

Microbiological and chemico-physical shelf-life and panel test to evaluate acceptability of liver mortadella

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Abstract

This study aimed to evaluate the shelf life of sliced cooked liver *mortadella* packaged in MAP (70-85% N₂, 15-30% CO₂) and stored in refrigeration (4°C) or slight thermal abuse (8°C) for up to 49 days (declared best before date 45 days). The proximate composition, aw, nitrites and NaCl content were determined at T0. Weekly, samples were submitted to microbiological [total viable count (TVC), lactic acid bacteria (LAB), *Enterobacteriaceae*, *Escherichia coli*, *Pseudomonas* spp., coagulase positive staphylococci, sulphite reducing clostridia, yeasts and moulds, *Listeria monocytogenes* and *Salmonella* spp.] and physico-chemical analyses [pH, colorimetric parameters, total volatile basic nitrogen (TVBN), thiobarbituric acid reactive substances (TBARs)], in parallel with consumer acceptability tests. The product characteristics (low salt and nitrites concentration, high aw and pH close to 6.5) were not efficient hurdles for microbial growth. No pathogens were detected in the samples; the initial TVC [5.4 Log colony forming unit (CFU)/g] increased rapidly, reaching values around 8 Log CFU/g at T14 for both the series, and was almost totally composed by LAB, leading to the acidification of the product (pH at T49=5.05 at 4°C and 5.24 at 8°C). The other microbiological parameters were below 2 Log CFU/g. The product showed a good protein and lipid stability (TVBN <33 N/100 g and TBARs <8 nmol/g at T49). The sensorial quality of liver *mortadella* was more affected by the storage time than by the temperature. An evident colour modification was detected after T35, when the product was also frequently rejected by the panellists, mainly due to odour. Thus, the shelf life of sliced cooked liver *mortadella* should be shortened below 30 days.

Introduction

Liver *mortadella* is a ready-to-eat cooked sausage very popular in Northern Italy (mainly Lombardy, Aosta Valley Region and Piedmont)

and many other countries (e.g. Germany, France, Switzerland; Villa, 2010). As reported by Cantoni *et al.* (1974), liver *mortadella* is obtained starting from dough made of pork meat with the addition of pork liver. The amount of liver added varies depending on the manufacturer, from 2-5% up to 15-25%. Depending on the proportion of liver used, the flavour of the product is more or less intense. In the production process, the ground meat and the fat are mixed coarsely, while the liver is finely cut separately. Pork rind, salt, spices, flavourings and binders could be also supplemented during the preparation. The resulting mixture is stuffed into natural or artificial casings. In fact, there are two variants of the liver *mortadella*: a raw version and a cooked one. Typically, the artificial casing is used in the cooked variant, while the natural for both the typologies.

This product contains high levels of fat and iron: as already reported for other liver products like pâté, a development of lipid and protein oxidation during refrigerated storage could lead to the degradation of the heme molecule and the release of iron, reducing the oxidative stability (Estévez *et al.*, 2004), thus resulting in unacceptable products.

From a microbiological point of view, the chemico-physical characteristics typical of liver *mortadella* such as low salt content, high pH value and high water activity (a_w), are singularly only small hurdles that are not able to inhibit the bacteria commonly associated with these products. Generally, the prevalent microflora present after production is lactic acid bacteria (von Holy *et al.*, 1991), but their number is too low to contrast the potentially pathogenic bacteria (e.g. *Listeria monocytogenes*, *Salmonella* spp., coagulase positive staphylococci) that could be transmitted through contaminated processing environment, instruments and manipulation by the operators. This aspect should be carefully considered since these products are very often handmade or processed in small factories (Paleari *et al.*, 2005).

Although it is very appreciated by consumers, a small number of scientific papers concerning liver *mortadella* has been published (Cantoni *et al.*, 1974; Paleari *et al.*, 2005), and no analytical data relating to the behaviour of the product during its shelf life are available.

Thus, the aim of the present study was to evaluate the shelf-life of sliced cooked liver *mortadella*, pre-packaged in modified atmosphere (MAP) and maintained at refrigerated conditions (4°C) and in thermal abuse (8°C); chemical-physical and microbiological parameters were considered in order to outline the changes during the shelf-life and to evaluate the suitability for consumption. A panel test was also evaluated in order to highlight the sensorial acceptability of the product.

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Key words: Liver mortadella; Shelf life; Oxidation; Lactic acid bacteria.

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Materials and Methods

Ingredients, production and chemical composition of liver mortadella

Liver *mortadella* samples were produced in a medium scale factory in the North of Italy. The recipe of the product is reported in Table 1. Briefly, raw meat (first-class lean pork) was minced and mixed with pork liver (up to 25%), fat and an aliquot of water, forming a stable matrix to which other commercial ingredients were added to produce the liver *mortadella*. All ingredients were mixed for 20 min, and the mixture was then stuffed under vacuum into a synthetic casing. The product was steam cooked at 75°C for 8 h taking care to reach an internal temperature of at least 69°C. After cooling down in appropriate refrigerated cell, *mortadella* was sliced in a white chamber and packaged under modified atmosphere (N₂ from 70 to 85%, CO₂ from 15 to 30%, maximum residue of O₂ <0.4%). The best before data assigned by the producer was 45 days. After the production, the samples were immediately transferred to the lab under refrigerated conditions.

Experimental design

At the lab, the samples were randomly grouped in two series, stored at refrigerated conditions (4°C) or in thermal abuse (8°C)

from T0 (day of the production) to T49 (time after the date by use assigned by the producer that was 45 days). After the production, at T0 the samples were submitted to the following determinations in duplicate: proximate composition (AOAC, 1990), water activity (Rotronic Hygromer Aw-DIO, Basserdorf, Switzerland), nitrites (Mirna and Hohmann, 1969) and salt content as total chlorides (Pearson, 1973). All the determinations were performed in duplicate. At all the settled sampling times (0, 7, 14, 21, 28, 35, 45 and 49 days from the production) the samples were submitted to microbiological and chemico-physical analyses and panel group's acceptance evaluation.

Microbiological analyses

For microbial counts, 10 g of each sample were homogenized in 90 mL of a diluent solution (0.85% NaCl and 0.1% tryptone), and serial 10-fold dilutions were prepared. Total viable count (TVC) was determined according to the ISO 4833 (ISO, 2003a) method. Lactic acid bacteria (LAB) were enumerated according to ISO 15214 (ISO, 1998) method. The number of *Enterobacteriaceae* was determined by the ISO 21528-2 (ISO, 2004) method. *Escherichia coli* were enumerated according to ISO 16649-2 (ISO, 2001) method. Coagulase-positive Staphylococci were determined by ISO 6888-1:1999 method (ISO, 1999). The count of spores of sulphite-reducing Clostridia was performed by ISO 15213 (ISO, 2003b) method, after pasteurization of the dilutions. Detection and enumeration of *Listeria monocytogenes* were performed according to AFNOR methods (AFNOR BRD 07/4-09/98 and AFNOR BRD 07/05-09/01, respectively; AFNOR, 1998). Detection of *Salmonella* spp. was performed according to ISO 6519 (ISO, 2012). Microbiological analyses were performed in duplicate.

pH determination

The pH of the products was determined by a pHmeter (Ghiaroni, mod. XS pH 6, Buccinasco,

Italy); 5 g of the product was homogenised with 20 mL of distilled water and the homogenate immediately subjected to pH determination in duplicate.

Lipid oxidation

Thiobarbituric acid reactive substances (TBARS) were determined in duplicate to evaluate the oxidative stability during storage according to Ke *et al.* (1984) in duplicate for each series.

Total volatile basic nitrogen

Total volatile basic nitrogen [TVBN; Reg. (EC) N. 2074/2005; European Commission, 2005] was determined on each series in duplicate after sampling for microbiological analyses.

Colour parameters

Colour parameters were determined on the surface of the slices of liver *mortadella* using a

Minolta Chromameter CR-200 (Minolta, Osaka, Japan) working at CIELab system. The L^* , a^* and b^* values, which describe the intensity of whiteness/brightness, red colour and yellowness, respectively, were taken at six locations on the cut surface immediately after opening the pack. Total colour differences (ΔE^*) between treated and control samples were calculated as: $\sqrt{(L1^*-L2^*)^2 + (a1^*-a2^*)^2 + (b1^*-b2^*)^2}$. A ΔE^* more than 2.3 means a variation hardly perceptible to the human eye, while ΔE^* more than 3.0 a variation well perceptible to the human eye.

Panel test

A panel test for the acceptability of liver *mortadella* maintained at 4 and 8°C was tested using a panel composed by 13 members. At all the sampling times, each panellist was presented with two slices of the product for each series and asked the questions: *would you eat*

Table 1. Recipe of liver *mortadella* analysed.

Ingredients	Quantity (%)
Meat	94.168
Salt (NaCl)	3.305
Red wine	1.309
Sucrose	0.377
Polyphosphates	0.377
Na ascorbate	0.094
Sodical (sodium nitrite 50%)	0.009
Grappa	0.075
Black pepper ½ grain	0.047
White pepper (powder)	0.094
Lemon	0.026
Cloves (powder)	0.005
Cinnamon (powder)	0.009
Cinnamon	0.006
Cloves	0.002
Drugs	0.009
Total	100.000

Table 2. Proximate composition, percentage of salt, nitrites, pH and water activity on day of the production.

	Present study Average±SD (n=2)	Italian liver mortadella (Cantoni <i>et al.</i> , 1974) Range	Dutch liver sausages (Cantoni <i>et al.</i> , 1974) Average	Finnish liver sausages (Krol, 1966) Average
Moisture (%)	45.50±0.09	33.70-36.30	50.96	53.30
Proteins (%)	14.95±0.55	14.30-17.70	14.20	14.80
Fat (%)	36.19±0.07	39.75-45.33	29.10	30.10
Ash (%)	3.38±0.02	6.10-6.50	3.40	1.80
Salt (%)	2.77±0.02	-	-	1.90
WPS (%)	5.74±0.08	-	-	-
Nitrites (ppm)	12.35±5.87	-	-	-
a_w	0.952±0.10	-	-	-
pH	6.47±0.10	-	-	-

SD, standard deviation; WPS, water phase salt; a_w , water activity.

it? If not, which is the reason (colour/odour/both)?

Statistical analysis

Chemical-physical results obtained during the different sampling time were subjected to statistical analysis through ANOVA test. A probability of $P < 0.05$ was considered as a threshold value for statistically significant differences.

Results

Chemico-physical characterisation

The proximate composition determined at T0 is shown in Table 2. The salt and nitrite concentrations, a_w and pH of the product are also reported: the observed values (low salt and nitrites concentration, high a_w and pH close to 6.5) were not singularly able to inhibit microbial growth.

Evolution of microbial populations and pH during the shelf life

In the samples analysed at the two different temperatures, *Listeria monocytogenes* was always absent in 25 g, as required by Reg. 2073/2005 for ready-to-eat products; also *Salmonella* spp. was absent in all the samples. High concentrations of total viable count and lactic acid bacteria (LAB) were found since T0 (TVC T0: 5.4 log CFU/g, LAB T0: 3.6 log CFU/g respectively) (Figure 1); generally the presence of microorganisms in a pasteurized meat product could be due to the possible inability of the heating core temperature reached during cooking to eliminate all the microflora present or to a post-contamination occurred during the slicing/packaging process. LAB counts showed a rapid increase (thus representing almost all the total viable count) since T14 at both the two temperatures (4°C: 7.9 ± 0.3 log CFU/g; 8°C: 8.4 ± 0.1 log CFU/g): these microbial loads could determine a souring of the product during the course of the shelf-life, due to the production of lactic acid. This was also confirmed by the trend observed for pH values: with the increase of LAB, a constant decrease of pH values was detected (from T0: 6.47 to T49 4°C: 5.05, 8°C: 5.24). All the other parameters considered (*Enterobacteriaceae*, *Pseudomonas* spp., *Escherichia coli*, coagulase positive staphylococci, sulphite-reducing clostridia, yeasts and moulds) resulted below the detection limit (2 Log CFU/g).

Lipid oxidation

Liver mortadella could be prone to lipid oxidation, as it is characterized by high fat and iron content. The level of TBARs was characterized by concentrations below 8 nmol/g (threshold suggested by Ke *et al.*, 1984) during all the period in both the series, showing a good oxidative stability (Figure 2). This could be possibly due to the presence of antioxidant compounds used as additives in the recipe (e.g. sodium ascorbate, sodium nitrite and

polyphosphates) and to the use of O_2 -free MAP during storage.

Total volatile basic nitrogen

TVBN content showed a gradual increase from T7, suggesting the occurrence of proteolytic activity related to bacterial growth (Table 3).

Colour parameters

Considering colour parameters, the samples maintained at 4°C were characterized by a sig-

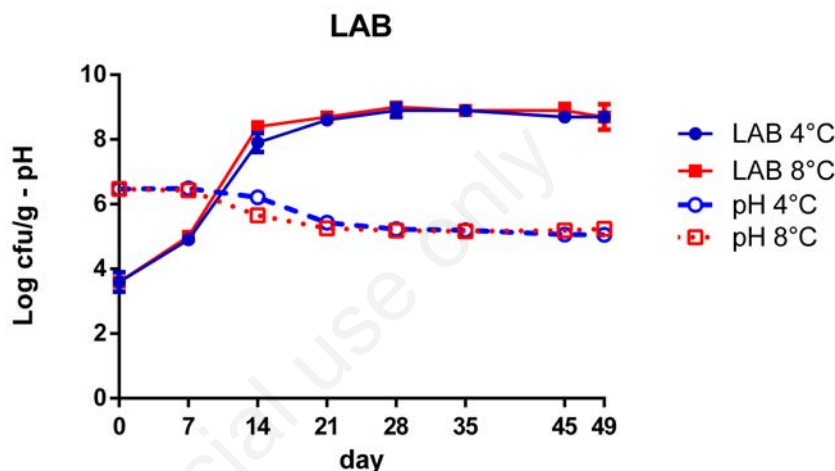


Figure 1. Lactic acid bacteria concentrations and pH.

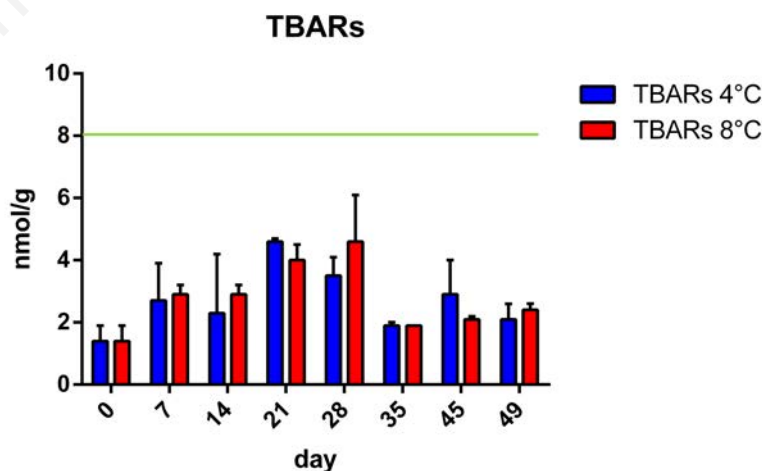


Figure 2. Thiobarbituric acid reactive substances.

Table 3. Total volatile basic nitrogen (mg N/100 g) detected in the two series during the trial.

	T0	T7	T14	T21	T28	T35	T45	T49
4°C	25.00±4.80	22.78±2.95	23.82±0	29.53±0.07	30.10±1.50	31.20±0.40	32.49±0.3	33.00±0.20
8°C	25.00±4.80	21.94±1.00	26.58±0.30	29.04±1.05	32.20±1.10	31.90±1.10	32.50±0	30.90±0.10

nificantly higher brightness (L) if compared to those maintained at 8°C (P=0.02). No significant differences were detected for the parameters a* and b* (P=0.15 and P=0.57, respectively) in the whole period between the two series. Considering the parameter ΔE , important index used to evidence the chromatic changes perceptible by the eye of consumer, an evident variation of colour was detected at the two temperatures after T35 (Figure 3).

Panel test

The panel test showed to accept liver *mortadella* samples maintained at 4 and 8°C was constantly high (>65%) till T28 in both the series; from T35 a rapid decrease of the acceptability was revealed, with a lower number of acceptable samples if compare to those rejected (data not shown). Considering the reasons for unacceptability of the products, at 4°C this was mainly related to the odour (50%), followed by colour (45%) and only a little part of the panellist rejected the products due to the changes detected in both colour and odour (5%). At 8°C the odour was responsible for 54% of the rejections, followed by colour (39%) and only 7% indicating both colour and odour as responsible for the unacceptability of the products.

Discussion

In the present study, the microbiological shelf-life of liver *mortadella* packaged under MAP was evaluated in optimal refrigerated conditions and in slight thermal abuse. As expected, the microflora that limited the shelf-life of this cooked sausage was composed by LAB. The loads of these microorganisms increased rapidly in both the series (4-8°C) reaching after only 14 days values above 7 Log CFU/g (7.9 and 8.4 Log CFU/g, respectively). Previous studies indicated in 7-8 Log CFU/g the threshold above which consumers generally reject a cooked meat product (Makela and Korkeala, 1987; Borch *et al.*, 1996). In particular, in cooked sausages, LAB should be considered as one of the major components of the microbial population, able to determine, depending on the species present, not only sensory changes (smell, taste) but also visual alterations (*e.g.* slime onto the surface, gas production) (Korkeala and Björkroth, 1997). As already stated previously for other cooked meat products, also in liver *mortadella* the presence of high numbers of LAB and the influence on the environment that they colonize during their replication (lactic acid production, acidic pH) played a key role due to their positive antimicrobial activity that limits the growth of potentially pathogenic and spoilage microorganisms.

A chemico-physical evaluation was also per-

formed considering parameters useful to characterize and highlight the evolution of the product during the storage. The results obtained from the determination of proximate composition, performed at T0, were compared with the data reported by Cantoni *et al.* (1974), referred to two different productions, one of cooked liver *mortadella* produced in Italy and one cooked liver sausage produced in Netherlands and with the data reported by Krol (1966) for Finnish liver sausages. In particular, the data reported by Cantoni *et al.* (1974), related to Italian cooked liver *mortadella*, whose percentage of liver employed is not reported, showed lower moisture content while the other parameters were in agreement with those observed in our study. The liver sausages manufactured in Netherlands showed mean values in agreement with ours, except for a lower fat content. Finally, the relative composition of the Finnish liver sausages (Krol, 1966) showed higher moisture content if compared to our products and lower salt content. Moreover, a lower fat content (18.5%) was revealed in the product studied by Krol (1966). The amount of nitrites in the product was far below the maximum allowed dose of 150 mg/kg of nitrites (E 249-250), provided for heat-treated meat products.

As described above, due to the presence of a high percentage of fat, the liver *mortadella* is a product that can be subject to lipid oxidation: the TBARS values indicated substantially an oxidative stability over time. Also TVBN could be considered stable overtime in both the series.

The colour of foodstuffs is one of the most important properties that influence the choice of the consumers at the point of sale: the samples kept at 4°C were characterized for the

entire trial by a significantly higher brightness index if compared to the samples maintained at 8°C while considering the index ΔE , which expresses the variation of colour over time perceptible from consumers' eye, no differences were detected between samples stored at 4 or 8°C, with a clear increase of this value only from T45.

The combination of product colour and odour characteristics was considered acceptable by the panel group until 28 days of storage. Considering the answers related to the attribute discriminated, the unacceptability mostly was caused by the odour, especially in thermal abuse, followed by colour. In particular, the product examined was characterized by a peculiar smell, with a very strong aroma, due to the percentage of pork liver in the recipe (25%), higher if compared to those reported in literature. In fact, starting at T0, this may have affected the acceptability of the products. Moreover, some lactic acid bacteria, besides producing lactic acid, also produce acetic acid and ethanol that may be responsible for an extreme acid odour, as reported before (Sinesio *et al.*, 2000).

Conclusions

The manufacturer assigned a best before date of 45 days to the liver *mortadella* analysed; from a chemico-physical point of view, the product showed perceptible colour changes just after T45, independently from the storage temperature. The TBARS were always stable and always below the limit suggested. This confirmed an excellent oxidation stability of the product, with the absence of any signal

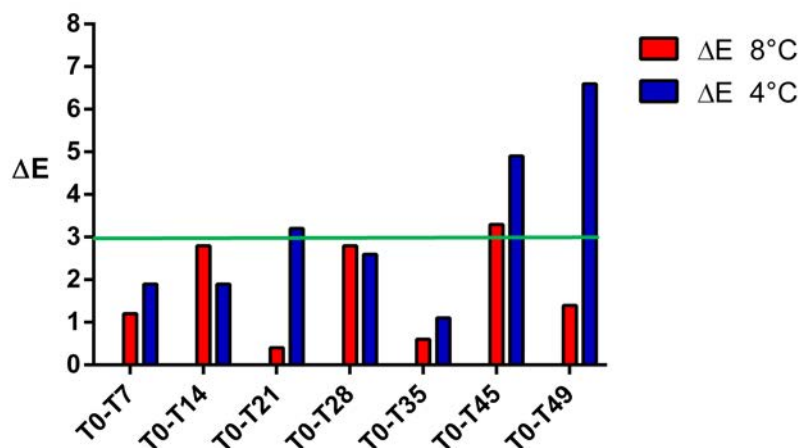


Figure 3. Colour parameters.

of rancidity of the fatty component, an important parameter for preservation of the product and the suitability for consumption. The increase of LAB, with the gradual lowering of the pH from T14, both at 4 and 8°C, may have contributed to the development of sensory alterations determining an overall decrease of the quality; on the other hand, it may have had positive consequences in terms of antimicrobial effect against different pathogenic and spoilage microorganisms (such as *L. monocytogenes*, *Enterobacteriaceae*, *Pseudomonas* spp., *Escherichia coli*, coagulase positive staphylococci, sulphite-reducing clostridia, yeasts and mould), as already reported by Shelef and Potluri (1995). In fact, microbiological analyses showed unsatisfactory results from T14 (clearly from T35), due to the high microbial loads. Finally, the acceptability was found to decrease from T35 as a higher number of panellists rejected the product. As a consequence we feel, on the basis of our results (microbiological criteria, TBARS, LAB loads and colorimetric indexes), that it would be reasonable to attribute a shelf life below 35 days (reasonably around 28-30 days) to the product.

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