the initial colour parameters of the tested samples were lower, while the metmyoglobin value resulted higher (Djenane *et al.*, 2003; Insausti *et al.*, 2001). Moreover, Hue values increased progressively during storage ($P<0.001$) and this increase showed to be negatively correlated to redness a* (Tab. 4), in agreement with Insausti *et al.* (1999). The a* value, a good indicator of colour acceptability, decreased only of 21% with respect to the initial value, reaching after 8 days a value of about 17, higher than the a* value reported as acceptable for beef steaks packaged in a CO enriched atmosphere by John *et al.* (2005). Conversely, b* (yellowness) and L* (lightness) parameters increased after 3 and 6 days, respectively, ($P<0.001$). L* changes could be affected by water loss, being these variables positively correlated, while b* increase could be related to the lipid oxidation. On the other side the a* decrease may result from the gradual formation of metmyoglobin on the meat surface, in agreement with Insausti *et al.* (2001) (Tab. 4).

Lipid oxidation increased during meat storage ($P<0.001$), even if the MDA level reached after 8 days was below the threshold value for rancidity (1-2 mg/kg meat); this could be due to the lower intramuscular fat content that characterizes the Podolian meat in comparison with other beef breeds (Djenane *et al.*, 2003; Cifuni *et al.*, 2004; Insausti *et al.*,2001).

Weight loss increased significantly to a maximum of about 3% after 8 days ($P<0.001$), a value comparable to that recorded by Cornini *et al.* (2005) for beef meat packed in protective atmosphere by using an expanded PLA tray heat sealed with a PLA film. Weight loss showed to be the only one parameter somehow influenced by the different packaging systems (Tab. 3), and the difference could be ascribed to the different WVTR properties of the PVC and Mater-Bi 2 films (Tab. 1). Several authors (Guignot *et al.*, 1994; Price and Schweigert, 1994) have highlighted a direct influence of pH on the charged groups of the myofibrils and thus on the water holding capacity, but in this study no correlation between water loss and pH was found (Tab. 4), as also reported by Insausti *et al.* (2001).

Table 1. Principal technical characteristics of packaging films.

Characteristics	Packaging film			
	PLA	Mater-Bi-1	Mater-Bi-2	PVC
Thickness (μm)	25	35	35	12
WVTR (g/m^2 24 h) (38°C; 100% R.H.)	175	408	842	34.1
OTR (cm^3/m^2 24 h) (23°C; 50% R.H.)	610	1246	2491	2940

All the microbiological in-

dices considered in this study increased constantly over the whole storage period, especially during the first 3 days of storage. Similar results were reported in a work on Podolian meat by Cannarsi *et al.* (2005), even if the initial values of psycrotrophics, *B. thermosphacta* and *Pseudomonas* spp were higher than those recorded in our meat samples.

The permissible maximum level of total bacteria in refrigerated meat ranges from 10^7 to 10^9 cfu/g; however, the correlation between bacterial numbers and sensorial spoilage is imprecise, thus making difficult to use bacterial levels as a spoilage index (Borch *et al.*, 1996). In fact Young *et al.* (1998) reported that the bacterial count at which meat could be considered spoiled (10^7 cfu/g) did not coincide with the panellists rejection of meat on the basis of colour, while Insausti *et al.* (2001) reported that meat samples with bacterial counts lower than from 10^7 cfu/g resulted unacceptable from a sensory point of view. Therefore, it is necessary, but not sufficient, to use microbiological indices for quality evaluation of meat; however, rather than the total count of bacteria, it is preferable to use as a spoilage indicator the growth of a specific spoilage bacteria, such as *Pseudomonas spp.* or *B. thermosphacta.*

CONCLUSION

Shelf-life evaluation of the fresh beef steaks stored at 4°C for 8 days highlighted that no significant differences existed among the various packaging systems, suggesting that for fresh meat packaging it could be possible to replace the PS tray/PVC film system, with a commercially viable solution, represented by an expanded PLA biopolymeric tray heat-sealed with a biopolymeric film, characterized by a negligible environmental impact in comparison with the use of synthetic plastic materials.

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SPOILAGE MECHANISMS EVALUATION TO PREDICT THE SHELF LIFE OF FRESH CHEESES

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ABSTRACT

This study was aimed to evaluate the spoilage mechanisms of fresh cheeses at different storage conditions. *Mozzarella* and *Ricotta* cheeses were stored at 5, 12 and 25°C and sampled periodically to evaluate sensorial, physical and chemical properties, proteolytic and oxidative reactions and microbial spoilage profile. The bacteria species were further identified. The dominant bacteria resulted some species of Coliforms and Pseudomonas causing proteolytic reactions. Shelf life was assessed at 8 days a 4°C storage.

Key words: fresh cheeses, microbial growth, shelf life, spoilage mechanism.

INTRODUCTION

Among the dairy products, fresh whey cheeses are very susceptible to microbial spoilage by Moulds, Yeasts, and *Enterobacteriaceae*.

Storage under aerobic conditions results in rapid spoilage, usually in less than 7 days. Additionally, soft and whey cheeses constitute a major food safety concern due to their lower hurdle effect compared with hard cheeses (Papaioannou *et al.*, 2005).

Numerous studies have underlined that fairly often, Mozzarella cheese is spoiled by *Pseudomonas* spp. growing on the cheese surface, mostly coming from water used during manufacturing (Cabrini and Neviani, 1983; Cantoni *et al.*, 2003a,b). Another factor limiting Mozzarella cheese shelf life is the presence of Coliforms (Rondinini and Garzaroli, 1990; Parisi, 2003a,b). The same studies demonstrated that proteolytic and lipolytic reactions also are of high importance in Mozzarella cheese preservation.

This study was aimed to evaluate the physical, chemical and microbiological spoilage mechanisms of fresh cheeses at different storage conditions.

MATERIALS AND METHODS

Ten *Mozzarella* under brine and ten *Ricotta* cheeses with the same manufacturing and packaging date, produced and packaged in pots by a Umbria (central Italy) cheese factory, were stored at 5°C, 12°C and 25°C and sampled periodically to evaluate sensorial, physical and nutritional properties, proteolytic and oxidative reactions and microbial spoilage profile. Sampling was performed at time 0 (trial beginning, immediately after packaging) and following the scheme reported in tables 1 and 2.

At each sampling time, pH, moisture content, total volatile nitrogen (TVN), peroxide value, rancidity test (Kreis test), Pseudomonas spp., Coliforms, Enterobacteriaceae, Mould, Yeast, coagulase-positive Staphylococci and *Listeria monocytogenes* were determined, beside sensorial evaluation. The analysis were carried out using ISO and AOAC methods. All the methods are validated and accredited following the ISO/IEC 17025. Sensory evaluation was performed by 5 trained people considering color, flavor, texture, and odor, on a hedonistic scale of 0 to 4 (0 = unacceptable; 1 = poor; 2 = fair; 3 = good; 4 = very good). The detected bacteria species were further identified using Apiweb (bioMérieux Corporate, 2008) and predictive shelf life was achieved following Arrhenius model.

Table 1 – Bacteria (cfu/g), TVN (mg/100 g) and pH results in Mozzarella

	5°C	12°C	25°C	5°C	12°C	25°C	5°C	12°C	25°C
day	**Coliforms**			**Enterobacteriaceae**			**Yeast**		
0	< 10			< 10			< 10		
2			~~$1.0 \cdot 10^7$~~			~~$3.0 \cdot 10^6$~~			~~$7.5 \cdot 10^4$~~
3	< 10	~~$5.4 \cdot 10^5$~~	~~$4.7 \cdot 10^8$~~	< 10	~~$6.8 \cdot 10^5$~~	~~$> 10^9$~~	< 10	~~$1.8 \cdot 10^5$~~	~~$1.0 \cdot 10^7$~~
5		~~$> 10^9$~~			~~$1.4 \cdot 10^8$~~			~~$8.5 \cdot 10^6$~~	
6	< 10			< 10			$3.0\ 10^4$		
7		~~$4.6 \cdot 10^7$~~			~~$5.0 \cdot 10^7$~~				
8	~~$1.2 \cdot 10^3$~~			~~$1.2 \cdot 10^3$~~			~~$1.5 \cdot 10^4$~~		
10	~~$4.3 \cdot 10^5$~~			~~$4.7 \cdot 10^5$~~			~~$4.0 \cdot 10^6$~~		
day	**Pseudomonas**			**Total Volatile Nitrogen**			**pH**		
0	< 10			< 10			5.88		
2			~~$7.5 \cdot 10^4$~~			~~4~~			~~5.91~~
3	$5.0\ 10^2$	~~$9.6 \cdot 10^5$~~	~~$> 10^{10}$~~	4	~~4~~	~~6~~	5.89	~~6.3~~	~~5.75~~
5		~~$3.9 \cdot 10^9$~~			~~4~~			~~5.76~~	
6	$6.0\ 10^5$			4			6.14		
7					~~3~~			~~5.78~~	
8	~~$1.1 \cdot 10^7$~~			~~3~~			~~5.94~~		
10	~~$6.0 \cdot 10^7$~~			~~6~~			~~5.92~~		

Table 2 – Bacteria (cfu/g), TVN (mg/100 g) and pH results in Ricotta

	5°C	12°C	25°C	5°C	12°C	25°C	5°C	12°C	25°C
day	**Coliforms**			**Enterobacteriaceae**			**Yeast**		
0	< 10			< 10			< 10		
2			$1.0\ 10^7$			$1.2\ 10^7$			$8.5\ 10^3$
3	< 10	< 10	$3.1\ 10^7$	< 10	$6.8\ 10^5$	$> 1\ 10^9$	< 10	$1.8\ 10^5$	$1\ 10^7$
5		$1.2\ 10^8$			$1.4\ 10^8$			$2.7\ 10^7$	
6	$1.0\ 10^2$			$1.0\ 10^2$			$6.0\ 10^4$		
8	$3.5\ 10^5$			$4.7\ 10^4$			$1.5\ 10^4$		
10	$1.7\ 10^3$			$1.9\ 10^3$			$3.2\ 10^6$		
day	**Pseudomonas**			**Total Volatile Nitrogen**			**pH**		
0	< 10			2			6.67		
2			$1.4\ 10^6$			8			5.48
3	$5.0\ 10^2$	$9.6\ 10^5$	$> 1\ 10^{10}$	4	4	10	6.65	6.68	5.39
5		$4.2\ 10^9$			6			6.30	
6	$5.0\ 10^5$			6			6.87		
8	$2.0\ 10^6$			4			6.80		
10	$8.3\ 10^7$			6			6.62		

RESULTS AND CONCLUSIONS

Only the significant results are shown in table 1 for Mozzarella cheese and in table 2 for Ricotta cheese. In the table boxes, one diagonal line corresponds to a shelf life going to end, based on sensory evaluations, and the presence of two diagonal lines indicates that the period went over the shelf life. *Listeria monocytogenes* and coagulase-positive Staphylococci were never detected (< 10 cfu/g); Kreis test was at all times negative.

On the basis of the results and sensory evaluations, the activation energy was set at 69.14 kj/mole and Q_{10} 2.81 and the shelf life was assessed at 8 days, for both mozzarella and Ricotta cheeses stored at 4°C.

The limits of acceptability were further assessed at 10^2 cfu/g for Mould, 10^4 cfu/g for Yeast, 10^6 cfu/g for Pseudomonas spp. and 6 mg/100 g for total volatile nitrogen, in both Mozzarella and Ricotta cheeses. The limits either for Coliforms or Enterobacteriaceae were set at 10^3 cfu/g in Mozzarella and 10^4 cfu/g in Ricotta. In agreement with several authors (Cabrini and Neviani, 1983; Cantoni *et al.*, 1983ab; Rondinini and Garzaroli, 1990; Parisi, 2003ab), Coliforms and Pseudomonas resulted the main bacteria involved in Mozzarella and Ricotta spoilage, with Pseudomonas, coming from water used during manufacturing, representing the highly prevailing one. These bacteria produce usually proteolytic and lipolytic enzymes that cause the decay of physical and chemical characteristics. In this study, however, only proteolytic reactions were revealed by chemical analysis.

As for microbial identification, Enterobacteriaceae were totally represented by Coliforms belonging to *Citrobacter freundii*, *Enterobacter amnigenus*, *Enterobacter*

cloacae and Pantoea in Mozzarella and to *Citrobacter freundii* and *Enterobacter cloacae* in Ricotta. The yeast were identified as *Rhodotorula mucillaginosa*, *Candida intermedia* and *Candida farmata* in Mozzarella and *Rhodotorula glutinis*, *Rhodotorula mucillaginosa* and *Rhodotorula minuta* in Ricotta. Finally Pseudomonas species were mainly *Burkholderia cepacia*, *Pseudomonas putida* and *Pseudomonas fluorescens* in both Mozzarella and Ricotta cheeses.

In conclusion, it emerges that the limiting factors for Mozzarella and Ricotta shelf life are environment and production hygiene, water used during manufacturing, and quality of raw materials. The improvement of these factors allows to extend the shelf life period, and to implement, during the commercial life, food safety and quality as stated by EC Regulations 2073/2005 and 852/2006.

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SHELF-LIFE OF EXTRA VIRGIN OLIVE OILS WITH LEMON AND ORANGE

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ABSTRACT

The shelf-life of "Peranzana" extra virgin olive oils flavoured with lemon and orange, was investigated over a 6 months storage period. Gourmet olive oils were prepared according to two different techniques: 1) olives milled together with each fresh citrus fruit, followed by the oil extraction, by using a three-phase system (industrial method); 2) preparation of flavoured oil by infusion (handling method). The following quality indices were monitored during storage: acidity, peroxide value, K_{232} and K_{270} indices, total phenolic content (TPC), antioxidant activity (AA) and volatile compounds (VC). Flavoured oils prepared by infusion were slightly more oxidised than the control, after the six months storage, particularly the orange oil. TPC in all samples decreased during storage, due to their high reactivity. The AA values of the flavoured oils, after an initial increase, were found to be similar to the control. The flavouring of extra virgin olive oils with lemon and orange, which leads to a great increase in volatile compounds, may be particularly appreciated by consumers, although it have a shorter shelf life.

Key words: citrus fruit, extra virgin olive oil, shelf-life, volatile compounds.

INTRODUCTION

Extra virgin olive oil is rich in monounsaturated fatty acids, essential fatty acids and nutritionally valuable minor compounds, such as phenolic elements. These latter possess antioxidant activity that delay the oxidative rancidity oil process and may also play an important role in the prevention of cardiovascular diseases

and cancer (Grande, 1985; Jacotot, 1994). Citrus fruits are also an important source of antioxidant compounds (Rapisarda *et al.*, 1998; Rapisarda *et al.*, 1999; Fernandez-Lopez *et al.*, 2004). In addition, citrus fruits such as lemon and orange also contain volatile compounds that could enhance the flavour of typical dishes. Few works have been published on the effects of the addition of citrus fruits to extra virgin olive oil. The aims of this work were: a) to evaluate the shelf-life of extra virgin olive oils flavoured with lemon and orange fruits produced using two different techniques; b) to evaluate change in their volatile content during the storage period.

MATERIALS AND METHODS

Industrial flavoured oils (IOL). Sixty kg of each fresh citrus fruit (whole lemons and oranges from the Gargano area) were separately added to 3 q olives of cv Peranzana and submitted to oil extraction by the three-phase system, obtaining lemon (IOL) and orange (IOO) flavoured oils. An extra virgin olive oil produced in the same period and representative of oil mill production was taken as the control (IOC).

Hand flavoured oils (HFO). Two hunfred g of minced peel of each fruit were separately added to 1 L Peranzana extra virgin olive oil, which was then packaged into dark glass bottles and stored at room temperature in the dark, with daily shaking. After 14 days of the infusion process, the lemon (HOL) and orange (HOO) flavoured oils were prepared by dilution to obtain products at 20g L^{-1} of citrus fruits concentration. The extra virgin olive oil without any fruit was used as another control (HOC).

Analyses and sampling. Acidity, peroxide value (PV), K_{232} and K_{270} were determined according to the European Standard Methods (EC 1989/03). Extraction of phenol fraction was performed according to a modified version of the method proposed by Montedoro *et al.*, (1992). Total phenolic content (TPC) was determined by spectrophotometry using the Folin-Ciocalteu reactive, and expressing the results as mg kg^{-1} equivalent of gallic acid. Antioxidant activity (AA) was determined by a modified version of the β-carotene method (Lee *et al.*, 1995). Volatile compounds (VC) were determined according to the method reported by Angerosa *et al.* (1997). For both typologies of flavoured oils analyses started in June and sampling was carried out at 0, 3 and 6 months. All analyses were carried out twice (three times for AA) and the results expressed as mean value ± standard deviation.

RESULTS AND CONCLUSIONS

At the start of the experiment, IFO showed lower acidity values than the control (Fig. 1). After 6 months IOL increased by about 50% whereas IOC and IOO did not change. Regarding HFO, no variation was observed over the period. PV and K_{232} of IFO showed the same control trend during storage (Figg. 2-3).

In contrast, HFO were slightly more oxidised than the control after 6 months and showed a smaller shelf life, particularly for HOO. Whit regard to K_{270}, a similar trend was found for all samples: the values increased after 3 months and remained fairly constant at 6 months (Fig. 4). At the beginning, TPC of IOL and IOO was about half of the control (Fig. 5).

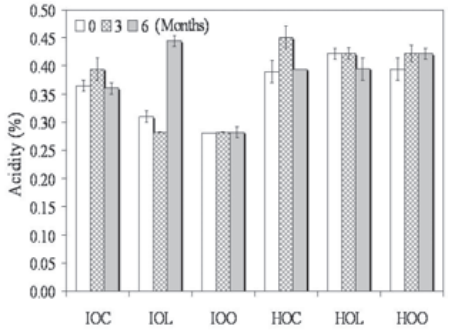

Fig. 1: Trend in acidity.

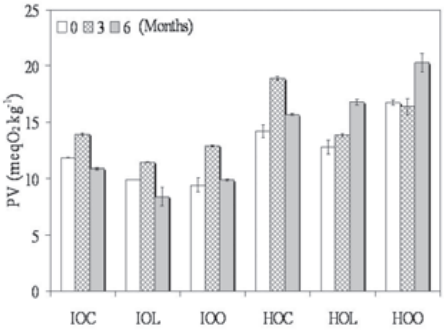

Fig. 2: Trend in PV.

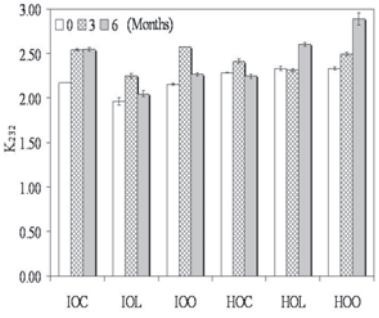

Fig. 3: Trend in K_{232}.

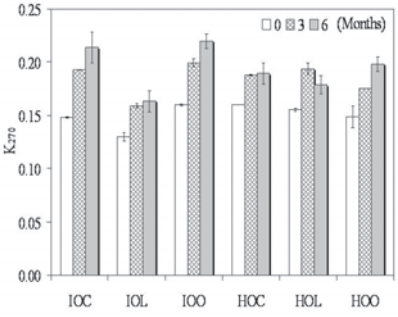

Fig. 4: Trend in K_{270}.

This is due to the addition of whole citrus fruits to olives in the milling phase, which results in a greater volume of vegetable water. This then leads to the accumulation of minor phenols in oil because they are lost in the vegetable water. As expected, TPC in all samples decreased during the storage period due to the high reactivity of phenols. The IOL sample showed a similar AA trend to the control, whereas IOO exhibited a constant decrease (Fig. 6). HOL and HOO showed an initial AA value higher than the control that successively decreased during storage. This is probably due to the presence of phenols deriving from citrus fruit that contribute to the increase of the initial antioxidant property. Flavoured oils showed a very high content of VC, which comes from the fruit peel (Fig. 7). IFO showed a higher con-

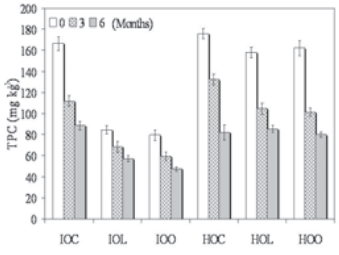

Fig. 5: Trend in TPC.

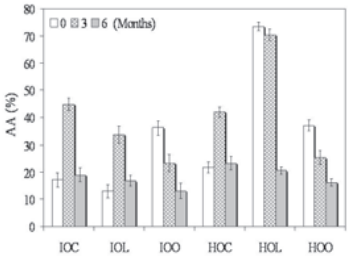

Fig. 6: Trend in AA.

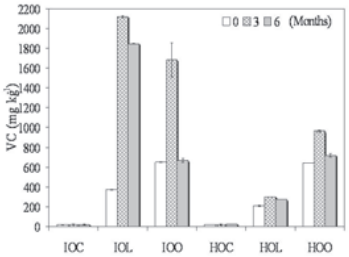

Fig. 7: Trend in VC.

centration of VC than the HFO after 3 and 6 months of storage, especially for lemon oil. In conclusion, the present work shows that the flavouring of extra virgin olive oil results in a very slight decrease of shelf life, but to a great increase of VC.

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SHELF LIFE OF SOME MONOVARIETAL EXTRA VIRGIN OLIVE OILS

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ABSTRACT

The shelf life of monovarietal extra virgin olive oils from some cultivars grown in experimental field of Daunia (Foggia, Italy) was studied. The following quality indices were considered during 12 months of storage: acidity, PV, K_{232} and K_{270}, total phenol content and antioxidant activity. Results showed that all samples belonged to category of "extra virgin" with the exception of Peranzana oil for acidity, but only at 12 months from production. Phenolic content and antioxidant activity decreased due to their reactivity with lipid radicals formed during storage.

Key words: antioxidant activity; extra virgin olive oil; monovarietal; phenols; shelf life.

INTRODUCTION

Lipid peroxidation can't be stopped but only slow down by some endogenous antioxidants (tocopherols and phenols) that improve the resistance of virgin olive oil to oxidative deterioration (Bendini *et al.*, 2007). Phenolic antioxidants interrupt the initiation and propagation stages of the oxidative chain reaction since they react with lipid radicals to form more stable products (Gomez-Alonso *et al.*, 2003), prolonging the shelf life of the product. It is well known that the phenolic content of virgin olive oil depends on several factors such as cultivar (Esti *et al.*, 1998), agronomic techniques used (Romero *et al.*, 2002), extraction technology (Angerosa *et al.*, 1996) and time and way of oil storage (Cinquanta et al., 1997). The present work reports the results of a study on shelf life and antioxidant properties of

monovarietal virgin olive oils obtained from native and newly cultivars introduced in Torremaggiore area, Apulia, Italy.

MATERIALS AND METHODS

Samples: Monovarietal virgin olive oils derived from olives of Cima di Melfi, Coratina, Frantoio, Leccino, Moraiolo, Nociara and Peranzana cultivars grown in experimental fields of Torremaggiore (Foggia, Italy) were taken. Olives of each variety were manually harvested at technological maturation and submitted within 36 hours to extraction of oil using an industrial pressure system. Oils were transferred into 1 litre glass bottles and stored in the dark at room temperature until analysis.

Chemical Analysis: Acidity, peroxide value (PV), K_{232} and K_{270} were determined according to the European Standard Methods (EC 1989/03). Extraction of phenol fraction was performed according to method proposed by Montedoro *et al.*, (1992) advisable modified, whereas total phenolic content (TPC) was determined by spectrophotometry using the Folin-Ciocalteu reactive, and expressing the results as mg kg^{-1} equivalent of gallic acid. The evaluation of the antioxidant activity (AA) was performed on the phenolic extract by means of the β-carotene/linoleate test (Lee *et al.*, 1995) with some modifications and expressed as Antioxidant Activity Coefficient (AAC). All analyses were carried out in duplicate (in triple for antioxidant activity) and the results expressed as mean value ± standard deviation.

RESULTS AND DISCUSSION

The figures 1-4 show the evolution of quality indices of oils during 12 months of storage. At production all the oils resulted belonging to "extra virgin" category, whereas during storage the evolution of quality indices were different for each-one.

Acidity values of all oils were always below the limits establish from European normative for extra virgin category (≤ 0.8 g/100g), except for Peranzana at the end of storage (0.84 g/100g). PV and K_{232} values (primary oxidation compounds) were found lower than the extra virgin limits reported in Regulation UE during storage (20 meqO_2/kg). Regarding the trend, they showed an initial increase in the first 6 months and than a decrease until the end of storage. This latter behaviour could

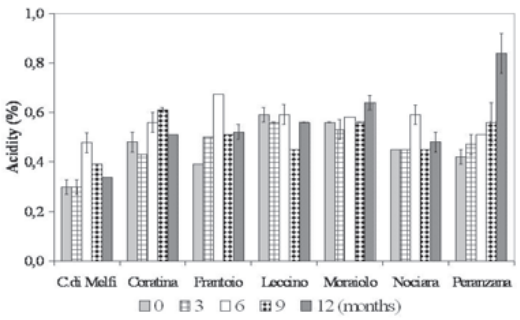

Fig. 1: Trend in acidity of oils during storage.

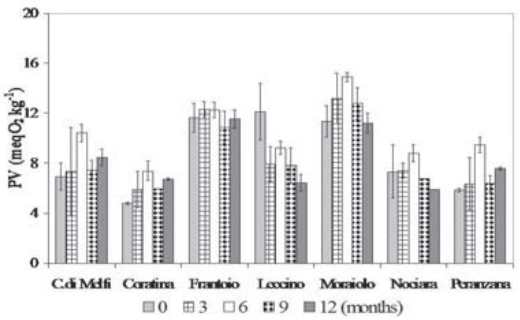

Fig. 2: Trend in PV of oils during storage.

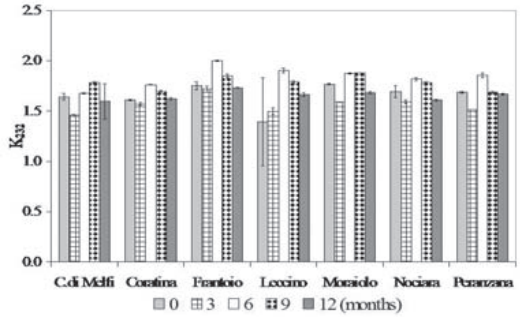

Fig. 3: Trend in K_{232} of oils during storage.

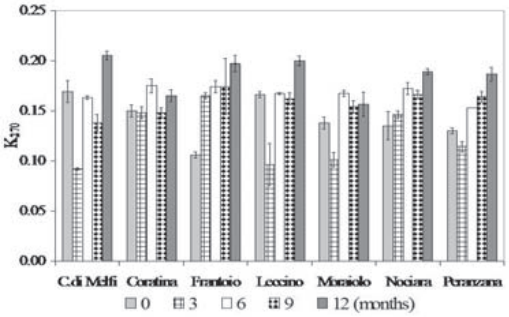

Fig. 4: Trend in K_{270} of oils during storage.

be explained by hydroperoxides decomposition that led to formation of aldehydes and ketones (secondary oxidation compounds). To confirm the formation of secondary oxidation compounds, almost all samples showed highest values in K_{270} after 12 months from production, even if to below the limits reported by Regulation UE (≤ 0.22).

The figures 5 and 6 show the evolution of TPC and AA of oils during storage.

All samples showed a decrease of phenolic content during storage (slight in the first 6 months and more marked at 9 and 12 months), according to that observed by *Cinquanta et al.* (1997) in some Molise oils. This behaviour is explained by high reactivity of phenols that are able to donate a hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation. Thus explains the resistance to oxidative stress of virgin olive oils, especially for those notorious rich in these compounds. Concerning the AA of oils, a certain relation with phenols content was observed. In fact, the trend of AA is almost similar to phenol content with high values until 6 months and low at 9 and 12 months due to the strong loss in phenol compounds.

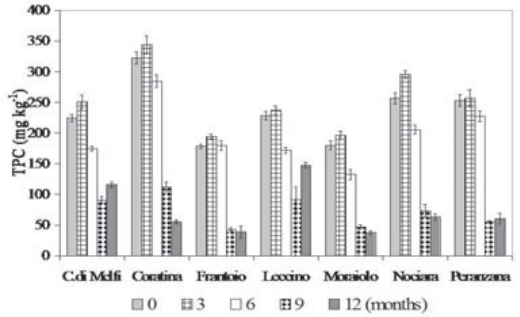

Fig. 5: Trend in phenolic content of oils during storage.

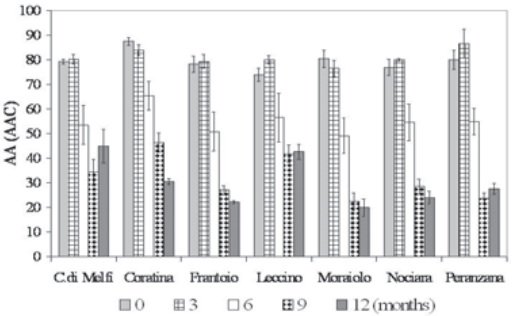

Fig. 6: Trend in antioxidant activity of oils during storage.

CONCLUSIONS

The monovarietal extra virgin olive oils investigated showed a good shelf life. In fact, all the samples belonged to "extra virgin" category along 12 months of storage with the exception of Peranzana oil, in relation only to acidity parameter but not much up the limit indicated by UE normative. The trend in phenol content and antioxidant activity showed a decrease, different for each-one, due to their reactivity with lipid radicals formed during storage.

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POLYPHENOLOXIDASE ACTIVITY OF MINIMALLY PROCESSED BABY ROMAINE LETTUCE (*LACTUCA SATIVA* L. CV. DUENDE) CULTIVATED UNDER DIFFERENT SALINITY CONDITIONS

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ABSTRACT

Fresh-cut fruit/vegetable salads are becoming increasingly popular for their convenience, fresh-like appearance, and the fact that they are generally free of additives. A major problem however, of fresh-cut produce is that it is subject to wounding through processing. Thus in fresh-cut produce the tissue is exposed to stress conditions which can result in the deterioration of cell wall structure and hence a reduction in shelf life. Enzymatic browning in leafy vegetables is considered one of the most important disorders, since it is easily detected by consumers, evident consequences on marketing.

We hypothesized that the cause of browning in fresh cut Baby Romaine lettuce, could be attributed to the degradative action of some endogenous enzymes such Polyphenoloxidase.

Polyphenoloxidase (PPO, E.C. 1.14.18.1) is a copper-containing enzyme which acts on phenols in the presence of oxygen, catalysing the oxidation of *o*-diphenols into *o*-quinones. Plant phenolics are readily oxidized by PPO, most often following tissue damage since PPO is suggested to act as a defensive enzyme. Although PPO has generally been recognized to be largely responsible for enzymatic browning of fruits and vegetables, it has not been clearly established, so far, whether a relationship among extent of browning, vegetal polyphenol content and enzyme (PPO) activity exists. Aim of the present work was to value the changes in polyphenoloxidase, as well as in total phenolic content, during 15 days of storage at 4 °C of minimally processed Baby Romaine lettuce (*Lactuca sativa* L. cv. Duende) cultivated under different salinity conditions (2,8 NaCl 4,8 NaCl dS/m; 2,8 $CaCl_2$ 4,8 $CaCl_2$ dS/m),

and harvest at different period (August and November 2006) in order to determine the most suitable agronomic condition for further processing.

Soil salinity increased PPO activity, in fact increasing in NaCl and $CaCl_2$ concentrations resulted in an increase in PPO activity, although $CaCl_2$ treatment seemed to have a stronger effect than NaCl one.

Key words: browning, polyphenoloxidase, salinity, $CaCl_2$, NaCl.

INTRODUCTION

Fresh-cut fruit/vegetable salads are becoming increasingly popular for their convenience, fresh-like appearance, and the fact that they are generally free of additives. A major problem however, of fresh-cut produce is that it is subject to wounding through processing. Thus in fresh-cut produce the tissue is exposed to stress conditions during processing which can result in cellular delocalization of enzymes and their substrates, leading to biochemical deteriorations such off-flavours, texture breakdown and browning.

Physiological disorders of lettuce (*Lactuca sativa* L., iceberg) that involve tissue discoloration (e.g. russet spotting, rusty brown discoloration, pink rib, etc.) continue to significantly reduce quality in fresh-cut lettuce (Saltveit 2004) and leads to development of off-flavours and losses in nutritional quality. Altered phenol metabolism is thought to be involved in this alteration (Saltveit, 2000). In tissue in which the initial level of phenolic compounds is low (e.g. lettuce leaves), browning only occurs after wound-induced increases in phenolic metabolism and the accumulation of precursor compounds. Agronomic condition could influence postharvest alteration of vegetable. In particularly soil salinity such as age of harvest could influence postharvest enzymatic activity of whole and processed vegetable as described by different authors (W. W. Kirk *et al.*, 2006; Demir Y. et al, 2003; Cliffe-Byrnes *et al.*, 2005). The Aim of the present work was to value the changes in Polyphenoloxidase, as well as in total phenolic content, during 15 days of storage at 4 °C of minimally processed Baby Romaine lettuce (*Lactuca sativa* L. cv. Duende) cultivated under different salinity conditions (2,8 NaCl 4,8 NaCl dS/m; 2,8 $CaCl_2$ 4,8 $CaCl_2$ dS/m), and harvest at different period (August and November 2006) in order to determine the most suitable agronomic condition for further processing.

MATERIALS AND METHODS

Plant material: Baby Romaine lettuce were field-grown in Southern of Sicily under different salinity conditions (2,8 NaCl 4,8 NaCl dS/m; 2,8 $CaCl_2$ 4,8 $CaCl_2$ dS/m) and harvest in two different periods (august and november 2006). In particular samples harvest in august were cultivated under 2,8 NaCl 4,8 NaCl dS/m; 2,8 $CaCl_2$ 4,8 $CaCl_2$ dS/m, while samples harvest in november were cultivated under 2,8 NaCl dS/m and 4,8 NaCl dS/m.

Minimally processed baby lettuce preparation: The leaves were cut into segments approximated the size of piece used in 'salad packs'. The pieces were washed with chlorinated water (0.01%) and then centrifuged to remove excess water. About 200g of cut tissue was packaged with a PET film and stored at 4°C for 15 days.

Baby Romaine lettuce samples were taken for analysis at 0, 3, 7, 10 and 15 days. The analyses were performed in triplicate.

Total Polyphenols: 10 g of each sample were added to 100 ml of extraction solvent (methanol with chloridric acid) and the mixture was homogenised for 180 minutes; the extracts were filtered with n°1 filter paper (Whatman). We extracted 2 ml diluted up to 20 ml with water for HPLC (60-70°); we added 4 ml of water, 1 ml of Folin & Ciocalteau reagent and, after 2 minutes, we added 4 ml of sodium carbonate 2 M. After 2 hours we reported the absorption range with a spectrophotometer Cary 1 E UV-Vis (Varian) at 550 and 850 nm range, with a maximum absorption at 760 nm, against blank (Waterman and Mole, 1994).

PPO extraction and assay: PPO was extracted from 10 g fresh lettuces in 40 ml 0.05 M phosphate buffer pH 7.0. The mixture was blended overnight at 4°C and then filtered and centrifuged at 4000 g for 10 min at 4°C. The supernatant was ultrafiltered with 50 KDa cut-off membrane (Millipore, Bedford, MA, USA) and the samples obtained were stored at temperature of –20 °C. The assay was conducted according to the method of Espin *et al.*, (1998). The enzyme activity, expressed in enzyme units per gram of fresh weight.

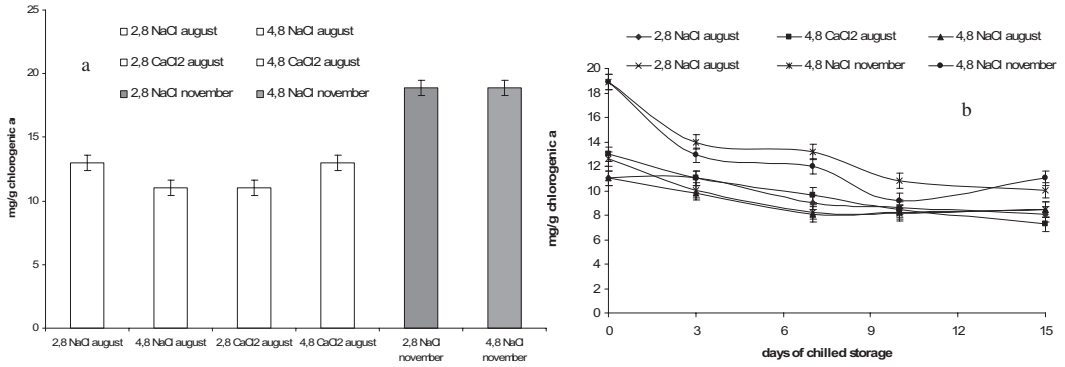

Fig. 1 Initial Polyphenols content (a) and evolution (b) in samples cultivated under different salinity conditions and harvest at different period (August and November).

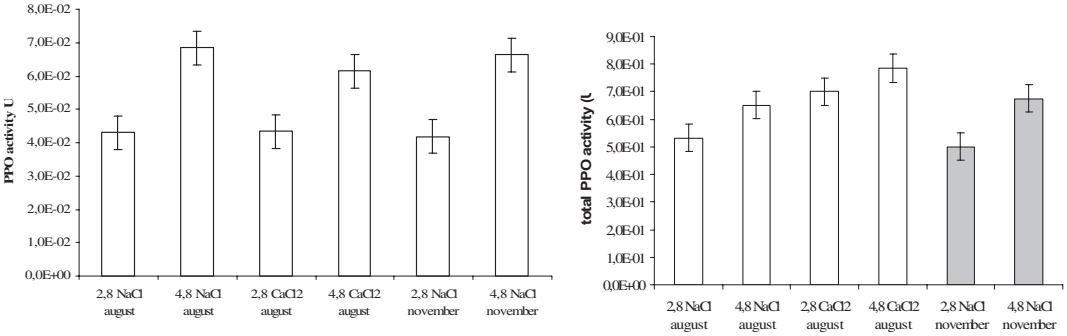

Fig. 2 Initial PPO activity and Total PPO activity during shelf-life in samples cultivated under different salinity conditions and harvest at different period (August and November).

RESULTS AND DISCUSSION

Total Polyphenols

The period of harvest (August and November) influenced total initial polyphenols content (Fig. 1), in fact samples harvest at November had the highest content one; the different salinity seemed have no effect on their initial content and evolution during shelf life (fig. 2), in fact in each sample total polyphenols decreased similarly during shelf-life.

PPO activity

Initial PPO activity in samples was only affected by different salinity concentration, in fact samples grown under 4,8 NaCl and 4,8 $CaCl_2$ showed similar and higher PPO activity, while the period of harvest seemed to be not significative (Fig. 3).

Soil salinity increased total PPO activity during shelf-life, in fact increasing in NaCl and $CaCl_2$ concentrations resulted in an increase in PPO activity, although $CaCl_2$ treatment seemed to have a stronger effect than NaCl one; The period of harvest seemed to be not significative (Fig 4).

*N*o correlation was found between Total Polyphenols variation and PPO activity during shelf-life.

CONCLUSIONS

Different salinity conditions (2,8 NaCl 4,8 NaCl dS/m; 2,8 $CaCl_2$ 4,8 $CaCl_2$ dS/m), and harvest at different period (August and November 2006) influenced PPO activity and total Polyphenol content of minimally processed Baby lettuce. In particular the period of harvest (August and November) influenced total initial polyphenols content, while soil salinity increased total PPO activity during shelf-life, particularly $CaCl_2$ treatment seemed to have a stronger effect than NaCl one. *N*o correlation was found between Total Polyphenols variation and PPO activity during shelf-life. All these results suggested that the best agronomic conditions to produce higher quality minimally processed Baby Lettuce included 2.8 NaCl salinity treatment and November harvest.

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LIGHT INDUCED CHANGES ON A MEAT MODEL SYSTEM

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ABSTRACT

The aim of this work was to investigate the effect of different light storage conditions on the quality evolution of a meat model system.

For the experimental phase, fresh minced beef meat was homogenized with a phosphate buffer in order to obtain a final solution with a pH of 7. To prevent the microbial growth during all the experimental time, chloramphenicol was added as preservative. The obtained model system was stored in clear glass vials under two different modified atmospheres. Vials were closed under 60 kPa of oxygen and 40 kPa of carbon dioxide.

During storage, the samples were exposed to constant temperature under lighting conditions produced by different light sources commonly used by retailers to illuminate meat products. In particular, a Cool white lamp (Osram Dulux El Longlife 30W/840) and a Nature® lamp (with high emission in the red region of the visible spectrum) were chosen. For each selected light source, the light intensity that reached the samples was recorded under real storage conditions. A series of sample was kept in the dark as reference. At different times, the samples were analysed in triplicate in order to monitor the changes occurred in the meat model system. The gas changes in the vial headspace, the amount of different myoglobin species and the colour (CIE L*a*b* parameters) of the model system were evaluated during time.

Results showed that the phenomena involved in colour changes were accelerated when the samples were stored under light. In fact, samples stored in the dark revealed a longer maintenance of the typical colour than solutions under Cool white and Nature lamps. The lighting caused a fast modification in chroma and hue indexes, especially when the cool white lamp was used. The major effect induced by the cool white light source was confirmed considering the evolution of the three forms of myoglobins.

INTRODUCTION

The appearance of fresh meat and meat products is the most factor that influ-

ences the customer selection. In fact, whereas food flavour and consistency affect subsequently purchase decisions, meat colour plays a direct role in the freshness perception and acceptability of the product at the purchase moment. The meat colour depends on the redox state of the myoglobin: the physiologically active species are the purple reduced pigment (deoxymyoglobin) and the oxygenate bright cherry-red form (oxymyoglobin), whereas the oxidized specie (metmyoglobin) appears brown-red (Mancini and Hunt M, 2005). During storage, meat is characterized by a continuous dynamic conversion among the above mentioned three different myoglobin states (Bekhit and Faustman, 2005; McKenna *et al.* 2005). However the appearance of the meat surface is not affected only by the real product colour but also by the lighting conditions where it is observed (Barbut, 2003). For this reason, light sources able to improve the natural colour of meat are generally used in stores. Nevertheless, the marketing choices do not often consider the potential damage induced on food by electromagnetic radiations. In fact, it is well known that light causes the deterioration of lipids, vitamins, proteins and natural pigments which results in off-flavours, loss of nutrients and colour fading (Bekbölet, 1990).

The aim of this work was to investigate the effect of different light storage conditions on the colour evolution of a meat model system.

MATERIALS AND METHODS

Sample preparation.

For the experimental phase, 100 g of fresh minced beef meat was homogenized with 1 l of phosphate buffer in order to obtain a final solution with a pH of 7.2. To prevent microbial growth during all experimental time, chloramphenicol was added to the solution as preservative (0.015 g/l). The samples were composed of 10 mL of model solution contained in 22 mL glass vials (clear in the UV-vis region of the spectrum) and conditioned under modified atmosphere (60 kPa of oxygen and 40 kPa of carbon dioxide) to simulate commercial packaging conditions.

Storage experiment and light exposure.

During storage, vials were stored horizontally on black turning plates inside thermostattted and ventilated cabinets for 9 days at constant temperature (4°C) under lighting conditions produced by different light sources commonly used by retailers to illuminate meat products. In particular, a cool white lamp (Osram Dulux El Longlife 20W) and a Nature lamp (Narva Nature 20W) were chosen. To avoid light reflecting phenomena, the internal walls of the cabinets were tinted black. For each selected light, illuminance and irradiance in the visible and UVA regions were measured under real storage conditions at the level of the samples by means a portable digital photoradiometer (Delta Ohm HD2102.2). The same number of samples was also conditioned in the darkness as reference.

Colour and myoglobin content determination

At different times, the samples were withdrawn and analysed in triplicate in order to monitor the colour changes occurred in the meat model system. The evolution of the colour of the samples was monitored by means of the CIE L*a*b* parameters calculated by the software Color version 3.00 (Perkin-Elmer Instruments) from the transmittance spectrum acquired in the visible region (380-780 nm) for supernatant solution obtained after centrifugation, filtration and dilution of the model

system. The transmittance spectrum of each sample was used to calculate some indexes related to the content of the myoglobin forms. In particular we considered the relationship between the transmittance (T%) at 572, 474 and 610 nm and the MetMb, DeoMb and OxyMb content respectively, referred to the T% at 525 nm due to the total pigment. In order to estimate the evolution of the myoglobin species, the following formula was applied:

$$Tx,525= (1-Tx)2/2*Tx) \ / \ (1-T525)2/2*T525)$$

where Tx corresponds to transmittance at a specific wavelenght (572, 474, 610).

RESULTS

CIELAB parameters show a colour fading of the model system during storage as a function of the lighting conditions.

If there were no marked trends in lightness (L*), a clear difference was observed in yellowness (b*), already after 40 hours of storage, and in redness (a*) after 140 h, among samples stored in the dark and under light (data not shown).

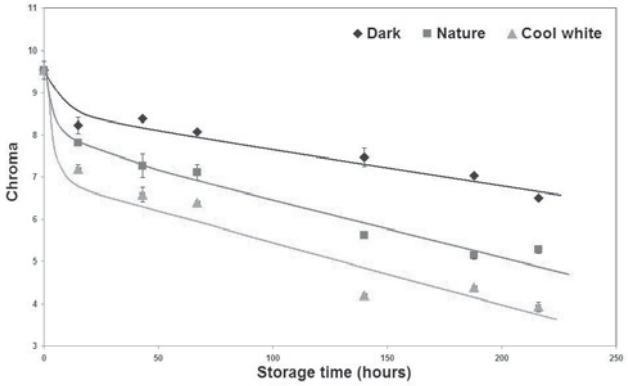

Fig. 1: Chroma trend during storage.

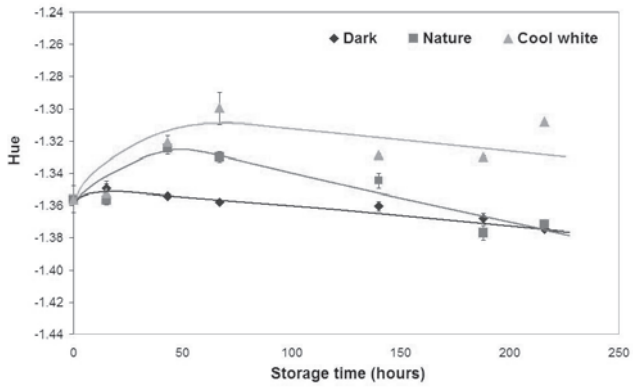

Fig. 2: Hue trend during storage.

The most noticeable influence of the light exposure was on saturation (chroma, C*). In fact, in Fig. 1 is evident the loss of saturation induced by light exposure, especially caused by the Cool white lamp. The major colour changes induced by this light source were evident also considering the hue trend (Fig. 2).

Oxymyoglobin content decreased during storage (Fig. 3) whereas the presence of deoxymyoglobin did not change significantly (Fig.4).

The decrease was more rapid in sample solutions stored in the light than in the dark, in particular in samples lighted with the Cool white lamp than those under Nature lamp.

Metmyoglobin content increased during storage in all samples in the first 40 hours and subsequently decreased in the model system stored under the Cool white lamp (Fig. 5). This different metmyoglobin trend probably indicates a more relevant degradative effect of-

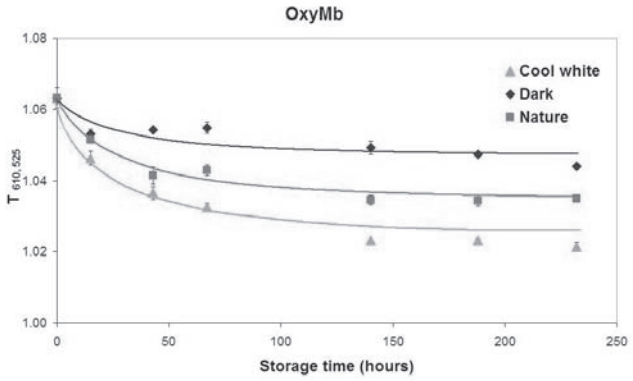

fig. 3: changes in oxymyoglobin content during storage

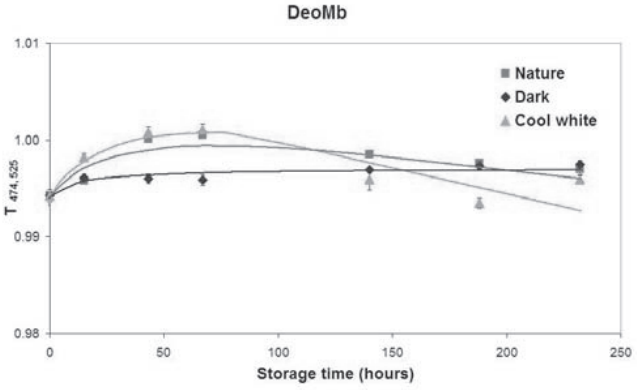

Fig.4: Changes in deossimyoglobin content during storage

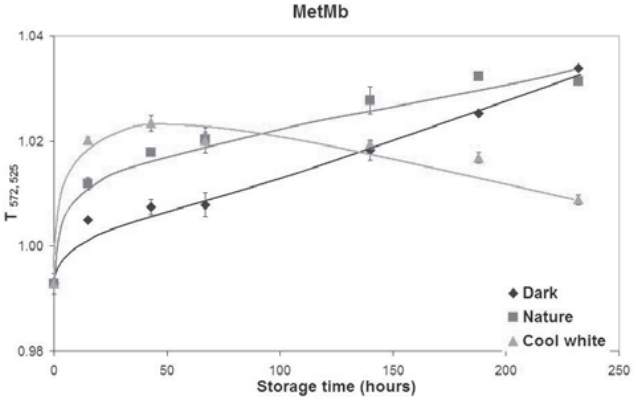

Fig. 5: Changes In Metmyoglobin Content during storage

fered by the Cool white lamp that promoted a fast degradation of the pigment that did not occur in samples stored under Nature lamp and also in the dark.

CONCLUSIONS

The two light sources used in this work, even though characterized by similar visible and UVA irradiance values, induced a different change on meat.

Probably, this phenomenon was observed because the wavelengths of the different lamp emission spectra played an important role in the conversion reactions of the myoglobin forms.

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BUFFALO MILK MINAS FRESH CHEESE WITH PROBIOTICS PROPERTIES

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ABSTRACT

The aim of this study was to produce fresh Minas-type cheese from buffalo milk and analyze its properties for up to 28 days in refrigerated storage. Five cheese-making trials were prepared. Minas-type fresh cheese is a great vehicle for *Bifidobacterium lactis* incorporation, as probiotic cultures were viable during storage and physico-chemical properties of probiotic products were similar to those of traditional cheeses. Thus, fresh Minas-type cheese made from buffalo milk is an excellent and viable alternative to improve buffalo milk and probiotic ingestion.

Key words: *Bifidobacterium lactis,* fresh cheese, physico-chemical properties, probiotic viability.

INTRODUCTION

Although several studies have tested the performance of a number of probiotic cultures in the production of different types of cheeses, few have dealt with fresh cheeses (Vinderola *et al.*, 2000; Buriti *et al.*, 2005a and Buriti *et al.*, 2005b) and no study has been carried out with buffalo milk fresh cheeses.

Minas is a typical Brazilian fresh cheese, normally prepared with cow milk, which presents high water activity, pH above 5.0, low salt content and absence of preservatives and, therefore, offers excellent conditions for survival and growth of probiotic strains (Buriti *et al.,* 2005a).

The aim of this work was to develop Minas fresh-type cheese of buffalo milk with probiotic properties; to evaluate physico-chemical properties and to determine the viability of the probiotic organisms during 28 days of refrigerate storage.

MATERIAL AND METHODS

Five pilot-scale buffalo milk Minas-type fresh cheese-making trials, denoted T1, T2, T3 T4 and T5, were performed in triplicate. Treatments: Cheeses T1 were manufactured with the addition of a probiotic culture composed of *Bifidobacterium lactis* (0.7% w/v); cheeses T2 were manufactured with the addition of traditional culture composed of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (1:1) (0.7% w/v); cheeses T3 were prepared with the addition of *B. Lactis* and traditional culture (both 0.7% w/v); cheeses T4 were prepared by direct acidi-

Table 1. Physico-chemical properties of cheeses during 28 days of refrigerated storage**.**

Treatment	1 day	14 day	28 day
pH			
T1	$6.46 \pm 0.03^{a.A}$	$6.40 \pm 0.05^{a.B}$	$6.11 \pm 0.18^{b.C}$
T2	$6.50 \pm 0.04^{a.A}$	$6.44 \pm 0.06^{a.B}$	$6.26 \pm 0.05^{b.B}$
T3	$6.47 \pm 0.03^{a.A}$	$6.54 \pm 0.03^{a.A}$	$6.53 \pm 0.01^{a.A}$
T4	$6.37 \pm 0.004^{a.B}$	$6.27 \pm 0.06^{b.C}$	$6.16 \pm 0.03^{b.C}$
T5	$6.37 \pm 0.02^{a.B}$	$6.23 \pm 0.05^{b.C}$	$6.19 \pm 0.02^{b.B.C}$
Titratable acidity (%)			
T1	$0.11 \pm 0.006^{b.A}$	$0.14 \pm 0.012^{a.b.A}$	$0.17 \pm 0.057^{a.A}$
T2	$0.06 \pm 0.032^{b.C}$	$0.15 \pm 0.016^{a.A}$	$0.15 \pm 0.026^{a.B}$
T3	$0.01 \pm 0.0009^{a.D}$	$0.01 \pm 0.0011^{a.B}$	$0.011 \pm 0.0014^{a.C}$
T4	$0.10 \pm 0.008^{b.B}$	$0.15 \pm 0.009^{a.A}$	$0.16 \pm 0.006^{a.A.B}$
T5	$0.11 \pm 0.014^{b.A.B}$	$0.14 \pm 0.004^{a.A}$	$0.18 \pm 0.10^{a.A}$
Moisture (%)			
T1	$62.56 \pm 5.34^{a.A}$	$60.31 \pm 2.12^{a.A}$	$59.58 \pm 3.36^{a.A}$
T2	$64.25 \pm 4.18^{a.A}$	$56.82 \pm 1.54^{b.B}$	$55.75 \pm 3.12^{b.B}$
T3	$62.59 \pm 1.09^{a.A}$	$61.15 \pm 2.86^{a.A}$	$61.53 \pm 2.06^{a.A}$
T4	$52.59 \pm 1.75^{a.B}$	$55.77 \pm 3.55^{a.B}$	$51.65 \pm 2.18^{a.C}$
T5	$53.83 \pm 2.92^{a.B}$	$45.48 \pm 3.32^{c.C}$	$49.67 \pm 1.63^{b.C}$
Aw			
T1	$0.97 \pm 0.0005^{a.C}$	$0.97 \pm 0.0054^{a.C}$	$0.97 \pm 0.0017^{a.C}$
T2	$0.97 \pm 0.0024^{b.C}$	$0.98 \pm 0.0040^{a.A.B}$	$0.97 \pm 0.0009^{b.C}$
T3	$0.99 \pm 0.0032^{a.A}$	$0.99 \pm 0.0045^{a.A}$	$0.99 \pm 0.0044^{a.A}$
T4	$0.98 \pm 0.0028^{a.B}$	$0.98 \pm 0.0063^{a.A.B}$	$0.98 \pm 0.0029^{a.A.B}$
T5	$0.99 \pm 0.0079^{a.A}$	$0.98 \pm 0.0033^{b.B.C}$	$0.98 \pm 0.0032^{b.B}$

T1: Cheeses with the addition of *B. lactis*
T2: Cheeses with the addition traditional culture;
T3: Cheeses with the addition of *B. lactis* and traditional culture;
T4: Cheeses with the addition lactic acid;
T5: Cheeses with the addition of *B. lactis* and lactic acid;
Superscripts letters classify means at row and capital letters classify means at column.

fication with lactic acid (Synth, São Paulo, Brazil; 0.25ml l–1 of a 85 g 100 g–1 food-grade solution) and T5 were manufactured by direct acidification with lactic acid plus the addition of *B. lactis* (0.7% w/v).

The cheeses were prepared according to Buriti *et al.* (2005a) and were packaged under vacuum in plastic bags and stored under refrigeration (7–9°C) for up to 28 days.

The samples were analysed by pH, titratable acidity, water activity (Aw) and moisture. Viability of *B. lactis* was monitored using the method developed by Grosso and Favaro-Trindade (2004). All determinations were carried out with 1, 14 and 28 days of refrigerated storage.

The data obtained were statistically analysed by SAS (Statistic Analysis System) (2001), version 8.02, using the GLM procedure and the following model: $Y_{ijk} = \mu + T_i + D_j + TD_{ij} + e_{ijk.}$ Where Y_{ijk} is the observed value for the variable in the *i*th treatment, the *j*th day; μ the general mean; T_i is the effect for treatment *i*; D_j is the effect for day *j*; TD_{ij} is the effect of interaction between treatment *i* and day *j*; e_{ijk} is the random effect for residual.

RESULTS AND CONCLUSIONS

All cheeses had suffered from slight acidification during storage, with the exception of those in T3, suggesting a synergic effect between the traditional culture and bifidobacteria (Table1).

Cheeses T4 and T5, elaborated under direct acidification conditions, had presented minor moisture, what means that sharp decrease in pH resulted in a larger loss of moisture. All treatments had high Aw values, in the range from between 0.97 to 0.99, during the period of storage, resulting in high susceptibility to the microbial spoilage.

No variation in *B. lactis* population was observed in any of the treatments analyzed during the period in storage (Table 2).

In order to exert the beneficial effects of probiotic foods, a minimum probiotic therapeutic daily dose of 10^8– 10^9cfu has been proposed, which corresponds to the daily intake of 100 g of a food product containing 10^6 up to 10^7cfu g^{-1} (Lee & Salminen, 1995). In the present study, the counts of *B. lactis* obtained for cheeses T1, T3 and T5 were always above the recommended levels (10^6–10^7cfu g^{-1}), thus satisfying this criteria established for a probiotic food.

Thus, fresh Minas-type cheese made from buffalo milk represents a good vehicle to introduce *B. lactis* and to improve consume of buffalo milk.

Table 2. Viability determined through counts (Log of cfu/g) in cheeses containing *B. lactis*.

Treatment	Mean values and standard deviation
T1	6.65 ± 0.25^b
T3	7.66 ± 0.13^a
T5	6.74 ± 0.30^b

T1: Cheeses with the addition of *B. lactis*
T3: Cheeses with the addition of *B. lactis* and traditional culture;
T5: Cheeses with the addition of *B. lactis* and lactic acid;
Superscripts letters classify means at column.

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TEXTURE AND SENSORY CHANGES OF A FRESH EWE'S CHEESE PACKED UNDER DIFFERENT MODIFIED ATMOSPHERES

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ABSTRACT

Shelf-life extension of fresh ewe's milk cheese packaged with three different MAP conditions was studied. Fresh cheeses were stored in barrier trays, hermetically sealed with a barrier to gas and water film. The gas mixtures used were: 20%CO_2/80%N_2, 30%CO_2/70%N_2 and 50%CO_2/50%N_2. Trays were stored at 4 °C and inspected at 0, 7, 14 and 21 days. Physical-chemical and microbiological analyses (pH, colour, dry matter, a_w, mesophilic micro-organisms, psychrotrops, total coliforms, Escherichia coli, Salmonella, Staphilococcus aureus and Listeria monocytogenes), gas composition inside the packaging (CO_2, O_2 and N_2), texture analysis (puncture test and Texture Profile Analysis-TPA) and sensory evaluation (acceptability test by scoring for colour, flavour, taste and texture) were carried out. Samples packaged with 30%CO_2/70%N_2 and 50%CO_2/50%N_2 had a shelf life of 21 days. Pathogens were not found during all the storage. Cheese stored with 30%CO_2/70%N_2 reached the best acceptability score to the sensory evaluation.

Key words: ewe's cheese; MAP; sensory analysis; shelf-life; texture.

INTRODUCTION

The increased consumer demand for fresh foods has resulted in the need to extend their shelf-life. The shelf-life extension, however, can not be attained with the use of thermal stabilisation and other techniques, such as product reformulation or use of additives, that are often unsuccessful or not liked by consumers.

The main cause of product quality loss is caused by micro-organisms, which are preferably controlled by the use of modified atmosphere packaging (MAP), which allows to stop or slow down microbial growth and not alter the overall quality of the product (Elena Gonzales-Fandos *et al.*, 2000; Frau *et al.*, 1999; Olarte and Gonzales-Fandos, 2001). Fresh cheeses have a very short life because of their pH, near to neutrality, the high a_w and the low salt content. Usually, the shelf-life of fresh cheese is only 7 days (Di Marzo *et al.*, 2006). There are few studies on the effect of MAP on the evolution of sensory and texture characteristics during storage of fresh cheeses (Fava *et al.*, 1993; Piergiovanni *et al.*, 1993; Maniar *et al.*, 1994). In fact, MAP can modify sensory and texture characteristics of the products during storage. In this research we examined a fresh ewe's cheese packaged under MAP with different gas mixtures to evaluate texture and sensory changes during storage.

MATERIALS AND METHODS

Fresh cheese was obtained by pasteurized ewe's milk, coagulated with calf liquid rennet and inoculated with probiotics. After 24 h of ripening it was packaged inside barrier to gas trays, containing 4 forms and wrapped with a barrier to gas and water film. Three batches were prepared with the following gas mixtures: 20%CO_2/80%N_2 (A), 30%CO_2/70%N_2 (B) and 50%CO_2/50%N_2(C), and stored at 4°C. The following analyses were carried out at 0, 7, 14 and 21 days: pH using a pHmeter Orion, mod. 710/A; colour with a Minolta colorimeter CR-300; dry matter (%) in a vacuum oven at a temperature of 70°C and water activity (a_w) using an electronic hygrometer (Rotronic, pbi International). Mesophilic micro-organisms, coliforms, *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, yeasts and moulds, were determined using the following media: PCA, VRBA, Chromogenic EC X-Gluc agar, Vidas SLM, Vidas DUO, Baird-Parker agar + RPF and PDA, respectively. Texture analyses were performed with a texturimeter TA.XT2i (Stable Micro Systems), equipped with a 6 mm cylindric probe (puncture test) and a 75 mm compression plate (TPA), respectively. The gas composition inside the trays was checked by a Dansensor gas analyser. Sensory evaluation was performed using an acceptability test, following the IDF standard 99B (1995), using a panel of eight judges from our laboratory. Judges used an hedonic scale from 1 to 7 (1= terrible; 7=excellent) to evaluate the following descriptors: colour, deformation, odour intensity, taste, aroma and consistency.

All data were submitted to two-way analysis of variance (ANOVA) using the soft-

Table 1. Values[1] of the physical-chemical parameters of the three cheese samples

Cheese	pH	dry matter (%)	a_w	L	a*	b*	Z
A	5.64a*	40.67b	98.41$^{n.s.}$	90.54a	-2.07b	10.01a	65.51$^{n.s.}$
B	5.55b	43.68a	98.33	90.33a	-2.05b	10.37b	64.71
C	5.67a	43.41a	98.53	89.79b	-2.24a	10.55c	63.47

[1] Data are the mean of the values obtained during the storage period.
* Data followed by different letters, for each column, significantly differ by LSD Fisher, $p \leq 0,05$. $^{n.s.}$ not significant

Table 2. Values[1] of the TPA parameters of the three cheese samples

Cheese	Hardness (g)	Cohesivene ss	Springiness (mm)	Chewiness (g · mm)
A	758.39c*	0,57 n.s.	6.64b	2824.65c
B	874.36b	0,57	6.92a	3423.74b
C	*1065.31a*	*0,56*	*6.83ab*	*3998.98a*

[1] Data are the mean of the values obtained during the storage period.
* Data followed by different letters, for each column, significantly differ by LSD Fisher, p ≤ 0,05. n.s. not significant

ware Statistica 6.0 for Windows, where the factors were the three batches and the days of storage. Means, when required, were separated according to LSD Fisher test, significance level $P \leq 0.05$.

RESULTS AND CONCLUSIONS

Physical-chemical changes: the sample B showed the lowest pH value during storage, while the sample A had the highest dry matter. No difference was found for the a_w in the three samples. The yellowness index Z did not differ significantly among the three samples (Table 1). *Microbiological analyses:* pathogens were not found in any samples throughout the whole experiment. The other micro-organisms increased during storage but they remained below the limits reported in the Reg. EEC n. 2073 (2005) for fresh cheeses. *Gas composition:* it was observed a decrease of N_2 % and an increase of CO_2 % for every sample and during storage. *Texture analysis:* hardness and chewiness values were higher in the sample C, during storage, while the cohesiveness remained constant in all the samples analysed (Table 2). Puncture test data showed a decrease for all the three samples during storage, but the B sample rupture force resulted significantly the highest (data not shown). *Sensory analysis:* a decrease in acceptability was observed during storage for all the samples. The sample A was above the acceptability score at 14th day of sampling, corresponding to 4 in the hedonic scale used in our test. Samples B and C reached the same results at 21th day of sampling.

MAP packaging resulted successful in doubling the shelf life of this fresh cheese.

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VOLATILE COMPOUNDS FOR THE EVALUATION OF FISH FRESHNESS DURING REFRIGERATION STORAGE

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ABSTRACT

In this study the volatile composition of some Mediterranean fish species have been studied. Fish samples were obtained from a local fisherman and there kept in ice up to five days; samples have been analysed every day since their capture and during the storage in ice. Volatile components have been extracted and analysed by HS-SPME-GC/MS; eighty-seven volatile components have been identified in each fish specie analysed and qualitative and quantitative differences resulted during the refrigeration storage. The data obtained have been statistically treated and correlated with those from chemical analyses.

Key words: chemical analyses, freshness, Mediterranean fish, SPME-GC-MS, volatile compounds.

INTRODUCTION

The consumer demand for high quality foods is surging and regarding fish and fishery products, freshness makes unquestionably the major contribution to their quality. Immediately after harvesting, the fish flesh initiate alterations which led to the degradation of the perceived quality. Volatile aroma compounds are generally considered to be important parameters for determining the flavour quality and spoilage index of fish and other seafood. (Hui-Zhen and Tung-Ching, 1997). Specifically, volatile aldehydes, ketones and alcohols which arise from microbial degradation and lipid oxidation are closely related to fish quality. (Lindsay, 1990). Although numerous sensory, chemical, microbiological, and instrumental tools exist for the determination of spoilage, methods for the reliable determination of freshness are few and not generally applicable. The freshness of fish has proven a difficult parameter to define by instrumental techniques (Olafsdottir *et al.*, 1997).

In this research the volatile composition of two Mediterranean fish species and

one mollusc specie have been studied during the refrigeration storage. The main objectives of this research were to develop a rapid and reliable method to monitor the quality of fresh Mediterranean fish and to identify volatile compounds that can be used as markers for freshness.

MATERIALS AND METHODS

Sampling

Two Mediterranean fish species, *Pagellus bogaraveo* (red sea-bream), *Trachurus trachurus* (horse-mackerel) and one mollusc specie, *Octopus vulgaris* (octopus), were analysed. The samples were supplied from a local fisherman and kept in ice up to five days. Samples have been analysed in duplicate immediately after their capture and during the storage in ice. Each species were sampled three time in a month.

Chemical analyses

Total volatile base-nitrogen (TVB-N) and trimethylamine (TMA) were determined according the Official Methods of Analysis (AOAC, 1999).

HS-SPME Procedure

The extraction of volatile compounds was done by a HS-SPME (headspace solid phase microextraction) using a DVB/CAR/PDMS fibre, with 50/30 μm film thickness (Supelco, Bellafonte, PA, USA). For each sample 4,5 g of fish, previously homogenized, were weighed into a 40 ml vial and suspended in 14 ml of saturated NaCl aqueous solution; the vial was kept at 35 °C with continuous internal stirring; equilibration time, 30 min; extraction time, 30 min. After sampling the SPME fibre was introduced into the GC injector, and was left for 3 min to allow the analyte thermal desorption.

GC-MS Analysis

A Varian 3800 gas chromatograph directly interfaced with a Varian 2000 ion trap mass spectrometer (Varian Spa, Milan, Italy) was used. Injector temperature, 260 °C; injection mode, splitless; column, 60 m, 0.25 mm i.d., 0.25 μm film thickness CP-Wax 52 CB (Chrompack Italy s.r.l., Milan, Italy); oven temperature, 45 °C held for 5 min, then increased to 80 °C at a rate of 10 °C/min, and to 240 °C at 2 °C/min; carrier gas, helium used at a constant pressure of 10 psi; transfer line temperature, 250 °C; ionisation mode, electron impact (EI); acquisition range, 40-200 *m/z*; scan rate, 1 μsec^{-1}. Compound identification, NIST library (NIST/EPA/NIH Mass Spectra Library, version 1.7, USA), linear retention indices (LRI) and authentic standard, where available.

Statistical Analysis

Statgraphic plus software, 5.1 version was used to perform statistical analysis of the data. One way analysis of variance (ANOVA) and Duncan's multiple range test were applied to the data to determine significant differences ($P < 0.01$) between the analyzed samples.

RESULTS AND CONCLUSIONS

In Table I the data regarding TVB-N, TMA are reported for the three species ana-

Table 1 - TVB-N and TMA average content (mg 100 g^{-1}) of the different fish species during storage at 4 °C.

	TVB-N			TMA		
Refriger. days	0	2	5	0	2	5
Octopus v.	8,59°	11,19b	14,38c	3,72a	3,85a	4,88b
Pagellus b.	15,03	15,04	15,78	-	-	-
Trachurus t.	17,42a	18,80a	23,13b	-a	-a	0,33b

Means with different letters (a-c) in the same row are significantly (P <0.05) different from each other for the refrigeration time.

lysed during the refrigeration storage. Significant increase for the observed parameters resulted during storage even if all of these are included in the range used for freshness definition.

Volatile compounds identified by GC/MS are listed in table II; table III reports for each species analysed, the volatile compounds which showed statistically significant changes by the ANOVA and Duncan's multiple range test ($P < 0.01$) during the refrigeration times. The significant components are aliphatic alcohols, aldehydes and ketones, mainly 2-ketones, 2-dienals and unsaturated alcohols. The methyl ketones (C_7-C_{11}) were probably formed from β-oxidation of

Table II – Volatile compounds identified in the analysed Mediterranean fish species

COMPOUNDS	LRI	COMPOUNDS	LRI	COMPOUNDS	LRI
Hexane	600	3-Methyl-1-butanol	1201	2-Ethyl-1-hexanol	1485
Heptane	700	Limonene	1202	Pentadecane	1500
Carbon disulphide	727	(E)-2-Hexenal	1221	2-Nonanol	1512
Dimethyl sulphide	744	6-Methyl-2-heptanone	1222	(E,E)-3,5-Octadien-2-one	1520
Acetone	813	Ethyl-toluene	1223	Benzaldehyde	1530
Butanal	872	(Z)-4-Heptenal	1228	β-Linalol	1540
Ethyl acetate	886	3-Octanone	1256	1,3-Tetradecadiene	1544
2-Butanone	902	Styrene	1261	1-Octanol	1552
3-Methyl-1-butanal	918	p-Cymene	1269	2-Undecanone	1599
2-Ethyl furan	936	2-Octanone	1281	Hexadecane	1600
1,3-Octadiene	937	1,2,3-Trimethyl benzene	1282	(Z)-2-Octen-1-ol	1610
Benzene	939	Octanal	1292	2-Decenale	1624
2-Pentanone	977	Tridecane	1298	4-Methyl-benzaldehyde	1625
Pentanal	980	1-Octen-3-one	1303	Butanoic acid	1634
1-Penten-3-one	1025	(E)-2-Penten-1-ol	1314	Ethyl decanoate	1637
Toluene	1039	2-5-0ctanedione	1325	Menthol	1638
2-3 Pentanedione	1058	(E)-2-Heptenal	1327	1-Nonanol	1653
Dimethyl disulphide	1074	6-Methyl-5-epten-2-one	1338	Acetophenone	1657
Hexanal	1082	1-Hexanol	1349	Heptadecane	1699
α-Pinene	1099	(Z)-3-Hexen-1-ol	1381	4-Ethyl-benzaldehyde	1714
3-Penten-2-one	1113	2-Nonanone	1389	(E,E)-2,4 Decadienale	1791
Ethyl benzene	1124	(E,E)-2,4-Hexadienal	1389	Hexanoic acid	1844
(E)-2-Pentenal	1132	Nonanal	1396	Heptanoic acid	1945
δ-3-Carene	1133	Tetradecane	1400	1,3-Ottadecadiene	1946
p-Xylene	1135	Ethyl octanoate	1434	Phenol	2009
1-Butanol	1145	(E)-2-Octenal	1438	Octanoic acid	2053
β-Pinene	1148	1-Octen-3-ol	1445	Nonanoic acid	2158
1-Penten-3-ol	1158	1-Heptanol	1451		
2-Heptanone	1180	Acetic acid	1464		
Heptanal	1184	(E, Z)-2,4-Heptadienal	1466		

Table III. Average content* of significant components of the Mediterranean fish species analysed during the storage on ice.

	Octopus vulgaris			*Pagellus bogaraveo*			*Trachurus trachurus*		
Refrigeration days	0	2	5	0	2	5	0	2	5
Hexanal	25a	29a	47b	699a	634a	1068b	726a	2021b	4241c
(E)-2-Pentenal	nd[1]	nd	nd	tr a	6b	21c	64a	129b	211c
1-Penten-3-ol	123a	213b	272b	61a	104b	257c	169a	855b	850b
2-Heptanone	tr a	tr a	46b	tr a	24b	42c	18a	43b	85c
3-Methyl-1-butanol	52a	195b	459c	tr a	34b	106c	nd	nd	nd
(E)-2-Hexenal	nd	nd	nd	tr a	tr a	16b	52a	88b	247c
Octanal	tr a	tr a	24b	76	78	89	138a	169b	223c
(E)-2-Penten-1-ol	74a	131b	154b	nd	nd	nd	164a	292b	290b
1-Hexanol	39a	67b	85c	43a	65ab	95b	nd	nd	nd
2-Nonanone	nd	nd	nd	tr a	19b	21b	49a	53ab	58b
1-Octen-3-ol	1772a	1899a	2019b	171a	284b	298b	270a	427b	444b
2-Ethyl-1-hexanol	66a	81a	149b	32a	58b	68b	4a	6a	356b
2-Undecanone	tr a	37b	53c	29a	40a	70b	tr a	21b	44c

*Arbitrary scale. Means with different letters (a-c) in the same row are significantly ($P < 0.05$) different from each other for the refrigeration time. nd: not detected; tr: trace (inferior to LOD).

their carbon chain followed by decarboxylation and they were also found in shellsfish. (Lindsay, 1990). 2-Dienals are connected with 2-ketones, whereas hexanal which derives from autoxidation of linoleic acid has been successfully used for evaluation of the oxidative state of red meat from different species (Durnford and Shahidi, 1998). The compounds present in the highest amount in all the analysed species were two alcohols, namely 1-penten-3-ol and 1-octen-3-ol, which are formed through autoxidation of unsaturated fatty acids (Sakakibara *et al.*, 1988). 1-Penten-3-ol was the most noticeable compound detected in rancid sardine oil (Peterson and Chang, 1982) and 1-octen-3-ol resulted the major volatile alcohols in various species, such as oyster, rangia clams, crab, praws, crayfish and sand-lobster (Kim *et al.*, 1994).

From table III a statistically significant increase resulted for the components considered during the refrigeration time even if, according the official methods, the freshness has been maintained during the period analysed.

From our results, the volatile compounds reported in table III, being correlated to lipid oxidation, could serve to evaluate the freshness of the Mediterranean fish considered: the same compounds have been indicated by different Authors in other fish species (Durnford and Shahidi, 1998; Hui-Zhen and Tung-Ching, 1997; Lindsay, 1990). Further analysis are necessary to determine the level of these compounds in spoilage fish samples in order to fix for each of them the range of amount well-matched with freshness.

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SUSCEPTIBILITY OF PLASTIC PACKAGES TO INSECT PESTS ATTACKS

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ABSTRACT

Packaging represents the ultimate defence of pasta producers against insect pests: even if the product comes out uncontaminated from the production line, this does not guarantee that it will reach the consumer in the same condition. The ability of some pests to pierce different plastic material is well known: insects are attracted by odors, therefore it is necessary to take in consideration the barrier properties to volatile compounds.

Pasta packages are characterized by numerous pinholes which are mechanically produced to avoid having swollen bags after sealing: these pre-existing holes are often exploited by most pests as a preferential entrance to packages, and they are detected by insects by following the outflow of attractive odors. Packaging, therefore, represents a critical point in the quality preservation of packed pasta. Insect pests are commonly present in the production and storage environments, and their presence is favoured by unappropriate prevention measures. Common pests that affect pasta and starchy products usually belong to the genera: *Tribolium*, *Sitophilus*, *Plodia* and *Rhyzopertha*.

The aim of the study was to evaluate the resistance to piercing of three plastic materials (polypropylene obtained with different processing techniques) by *Tribolium* spp., *Sitophilus* spp. and *Rhyzopertha dominica*, and the relative ability of each pest to enter the packages. Two tests were carried out, the first one was aimed at evaluating the resistance of the plastic materials, the latter evaluated the ability of the species tested to enter pasta bags.

Results confirmed the good resistance of PP to the penetration of insect pests, and highlighted the highest penetration ability of *Sitophilus* spp. The optimization and suitable design of the packaging system, which should consider the reduction of the hole diameter, would allow to reduce the risk of pests penetration.

Key words: Insect pests, Packaging, Pasta, Piercing, Polypropylene.

INTRODUCTION

Packaging represents the ultimate defence of pasta producers against insect pests: even if the product comes out uncontaminated from the production line, this does not guarantee that it will reach the consumer in the same condition. Stored-product insects can enter packaged goods during transportation, storage in warehouses or in retail stores. In any case, consumers who come across contaminated packages, hold the manufacturer responsible for the inconvenient, with severe consequences on the image of the company, even if the responsibility is up to a third part. Insect that attack packaged products can be classified as "penetrators" or "invaders" (Highland, 1984): the former insects, which are able to bore holes through packaging materials, include *Sitophilus* spp., *Rhyzopertha dominica*, *Plodia interpunctella*, *Lasioderma serricone* and *Stegobium paniceum*; on the other hand, invaders are insects that can enter packages only through existing holes: this is the case of *Tribolium* spp., *Cryptolestes ferrugineus* and *Oryzaephilus* spp. (Highland, 1991). This categorization, however, is artificial, as penetrators also enter packages through existing holes, and invaders can, under certain circumstances, penetrate packaging materials: this is the case of *T. confusum*, which will act as penetrator when confined without food (Cline, 1978a; Cline, 1978b; Bowditch, 1997). Moreover, the ability of species to penetrate materials may vary between life stages and depending on the material considered (Cline, 1978a; Cline, 1978b; Bowditch, 1997). It is well known that insects are attracted into packages by odors, and the use of materials with high barrier to aroma compounds is one of the milestones in the prevention of infestations by pests; unfortunately data on aroma permeability of packaging materials are lacking, and the choice of suitable materials can only be helped by O_2, CO_2 and water vapour permeability data, available among the performance characteristics of materials. Pasta packages are characterized by numerous pinholes which are mechanically produced to avoid having swollen bags after sealing: these pre-existing holes are often exploited by most pests as a preferential entrance to packages, and they are detected by insects by following the outflow of attractive odors. Packaging, therefore, represents a critical point in the quality preservation of packed pasta. Insect pests are commonly present in the production and storage environments, and their presence is favoured by inappropriate prevention measures. Common pests that affect pasta and starchy products usually belong to the genera: *Tribolium*, *Sitophilus*, *Plodia* and *Rhyzopertha.*

The aim of the study was to evaluate the resistance to piercing of three plastic materials (polypropylene obtained with different processing techniques) by *Tribolium* spp., *Sitophilus* spp. and *Rhyzopertha dominica*, and the relative ability of each pest to enter the packages. Two tests were carried out, the first one was aimed at evaluating the resistance of the plastic materials, the latter evaluated the ability of the species tested to enter pasta bags.

MATERIALS AND METHODS

The research evaluated the ability of the tested insects to enter pasta packages by piercing or using the pre-existing pinholes. Three polypropylene films were used, all

supplied by Rotocalco Mediterranea s.r.l.: bioriented PP laminated with co-extruded PP (thickness 25 mm + 30 mm) (A), acrylic-coated PP (thickness 25 mm) (B), and bioriented PP laminated with cast PP (thickness 25 mm + 30 mm) (C).

Two trials were carried out: the former aimed at evaluating the resistance of packaging materials to perforation, the latter aimed at evaluating the susceptibility of pasta pouches to pest invasion. For both tests, insects were previously left unfeeded for 36 h.

Test 1. Five insects for each of the species tested were confined without food in cylinders (height 5,3 cm, diameter 3 cm), which were sealed with the test material. Three replicates for each film were performed. Each cylinder was placed inclined by 45° into a container being completely covered with pasta. In order to validate the effectiveness of the method, the same trial was performed using Kraft paper instead of PP, as the former offers limited resistance to insect attacks.

Test 2. The ability of pests to penetrate pasta pouches during shelf-life was assessed. This test simulated the real packaging and storage conditions, and consisted in packing pasta in 250 g pouches realized with pinholes having the same diameter as those present in commercial packages. Each pouch was placed into plastic boxes (27 × 6 cm) where 10 insects for each tested species were introduced, simulating a large infestation. Observations for insect penetration were performed at 7-minute intervals in the first 45 minutes; afterwards the frequency of examinations was one every 20 minutes. The trial, carried out in triplicate, ended when the first insect managed to enter the packages.

Both tests were performed at a controlled temperature of 27 ± 2°C and at 70% relative humidity.

RESULTS AND DISCUSSION

In the first test, carried out for 27 days, all materials showed numerous superficial abrasions by microscopic observations, as to testify the piercing trials made by insects. However, pests were not able to pierce the materials. Cline (1978b), in a study on the resistance of flexible packaging materials to insect attacks, classified PP as the most resistant, followed by polyester, aluminium, PVC, paper, polyethylene and cellophane. The result observed has to be attributed only to the resistance opposed by the materials to the insects attacks, as the test carried out with Kraft paper, maintaining the same conditions as the tests performed with the tested materials, allowed to validate the effectiveness of the technique used. In the latter test, Kraft paper was pierced by *R. dominica* after 24 hours, by *Sithophilus* spp. after 60 hours and by *Tribolium* spp. after 5 days.

The results relative to the second test are reported in Table 1, which reports the time required for each insect species to penetrate the pouches. *Sithophilus* spp. detected the food source most promptly, as 80% of the insects penetrated the pouches within 45 minutes. For what concerns *Tribolium* spp., this species showed a typical behaviour, which consisted in the association of insects in the effort of piercing the material.

Table 1. Time required (minutes) for the penetration of insect in pouches made with different plastic materials.

	Sitophilus spp.	*Tribolium spp.*	*Rhyzopertha dominica*
A	10	100	120
B	13	100	120
C	18	100	140

Results confirmed the good resistance of polypropylene to the penetration of insect pests, not depending of the association with other materials in a laminated structure. The tests especially highlighted differences in the piercing ability of insects, with the highest penetration ability of *Sitophilus* spp. The optimization and suitable design of the packaging system, which should consider the reduction of the hole diameter, would allow to reduce the risk of pests penetration.

Insect-proof packaging can help reduce the employment of insecticides in order to reduce the risk of food losses due to insect contamination. The experimental trials performed in the present research represent useful tools for testing the resistance to insects and choosing the best materials intended for the packaging of pasta, cereals and other products subject to insects attacks, such as bakery products, powdered milk, tea etc.

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TESTING PROCEDURE OF SHELF LIFE OF PRODUCT PACKAGES IN A MIXTURE OF GASES

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ABSTRACT

The efficacy of the packaging in a mixture of gases depends on a multitude of factors associated both with the packaged product, packaging material applied, structural form of the packaging, composition of the gas mixture in the packaging and with the microclimatic conditions of storage, which have to be taken into account all at the same time, in order to ensure tangible benefit both for the producer and distributor, and more importantly, for the consumer.

That approach is related to the consumer policy adopted, in accordance with which the requirements and expectations of the customers ought to be treated as the point of reference for the durability of products as declared by the producer. The extent to which those requirements and expectations are met, combined with the technological possibilities in the area of the initial level of product quality and of the packaging systems applied, determines the durability of the packaged products.

The aim of this work was to propose the procedure of research on the MAP packaged products, taking into account the product-cryptoclimate-packaging-environment conditions configuration. This procedure is a way to determine and monitor the shelf life of packaged products.

Key words: estimation of shelf life, modified atmosphere packaging (MAP), product-cryptoclimate-packaging-environment conditions configuration.

INTRODUCTION

Modified atmosphere packaging is one of the modern packaging systems which extend the shelf life of the packaged products. On the basis of the studies of related publications, and of author's own research completed so far, we can state that the efficacy of the packaging in a mixture of gases depends on a multitude of factors associated both with the packaged product, packaging material applied,

structural form of the packaging, composition of the gas mixture in the packaging and with the microclimatic conditions of storage, which have to be taken into account all at the same time, in order to ensure tangible benefit both for the producer and distributor, and more importantly, for the consumer (Robertson, 2000; Ucherek, 2004).

An analysis of the research completed to date, the interpretation of the outcome of own research, and consultation with the representatives of the industry, revealed a need to develop procedures of testing the durability of MAP packaged products, taking into account the configuration: product-cryptoclimate-packaging-environment conditions.

On the basis of an analysis of the main assumptions presented in the trade publications and implemented in the research conducted by several authors (Ucherek, 2007), the author has proposed a procedure of research on the durability of products packaged in modified atmosphere, presented on fig. 1.

THE PROGRAM OF RESEARCH ON THE SHELF LIFE OF MAP PACKAGED PRODUCTS

In compliance with the recommendations of IFST, regardless of the kind of the packaged product and packaging system applied, the point of departure in the research on the durability of food is the level of the selected, product-specific quality parameters, illustrating the extent of the quality changes in the course of storage. That is why in the first phase of research the attention was focused on the scope and intensity of the changes of quality parameters of a product stored in varying micro-climatic conditions, in order to construct mathematical models describing the above relationships while taking into consideration the product-cryptoclimate-packaging-environment conditions configuration (Lisińska-Kuśnierz, *et al.*, 2006; Shelf Life of Foods, 1993).

The selection of the parameters characterising the quality of the packaged products is to be done on the basis of the recommendations of the Company Normalization Document as well as on the basis of the findings of the research pertaining to the group of products presented in the trade publications.

Considering the necessity to take into account the mutual interactions of the packaged product, components of the gas mixture in the packaging, and the packaging material, it was important to know the extent and dynamics of the changes of the oxygen content in the packaging occurring in the course the storage in varying microclimatic conditions, the functional qualities of the packaging materials, and to develop models of the above changes as a function of the time.

According to the research on the durability of MAP packaged products such as: meat, meat preserves, fish preserves, vegetables, fruit, rye bread products and ground coffee, the efficacy of the system of packaging in a mixture of gases, applied with the purpose of preserving the adequate level of quality of the product, depends on many factors. That is why, as a next stage of the considerations, one needs to estimate the influence of the selected factors on the level of changes of the quality parameters of the packaged products, in order to identify the factors determining their quality (Man *et al.*, 2000; Steel, 2004).

Next, in order to propose a methodology of preliminary determination of the products durability on the basis of the accelerated tests, we analysed the possibilities of using short-term storage in an increased temperature and relative humidity of

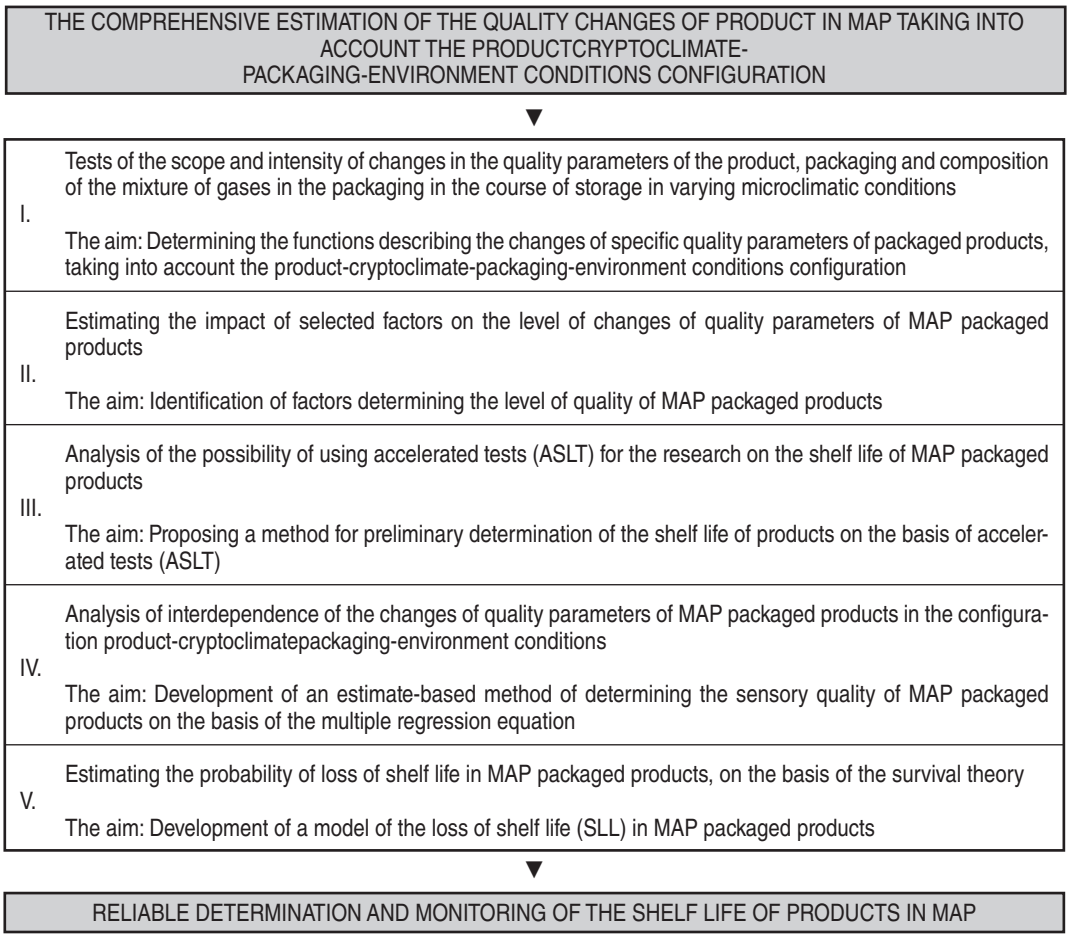

Fig. 1. The procedure of research on the shelf life of products packaged in modified atmosphere Source: autor's work.

the air for the examination of the durability of the packaged products. Such tests could replace the extensive storage in normal conditions.

On the basis of the published findings of the research on the durability of the products packaged in a mixture of gases, for the purposes of our work we assumed that the durability of the packaged product would be determined on the basis of the evaluation of the sensory quality. That is why we analysed the dependence of the changes in the sensory quality from the changes of the other quality parameters of the packaged products, taking into account the product-cryptoclimate-packaging-environment conditions configuration, so as to develop an estimate-based method of assessment of the sensory quality of the MAP packaged products, on the basis of the multiple regression equation. The above research undertaking was the result of the willingness to identify an objective method where the tests performed by a team of experts, requiring necessary preparations, could be replaced by laboratory tests. The need to

implement such solutions constituted the topic of several publications (Mazza *et al.*, 2001, Irwin *et al.*, 2004).

An important piece of information in the effort to solve the problem of the packaged products' durability is the possibility to estimate the probability of durability loss depending on specific forecast factors. For that purpose, on the basis of suitable mathematical tools used in the survival analysis in the economic sciences, matrices of durability loss were developed, and then a durability loss model, which was symbolically termed as SLL Model – Shelf-Life Loss of MAP packaged products. The above methods can be useful in the decision-making process concerning for example the selection of a specific structural form of the packaging or the MAP packaging process parameters. The implementation of the above partial objective of the research is directly connected with the innovative approach to the problem of determining the durability of the packaged products, as presented in the trade publications (Ucherek, 2007, Kader *et al.*, 2000). That approach insists on combining the research methods used in experimental, economic and social sciences – on the grounds of the interdisciplinary character of the product durability issue.

Because of an important number of the empirical data collected and of the necessity to perform many computations, and in order to obtain a better efficiency of the analysis of the findings, at the next stage of the considerations the author decided to support the experimental research with computers. For that purpose, a dedicated software application ought to be developed on the basis of the research findings. Such software would facilitate the determination and monitoring of the MAP products durability.

The above procedure was applied to MAP packaged peanuts, and the findings of the research as well as the statistical and topic-related interpretation have already become the subject of another publication by the author.

CONCLUSION

Determining and monitoring of the durability of packaged products can become effective tools in the development of the market strategy for a given product. As a consequence, the economic losses connected with the spoiling of a food product prior to the 'best before' date are limited or completely eliminated. The consumers' interests are also protected. Of course, in a growing competition environment, a high reliability and confidence in the product and producer are also very important.

On the basis of the above considerations we can affirm that the proposed procedure of research on the MAP packaged products, taking into account the product-cryptoclimate-packaging-environment conditions configuration, is a way to determine and monitor the durability of packaged products.

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PINEAPPLE SHELF LIFE EVALUATION USING AN ELECTRONIC NOSE

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ABSTRACT

In this study we have investigated the applicability of a commercial e-nose in the monitoring of the quality of a ready to use fresh cut fruit (packaged pineapple slices) with a short shelf life. The pineapple trays were taken at the beginning of their commercial life and stored at three different temperatures (5.3, 8.6 and 15.8°C) for 6-10 days in order to apply two analytical approaches: 1) a classical monitoring by means of a series of sensoristic analysis on samples taken at various stages of storage; 2) a continuous method with the e-nose probe directly in contact with the headspace of the pineapple. The results showed that the e-nose was able to discriminate between several samples and to correlate the changes in volatile compounds to a quality decay due to different time-temperature storage conditions. In particular, for each storage temperature, a transition function was applied in order to interpolate the PC1 score trend versus the storage time. The second derivative of the transition function was calculated to estimate the stability time. Results revealed that freshness of fruit was maintained for about 5 days at 5°C, 3 days at 9°C and 1 day at 16°C. Moreover, from the time-temperature tolerance chart, a Q_{10} value of 4.48 was derived. These data were confirmed applying the continuous method, where the headspace around the fruit was automatically monitored during the preservation of slices in a storage cell at controlled temperatures. In this case, we observed that, in correspondence with the stability time, a clear change in normalised sensor signals occurred and also this kinetics was parameterized using a second derivative function. With this approach, the freshness of food was maintained for about 5 days at 4°C, 2 days at 8°C and 1 day at 16°C.

Key words: e-nose, freshness, minimally processed fruit, pineapple, shelf life.

INTRODUCTION

There is a growing emphasis and consensus that e-nose is very effective not only for the quality control of the foodstuffs but also for shelf life investigations. The increasing use of the e-nose in studies aimed investigating the evolution of volatiles during storage is explained by several advantages: simple and fast approach,

non-damaging analysis, no need for sample preparation, the automatic collection of the data and its multivariate statistical elaboration in real time (Benedetti *et al.* 2005). Aim of this work was to monitor the evolution of the volatile compounds released from ready to eat pineapple during storage using an e-nose. In the past, pineapple was considered an "exotic" fruit but now is widely diffused in our food habits, especially for its characteristics of durability and ripening that can be modulate. Aroma of fresh pineapple is very characteristic and appreciate. It is due to the prevalence of esters, with methyl 2-methylbutanoate, methyl 3-(methylthio)-propanoate, methyl butanoate, methyl hexanoate, ethyl hexanoate and ethyl 3-(methylthio)-propanoate, as well as 2,5-dimethyl-4-methoxy-3(2H)-furanone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone as major constituents (Elss *et al.* 2005).

Fig. 1: Device for the continous e-nose approach.

MATERIALS AND METHODS

Samples were servings of 250 g of pineapple slices (cv. Golden Ripe, Costarica) packaged in PVC trays welded with a microholed film.

The packages, provided by a local producer, were taken at the beginning of their commercial life and stored at three controlled temperatures. At different storage times, chemical-physical, microbiological (TBC) and colorimetric measurements were performed. Moreover, the aroma compounds released from the fruit were evaluated using an electronic nose (PEN 2 model, WMA Airsense Analitics Inc., equipped with 10 MOS sensors, differently selective to volatile compounds) following two analytical approaches. In the classical approach, at each storage time and for each temperature, 13 g of shredded fruit were placed in air tight vials, hermetically sealed and stored at -20°C until analysis. Then, the vials were equilibrated and analysed at room temperature in standardized conditions (Benedetti *et al.* 2005). Samples were analysed in triplicate from each time-temperature combination.

In the continuous survey, 100 g of fresh pineapples slices were placed in a suitable glass cell (fig.1), equipped with an inlet (flushing air) and an outlet (sampling probe), assembled in a thermostatic incubator. Signals were recorded, for 6-10 days at three controlled temperatures. Data treatment was performed by means of PCA, Cluster Analysis and modelling of signal or PC1 trends (Riva *et al.* 2002) .

RESULTS AND DISCUSSION

Use of the e-nose combined with multivariate statistical elaboration (PCA and CA- Ward method, square Euclidian distance) was able to discriminate between the different samples. The two first principal components represented 93% of the total variance and their biplot (loadings–sensors and scores–samples, figure 2) allowed a separation of the samples according to the storage conditions. Samples

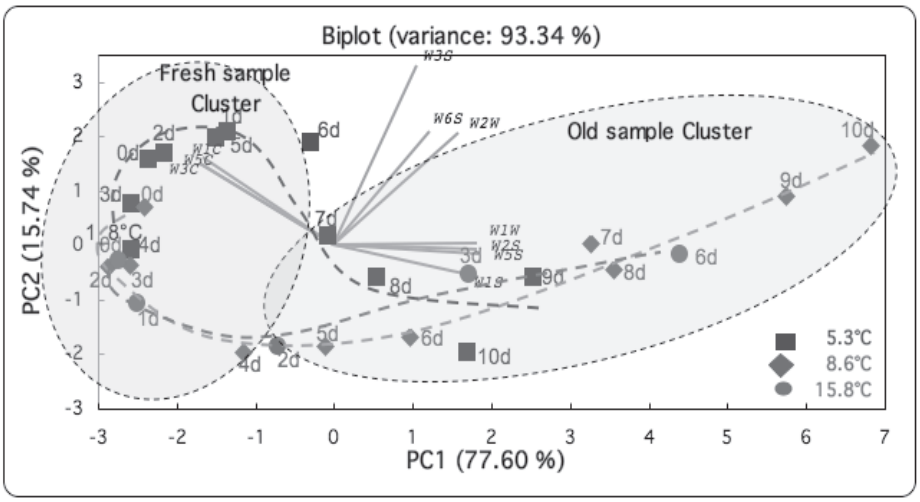

Fig. 2: PCA-Biplot inclusive of cluster analysis, e-nose classical approach

were distributed along PC1 and PC2 according to the storage time and storage temperature, respectively. Figure 2 includes also the results of a cluster analysis.

As one notices, two groups of samples can be identified and classified as follows along the first principal component: "fresh" corresponding to 0-7 days at 5.3°C, 0-4 days at -8.6°C and 0-1 days at 15.8°C; "old" for other storage conditions.

As a consequence, for each storage temperature, a transition function was applied in order to interpolate the PC1 score trend versus the storage time. The second derivative of the transition function was calculated to estimate the time related to maximum acceleration of the volatile decay process (stability time, Figure 3). Tests performed showed also that the W1C sensor (sensitive to aromatic compounds) identified the fresh samples, whereas the W1S sensor (sensitive to hydrocarbon compounds) recognized the volatile fingerprint of the older products.

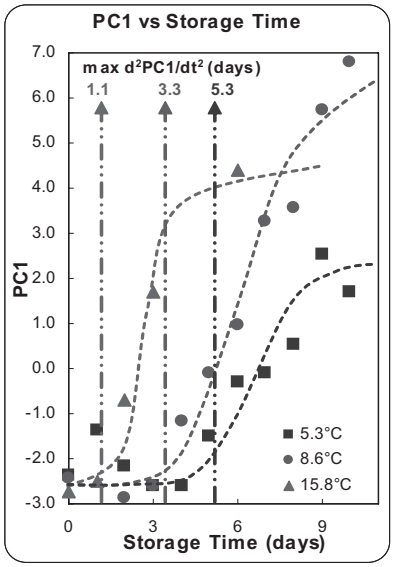

Fig. 3: PC1 vs time modelling, e-nose classical approach

Results were confirmed applying the continuous method, i.e with headspace monitoring by e-nose during air flushing around pineapple slices.

In this case, we observed that, in correspondence with the fresh aroma loss, a change in many sensor signals appears. In order to parameterize the changes, response of each sensor during time was normalized and the average of normalized data were considered as "raw" index of aroma trend.

For each storage temperature, a clear transition kinetics was recognized (Figure 4) and also this trend was parameterized: the maximum of the second derivative func-

tion (i.e the maximum acceleration of the normalized signal change) allowed to obtain a stability time as a function of the temperature.

Stability times measured with the two e-nose methods (Figure 3 and 4) were similar: differences could be attributed to intrinsic variability of fresh fruit at the beginning of storage.

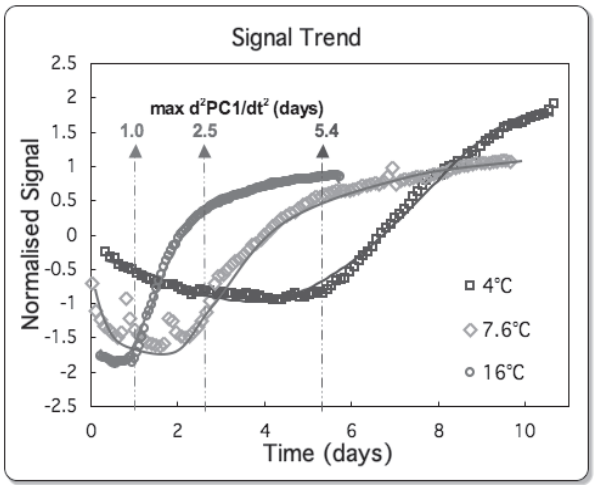

Fig. 4: Normalised signal trend and correspon-ding stability times, e-nose continuous approach

As known, microbiological indices are good predictors of freshness loss. Pineapple slices, as many fresh fruits, show a characteristic microflora of acid lactic bacteria and yeasts: in ready to eat products the usual pre-treatments (peeling, washing, cutting) poorly influence or could also enhance their biological activity. The growth of these microorganisms leads to acidification, texture loss, starting of fermentation. As reported in a parallel work (Di Egidio *et al.* 2008), microbial growth kinetics (TBC) have been parameterized on the same samples, in order to define a stability time defined as the time required to observe 5 x106 CFU/g counts, in absence of coliforms or other pathogenic microorganisms. Therefore, time-temperature tolerance chart was applied to define the expected shelf life on the basis of e-nose observations and on a microbiological survey. Figure 5 shows the corresponding results and includes the Q10 values (n-fold decrease of the shelf life for a 10 °C temperature increase). On the basis of these results, freshness of ready to eat pineapples slices is maintained for about 5 days at 4°C, 2 days at 8°C and 1 day at 16°C.

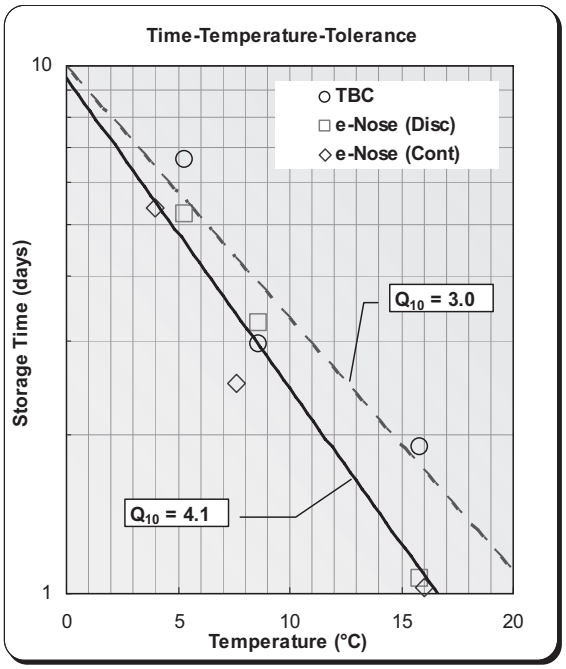

Fig. 5: Time-temperature tolerance chart of ready to use pineapple

CONCLUSIONS

Shelf life studies require in the practice a fast and pragmatic approach: when the safety is assured, the sensory quality decay can be monitored and parameterized. In this perspective, as demonstrated in this work, the electronic nose can easily contribute to take into ac-

count the complex evolution of the aroma profile. Results obtained confirmed the effectiveness of e-nose in the monitoring the shelf life of the minimally processed pineapple and were in agreement with the microbial growth. The lower stability times and higher Q10 values obtained with e-nose are a well and careful estimate of pineapple freshness decay. An interesting future development could be an application in-line of the continuous e-nose method in order to monitor the evolution of volatile compounds during storage in commercial conditions.

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EVALUATION OF SHELF LIFE OF BAKERY PRODUCTS BY IMAGE ANALYSIS

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ABSTRACT

Plastic films today, are widespread and involved in different roles. Just one of these will see them used directly in contact with foods as primary packaging. Producers try to reach the most suitable kind for all purposes, working on different mixtures, new coupled, density, thickness, and expecially on water vapour transmission (WVT) and oxygen permeability (OP): the two most important properties for fresh bakery products.

The appearance of baked products is an important quality attribute, correlating with product flavour and influencing the visual perceptions of consumers and hence potential purchases of the product.

Consumers look at the quality as major factor for selecting a product and among the main characteristics related to it they considered texture, taste and surface colour.

Two different plastic films, polypropylene (PP) and lacquered acrylic (LA) films having two main differences, cost and oxygen permeability were tested on a typical bakery product: a “muffin” having an ordinary shelf life of 7 weeks.

The aim of this study was to understand the main changes caused in the external appearance of a muffin, comparing image analysis and chemicals between samples packed differently. Muffins were tested for 12 weeks or rather.

Image analysis and chemical analysis as lipid oxidation, loss of weight, water activity and dry matter, were carried out on both samples.

Muffins packaged in the two polymeric films used, showed the same chemical attitude, As one of the main goals of this research we could suggest to the muffin’s producer to use the cheapest polimeric film, looking at the economical benefit.

Key word: bakery products, colour measurements, image analysis, packaging, shelf life.

INTRODUCTION

Bakery products were commonly purchased by a wide range of consumers for their nutritional qualities, their palatability and their easy availability.

The appearance of baked products is an important quality attribute, correlating with product flavour and influencing the visual perceptions of consumers and hence potential purchases of the product, both internal and external appearance contribute to the impression of the products quality (Brosnan T., Sun D. W., 2004).

Consumers look at the quality as major factor for selecting a product and among the main characteristics related to it they considered texture, taste and surface colour (Mandala I. G. *et al.*, 2006).

Many studies carried out the utility of image analysis to analyse visual characteristics in fruits and vegetables (Tao Y. *et al.*, 1995), but also in bakery products (Abdullah Z. M., *et al.*, 2000).

Many instruments for commercial colour measurements are designed for quality control but they required homogenized samples to achieve uniform colour giving back a food sample no longer useable for other purpose.

The aim of this study was to understand the main changes caused by storage, in the external appearance and in chemical analysis.

MATERIALS AND METHODS

Two different polypropylene film were used to pack our samples. A polypropylene thermo-welding film (PPTH) and a polypropylene acrylic lacquered film (PPAL), both were provided by CIELLE Imballaggi, Italy.

Packed muffins in PPTH and in PPAL were kindly supplied by a local food industry.

Lipid oxidation (Pensel, N., 1990), loss of weight, water activity, dry matter and moisture were determined.

The evaluation of morphological and colorimetrical properties of muffins was carried out through a computerized system of image analysis with the following devices: digital camera (Nikon Coolpix S4), personal computer Pentium 4 and operating system Windows XP Professional. The acquired images (at fix distances 40 cm from the muffins inside a black box) were studied by the image analysis software KS-400 V.3.0. This software can be adapted for specific purposes choosing between different kinds of algorithm available to achieve Macros of image analysis.

72 samples were analysed (six replicates for 12 weeks) and the developed macro Muffin measured the following features: area (mm2), diameter maximum and minimum (mm), perimeter (mm), shape and roundness factor, mean grey value and standard deviation of grey value.

RESULTS AND CONCLUSIONS

T-test were performed on all data collected each week. Values was under the Tcr (4.303, n=2) so they were considered equivalent and results elaborated as replicates.

Changes in weight loss and lipidic oxidation were negligible, moisture had a decrease between the first and the second week and activity water show an high decrease during the first week followed by a more stable and constant trend (data

not showed).

In Figure 1 is reported the muffin at time zero and after 12 storage's weeks. Each colour channel histogram of the RGB images are below each muffin. It is evident a great difference on each colour channel corresponding to a different storage's time.

As one of the main goals of this research we could suggest to the muffin's producer to use the cheapest polimeric film, looking at the economical benefit.

From another point of view, we observed and took into account the interesting and objective results that came from the image analysis. We have observed a contiunous discoloration and lost of brightness from time zero to the end of the monitoring.

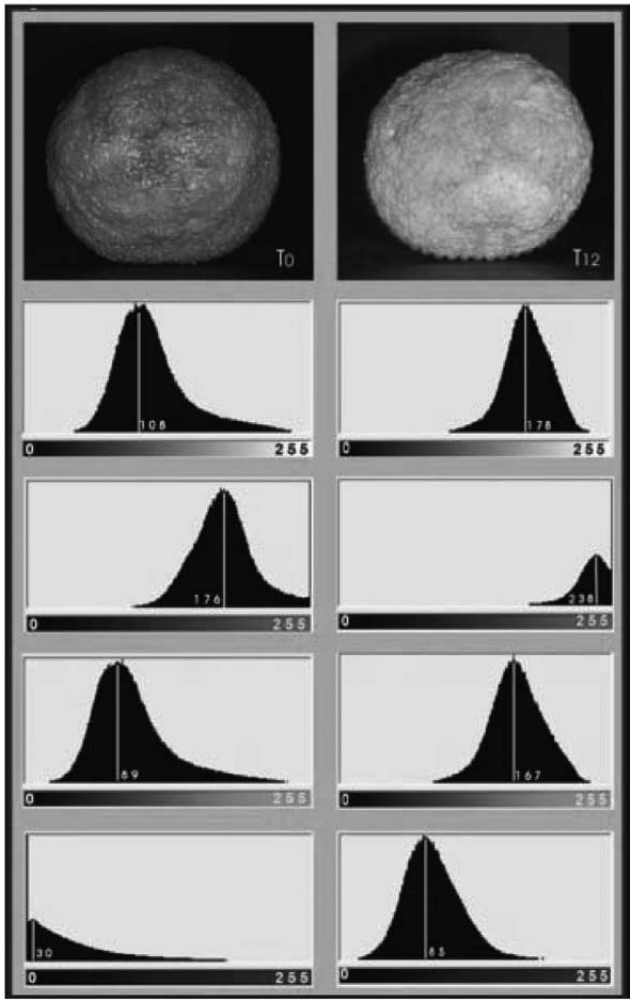

Figure 1. Rappresentation of colour channel histograms of the muffin at the beginning and at the end of the monitoring.

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THE EFFECT OF PACKAGING ON THE SENSORY PARAMETERS OF INDUSTRIAL BREAD DURING SHELF LIFE

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ABSTRACT

Samples of two different commercial lines of industrial white bread (fresh and long life) made from Sicilian durum wheat (cv Simeto) were evaluated by instrumental and sensory analyses to determine their shelf life with respect to the packaging used. Sensory analysis was carried out by a panel of trained judges who evaluated the samples daily (fresh line) and weekly (long life). The data collected was statistically analyzed by Analysis of Variance and Principal Component Analysis. It may be concluded that the correlation between sensory and instrumental parameters can be used as a tool to establish the shelf life of this bread and its potential trade in a modern market.

Key words: bread, shelf-life, sensory analysis, instrumental parameters.

INTRODUCTION

The industrial bread constitutes a valid alternative to fresh bread that in our country still has great appeal but suffers a continuous relative decrease, own to the difficult daily purchase of the handcraft bread. In the sector of the fresh bread in Italy are present handcraft bakery (63%), industrial bread making (25%) and bakeries “in-store” (12%) with a production over 250 types of bread and a market that in 2007 amounted to 3.5 million tons in volume and 6 million of Euro in value, with a per-capita consumption of 55 annual kilograms. Since 1976 a cooperative of durum wheat producers operates, in the province of Enna increasing the produc-

tion from 3.700 t (2000) at more than 6.000 t (2007). In order to wide the Sicilian industrial bread market is useful to study, by sensory and instrumental parameters, the aging process responsible for the loss of the sensory characteristics related to packaging employed.

MATERIALS AND METHODS

"Fresh" loaf (SSL), in slices, daily produced with a *shelf life* of five days is packaged (Cryovac Sealed Air Corporation) in polyolefin micro perforated film (OPT1330).

For "long life" bread (LSL), with a *shelf life* of 40 days, different packaging (Cryovac Sealed Air Corporation) were used: a multistrate coexstrused film for the top (TM PLY T9250 permeability O2 45 bar cm3/m2 24 h, and moisture vapor trasmission rate (MVTR) <=10) and a multistrate coexstrused film for the base (TM PLY T6010 B permeability O2 1 bar cm3/m2, 24 h and MVTR <=10).

The fresh bread controls have daily been performed for five days and replied for three following weeks while for "long life" product, in triple, the samples of different breads for six weeks (wk) have been analyzed.

Humidity %, degree of acidity, digital image analysis, resistance to cut and sensory profile have been determined on the samples. Determination of humidity % and degree of acidity were performed according to AOAC methods (1990). Digital image analysis (expressed as pores area percentage) was carried out with scanner (Cannon), PC Pentium Windows XP Professional and software ImageJ. The resistance to cut was determined, by Warner Bratzler (Instron 4411) expressed in kg/cm2 on cube of bread.

In order to define the attributes characterizing the product the sensory profile (UNI 10957, 2003) was constructed by a trained panel of 12 judges. Assessors were requested to evaluate in the sensory laboratory (UNI ISO 8589, 1990) of Food Technology Section of DOFATA) equipped with a specific software (*FIZZ Biosystèmes)* the intensity of the twenty sensory descriptors selected on the basis of the frequency (%) by assigning a score between 1 (absence of the sensation) and 9 (extremely intense).

RESULTS AND DISCUSSION

The sensory data (at t1, t2, t3, t4, t5 for fresh bread and at wk1, wk4, wk6 for long life product), for each attribute were submitted to Analysis of the Variance (ANOVA) with samples, judges, replicates and their respective interactions JxS, SxR and JxR, as effects. The significance of these effects was tested with F test. Principal Component Analysis (PCA) was also applied to sensory and instrumental means values (except digital image analysis results) in order to interpret the differences among the samples. Data analysis was carried out by statistic software *Statgraphics plus*.

The results of ANOVA for fresh bread (SSL) and long life bread (LSL) show significant differences among samples for many of the attributes evaluated. The judges introduce significant differences for all attributes considered and the replicates show a good reliability for all attributes. The interaction JxS reveals significant differences for the attributes, the interaction SxR shows a good homogeneity of the samples during replicates, finally, the interaction JxR underlines a good reliability

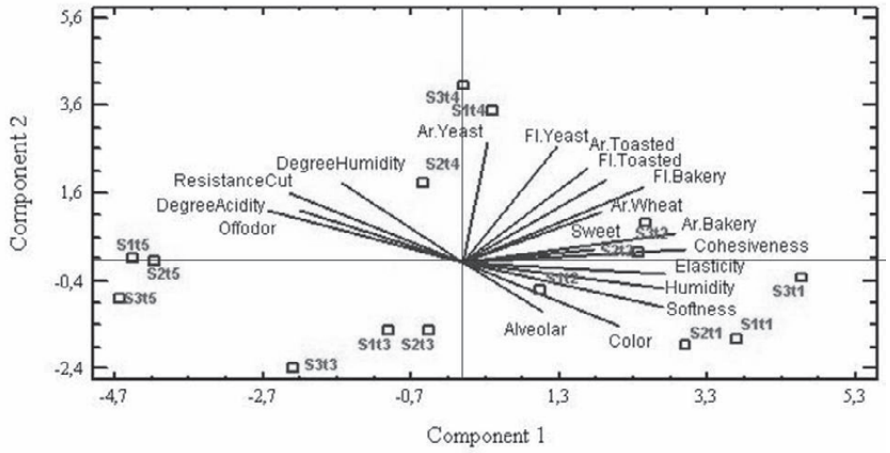

Figure 1. Biplot of short shelf life bread (SSL).

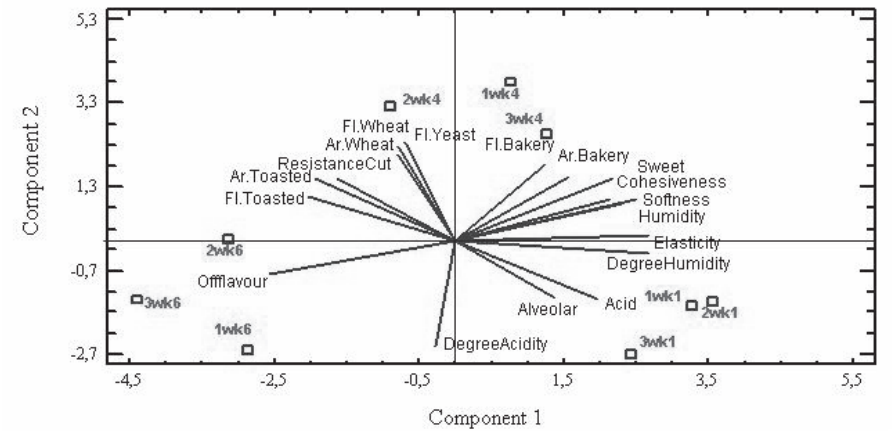

Figure 2. Biplot of long shelf life bread.

of the answers furnished by the judges. Considering the samples SSL only at t1, t3 and t5 of *shelf life,* the bread shows sensory loss in eleven (Color, Alveolar crumb structure, Elasticity and Humidity, Aroma bread, Sweet, Softness, Cohesiveness and Flavour bread, toasted and wheat) of twenty attributes as from t3. For the long life bread, sensory results show, after 4 weeks of *shelf life,* significant differences for nine attributes (Alveolar crumb structure, Elasticity, Acid, Aroma bread, Aroma and Flavour toasted, Aroma and Flavour wheat and Flavour yeast).

Sensory and instrumental data were performed to Principal Components Analysis (PCA), to observe samples positions on the multidimensional space relating to parameters examined. The figure 1 shows the Bi-plot overlapping of Score plot (six samples, three replications, in red) and Loading plot (3 physico-chemical parameters and 16 sensory attributes) for the fresh samples (SSL) with 66,24% of explained variance by the first two Principal Component.

Along the first component (PC1) (explain 46,78% of variance), the samples at t1

and t2 are positioned on the lower right corner, correlated positively to Elasticity, Humidity, Softness, Cohesiveness, Aroma and Flavour bread, while the samples at t5 are on the left correlated positively to Off-odor, Off-flavour, degree of acidity and resistance to cut. Along the PC2 (19,46% of variance) are positioned the samples at t3 (in lower) and t4 (in upper). Bi-plot of long life samples (LSL) at wk1, wk4, wk6 (figure 2) shows an explained variance of the first two PC of 79,49% (PC1 explains 45,90%). The samples at wk1 were in lower right corner, characterized by an high intensity of Alveolar structure, Acid and Humidity%, and in lower left corner were positioned the samples of wk6 that show an high intensity of Off-flavour. The forty week of storage constitutes a critical point for the *shelf life* of this product.

Regarding instrumental data only the humidity % of the samples SSL increase as from t2, own to micro perforated film used. The values of degree of acidity are unchanging for all samples. Regarding the resistance to cut data, LSL samples show an higher value as from wk2 keeping values high, while the samples SSL as from t3. Digital image analysis didn't give any useful information to be correlated to aging process.

CONCLUSION

A large vocabulary of twenty sensory attributes was developed for these industrial bread related to packaging showing a good prediction capacity for the *shelf life* of bread. These results can be successful used in bread making to improve bread quality. In future research we may able to study the influence of other packaging on these industrial breads using this set of objectively determined attributes and relate them to other instrumental information.

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IMPROVED SHELF LIFE OF PLUM BELONGING TO SICILIAN GERMOPLASM

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ABSTRACT

Problems in the post-harvest supply chain represent a critical point in the field of agrofood marketing and the commercial achievement of a product can not be separated from these issues. Plum are considered to have a climacteric-ripening pattern, in which ethylene is the hormone responsible for ripening. The objective of this study was to increase the condition of storage and the shelf life of two plum cultivars belonging to the Sicilian germoplasm already (Sanacore and Ariddo di Core) using low temperature (°C) and a modified atmosphere through the Mini Airform (MA) 350 Pallet Bag system (Tectrol). Qualitative destructive analysis to determine weight losses, total sugar, and acidity titratable were made to compare samples stored in MAP and test manteined in normal atmosphere at 0°C.

Key words: modified atmosphere, plums, post harvest, quality, shelf life.

INTRODUCTION

Plum, like other stone fruits, are considered to have a climacteric-ripening pattern, in which the hormone responsible for triggering the ripening and senescence processes is the ethylene. One of the main consequences of this behaviour is a reduced shelf life, commercial problems limiting the shipping and a decrease in quality parameters such as fruit turgidity, firmness, coloration and the occurrence of decay and off-flavours (Giovannoni, 2001). Although cold and controlled atmosphere storage (at 0°C) is beneficial in extending the postharvest life of plums, its benefits may be limited by the development of brown rot caused by *Monilinia laxa* and physiological disorders such as internal breakdown and gel breakdown, attributed to chilling injury (Truter *et al.*, 1994; Crisosto *et al.*, 2004). Plum con-

sumer acceptance and market life are highly dependent on harvest date, and plums should be marketed and consumed within their potential market life (Zuzunaga *et al.*, 2001; Crisosto *et al.*, 2004) but, depending on the cultivar, they may only have a commercial life of 2–6 weeks (Abdi *et al.*, 1997).

Our aim is to prolong shelf life through the application of an innovative conservation method of two plum cultivars belonging to the Sicilian germoplasm: Sanacore and Ariddo di Core, and to evaluate the effects of conservation on maturity level through the evaluation of parameters usually used to determine the maturity of fruit, such as fruit size, skin colour, flesh firmness, soluble solids or acidity (Robertson *et al.*, 1991; Crisosto, 1994). These cultivars were chosen because they are valuable products that till now have a limited marketing circuit. Considered conservation condition were low temperature (°C) and modified atmosphere through the Mini Airform (MA) 350 Pallet Bag system (Tectrol). This system, generally used for long transport in a short time, was used to stock pallet in a refrigerating cell for a long storage.

MATERIAL AND METHODS

Sanacore and Ariddo di Core plums were harvested on 16th August at a commercial orchard in Monreale – Palermo. Fruits were transported by ship to a packaging house in Piedmont near Cuneo to store with the modified atmosphere. Both cultivars were cooled and stored for 1 day at 0°C in pallet in a commercial plastic box, then the Mini Airform (MA) 350 Pallet Bag (Tectrol) was used. It is a System of the TransFresh Corporation® used in the application of controlled and modified atmosphere inside a pallet, covered with specific bags in PE for the protection of perishables in the commercial trade. The system is a flow through type that operates with an high and low-pressure side, using carbon dioxide gas. Air present in the envelope is partially removed and substituted with CO_2. A manual timer regulate both the time of evacuation and the filling with carbon dioxide. For plums was used a time of 14“ for evacuation and 31,8“ for CO_2. Modified atmosphere composition was monitored during the whole period.

Under these conditions the estimated composition of the atmosphere was as follows: N_2 71.7%, 18% O_2 , 10.3% CO_2. Fruits under the CO_2-rich atmosphere were stored at 0°C for 52 days. As comparison, samples of 30 fruits for three box were stored as test under air at 0°C. At start and after storage were measured the weight losses (%), soluble solids content by refractometer readings (°Brix), acidity (meq/l) and pH, % of gasses (O_2 and CO_2) inside the Tectrol envelop was monitored by the gas analyzer Combo models.

RESULTS AND CONCLUSION

The storage at low temperature under modified atmosphere (m.a.) positively influences the shelf life of the selected plums. The weight losses (%) for all samples stored inside the Tectrol System respect to the TEST samples stored in air were considerably lower. In particular cultivar Sanacore shows larger weight losses in respect to Ariddo di Core (fig. 1). The Tectrol System storage does not influence the evolution of total carbohydrates content except for the cultivar Ariddo di Core: for samples stored in m.a., soluble solids increased because of the reduction in water

content in plums during storage (Tab.1).

Table1. Evolution of total carbohydrates (°Brix).

Cultivar	START 20.08.07	TEST A.N. 13.10.07	SAMPLE A.M. 13.10.07
Sanacore	17.29	17.40	16.39
Ariddo di Core	18.10	15.89	19.10

Analysis of modified atmosphere composition show that the % CO2 trend isn't constant as a function of time, infect the concentration of gas decreases after two weeks at minimum value of 3%. In this case to maintain the balance of gasses it was necessary to fill again CO_2 in the bag as reported in fig.2. The response of fruits to CO_2 levels is dependent upon the sensitivity of the commodity, the temperature, the duration of exposure and the rate of permeability of film bag.

Titrable acidity for cultivars Ariddo and Sanacore is lower after storage under m.a. as well as for samples stored in air. In particular Ariddo di Core shows best results in respect to Sanacore, infact Ariddo is characterized by a higher acidity than Sanacore (fig.3 and 4). Considering these results, the Tectrol System could

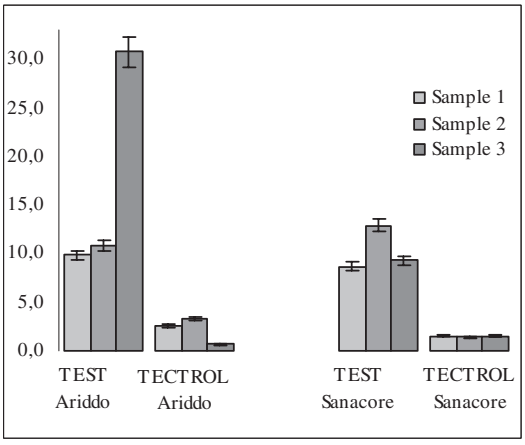

Fig.1-Weight losses % on cv Ariddo di Core and Sanacore after storage (52 days) in A.N. and M.A.

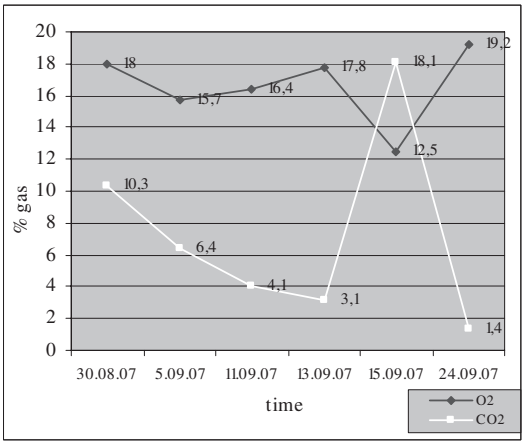

Fig.2-Evolution % of gasses inside the Tectrol System.

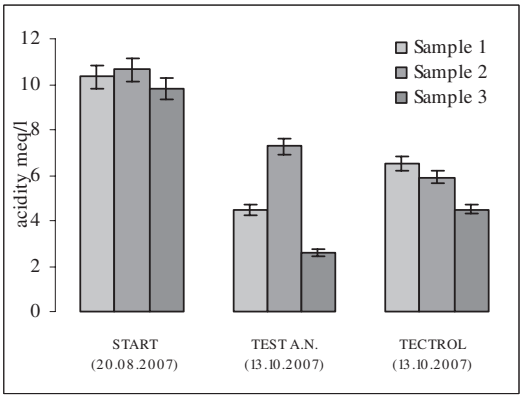

Fig.3 - Evolution of acidity in cv Ariddo di Core.

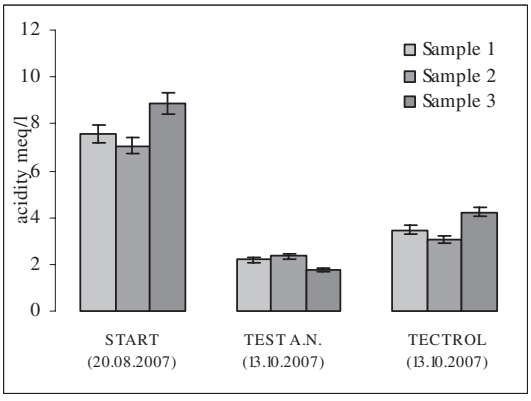

Fig.4 –Evolution of acidity in cv Sanacore.

be considered a promising solution to satisfy the fresh market demand, focused on the destagionalization and the diversification of consumption. In this experiment low temperature with modified atmosphere was able to prolong the shelf life of fruits for 52 days preserving the most important qualitative parameters. The limitative aspect of this method is the manual control in the monitoring of gasses inside the pallet, but this operative aspect could be solve with an automatisation of the system, then best results can be obtained with the improvement of the supply chain (storage in the same area of the harvest).

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EVALUATING *PSEUDOMONAS* GROWTH THROUGH THE ANALYSIS OF THE GAS OF THE HEAD SPACE

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ABSTRACT

A gas analyzing approach of the head space is proposed and the Gompertz equation was used to evaluate the Minimum Detection Time to attain an increase of CO_2 or a decrease of O_2 in the head space of 3% (MDT_1 and MDT_2, respectively), due to *Pseudomonas* population. The results pointed out that both MDT_1 and MDT_2 showed a good correlation with *Pseudomonas* number (0.921 and 0.997, respectively). However, these values were influenced by the substrate used for the assays.

Key words: *Pseudomonas* spp., headspace, gas analyzing approach, mozzarella cheese.

INTRODUCTION

The psychrothrophs from milk include both Gram positive and Gram negative bacteria, comprising *Pseudomonas*, *Aeromonas*, *Enterobacter*, *Klebsiella* and other coliforms, *Bacillus*, *Clostridium* and *Micrococcus* (Munsch-Alatossava and Alatossava, 2006). Among the cited genera, we could consider *Pseudomonas* (namely *P. fluorescens*, *P. putida*, *P. fragi*, *P. putrefaciens* and less frequently *P. aeruginosa*) as the most important one for its spoiling significance for milk and dairy products (Sinigaglia *et al.*, 2008). Nowadays, the evaluation of *Pseudomonas* spp. is carried out through the use of selective media; however, the required time for the analysis is too long for the dairy industry. Therefore, this paper focused on the possibility of using an alternative approach for enumerating *Pseudomonas* counts in order to reduce the time to attain the results; a gas analyzing approach was proposed, based on evaluation of the Minimum Detection Time and on its correlation with the viable count in plates.

MATERIALS AND METHODS

Microorganisms. This study focused on three strains of *Pseudomonas* spp.: PSE 5 and PSE 8, isolated from mozzarella cheese and identified as *P. fluorescens* and *P. putida* respectively, and *P. fluorescens* DSMZ 50090. The strains were maintained at 4 °C on PCA slants (Oxoid) and grown separately in PC broth (glucose, 1g/L; yeast extract, 2.5 g/L; tryptone, 5 g/L) at 25 °C for 48 h.

Assays. The experiments were performed in a model system, consisting in sterile vials (volume 20 mL, DANI Instruments, Milan, Italy), containing 10 mL of broth and hermetically sealed with a butyl cap and a metal ring. Three different experiments were carried out: 1) Effect of the initial cell number (3-6 log cfu/mL) on the production/consumption of CO_2/O_2. The assays were performed on a single strain (PSE 8) in PC broth. 2) Influence of the strain. The assays were performed separately on the three strains (initial inoculum, 4 log cfu/mL); PC broth was used as medium. 3) Influence of the medium. The experiments were performed into 4 different media, inoculated with a microbial cocktail of the three strains (initial inoculum, 4 log cfu/mL). The media used were: PC broth, PSE broth (tryptone, 10g/L; K_2SO_4, 10g/L, $MgCl_2*6H_2O$, 2.99 g/L; cefaloridine, 50 mg/L; fucidine, 10 mg/L), Skim Milk (SM) (Oxoid) and Skim Milk Agar (SMA) (Agar, 1.2% w/v). The samples were stored at 25 °C and at regular time intervals the content of O_2 and CO_2 in the head space was evaluated through an head space gas analyzer Checkmate II (PBI Dansensor, Ringsted, Denmark).

Data modeling and statistical analyses. The analyses were performed on two independent batches, labeled as A and B. Data of CO_2 and O_2 were modeled through a positive and a negative Gompertz equation, re-parameterized by Corbo *et al.* (2006), in order to evaluate the time to attain an increase of CO_2 or a decrease of O_2 in the head space of 3% (these parameters were labeled as MDT_1 and MDT_2, respectively) (MDT, Minimum Detection Time).

RESULTS AND CONCLUSIONS

In a previous paper (Bevilacqua *et al.*, 2007), the possibility of monitoring coliforms of mozzarella cheese through a gas analyzing approach was proposed; in the present research this method was used as a mean to evaluate the counts of *Pseudomonas* of dairy origin. Different assays were carried to "weight" the elements involved in the production of CO_2 and O_2 consumption in the head space. The 1st experiment was performed to evaluate the influence of the initial cell count. Both CO_2 production and O_2 consumption showed a sygmoidal kinetic; therefore, data were modeled through a positive and a negative Gompertz functions, respectively, and MDT values were evaluated. As one could infer from the figure 1, MDT_1 and MDT_2 were affected

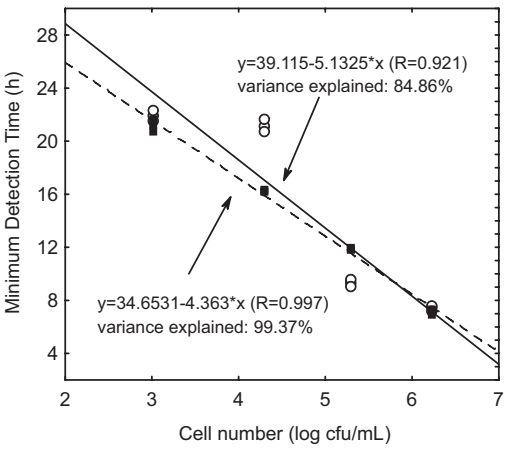

Figure 1: Correlation MDT_1/MDT_2 *vs* cell counts of *Pseudomonas* spp. (strain PSE 8).

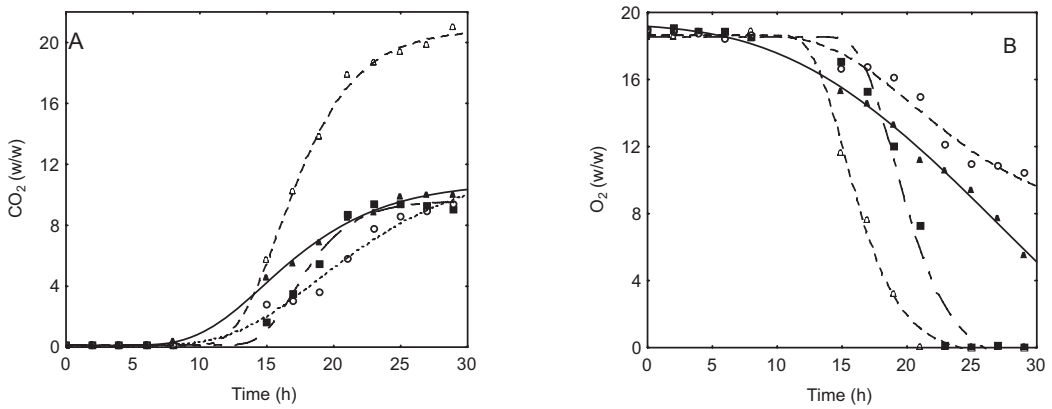

Figure 2: Evolution of CO_2 (A) and O_2 (B) in the head space of the model system, inoculated with a cocktail of *Pseudomonas* spp. (PC broth, Δ; PSE broth, ■; Skim milk, ○; Skim milk agar, ▲).

by the initial cell counts of *Pseudomonas*. The correlation was significant (84.86% of variance explained) for MDT_1 value and highly significant (99.37% of variance explained) for MDT_2. *Pseudomonas* strains seemed to affect only in a slight manner the kinetic of CO_2 and O_2 in the head space; therefore, we could suggest that the differences recovered were probably due to an intrinsic variability of the samples rather than to a real difference of the microbial targets (data not shown). Figure 2 reports the influence of the media on CO_2 (A) and O_2 evolution (B). When PC broth was used as medium, the CO_2 attained at the plateau was ca. 20%; otherwise, in PSE broth, Skim Milk and Skim Milk Agar the maximum amount of CO_2 recovered at the end of the running time was approximately 12-13%. These results were confirmed by the consumption of O_2; in PC broth, in fact, oxygen was at the undetectable level after 24 h. On the other hand, in PSE broth the cocktail experienced a longer MDT value (17.36 h) and in the media containing skim milk after 30 h the amount of O_2 was 5.51% (skim milk+agar) and 10.45% (broth). The results of this paper could be considered as the 1st approach for the development of a rapid method for the enumeration of pseudomonads in milk and dairy products and pointed out that the consumption of oxygen is significantly related with the initial contamination of the microbial target; moreover, regarding the substrate to be used in the model system we could propose both PC broth and PSE broth, as they resulted in an higher rate of O_2 decrease.

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EVALUATION OF SHELF LIFE OF MINIMALLY PROCESSED PINEAPPLE BY USING CONVENTIONAL AND ALTERNATIVE METHODS

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ABSTRACT

The aims of this work were: 1) to investigate the freshness decay of minimally processed pineapple stored at different temperatures (5.3, 8.6 and 15.8 °C) by applying non-destructive methods (NIR spectroscopy and electronic nose) compared with conventional methods; 2) to model the freshness decay kinetics in order to determine the maximum acceptability time. The fresh cut pineapple samples were analysed at different times according to the storage temperature. The quality decay of this fruit was evaluated by using both an electronic nose and NIR spectroscopy. The classical modelling of the total bacterial count (TBC) by using the Gompertz equation allowed the setting of a stability time above which the pineapple was no longer acceptable. Principal components analysis, applied to both non-destructive methods, showed a clear discrimination between "fresh" and "old" samples, and gave at each temperature the evaluation of a stability time. All the stability times obtained by microbiological analysis, electronic nose and NIR spectroscopy allowed the building of a time-temperature stability plot and the evaluation of Q_{10} mean value (3.6-3.8). According to our results the shelf life prediction of fresh cut pineapples stored at 5.3 ranges from 5 to 6 days, those stored at 8.6 °C between 2 to 3 days, and only 1 day for the samples stored at 15.8 °C. Overall, these results show that non-destructive methods could support the conventional techniques in studying the shelf life of minimally processed fruits.

Key words: minimally processed fruits; pineapples; shelf life; NIR spectroscopy; electronic nose.

INTRODUCTION

The market sales of ready-to-use fresh vegetables and fruits have grown rapidly in the recent years as a result of changes in consumer attitudes. Ready-to-use fresh fruits have a shelf life up to some days, that depends on both product quality (i.e. raw material quality and process operating conditions) and storage conditions (i.e. temperature, atmosphere, packaging). Predictive models are a useful tool both to understand degradation phenomena during shelf life and to optimise storage conditions, especially when kinetics phenomena are modelled under dynamically varying conditions. Several studies have been carried out to monitor the shelf life of minimally processed fruits and vegetables using chemical, physical and microbiological indices (González-Aguilar *et al.*, 2004, Klaiber *et al.*, 2005). Some of these methods are time consuming and are hardly practised during the storage of products. Recently the development of multivariate statistical techniques has focused more attention on rapid and non-destructive methods to investigate the freshness decay of the food product. Near infrared spectroscopy (NIR) and electronic nose (EN) are probably the most powerful and convenient analytical tools, to monitor the modifications occurring during shelf life. In particular, in the NIR region (between 750 and 2500 nm), vibration and combination overtones of the fundamental O-H, C-H and N-H bounds are the main recordable phenomena (William and Norris, 2001). The EN is a device equipped with an array of weakly specific and broad-spectrum chemical sensors that mimic the human olfactory perception and provide a digital fingerprint of the odorant, which can be analysed with an appropriate statistical software.

The aim of this work was to investigate the freshness decay of minimally processed pineapple stored at different temperatures by applying non-destructive methods (electronic nose and NIR spectroscopy) compared with conventional methods and to build a model for predicting the maximum acceptability time as a function of storage conditions.

MATERIALS AND METHODS

The fresh cut pineapple samples (cv. *Golden Ripe*, Costarica) were supplied by the manufacturer at the beginning of their commercial life and analysed at different times according to the storage temperature. Average temperatures measured by the recording devices (TB Econorma S.a.s Treviso, Italy) during storage were 5.3, 8.6 and 15.8 °C. Samples stored at 5.3 and 8.6 °C were analyzed every day up to 10 days, while those stored at 15.8 °C up to 6 days.

At each time (depending on temperature) NIR spectra were collected by using a FT-NIR spectrometer (MPA, Bruker Optics, Ettlingen, Germany) equipped with an integrative sphere (12500-3900 cm^{-1}, resolution: 32 cm^{-1}). For each sample 7 spectra were collected and the average of the results was used for the analysis. The evaluation of e-nose was carried out by using a commercial portable electronic (PEN 2 model, Win Muster Airsense). At each storage time and for each temperature, 13 g of sample were placed in 45 mL glass vials, sealed with PTFE/silicone septum and a screw cap. The vials were stored at -20 °C until the time of the analysis. Then, the vials were equilibrated at 23 ± 1 °C for 80 minutes and analyzed at the same temperature. Each sample was evaluated 3 times and the average of the results was used for the statistical analysis.

At the same time, the total bacterial count (TBC), soluble solids content, pH and acidity were measured. Both NIR and electronic nose datasets were analysed by using principal component analysis (PCA) in order to detect unusual or outlying samples and to uncover pineapple modification during storage at each temperature (The Unscrambler, versione 9.7, Camo, Inondhcim, Norway). The values of the PC scores and the microbiological data were modelled as a function of time to identify the stability times during storage at each temperature (Table Curve Software, v. 4.0, Jandel Scientific, San Rafael, CA, USA).

RESULTS AND DISCUSSION

The Fourier Transform near infrared (FT-NIR) spectra of minimally processed pineapple collected during storage are dominated by some peaks at 10244, 8454, 6996, 5623 and 5191 cm^{-1}. Absorbance at 10244, 6996 and 5191 cm^{-1} are due to the bands rising from OH stretching and bending vibrations of water, while the peaks at 8454 and 5623 cm^{-1} rise from C-H stretching vibration of sugar and organic acids (William and Norris, 2001).

A preliminary examination of the spectra was performed by PCA applied to the second derivative of the spectra in the range 11000-3900 cm^{-1}. Examining the score plot shown in Figure 1, in the area defined by the first two principal components, a satisfactory sample distribution was found according to the storage conditions. In particular, the first component (98% of the total variance) was able to separate the sample between "fresh" and "old" and to identify a shelf life threshold, corresponding to 5 days for sample stored at 5.3 °C, 3 days for samples stored at 8.6 °C and less than 1 day for samples stored at the highest temperature. The loading intensity on PC1 and PC2 plotted against wavenumbers showed the water, sugar and acidic component absorption bands as principal wavenumbers explaining variation during the storage of minimally processed pineapple. To better describe the spectral change undergone during storage at each temperature, the PC1 scores were plotted against time and modelled using a sigmoid function. The time of maximum acceleration of the process, corresponding to the minimum value of the second derivative, was calculated and was associated with the stability time. This time (5 days at 5.3°C; 3.4 days at 8.3°C and 1.1 day at 15.8 °C) corresponds to highest degradation rate, responsible for "freshness" loss with a negative influence on the product quality and appearance during trading (Figure 2). Storage beyond this point results in a product which rapidly will be no longer acceptable.

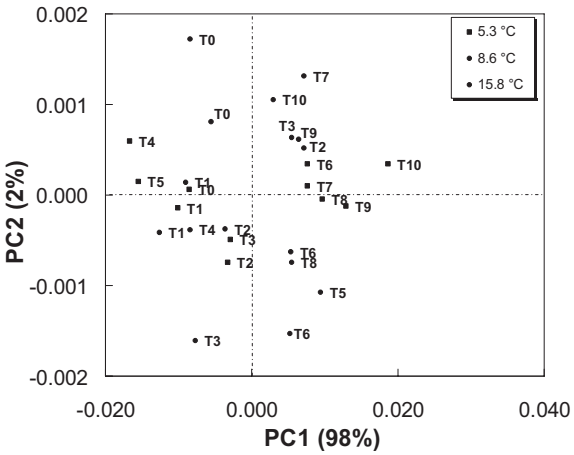

Figure 1: PCA applied to the second derivative of minimally processed pineapple

The stability times measured by using FT-NIR spectroscopy allowed the building of a time-temperature tolerance chart, obtaining a Q_{10} value equal to 4.38. As reported in

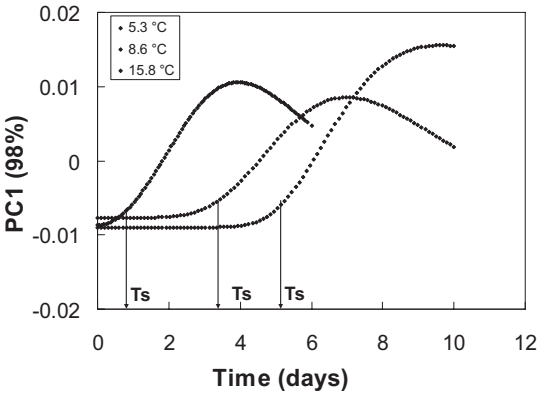

Figure 2: PC1 scores versus time obtained by FT-NIR spectroscopy samples stored at 5.3°, 8.6° and 15.8 °C.

a parallel work (Riva and Torri, 2008), the separation of samples on the basis of storage condition was also confirmed by the electronic nose. Results obtained by PCA applied to correlation matrix of the entire data set showed a good sample separation along PC1 and PC2, identifying two groups of samples. The samples stored up to 7 days at 5.3 °C were considered "fresh", just as those stored up to 4 days at 836 °C, and 1 day at 15.8. All the other samples were considered "old". As for NIR spectroscopy, the PC1 scores were plotted against time, modelled with a transition function, whose second derivative was calculated. For each temperature, the time related to the maximum acceleration of the decay process of volatile compounds was calculated, and was associated with the stability time (5.3 days at 5.3°C; 3.3 days at 8.3°C and 1.1 day at 15.8 °C). The stability times at each temperature, obtained by NIR and EN methods, were confirmed by the microbiological analysis, that are good predictors as a rule of freshness loss in fresh product. In particular, ready to use pineapple samples are characterized by acid lactic bacteria and yeasts development. The growth of these microorganisms determines the product acidification caused by a fermentation process and the texture loss. The total bacteria count (TBC) was modeled by using Gomperzt equation (Zwietering *et al.*, 1990) in order to define a stability tome at each temperature. Considering as th threshold a TBC of $5*10^6$ CFU/g, as indicated by the Fresh legal value in a ready to use products with no coliforms or other pathogenic microorganisms, the stability times were determined on the abscissa axis as the point where the French legal requirement intersected each one of TBC lines. The maximum acceptability time for each temperature was 6.7 days for the storage at 5.3 °C, 2.9 days at 8.3°C and 1.9 days at 16.5 °C. The time-temperature stability plot (fig. 3) and a Q_{10} mean value (3.6-3.8) was obtained by using all the stability times from NIR, EN and microbiological analysis. According to our results the shelf life prediction of fresh cut pineapples stored at 5.3 ranges from 5 to 6 days, those stored at 8.6 °C between 2 to 3 days, and only 1 day for the samples stored at 15.8 °C.

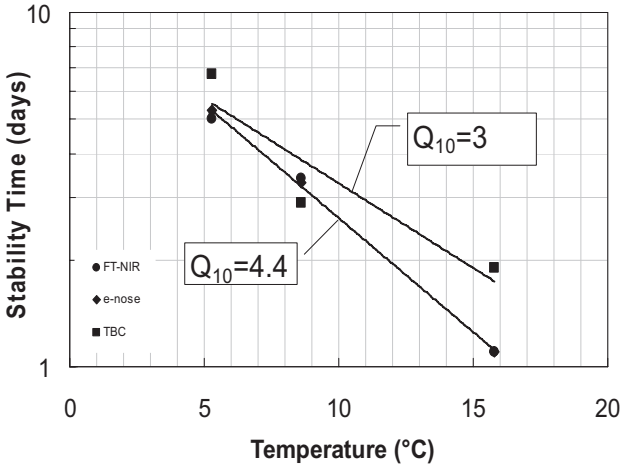

Figure 3: Time-temperature tolerance chart of ready to use pineapple by using FT-NIR data, electronic nose and microbiological analysis.

CONCLUSIONS

In this work, shelf life of minimally processed pineapple was measured with a "classical" modelling approach to obtain time-temperature tolerance charts. Results of this study can be employed to estimate real exposure conditions and quality decay during marketing. Overall, these results show that non-destructive methods, such as NIR and EN could support the conventional techniques in studying the shelf life of minimally processed fruits. In particular, the FT-NIR could be applied to monitor the molecular modification, while EN to evaluate the aroma profile during storage. The lower stability times and higher Q_{10} values obtained with FT-NIR and EN gave a good assessment e of pineapple freshness decay, slightly anticipating microbial decay and thus rendering the use of fresh-cut fruit safe.

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PRELIMINARY VALIDATION OF A NEW INSTRUMENT FOR STUDYING FOOD OXIDATIVE STABILITY

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ABSTRACT

Fat oxidation is one of the most serious problems occurring during storage of foods, causing a shortage of their shelf-life. In order to optimize food formulation, to predict the shelf life or to choose the best packaging solution, the food industry is very interested in easy, quick and reliable solutions able to estimate the oxygen sensitivity of a product. The degree of lipid oxidation can be measured by chemical or physical methods as well as stability tests, which measure the stability of a fatty food under conditions that attempt to accelerate the normal oxidation process.

In the field of accelerated shelf-life testing, OXITEST, a new instrument developed by Velp Scientifica, permits food stability to be investigated: it is based on the absolute pressure change in a closed and thermostatted room, assumed as the oxygen uptake by reactive substances.

One of the advantages of this technique is that food stability against rancidity can be measured directly on whole foods (solid, liquid, doughy) without the need to perform preliminary separation of the fat. Moreover, the information obtainable from instrument response regard not only the induction period (IP) of autoxidation process but also the rate and acceleration of the autoxidation process itself and, finally, the amount of oxygen consumed by the product (in specific accelerated conditions). The aim of this work was to propose a standard operating procedure in order to verify the reliability of the measuring system under normal laboratory conditions and to investigate one of the possible applications of OXITEST.

The validation study was carried out using a blank model system with increasing concentrations of fatty acids. Within the linear working range of the induc-

tion period, the limits of detection (LOD) and quantification (LOQ), and the limit of repeatability (r) were estimated. In this way a standard operating procedure is proposed in order to verify the accuracy of the measuring system under normal laboratory conditions.

A preliminary comparison between OXITEST and OSI technique was also performed, with the aim to evaluate the accuracy of the new technique versus a validated method. The comparison was performed using vegetable oils of various botanical origins with different oxidation levels. The induction periods obtained by both OSI and OXITEST methods were compared and the correlation was verified deducing the Pearson product moment correlation coefficient. The results obtained indicate OXITEST as a reliable alternative to validated methods.

Key words: fat oxidation, oil, shelf-life, accelerated test, validation, OXITEST.

INTRODUCTION

The main cause of deterioration of lipids and lipid containing foodstuffs is lipid autoxidation. The degree of lipid oxidation can be measured by chemical or physical methods that are often time consuming, expensive and require very trained people (Laubli *et al.*, 1986). The level of oxidation of fatty foods can be also determined using tests which measure the stability under accelerated conditions (for example at elevated temperature) in order to obtain a faster autoxidation in a few hours instead of weeks or months. Generally, these tests allow to obtain an oxidation curve, characterized by an Induction Period which measures the time required to reach an end point of oxidation corresponding to either a level of detectable rancidity or a sudden change in the rate of oxidation (De La Presea-Owens *et al.*, 1995).

In the field of accelerated tests, Oxitest, a new instrument developed by Velp Scientifica, permits food oxidative stability to be investigated. Differently from similar and already validated methods (Farhoosh, 2007), such as Rancimat (Metrohm) and Oxygen Stability Index (OSI Omion Inc.), the easiness to use, due to the possibility to perform the analysis directly on the food (liquid, solid or pasty) without previous fat separation, represents the most important Oxitest benefit. The information obtainable from instrument response regard not only the Induction Period (IP) of the autoxidation process, but also the rate and acceleration of the autoxidation itself. The amount of oxygen consumed by the food product during oxidation can be obtained as well.

The aim of this work was to propose a standard operating procedure in order to verify the reliability of the measuring system under normal laboratory conditions and to investigate one of the possible applications of OXITEST. For this reason the following steps were considered: evaluation of some parameters of validation in accordance with the Eurachem Guidelines, to test the ability of OXITEST in revealing adulteration of vegetable oils.

MATERIALS AND METHODS

Hardware description: Oxitest (Velp Scientifica, Usmate (MI) – Italy) can operate by accelerated conditions of temperature (room-110°C) and oxygen or air pressure (0-8 bar). The following operating conditions have proved to be the most suitable to

test most of the food samples: 90°C and 6 bar of oxygen. The instrument measures the absolute pressure change inside two independent, closed and thermostatted rooms (A-B), monitoring the oxygen uptake by reactive substances. Inside the rooms are introduced the titanium plates containing the sample and, in some cases, one or more spacers. An external cover, provided with screws and a discharged tap, permit the hermetic seal of the reactor and allow the operator to eliminate the final residual atmosphere from the room.

Software description: a dedicated software lets the operator follow and record the oxidative process inside the two reaction chambers and calculate the IP by graphical or least square methods. At the end of oxidation test, for each sample, it is possible to obtain a Test Report with the sample Induction Period and the analysis details.

Validation: two model systems were setting by the addition of growing concentrations of fatty acids with a different degree of insaturation to a blank with high oxidative stability.

The exactness was evaluated on a different matrix: commercial seed oil samples were testing both Oxitest and an already validated techniques (OSI).

Study on vegetable oils: two different types of Italian commercial oils was used for this investigation. Increasing concentrations of seed oil were added to extra-virgin olive oil.

RESULTS

This preliminary study was based on the estimation of the following quality parameters deduced from Eurachem guidelines: the linear range, the detection and quantification limits, the measure's repeatability and the exactness of the method.

The linear range was evaluated adding growing concentration of fatty acids to the blank. The oxidation curves changed with the increasing of fatty acid. In particular, the IP tended to extend progressively and proportionally to the concentrations of the analytes. The LOD and LOQ were calculated (Figure 1).

Oxitest repeatability was evaluated by using the same model systems and all the analyses were made in homogeneous conditions (operator, instrument, etc). The variances, relative to the average IP of highest and lowest concentration levels of both fatty acid model systems, were compared by F-test. The dispersion of the single data around the average was not influenced by the variation of the analyte concentration (data not shown).

The exactness was evaluated comparing the response of Oxitest with those obtained by an already validated method (OSI). In particular, vegetable oils were tested and the average IP were worked out with the Pearson's correlation coefficient. A good correlation coefficient, as shown in Figure 6, was found (r=0,97855).

	LOD (%)	LOQ (%)
Blank + fatty acid (low degree of insaturation)	4,8	14,5
Blank + fatty acid (high degree of insaturation)	3,0	10,0

Figure 1: detection and quantification limits for the two model systems

Another important information can be obtained from the oxidation curve: the oxidation rate. It is usually estimated on the curve after the

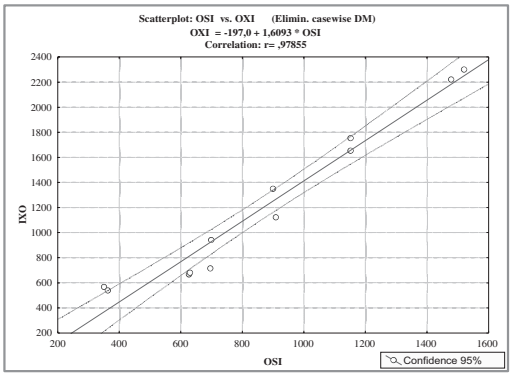

Figure 6: Pearson's correlation between Oxitest an OSI

Induction Period (data not shown).

Induction Period. The curves were modeled with a sigmoidal equation by using a mathematical software (data not shown).

In the field of edible vegetable oils the identification of adulterations is one of the possible applications of the Oxitest. In order to investigate this ability of the instrument, different samples of Italian extra-virgin olive oil were mixed with increasing amounts of Italian seed oil and then tested by Oxitest. The addition of increasing concentrations of seed oil to the extra-virgin olive oil causes a proportional shortening of the mixture

CONCLUSIONS

The detection and quantification limits found for the Oxitest, the high degree of correlation with OSI method and the easiness to use, make Oxitest competitive towards similar techniques. Oxitest, already used for the quality control of suppliers and food raw materials, can be used satisfactorily on finished foods as well. The ability of Oxitest in revealing adulterated oils has been pointed out and confirmed as one of the important possible applications, being the authenticity of products labelled as extra-virgin olive oil of paramount importance from the standpoints of both commercial value and health aspects.

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CONSUMER BEHAVIOUR ABOUT FRESH FISH: THE CASE OF SLICED RED TUNA

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ABSTRACT

This survey has conducted in Sicily, an island situated in Mediterranean, where the fresh fish consumer has an important role in the main meal. In fact, given the growing segmentation of demand, the aim of this analysis is to take over the purchasing behaviour about consumer of fresh fish, focusing on sliced red tuna, to offer a contribution to the definition of the strategic choices of distribution enterprises. In particular, through the research it has been possible to detect: the reason that to bring people to prefer fresh fish, specially sliced red tuna, rather than other fish of the same merchant use; what is the place where they prefer to buy it or eat it, for example retail, fish market, local market, restaurant, etc., and why they choose some place instead of another, putting attention on qualitative perception of product.

In the purpose to value the relations which link the purchasing choices with red tuna and to examine the consumption aspects about individual motivations e values, the research is conducted using *ad hoc* structured questionnaire to a sample of habitual consumers of sliced fish.

In particular the study has been subdivided in two parts: in the first one, considering that a product is distinguishing from another for the place where it is sold, the survey aims to take over the preference about purchasing or consumer place of fish fresh; the second part underlines the different perception that consumers, belong to varieties professional categories (housewives, professional man, employee, etc.), have about the qualitative aspect of fish, with particular attention to red tuna.

Key words: consumer behaviour, purchasing places, red tuna.

INTRODUCTION

In the recent years the fresh fish consumer is increasing in Italy and in particu-

lar in Sicily, obtaining an important role in the main meal. The reasons for this growing could be found in diffusion process of modern distribution systems, in the food crisis (such us BSE, Bird flu, etc.) which have brought the consumer to eat alternative products, such us fish, and to care about qualitative foods. Then, large-scale retail trade has focused attention on fish products through specialised space, in which consumer can find fresh and frozen fish. Moreover, in recent years, the raw fish consumer is increasing by internationalization food consumers with diffusion of Asiatic catering, such as *sushi* and *sashimi*, among which red tuna have a main role and in particular that caught in Atlantic and in Mediterranean, for its qualitative aspects.

For this reason it seems to be interesting to take over the purchasing behaviour about consumer of fresh fish, focusing on sliced red tuna, also to offer a contribution to the definition of the strategic choices of distribution enterprises. The central purpose of this article is to examine the reason to bring people to prefer fresh fish, specially sliced red Tuna, rather than other fish of the same merchant use, and what is the place where they prefer to buy it, putting attention on qualitative perception of product; in particular this paper has been subdivided in two parts: the methodology is outlined in the first one to explain the method of investigation, to indicate the period wherein the survey was conducted and to give information about the sample interviewed. In the second part the results are discussed to take over the preference about purchasing or consumer place of fresh fish and the perception that consumers have about the qualitative aspect of this, with particular attention to red tuna.

MATERIALS AND METHODS

The survey about fresh red tuna consumption is conducted in Sicily in 2007 using *ad hoc* structured questionnaire to a sample of people, with face to face interview at large-scale retail trade in cities of Palermo and Catania. The questionnaire-schedule has given the opportunity to take qualitative and quantitative information about social-economic and cultural characteristics of interviewed persons and about their purchasing behaviour and their qualitative perception on product and its price, also the distribution trade characteristics. In particular, using closed and multiple test procedures, the persons can choose, among a different code options, those better for their opinion, position or behaviour modality (Berni, *et al.*, 1995).

Through the interview it has been possible to take over: what kind of sliced fresh fish product the consumer often eats; the frequency purchasing; the purchasing and consumer place. In particular the first part of questionnaire is about qualitative perception of red tuna and its identification, focused on the motivations to bring the consumer to put attention on the same fish. Then it took over: the origin place of tuna and the reasons induce to favour the consumer of red tuna rather than other kind of fish; purchasing place and behaviour, also price perception of same one. Moreover the research has supplied information on social and economic characteristics of consumers, in particular these data are in the last part of questionnaire.

RESULTS AND CONCLUSIONS

The survey has shown about social and economic characteristics that the

interview sample is in great measure female gender (60%), while the 40% is male sex.

In relation to educational qualification the interviewed consumer has a medium cultural level, in fact the 31.2% of their has "junior high school", the 41.7% has "senior high school", the 27.1% has "academic degree". With regard the kind of profession the 24.6% of person is "employee", followed by "freelancer" with 17.1%, while the 16.6% has indicated the category "Other" and, at the end, the housewives with 16.0%. The framework of interviews characteristics has been completed with the variable income that is on medium-low level, in fact the 54.2% of consumes earns "less than 20 thousands euro", the 33.7% "between 20 and 40 thousands euro" and the 9.4 "more then 40 thousands euro", while the 2.6% of interviewed has not given information.

In order to achieve the analysis on red tuna consumer, it needed to identify the fresh fish consumer, which are 85% of interviewed and, among them, those that eat sliced fish. In the matter of fresh sliced fish much consumed, the study notices that the 31.7% of sample consumes "other" kind of fish instead of those mentioned in questionnaire, in particular the species has been frequently indicated are: mullet, cod, bass and black sea bass, sardines, etc. From these results it is possible to say that often the consumer chooses kind of fish less precious and cheaper or fish culture. The other part of sample interviewed eats for the 20.6% swordfish, the 12.3% salmon, the 11.1% red tuna, the 9.5% groper, the 8.3% greater amberjack and, at the end, the 6.5% eats "other kind of tuna". Among interviewed persons the 39.6% is consumer of red tuna, while the 60.4% not eats fish belong to kind of tuna. For this reason the survey is about the first aliquot of consumers.

The motivations to bring examined consumer to favour red tuna is about "nutritional characteristics" for the 35.6%, "organoleptic quality" for the 37.8%, "culinary quality" for 14.4% and "easiness of cooking" for 12.2%. In regard to the reason to induce consumer to prefer red tuna to other kind of fish, the interviewed persons have indicated: the "taste" for 24.3%; "consistence" for 19.1%; the "colour" for 17.7% ; because tuna is "more digestible" for 13.9%, "more convenient" for 12.6%, because "has not fat" for 12.4%. Moreover the study shown that, during the purchasing process, the consumer is shaped by "freshness of product" for 37.1%, although the consumer, in many cases, is not able to recognize it, by "additional information about product" for 21.1%, by "geographic origin" for 21.0% and by "promotional offers" for 20.8% . About purchasing place, the survey evidences that the consumer uses to buy fish at specialized retail for 64.4%, maybe because he attribute more reliability to retailer upon which he put his trust, and , so, more quality to product. An other important aspect by considering is the limited diffusion of buying at large-scale retail trade, in fact only the 12.2% of sample purchases at this kind of retail, while the 16.7% at corner market and 6.7% prefers buying directly at port.

In sum the results of this analysis bring to light a new consumer profile that is influenced by other aspects than the price, as well as it was before, which are organoleptic and nutritional characteristics of product. Moreover, based on these data the quality seems to be a decisive characteristic for the choice of product, in particular about red tuna, in spite of it is noteworthy underline that often the consumer doesn't know the necessary element to individuate and appreciate the quality. For this reason it is important that the retailers, specially large-scale retail trade, take effective strategy about product preservation, investing in research and cooperating with university, in order to provide incentives the development of

modern and efficient technology for shelf life, because the product qualitative play a relevant role in its purchasing, in particular for fish. At the end, in this framework the information have a main part and, for this, effective communication, not only about product but also about political strategy, can contribute to link consumer with enterprises making him more aware about product quality.

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