5. APPENDIX

5.1 Contributions to national and international congress

V. Bono (2012) Recovery of fibrous fractions from wine industry by-products and their use in baked goods. Atti XVII Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Bologna, Cesena, 19-21 September, 2012

V. Bono (2012) Recovery of fibrous fractions from wine industry by-products and their use in baked goods. **POSTER**: XVII Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Bologna, Cesena, 19-21 September, 2012

C. Alamprese, S. Grassi, C. Picozzi, **V. Bono**, E. Casiraghi (2012), Applicazione di tecniche spettroscopiche IR al monitoraggio della produzione di latti fermentati. Atti 5° Simposio Italiano di Spettroscopia NIR, Agripolis, Legnaro (PD) 26-28 Settembre 2012.

C. Alamprese, S. Grassi, C. Picozzi, **V. Bono**, E. Casiraghi (2012), IR spectroscopy for monitoring the production of lactic acid fermented milks. **POSTER**: 5° Simposio Italiano di spettroscopia NIR. Agripolis, Legnaro (PD) 26-28 Settembre 2012.

V. Bono (2013). The use of fibrous fractions from grape skins in breadmaking. Atti del XVIII Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Padova, Conegliano, 25-27 September, 2013

V. Bono (2013). The use of fibrous fractions from grape skins in breadmaking. **POSTER**: XVIII Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Padova, Conegliano, 25-27 September, 2013

V. Bono, E. Casiraghi, G. Giovanelli (2013). Evaluation of phenolic content of wine by-products by NIR spectroscopy. Atti convegno NIR 2013 - 16th International Conference on Near Infrared Spectroscopy. La Grande-Motte (France), 2-7 giugno 2013.

V. Bono, E. Casiraghi, G. Giovanelli (2013). Evaluation of phenolic content of wine by-products by NIR spectroscopy. **POSTER**: NIR 2013 - 16th International Conference on Near Infrared Spectroscopy. La Grande-Motte (France), 2-7 giugno 2013.

V. Bono, M. Mariotti (2013). Effects of the addition of fibrous fractions recovered from grape skins on wheat dough and bread quality. **POSTER**: Cereals & Europe Spring Meeting 2013, Leuven, Belgium, 29-31 Maggio 2013.

S. Grassi, C. Alamprese, **V. Bono**, C. Picozzi, R. Foschino, E. Casiraghi, J.M. Amigo (2013). MCR applied to milk lactic acid fermentation monitoring. Atti convegno NIR 2013 - 16th International Conference on Near Infrared Spectroscopy. La Grande-Motte (France), 2-7 giugno 2013.

S. Grassi, C. Alamprese, **V. Bono**, C. Picozzi, R. Foschino, E. Casiraghi, J.M. Amigo (2013). MCR applied to milk lactic acid fermentation monitoring. **POSTER**: NIR 2013 16th International Conference on Near Infrared Spectroscopy. La Grande-Motte (France), 2-7 giugno 2013.

L. Azzini, **V. Bono**, C. Cappa, M. Lucisano, M. Mariotti, M. Romani, S. Feccia, A. Marti, M..A. Pagani (2014). Suitability of Near Infrared Spectroscopy to study the effect of water management on rice characteristics and cooking behavior. **POSTER and FLASH PRESENTATION**: NIR ITALIA 2014 - 6° Simposio Italiano di Spettroscopia NIR. Modena 28-30 maggio.

V. Bono (2014). Characterization of fibrous fractions from wine industry by-products and their use in baked goods. Atti del XIX Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Bari, Bari, 24-27 September, 2013.

V. Bono (2014). Characterization of fibrous fractions from wine industry by-products and their use in baked goods. **ORAL PRESENTATION**: XIX Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Bari, Bari, 24-27 September, 2013.

M. Lucisano, L. Azzini, **V. Bono**, C. Cappa, S. Feccia, M. Mariotti, A. Marti, M. A. Pagani, M. Romani (2014). Can irrigation conditions influence characteristics and cooking behaviour of rice? **ORAL PRESENTATION**: 4TH International rice congress. Thailandia, 27-31 October 2014.

M. Lucisano, L. Azzini, **V. Bono**, C. Cappa, S. Feccia, M. Mariotti, A. Marti, M. A. Pagani, M. Romani (2014). Can irrigation conditions influence characteristics and cooking behaviour of rice? Atti del convegno: 4TH International rice congress. Thailandia, 27-31 October 2014.

Recovery of fibrous fractions from wine industry by-products and their use in baked goods

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1. State of the Art

A relevant challenge for innovation is the development of foods with optimal dietary fiber and antioxidant contents. Scientific studies have demonstrated that a high fiber intake is associated with body weight control and a reduced risk of diseases such as colon cancer, diabetes and atherosclerosis (Bingham et al., 2003; Dana, 2005). To this regard, by-products from vegetable foods processing (e.g. grape, apple and tomato) are potential advantageous sources since they combine the presence of large amounts of dietary fibers and natural antioxidants. Grape pomace contains many substances which have beneficial health effects such as fibers (17-21%), tannins (16-27%), polyphenolic compounds (2-6.5%), lipids (7-12%), sugars (3%) and tartaric acid (Vermerris et al., 2006). However, grape stalk is currently distributed as fertilizer, while grape marcs are principally sold to distilleries for alcohol production, skins and seeds after distillation are used for tartaric acid and oil extraction, respectively. Some applications have considered the use of these by-products as food ingredients, after drying and milling (Sudha et al., 2007). Besides the optimization of processing technologies to convert them into multi-functional ingredients, adding value to by-products through the development of novel foods is a strategic research area. To this regard, bread is an interesting model for studying the rheological, macro- and micro-structural effects of fiber fortification on a food matrix (Mariotti et al., 2006).

2. PhD Thesis Objective and Milestones

This **PhD research project focuses** on the recovering of antioxidant- and fiber-rich skin powders from wine industry by-products. By-products will be evaluated also by NIR-MIR spectroscopy, to the create a predictive quality model. Finally, the use of wine making by- products for the production of baked goods of high technological and nutritional quality will be optimized.

Table 1. Gantt diagram

Activity	Months																																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
A1) Collection of by-products	■	■	■										■	■	■										■	■	■									
A2) Recovery and characterization of skins powders		■	■	■	■				■	■				■	■	■	■				■	■				■	■	■	■							
A3) Development of non destructive methods for screening			■	■	■	■	■	■							■	■	■	■	■	■																
A4) Skin powders use in baked goods formulations						■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		
1) Optimization of skin powders addition in dough						▒	▒	▒	▒	▒	▒	▒			▒	▒	▒	▒	▒								▒	▒								
2) Baking-test of innovative formulatons										▒	▒	▒	▒			▒	▒	▒	▒	▒	▒	▒	▒	▒	▒		▒	▒	▒	▒	▒	▒				
3) Baked goods quality evaluation during shelf-life												▒	▒	▒					▒	▒	▒	▒	▒	▒	▒	▒				▒	▒	▒	▒	▒		
A5) Thesis and paper preparation	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

The PhD project will be divided into the following activities
(Table 1)

A1. Collection of wine industry by-products
Batches of white and red grape pomace of different varieties will be obtained from wine-makers. Skins will be separated from the seeds.

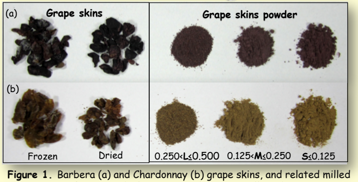

Figure 1. Barbera (a) and Chardonnay (b) grape skins, and related milled fractions (L, large; M, medium; S, small; mm)

A2. Recovery and characterization of grape skin powders
The dried products obtained will be finely milled. Chemical composition, water binding capacity, emulsifying properties, emulsion stability, color, antioxidant contents, antioxidant activity and other properties will be evaluated. The effects of different particle sizes of the various powders (Figure 1) on these properties will be also determined.

A3. Development of rapid, non destructive methods for screening
NIR and MIR spectroscopy will be used as rapid and non destructive methods to evaluate the quality of wine industry by-products.

A4. Applications of skin powders for the production of innovative baked goods
1) Effects of the presence of increasing levels of skin powders on dough networking
2) Baking of innovative formulations and evaluation of the final products
3) Baked goods quality evaluation during storage at controlled conditions

1) The properties will be investigated by farinographic test, rheofermentographic test, Image Analysis techniques, and fundamental rheological measurements to investigate the dough behaviour during mixing and leavening.

2) Baked goods formulations enriched with the grape skin powders will be baked. The final products will be characterized by means of different techniques

3) Selected samples will be submitted to accelerated shelf-life tests, and their quality during storage will be evaluated.

3.References

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XVII PhD Workshop 2012

On the Developments in the Italian PhD Research on Food Science Technology and Biotechnology
19-21 Settembre, 2012. Campus di Scienze degli Alimenti, Cesena

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IR spectroscopy for monitoring the production of lactic acid fermented milks

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INTRODUCTION

Lactic acid fermented milks are very popular products, appreciated for their sensory and healthy properties.

Only a thorough understanding and monitoring of the fermentation process can reduce the risk of product failure and assure quality reproducibility. Hence, one of the main requirements of the food industry is the availability of a rapid and non-destructive method providing real time information in order to assure an effective control at all stages of the process.

Therefore, **the aim of this study** was to evaluate the suitability of IR spectroscopy, joined with Partial Least Square Regression, as a tool for the simultaneous determination of the commonest fermentation indexes.

MATERIALS and METHODS

Skim milk powder was reconstituted to 10% (w/v) in distilled water, heat-treated and inoculated with approximately 10^6 cfu/mL of single or mixed (1:1) cultures of ***S. thermophilus*** and ***L. bulgaricus***, previously isolated from a commercial culture (Danisco A/S, Denmark). Milk fermentations were carried out in duplicate, for 7.5 h, at 37°C, 41°C and 45°C.

FT-NIR spectra were collected in continuous in diffuse transflectance (12,000-4,500 cm^{-1}; resolution 16 cm^{-1}; 64 scan), by using an MPA spectrometer (Bruker Optics, Italy) equipped with a fiber-optic probe.

FT-IR spectra were collected (4,000-700 cm^{-1}; resolution 4 cm^{-1}; 16 scan) every 45 min, by a Vertex 70 spectrometer (Bruker Optics, Italy) fitting a multiple reflection ATR cell with a germanium crystal.

Every 45 min, **pH**, **titratable acidity**, **microbial counts**, and **metabolite concentration** (galactose, lactic acid, and lactose) were also evaluated by means of conventional methods.

PLS (Partial Least Square) regression models were developed using pre-treated (1st derivative, Savitzky-Golay method) FT-NIR and FT-IR spectra. A cross-validation procedure with 10 CV was applied.

RESULTS and DISCUSSION

Fermentative trials were monitored through IR-spectroscopy and traditional methods: microbial plate counts, pH, titratable acidity and metabolites (galactose, lactic acid and lactose). The obtained results were used to develop PLS regression models.

As regards the FT-NIR spectra, only the region from 8,900 to 5,555 cm^{-1} was selected for PLS models (**Fig. 1a**). The main absorption peaks (7,150 cm^{-1}; 6,800 cm^{-1}) are due to the combination and first overtone bands of O-H bond, respectively [1].

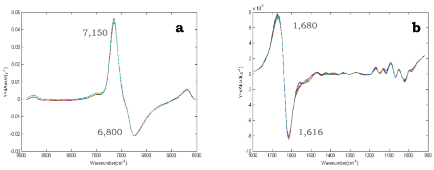

Figure 1. FT-NIR (a) and FT-IR (b) reduced spectra, after transformation in first derivative

The fingerprint region (1,800 - 970 cm^{-1}) of FT-IR spectra was used in PLS models (**Fig. 1b**). Peculiar absorption peaks were observed around 1,680 cm^{-1} and 1616 cm^{-1}, corresponding to the O-H bond of water and N-H bond of proteins, respectively [2-3].

Statistical parameters of calculated PLS models are reported in **Table 1**. In some cases, data originated from all the fermentation trials were used for regression; in other cases (as specified in the Table), better models were obtained selecting partial datasets on the basis of the tested inoculum.

Table 1. Statistical parameters of the PLS model calculated with FT-NIR and FT-IR data, for the prediction of fermentation monitoring indices.

		FT-NIR data					**FT-IR data**				
			Calibration		**Cross-validation**			**Calibration**		**Cross-validation**	
Parameter	**Range (Min-Max)**	**LV**	**R^2**	**RMSE**	**R^2**	**RMSE**	**LV**	**R2**	**RMSE**	**R2**	**RMSE**
pH	3.67 - 6.51	13	0.9639	0.1701	0.9327	0.2345	11	0.9762	0.1374	0.9542	0.1901
TA Lb (% ac. lattico)	0.13 - 1.18	9	0.9485	0.0203	0.8358	0.0364	9	0.9915	0.0081	0.9514	0.0020
TA St (% ac. lattico)	0.13 - 1.18	7	0.9451	0.0014	0.9100	0.0180	10	0.9743	0.0095	0.8548	0.0032
Lactose Lb+St (g/L)	3.02 - 8.05	16	0.9671	0.7263	0.8221	1.7155	20	0.9969	0.2240	0.7006	2.2269
Galactose (g/L)	0.02 - 3.45	13	0.8525	0.6435	0.8243	0.7601	13	0.8936	0.5401	0.7755	0.7879
Lactic Acid (g/L)	n.d. - 2.48	15	0.9307	0.5444	0.8556	0.7887	11	0.9632	0.3885	0.9233	0.5635
L. bulgaricus (log CFU/mL)	6.00 - 8.65	15	0.9457	0.2024	0.8566	0.3315	11	0.9247	0.2378	0.8204	0.3662
S. thermophilus (log CFU/mL)	6.35 - 8.65	13	0.8342	0.2886	0.6755	0.4067	9	0.9613	0.1385	0.7914	0.3296

LV, latent variables; R^2, correlation coefficient ; RMSE, root mean square error; Lb, *L. bulgaricus* inoculum; St, *S. thermophilus* inoculum; Lb+St, mixed inoculum, n.d., not detectable

High correlation coefficients and little standard errors were obtained in calibration for all the PLS models. The best results in prediction were obtained for pH, TA and lactic acid, the reference parameters typically required in fermentation monitoring.

CONCLUSIONS

The study demonstrated that IR spectroscopy, in combination with chemometric techniques, has potential to become a useful tool in monitoring of lactic acid fermentations. The major advantage of the presented approach is the simultaneous prediction of quantitative process variables which otherwise are not available on-line. The development of more robust models, based on industrial productions, is required.

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POSTER: 5° Simposio Italiano di spettroscopia NIR. Agripolis, Legnaro (PD) 26-28 Settembre 2012.

The use of fibrous fractions from grape skins in breadmaking

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Introduction

In recent decades, for economics as well as environmental reasons, there has been a continuous and growing emphasis on recovering, recycling and upgrading food wastes (Garau *et al*., 2007). Among others, one reason is the presence of natural bioactive compounds, such as dietary fibre (DF) and antioxidants, which could be of high value in the pharmaceutical, cosmetic and food industries (Femenia, 2007). The intake of these substances, in fact, has been related to important nutritional and health benefits. Thus, the importance of an adequate consumption of DF in our daily diet has promoted the development of fibre-rich products with added nutritional value. In particular, a growing interest has been observed in the exploitation of the residues generated by the wine-industry. This research aimed to investigate the **potential use of grape-skins** deriving from the wine-industry, as a source of dietary fibre and polyphenols in breadmaking. In particular, **two grape** (*Vitis vinifera* L.) varieties were considered: **Barbera** (red wine) and **Chardonnay** (white wine). Bread represents an interesting model system for studying the rheological, macro- and micro-structural effects of fiber fortification on a food matrix, and it also characterized by a worldwide consumption. It could thus represent a potential vehicle for important amounts of fiber and antioxidants.

Materials and Methods

Grape Skins (GS)
↓
Dried GS
(60°C)(a_W≤0.3)
↓
Milled GS
(controlled conditions)
↓
SAMPLES
↓

- **3 fibrous fractions*** from **Chardonnay** (**ChL**, **ChM**, **ChS**)
- **3 fibrous fractions*** from **Barbera** (**BaL**, **BaM**, **BaS**)
- **wheat flour** (**WF**)

powders particle sizes (mm): 0.250≤large (L)**<0.500; 0.125≤**medium (M)**<0.250; **small (S)**<0.125*

Raw Materials

6 MIXTURES
(10% integration level)
+
1 CONTROL (**WF**)

7 BREAD DOUGHs
from the following mixtures:
90WF-10ChL; **90WF-10ChM**; **90WF-10ChS**;
90WF-10BaL; **90WF-10BaM**; **90WF-10BaS**;
and from **100WF**

7 BREADS

Raw materials characterization: colour; moisture; protein; ash; fat; water binding capacity; soluble and insoluble dietary fibre; polyphenols; antioxidant activity

Mixtures and doughs evaluation: viscoamylographic test (Brabender® MVA); farinographic test (Brabender® Farinograph); rheofermentographic test (Chopin® Rheofermentometer)

Bread characterization: baking losses, weight, height, specific volume, crust and crumb colour, crumb holes geometrical features, liking test (on fresh breads); weight, moisture, aw, crumb softness (during storage)

Results and Discussion

RAW MATERIALS

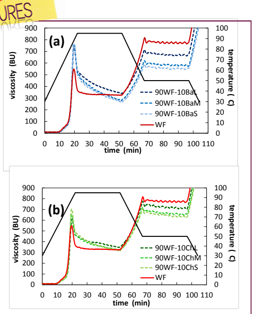
Tab. 1. Wheat flour and grape skins (GS) powders composition

Variables	Ch_L	Ch_M	Ch_S	Ba_L	Ba_M	Ba_S
Moisture (g/100g)	6.2^a ± 0.1	6.4^a ± 0.1	6.4^a ± 0.3	4.3^b ± 0.2	3.6^a ± 0.1	3.4^a ± 0.2
Fat (g/100 g d.b.)	4.2^a ± 0.2	5.9^b ± 0.3	7.3^c ± 0.2	4.1^a ± 0.2	6.8^b ± 0.2	7.7^c ± 0.3
Protein (g/100g d.b.)	9.8^a ± 0.4	10.4^{ab} ± 0.1	11.0^b ± 1.0	8.2^a ± 0.5	9.6^b ± 0.3	9.1^b ± 0.1
Total dietary fiber (g/100g d.b.)	51.7^a ± 2.0	54.4^a ± 0.5	51.8^a ± 4.1	57.5^a ± 3.4	54.1^a ± 3.4	54.8^a ± 1.6
Insoluble dietary fiber (IDF) (g/100g d.b.)	44.0^a ± 2.2	44.9^a ± 0.3	42.8^a ± 3.1	53.8^c±4.1	41.8^b ± 1.2	36.1^a ± 3.1
Soluble dietary fiber (SDF) (g/100g d.b.)	7.7^a ± 0.5	9.4^b ± 0.6	9.2^b ± 1.0	6.5^a ± 1.0	12.9^b ± 2.8	20.2^c ± 2.3
Ash (g/100g d.b.)	3.5^a ± 0.2	4.3^a ± 0.2	5.1^b ± 0.2	3.7^a ± 0.4	6.9^b ± 0.3	8.9^c ± 0.1
Water binding capacity (g H_2O/g d.b.)	2.9^a ± 0.1	3.3^a ± 0.3	2.9^a ± 0.1	2.7^a ± 0.1	2.6^a ± 0.2	2.2^a ± 0.1

Note: in the same raw, within the same GS variety, values followed by different letters are significantly (P<0.05) different.

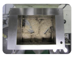
Interesting differences were evidenced both between the two varieties and within the same variety, among the various GS powders.

MIXTURES



When the temperature exceeded **60°C**, the **viscosity** of the systems **increased** rapidly and **to a greater extent** in the presence of **GS powders** (Fig. 1). Therefore, their constituents seem to be able to act in a **synergistic** way with the swelling of WF starch granules.

DOUGHS



Farinographic test: during mixing the replacement of WF with 10% of GS powders was associated with an **increase** in the **farinographic water absorption** values, mainly due to the high fiber content of the powders; at the same time, this supplementation was not associated with a severe deterioration of the technological characteristics of the wheat flour.

Rheofermentographic test: during leavening the presence of GS powder determined a lower development of the dough during proofing and a earlier time of dough porosity appearance, as expected. Indications on **short-term fermentation times** were obtained, in order to avoid both a breakdown of dough structure and an excessive CO_2 release.

BREAD

Fig. 2 Images of bread obtained from (a) 100 WF, (b) 90 WF – 10 BaL, (c) 90 WF – 10ChL.

Bread characteristics: Breads were characterized both just after the production and during storage for 48h at controlled conditions (25°C, 60% RH). The effects of GS powders supplementation were **positive and encouraging** in spite of the high GS powders supplementation adopted (10%, flour basis). In particular, the **specific volumes** of the composite breads (2.46-2.87mL/g vs. 3.65±0.25mL/g for 100% WF bread)(Fig. 2, Fig. 3) and the **crumb hardness** of the fresh products (0.32-0.80N *vs.* 1.01±0.24N for 100% WF bread, at 25% deformation level) are here underlined. The **greater crumb softness** exhibited by the fresh products was maintained **during** the whole investigated **storage** period (Fig. 4, Fig. 5).

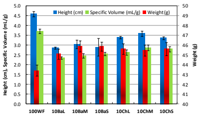


Fig. 3. Breads weight, height and specific volume

Positive and encouraging results were obtained, highlighting the potential use of wine industry grape skins (in which large amounts of dietary fiber and antioxidants are present) **for the production of innovative and nutritionally interesting baked goods.**

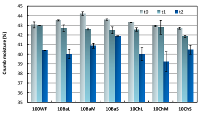


Fig. 4. Crumb moisture variations during storage

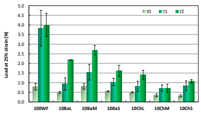


Fig. 5. Crumb hardness increase during storage

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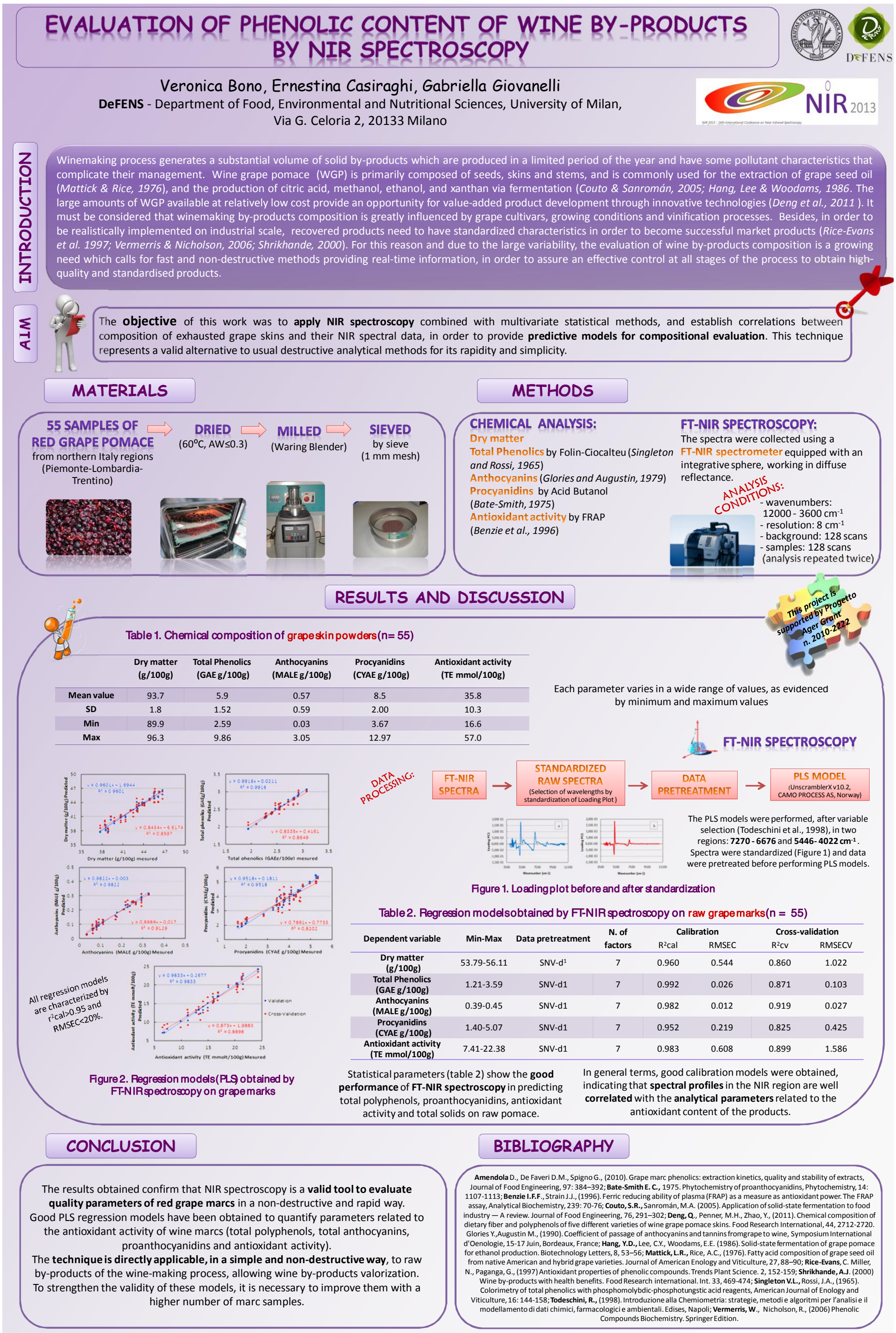

EVALUATION OF PHENOLIC CONTENT OF WINE BY-PRODUCTS BY NIR SPECTROSCOPY

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INTRODUCTION

Winemaking process generates a substantial volume of solid by-products which are produced in a limited period of the year and have some pollutant characteristics that complicate their management. Wine grape pomace (WGP) is primarily composed of seeds, skins and stems, and is commonly used for the extraction of grape seed oil (*Mattick & Rice, 1976*), and the production of citric acid, methanol, ethanol, and xanthan via fermentation (*Couto & Sanromán, 2005; Hang, Lee & Woodams, 1986*. The large amounts of WGP available at relatively low cost provide an opportunity for value-added product development through innovative technologies (*Deng et al., 2011*). It must be considered that winemaking by-products composition is greatly influenced by grape cultivars, growing conditions and vinification processes. Besides, in order to be realistically implemented on industrial scale, recovered products need to have standardized characteristics in order to become successful market products (*Rice-Evans et al. 1997; Vermerris & Nicholson, 2006; Shrikhande, 2000*). For this reason and due to the large variability, the evaluation of wine by-products composition is a growing need which calls for fast and non-destructive methods providing real-time information, in order to assure an effective control at all stages of the process to obtain high-quality and standardised products.

AIM

The **objective** of this work was to **apply NIR spectroscopy** combined with multivariate statistical methods, and establish correlations between composition of exhausted grape skins and their NIR spectral data, in order to provide **predictive models for compositional evaluation**. This technique represents a valid alternative to usual destructive analytical methods for its rapidity and simplicity.

MATERIALS

55 SAMPLES OF RED GRAPE POMACE from northern Italy regions (Piemonte-Lombardia-Trentino) → **DRIED** (60°C, AW≤0.3) → **MILLED** (Waring Blender) → **SIEVED** by sieve (1 mm mesh)

METHODS

CHEMICAL ANALYSIS:

Dry matter
Total Phenolics by Folin-Ciocalteu (*Singleton and Rossi, 1965*)
Anthocyanins (*Glories and Augustin, 1979*)
Procyanidins by Acid Butanol (*Bate-Smith, 1975*)
Antioxidant activity by FRAP (*Benzie et al., 1996*)

FT-NIR SPECTROSCOPY:

The spectra were collected using a **FT-NIR spectrometer** equipped with an integrative sphere, working in diffuse reflectance.

ANALYSIS CONDITIONS:
- wavenumbers: 12000 - 3600 cm^{-1}
- resolution: 8 cm^{-1}
- background: 128 scans
- samples: 128 scans

(analysis repeated twice)

RESULTS AND DISCUSSION

Table 1. Chemical composition of grape skin powders (n= 55)

	Dry matter (g/100g)	Total Phenolics (GAE g/100g)	Anthocyanins (MALE g/100g)	Procyanidins (CYAE g/100g)	Antioxidant activity (TE mmol/100g)
Mean value	93.7	5.9	0.57	8.5	35.8
SD	1.8	1.52	0.59	2.00	10.3
Min	89.9	2.59	0.03	3.67	16.6
Max	96.3	9.86	3.05	12.97	57.0

Each parameter varies in a wide range of values, as evidenced by minimum and maximum values

FT-NIR SPECTROSCOPY

DATA PROCESSING: FT-NIR SPECTRA → STANDARDIZED RAW SPECTRA (Selection of wavelengths by standardization of Loading Plot) → DATA PRETREATMENT → PLS MODEL (UnscramblerX v10.2, CAMO PROCESS AS, Norway)

The PLS models were performed, after variable selection (Todeschini et al., 1998), in two regions: **7270 - 6676** and **5446 - 4022 cm^{-1}**. Spectra were standardized (Figure 1) and data were pretreated before performing PLS models.

Figure 1. Loading plot before and after standardization

All regression models are characterized by $r^2cal>0.95$ and RMSEC<20%.

Figure 2. Regression models (PLS) obtained by FT-NIR spectroscopy on grape marks

Table 2. Regression models obtained by FT-NIR spectroscopy on raw grape marks (n = 55)

Dependent variable	Min-Max	Data pretreatment	N. of factors	Calibration		Cross-validation	
				R^2cal	RMSEC	R^2cv	RMSECV
Dry matter (g/100g)	53.79-56.11	SNV-d^1	7	0.960	0.544	0.860	1.022
Total Phenolics (GAE g/100g)	1.21-3.59	SNV-d1	7	0.992	0.026	0.871	0.103
Anthocyanins (MALE g/100g)	0.39-0.45	SNV-d1	7	0.982	0.012	0.919	0.027
Procyanidins (CYAE g/100g)	1.40-5.07	SNV-d1	7	0.952	0.219	0.825	0.425
Antioxidant activity (TE mmol/100g)	7.41-22.38	SNV-d1	7	0.983	0.608	0.899	1.586

Statistical parameters (table 2) show the **good performance** of **FT-NIR spectroscopy** in predicting total polyphenols, proanthocyanidins, antioxidant activity and total solids on raw pomace.

In general terms, good calibration models were obtained, indicating that **spectral profiles** in the NIR region are well **correlated** with the **analytical parameters** related to the antioxidant content of the products.

CONCLUSION

The results obtained confirm that NIR spectroscopy is a **valid tool to evaluate quality parameters of red grape marcs** in a non-destructive and rapid way. Good PLS regression models have been obtained to quantify parameters related to the antioxidant activity of wine marcs (total polyphenols, total anthocyanins, proanthocyanidins and antioxidant activity). The **technique is directly applicable, in a simple and non-destructive way**, to raw by-products of the wine-making process, allowing wine by-products valorization. To strengthen the validity of these models, it is necessary to improve them with a higher number of marc samples.

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MCR-ALS applied to milk lactic acid fermentation monitoring

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INTRODUCTION

Fermentation is one of the earliest methods adopted to obtain value-added milk products with an extended shelf-life. Despite its old manufacturing tradition, the dairy industry needs continuous improvement of methods providing real time information about the progress of the process. Among the most promising techniques, near-infrared (NIR) spectroscopy represents a fast and non-destructive alternative, able to simultaneously detect, after calibration, the main compounds involved in the fermentation process and to describe the trend of the process (Bock et al., 2008).

Soft-modelling methods, such as the multivariate curve resolution optimized by alternating least squares (MCR-ALS), demonstrated to be the most powerful method to describe the phenomena occurring in any kinetic reaction (Garrido et al., 2008) and seems to be suitable to extract information from FT-NIR spectra acquired during fermentation processes (Gonzàlez-Sàiz et al., 2008).

AIM

The purpose of the current work is to investigate the capability of **MCR-ALS** applied to **FT-NIR spectra** to extract in-line relevant information about **milk lactic acid fermentation dynamics**.

MATERIALS and METHODS

Skim milk powder was reconstituted to 10% (w/v) in distilled water, heat-treated and inoculated with approximately 10^6 cfu/mL of single or mixed (1:1) cultures of *S. thermophilus* and *L. bulgaricus*, previously isolated from a commercial culture (Danisco A/S, Denmark). Milk fermentations were carried out in duplicate, for 7.5 h, at 37˚C, 41˚C and 45˚C.

FT-NIR spectra were collected in continuous in diffuse transflectance (12,000-4,500 cm^{-1}; resolution 16 cm^{-1}; 64 scan), by using an MPA spectrometer (Bruker Optics, Italy) equipped with a fiber-optic probe.

Curd development was monitored on-line using a Physica MCR 300 rheometer (Anton Paar GmbH, Graz, Austria) through a dynamic oscillatory test (constant 1% strain, 1 Hz frequency).

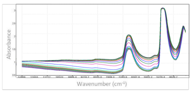
MCR-ALS analyses were performed by means of MCR-ALS software (http://www.mcrals.info/) implemented in MatLab v. 7.4 (The Mathworks Inc., Cambrige, UK). The spectra matrix (**D**) is decomposed by MCR-ALS into two sub-matrices, concentration, **C** ($M \times F$), and spectra profiles, $\mathbf{S}^T$ ($F \times N$).

ALS optimization:

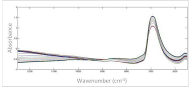
- Convergence criterion: 0.1%;
- Number of significant components: 3;
- The initial estimates: 3 spectra from D recorded at the beginning, middle and end of a representative fermentation batch;
- Constraints: non-negativity to **C** and $\mathbf{S}^T$, and unimodality to **C**.

RESULTS and DISCUSSION

FT-NIR SPECTRA

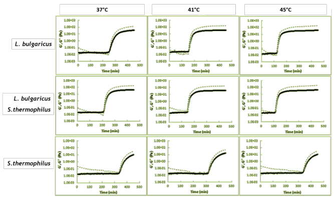


Spectral range reduced to 8,900-5,555 cm^{-1}
PRE-TREATMENT: SNV



FT-NIR spectra (12,500-4,500 cm^{-1}) collected during one of the fermentation processes performed at 41°C with the mixed starter culture (1:1) and reduced spectra (12,500-5,555 cm^{-1}) pre-treated by SNV.

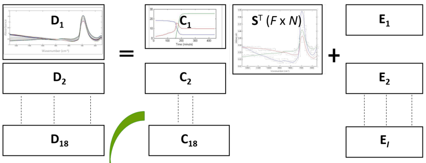
RHEOLOGICAL MEASUREMENTS



Rheological behaviours for one representative fermentation trial for each combination temperature-inoculum. Dash thin lines (--) represent the G' modulus and solid thick lines (—) the G'' modulus.

MCR-ALS

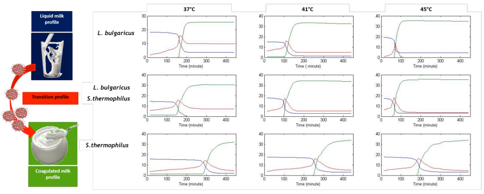
Depiction of MCR-ALS decomposition



99.9% of explained variance; %LOF: 0.63665; S.D. residuals <0.007

Concentration profiles

The obtained profiles contained information about the main changes occurring in the food matrix.



MCR-ALS pure component spectra: blue profile describes the liquid-like behaviour of milk, green profile reflects the solid-like behaviour of coagulated milk and the red profile represents the middle passage in the biotransformation.

CONCLUSIONS

MCR-ALS applied to FT-NIR spectra for monitoring milk fermentation provided a possible automated control system, which can be implemented in industrial productions to describe curd formation and, as a consequence, to define the end point of the fermentation.

In particular the comparison of MCR concentration profiles with the rheological measurements showed that is possible to follow the viscoelastic behaviour of curds, no matter the operative conditions used. Actually gel formation is the main physical change characterizing this biotransformation and even if it is extremely relevant in final product quality and customers' acceptance, there are no on-line systems available for its monitoring.

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Effects of the addition of fibrous fractions recovered from grape skins on wheat dough and bread quality

DeFENS

UNIVERSITÀ DEGLI STUDI DI MILANO
FACOLTÀ DI AGRARIA

C&E Spring Meeting 2013
May 29-31, 2013
Leuven, Belgium
UNLOCKING THE FULL POTENTIAL OF CEREALS: CHALLENGES FOR SCIENCE BASED INNOVATION

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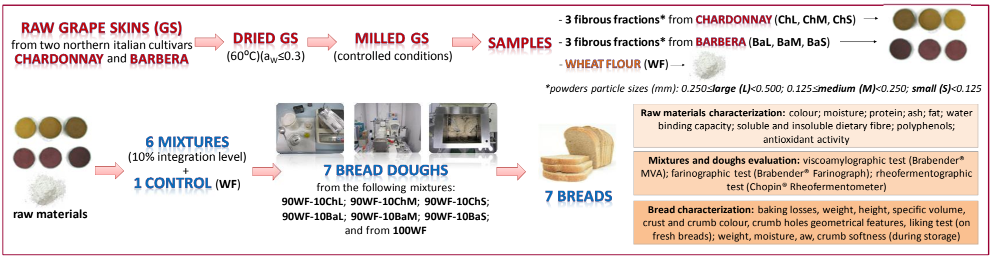
Introduction

Grape (*Vitis vinifera*) is one of the world's largest fruit crops, with an approximate annual production of 58 million metric tons (FAO, 2001). Grape pomace consists mainly of skins and seeds, and accounts for about 20% of the weight of the grape processed into wine. **Grape skins and seeds** are increasingly being used to obtain **functional food ingredients**, such as natural antioxidants and dietary supplements (Bagchi et al., 2000; Girard and Mazza, 1998; Saura-Calixto and Larrauri, 1999; Shrikhande, 2000). The major constituents of grape pomace, skins and seeds, in fact, is characterized by high polyphenolic and dietary fibre (DF) contents (Bravo and Saura-Calixto, 1998; Valiente et al., 1995) and the **large amounts** of grape pomace available at a relatively low cost is an interesting opportunity for the development of value-added products through innovative technologies.

To this regard, **bread** represents an interesting model system for studying the rheological, macro- and micro-structural effects of fiber fortification on a food matrix (Mariotti et al., 2006). One of the main directions in the cereal sector, in fact, is the use of wheat flour or flours deriving from other cereals with various 'healthy ingredients' in order to originate finished products having and improved nutritional quality. The development of foods having an optimal composition in terms of dietary fibre and antioxidant components is a relevant challenge for food innovation.

Materials and Methods



RAW GRAPE SKINS (GS)
from two northern italian cultivars
CHARDONNAY and **BARBERA**

DRIED GS
(60°C)(a_W≤0.3)

MILLED GS
(controlled conditions)

SAMPLES

- **3 fibrous fractions*** from **CHARDONNAY** (**ChL, ChM, ChS**)
- **3 fibrous fractions*** from **BARBERA** (**BaL, BaM, BaS**)
- **WHEAT FLOUR** (**WF**)

powders particle sizes (mm): 0.250≤large (L)**<0.500; 0.125≤**medium (M)**<0.250; **small (S)**<0.125*

raw materials

6 MIXTURES
(10% integration level)
+
1 CONTROL (**WF**)

7 BREAD DOUGHS
from the following mixtures:
90WF-10ChL; **90WF-10ChM**; **90WF-10ChS**;
90WF-10BaL; **90WF-10BaM**; **90WF-10BaS**;
and from **100WF**

7 BREADS

Raw materials characterization: colour; moisture; protein; ash; fat; water binding capacity; soluble and insoluble dietary fibre; polyphenols; antioxidant activity

Mixtures and doughs evaluation: viscoamylographic test (Brabender® MVA); farinographic test (Brabender® Farinograph); rheofermentographic test (Chopin® Rheofermentometer)

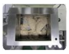
Bread characterization: baking losses, weight, height, specific volume, crust and crumb colour, crumb holes geometrical features, liking test (on fresh breads); weight, moisture, aw, crumb softness (during storage)

Results and Discussion

Table 1. Wheat flour and grape skins (GS) powders composition

	WF	BaL	BaM	BaS	ChL	ChM	ChS
Moisture (%)	14.28 ± 0.02	4.27 ± 0.24	3.64 ± 0.13	3.44 ± 0.21	6.20 ± 0.09	6.37 ± 0.07	6.44 ± 0.27
Protein (%db)	14.70 ± 0.18	8.23 ± 0.50	9.61 ± 0.31	9.13 ± 0.10	9.77 ± 0.39	10.41 ± 0.14	11.04 ± 0.95
Ash (%db)	0.63 ± 0.01	3.74 ± 0.35	6.92 ± 0.28	8.97 ± 0.03	3.52 ± 0.24	4.33 ± 0.23	5.10 ± 0.22
Fat (%db)	1.36 ± 0.08	4.07 ± 0.16	6.78 ± 0.21	7.71 ± 0.29	4.22 ± 0.21	5.89 ± 0.28	7.32 ± 0.21
WBC (gH_2O/g db)	0.90 ± 0.01	2.69 ± 0.07	2.60 ± 0.15	2.23 ± 0.05	2.95 ± 0.09	3.28 ± 0.31	2.89 ± 0.05
Soluble dietary fiber (g/100g db)		6.5 ± 1	12.9 ± 2.8	20.2 ± 2.3	7.7 ± 0.5	9.4 ± 0.6	9.2 ± 1.1
Insoluble dietary fiber (g/100g db)		53.8 ± 4	41.8 ± 1.2	36.1 ± 3.0	44.0 ± 2.2	44.9 ± 0.3	42.8 ± 3.1

Interesting differences were evidenced both between the two varieties and, within the same variety, among the various GS powders.



Dough behavior during mixing: the replacement of WF with 10% of GS powders was associated with an **increase** in the **farinographic water absorption** values, mainly due to the high fiber content of the powders; at the same time, this supplementation was not associated with a severe deterioration of the technological characteristics of the wheat flour.

Dough behavior during leavening: the presence of GS powder determined a lower development of the dough during proofing and a earlier time of dough porosity appearance, as expected. Indications on **short-term fermentation times** were obtained, in order to avoid both a breakdown of dough structure and an excessive CO_2 release.

When the temperature exceeded **60°C**, the **viscosity** of the systems **increased** rapidly and **to a greater extent** in the presence of **GS powders** (**Fig. 1**). Therefore, their constituents seem to be able to act in a **synergistic** way with the swelling of WF starch granules.

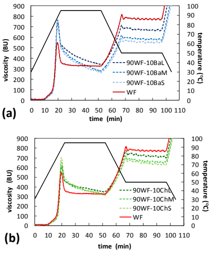


Fig. 1. Viscoamylographic profiles of the mixtures containing (a) **Ba** or (b) **Ch** grape skins powders

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Utilizzo della spettroscopia NIR per valutare l'effetto delle condizioni d'irrigazione sulle caratteristiche del riso, prima e dopo cottura

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Introduzione

L'**adattabilità del riso** (*Oryza sativa* L.) a **diverse condizioni irrigue** ha permesso lo sviluppo di metodi di coltivazione a diversa intensità dell'uso di acqua (Bouman *et al.*, 2001). Le diverse condizioni di irrigazione, tuttavia, potrebbero non solo influenzare lo stato nutrizionale della pianta ma anche le caratteristiche compositive della cariosside (rapporto amilosio/amilopectina, quantità di amido, profilo di gelatinizzazione) e, di conseguenza, il comportamento in cottura del riso lavorato (Ahmad *et al.*, 2009; Cheng *et al.*, 2003).

Scopo

L'obbiettivo di questa ricerca è stato quello di valutare le eventuali variazioni delle caratteristiche merceologiche, chimiche e reologiche dovute alle **diverse tecniche colturali** applicate a differenti varietà di *Oryza sativa* L. Lo studio è stato condotto attraverso l'utilizzo sia di tecniche tradizionalmente utilizzate per la caratterizzare del riso che mediante **l'utilizzo della spettroscopia NIR**, come tecnica rapida e non distruttiva per la valutazione delle caratteristiche del riso prima e dopo cottura.

Materiali e Metodi

4 varietà

3 sistemi di coltivazione caratterizzati da un uso via via più intenso dell'acqua:

- **DRY**, sommersione in 3°-4° foglia;
- **IRR**, semina interrata e irrigazione turnata;
- **FLD**, semina in acqua e sommersione continua.

Metodi

Resa globale, resa in chicchi interi e difetti: metodo ENR; **Contenuto in Azoto**: ISO 20483:2006 // **Contenuto in Amilosio**: UNI EN ISO 6647-1:2008; **Caratteristiche biometriche**: Image analysis, UNI 11106:2004; **Pasting properties**: Brabender® Micro-Visco-Amilografo; **Tempo di gelatinizzazione**: UNI ISO 14864:2004 // **Texture**: UNI 11301:2008; Aquisizioni **FT-NIR** in riflettanza diffusa (8 cm^{-1}).

Risultati e Discussione

I **profili viscoamilografici** del riso non hanno mostrato differenze in funzione delle tre condizioni di coltivazione: le diversità tra i tracciati sono infatti associabili alle caratteristiche quantitative e qualitative dell'amido delle quattro varietà, strettamente influenzate dal genotipo.

Rese e difetti

I diversi sistemi di coltivazione non hanno influenzato la **resa globale** di tutte e quattro le varietà (Figura 1). La **resa a granello intero** è risultata, invece, significativamente ($p<0.05$) inferiore nel secondo anno; solo per la varietà Gladio i sistemi di irrigazione non hanno modificato questo parametro.

Figura 1. Resa globale

I sistemi a ridotto regime idrico, inoltre, sembrano non aver influenzato la presenza di **chicchi macchiati e gessati**, difetti che riducono il valore merceologico del prodotto (Figura 2). Anche per le **caratteristiche biometriche**, non sono state registrate differenze in funzione della quantità di acqua somministrata durante la coltivazione.

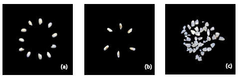


Figura 2. Grani gessati (a); grani macchiati (b); grani rotti (c)

Contenuto di azoto e amilosio

Il tenore in **azoto** è fortemente influenzato dall'annata di coltivazione: in tutte le varietà è significativamente ($p<0.05$) maggiore nel primo anno.

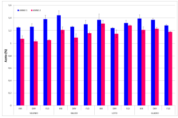
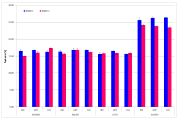


Figura 3. Contenuto in azoto (%)

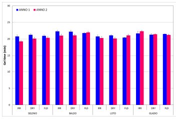


Come atteso, in entrambi gli anni di coltivazione il tenore di amilosio risulta maggiore nella varietà Gladio.

Figura 4. Contenuto in amilosio (%)

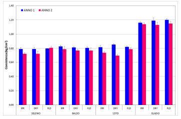
Comportamento in cottura

L'annata di coltivazione non sembra aver influito sul **tempo ottimale di cottura** (Figura 5):



solo per le varietà Selenio e Baldo tale indice risulta maggiore nel primo anno, mentre non è stato riscontrato un *trend* univoco per Loto e Gladio.

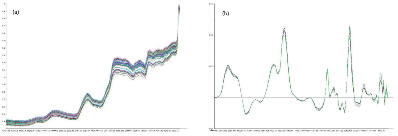
Figura 5. Tempo di gelatinizzazione (min)



La **consistenza** del chicco dopo cottura risulta maggiormente influenzata dalla varietà: come atteso sulla base del livello di amilosio, tali valori sono risultati nettamente superiori per la varietà Gladio

Figura 6. Consistenza del chicco dopo cottura (kg/cm^2)

Spettroscopia FT - NIR



Spettri grezzi di tutti i campioni di granella e spettri dopo trattamento di SNV, First Derivative.
Il *range* spettrale considerato utile per i successivi trattamenti è quello compreso tra 9083-4223 cm^{-1}.

Figura 7. Spettri grezzi (a) e dopo pretrattamenti (b): taglio spettrale (9083-4223 cm^{-1}), SNV e derivata prima

Le PCA sono state ricavate sia considerando unicamente gli spettri NIR (Figura 8) sia abbinando agli spettri NIR alcune variabili (Figura 9): con entrambe le valutazioni è stato possibile **suddividere i campioni** non solo in funzione **dell'annata di coltivazione**, ma anche in funzione **della varietà**, seppur con una minore discriminazione. Prendendo in considerazione ogni singola varietà, non sono state riscontrate differenze tra le tecniche di coltivazione.

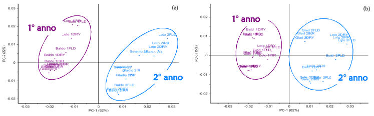


Figura 8. Score Plot PCA ottenuta dall'elaborazione degli spettri: (a) PC1+PC2; (b) PC1+PC3

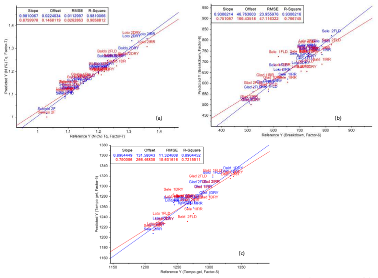


Figura 10. Rette di regressione in calibrazione e cross-validazione tra gli spettri FT-NIR e l'azoto (a), il *breakdown* viscoamilografico (b) e il tempo di gelatinizzazione (c)

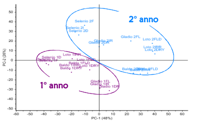


Figura 9. Score Plot PCA ottenuta dall'elaborazione degli spettri e degli indici qualitativi considerati

Valori di R^2cal superiori a 0,89 – associati a valori modesti dell'errore standard (RMSE) - sono stati ottenuti in calibrazione e cross-validazione per il tenore in azoto, *breakdown* viscoamilografico e tempo di gelatinizzazione.

Conclusioni

- **Nessuna** delle tre condizioni di **coltivazione** sembra aver **influenzato in maniera univoca** le caratteristiche merceologiche, compositive e reologiche delle varietà di riso nei due anni considerati.
- La mancanza di diversità imputabili alle tecniche colturali è stata ben evidenziata anche dalla **spettroscopia NIR**, in quanto tale tecnica ha suddiviso i campioni in due gruppi (ciascuno contenenti le quattro varietà), corrispondenti alle due annate di coltivazione. La spettroscopia NIR, rappresenta quindi una **tecnica idonea** per la previsione di alcuni parametri caratterizzanti la **qualità del riso**, normalmente valutati con metodi lunghi e laboriosi.

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POSTER and FLASH PRESENTATION: NIR ITALIA 2014 - 6° Simposio Italiano di Spettroscopia NIR. Modena 28-30 maggio.

5.2 List of papers

S. Grassi, C. Alamprese, **V. Bono**, C. Picozzi, R Foschino, E. Casiraghi (2013). Monitoring of lactic acid fermentation process using FT-NIR spectroscopy. Accepted for publication in: Journal of Near Spectroscopy.

S. Grassi, C. Alamprese, **V. Bono**, E. Casiraghi, J.M. Amigo (2013). Modelling Milk Lactic Acid Fermentation Using Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS). Accepted for publication in Food and Bioprocess Technology.