3. Development of baked goods enriched in fibrous fractions from wine-industry by-products

3.1 INTRODUCTION

It has been already proved that the advantages of using by-products in many food industry applications, such as bakery products, dairy products, meat products, fish products and so on, are due to the multiple properties of these ingredients, together with costs and environmental benefits. I Many studies are reported in the scientific literature about the advantages of adding dietary fibes obtained by industrial by-products in many foods (Blecker et al., 2001; Garcia et al., 2002; Sangnark and Noomhorm, 2004; Borderias et al., 2005), but at the same time they underline the large variability of fibres' properties and bioactive compounds (Elleuchet al., 2011). Furthermore, it appears that, to successfully develop fibre-enriched foods, it is necessary both to optimize the by-products recovery technologies and to closely evaluate the effects of the new ingredient incorporation, particularly in terms of sensory modification and micro- and macro-structural changes of the enriched products. In fact, fibre supplementation - in the case of a leavened product such as wheat bread - usually weakens the gluten network reducing the baking quality and decreasing bread volume (Mariotti et al., 2006). In accordance with this, Wang et al. (2002) underlined a decrease of the specific loaf volume but a higher softness of the bread crumb when using carob fibre, inulin and pea fibre. On the opposite, Sabanis et al. (2009) reported an improvement of loaf volume and crumb softness of gluten-free breads by adding dietary fibre from maize and oats, characterized by a fine particle size (99% were smaller than 100μm); the reason could be their lower perturbation of the dough organization in a system not containing gluten.

Even if the fortification of baked products is a convenient way to deliver beneficial compounds, such as fibres, to a large number of people, further studies have to be carried on. In fact, literature contains many reports about additions of dietary fibres to baked goods (e.g. Wang et al., 2002; Sangnark and Noomhorm, 2004; Mariotti et al., 2006) but few of them consider the application of fibres, from vegetable by-products for instance, in gluten-free bread (dedicated to a specific diet).

In this context, the winemaking by-products (grape pomace and grape seeds) represent interesting ingredients for the production of baked goods, also because they contribute to the flavor and functionality.

3.2 BREAD: STRAIGHT-DOUGH METHOD

3.2.1 MATERIALS AND METHODS

3.2.1.1 Grape skins, wheat flour, and bread mixtures

One red wine grape skins (**RWGS**) and one white wine grape skins (**WWGS**) were used in this study: *Vitis vinifera* L. cv. Barbera (**Ba**) and cv. Chardonney (**Ch**), respectively. Both were acquired from wine-makers and partners involved in Valorvitis (AGER project grant n. 2012-2222). The skins were oven-dried at 54°C until constant weight, milled, and sieved through an automatic sifter 10min; 6amplitude; Octagon Digital, Endecotts LtD, GB) equipped with three different certified sieves (openings: 125, 250 500 μm). The following grape skins powders fractions were obtained: 0.250 ≤ large (**L**) ≤ 0.500mm; 0.125 ≤ medium (**M**) ≤ 0.250mm; small (**S**) ≤ 0.125mm (Fig. 2). Six different grape skins powders (GSPs) were thus obtained: BaL, BaM, BaS; ChL, ChM, ChS.

Fractionation was performed both to allow a rapid and homogeneous dispersion of the powders into the food matrix and to evaluate the potential influence of particle size on the physical-chemical properties of the sample. The GSPs were then vacuum packaged (Reepack S.r.l., Seriate, BG, Italy), and stored in the dark at 4°C, until characterization.

For bread production, a commercial wheat flour (**WF**) was provided by Molino Valente (NOVA S.p.A, Felizzano, AL, Italy). The wheat flour, too, was stored vacuum packaged in the dark at 4 °C.

WF and the various GSP were mixed for 20 min at speed 1 in a Hobart N-50 Mixer (Troy, Ohio) before being used, in order to guarantee their homogeneous distribution in the final mass.

In addition to WF (reference), the following 6 mixtures were used in breadmaking:

1) BaS: 90% WF + 10% BaS;
2) BaM: 90% WF + 10% BaM;
3) BaL: 90% WF + 10% BaL;
4) ChS: 90%WF + 10% ChS;
5) ChM: 90% WF + 10% ChM;
6) ChL: 90%WF + 10%ChL.

The percentage of GSPs addition has been select on the basis of the results obtained by Hoye & Ross C.F. (2011). Fresh yeast (Lesaffre Italia S.p.A., S. Quirico, Trecasali, PR, Italy), sucrose (Eridania Sadam S.p.A., BO, Italy), salt (Italkali - Società Italiana Sali Alcalini S.p.A., PA, Italy), and extra virgin olive oil (Monini S.p.A., Spoleto, PG, Italy)(informazioni), were also used for the breadmaking process. according to the recipe reported in Tab. 20.

Table. 20 Bread recipe

INGREDIENTS	Amounts (g)
Mixture (90%WF[1]+10%GSP)	300
Oil[2]	6
Fresh yeast[3]	9
Sugar[4]	18
NaCl[5]	6

[1] Molino Valente, NOVA S.p.A (Felizzano AL, Italy); [2] Monini S.p.A., Spoleto, PG, Italy; [3] Lesaffre Italia S.p.A., S. Quirico, Trecasali, PR, Italy; [4] Eridania Sadam S.p.A., BO, Italy; [5] Italkali - Società Italiana Sali Alcalini S.p.A., PA, Italy.

3.2.1.2 Bread mixtures rheological properties

a) Pasting properties

The wheat flour, the 6 different grape skins powders, as well as the 7 composite mixtures, were all evaluated in terms of pasting behavior by means of a Brabender® MicroViscoAmylograph (MVA; Brabender OHG, Duisburg, Germany), that provides an accurate control of the time-temperature and shear profile applied. GSPs measurements required the setting up of a new method for the preparation of the sample. After some preliminary trials, the following amounts were used for the test: 25g of GSP in a total volume of 105mL. In the case of WF and of the six composite flours, the standard procedure was adopted for sample preparation: 15g of GSP in 100mL distilled water, scaling both flour and water weight on a 14% sample moisture basis. The suspensions were subjected (stirring at 250 min^{-1} and using a 300 $cm \cdot g_f$ cartridge) to the following standard temperature profile: heating from 30°C up to 95°C, holding at 95°C for 30min, cooling from 95°C to 50°C for 30min and cooling to 30°C. A heating/cooling rate of 1.5°C was applied. The following indices were considered (when possible, in relation to the pasting profile): gelatinization temperature (temperature at which an initial increase viscosity occurs; °C), peak viscosity (maximum paste viscosity achieved during the heating cycle; BU), final viscosity (paste viscosity achieved at the end of cooling cycle; BU) and setback (index of viscosity increase during cooling, corresponding to the difference between final viscosity after the holding period at 95°C; BU) (Mariotti et al., 2005).

b) Farinographic properties

The mixing properties of the 7 different bread mixtures were examined with a Brabender® Farinograph (Brabender, Duisburg, Germany). Because of a limited availability of GSPs, the tests were initially conducted with a small farinograph bowl (50g), in duplicate. Subsequently, adopting an appropriate conversion, the test was performed in a traditional 300g farinographic bowl. The following indices were taken

into account: water absorption (%), consistency (BU), development time (min), stability (min), tolerance index (MTI), time to breakdown (min), farinograph quality number.

c) Rheofermentographic properties

Dough development and the gas volume from the yeast activity were measured with a rheofermentometer (Chopin, Tripette & Renaud, Villeneuve La Garenne Cedex, France). The dough was prepared in the farinograph bowl by mixing, for 15 min at 30°C, the same ingredients used for simple doughs or for bread doughs. For simple doughs, wheat flour (or the mixture; 300g) were mixed with 8.4g compressed yeast (previously dissolved in water), 6g salt and the remaining water, according to the farinographic absorption value. For bread doughs production, WF and the 6 composite mixtures (300g) were mixed with 9g compressed yeast (previously dissolved in water), 18g sugar, 9g salt, 6g oil and the remaining water, according to the farinographic absorption value. The rheofermentographic test was performed for 3h at 30°C on 100g portion of the dough. The following parameters were taken into account: H_m, dough maximum height; h, dough development at the end of the test; $(H_m/h)/Hm$, decrease of the development of the dough at the end of test than maximum developing recorded; T_1, time at which the dough reaches the maximum height.

H'm, maximum height of the curve; T'1, time required to obtain H'm; Tx, time of dough porosity appearance; CO_2tot, total volume of gas produced; CO_2PR, volume of gas released during leavening; CO_2RL, volume of gas still present in the dough at the end of test; R_c, gas retention coefficient.

d) Image analysis

The image analysis was performed both on the dough on both slices of bread. In order to follow its structural changes during proofing, and its leavening kinetics, the dough was immediately collected from the farinographic bowl at the end of the mixing period, 6 aliquots (10 g each) were taken from the mass, rounded into a ball shape, placed each into a Petri dish, and put into a thermostatic chamber (30°C x 3 h). Images of the Petri dishes containing the leavening doughs were then acquired at fixed times: 0, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, and 180min. Therefore, for each formulation, 90 images were acquired, and a dataset of 630 doughs images, in total, was analyzed. The whole procedure was repeated two times.

Acquisition process

For the acquisition of dough images, during the acquisition process, performed with a flatbed scanner (Epson Perfection 3170 Photo, Seiko Epson Corp., Japan), samples were covered with a black box in order to prevent loss of light, and images were

acquired at a resolution of 300 dpi (dots for inch) and at a color depth of 24 bits. The captured images were then saved in uncompressed. TIFF format. To create the final dataset of images shown in Fig. 37, a region of interest (ROI) of 254 * 161 pixel was extracted from each single image. The ROI images were converted to 8 bit grey scale and subjected to spatial calibration before the image analysis processing.

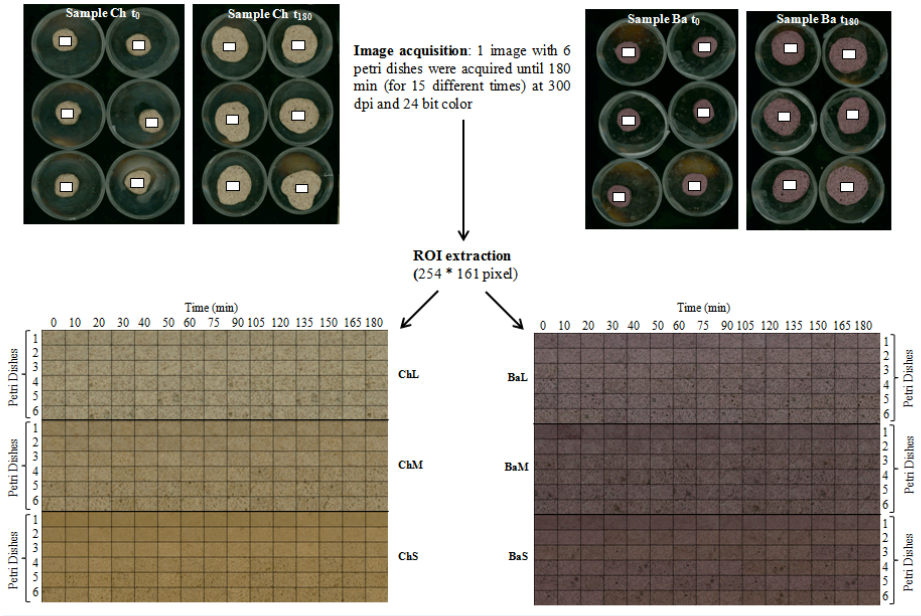


Figure 37 Construction of the whole image data set used for dough samples image texture analysis evaluation. On the left: images of samples from Chardonnay variety, with the tree different particle sizes: ChL, ChM and ChS; on the right, images of samples from Barbera variety, with the tree different particle sizes: BaL, BaM and BaS.

Dough leavening kinetics

The changes occurring in the doughs during leavening were determined following the variations in some of its morphological descriptors: diameter (mm), perimeter (mm), area (mm^2), and aspect (a shape descriptor). In order to extract these features, the .TIFF images of the doughs were processed by means of a dedicated software (Image Pro-Plus 4.5.1.29, Media Cybernetics Inc,MD, USA). In addition, according to the method proposed by Mariotti et al. (2010), the percentage of dough increasing area (IA, %), at each fixed checking time, was calculated as follows:

$$IA\ (\%) = \frac{A(tx) - A(t0)}{A(t0)} * 100$$

where $A_{(t)}$ is the mean area of the doughs in the 6 Petri dishes, measured at the prefixed times (t_x) during leavening, and $A_{(0)}$ is the mean area of the doughs in the 6 Petri dishes at the beginning of the proofing period (t_0). The dough expansion relative to the initial dimension was used to eliminate the effect of the different initial dimension of the various doughs. IA (%) values were then plotted as a function of proofing time, and the kinetics of the resulting curves were studied.

Surface texture analysis

The assessment of surface texture features was carried out by means of two different image texture analysis methods (Gray level co-occurrence matrix; Angle Measure Technique).

Gray level co-occurrence matrix

The gray level co-occurrence matrix (GLCM) is one of the mostly used statistical texture analysis method, and it is based on statistical calculations on the second-order histograms of the gray scale images (Haralick et al., 1973). It provides information about the distribution of gray-level intensities with respect to the relative position of the pixels with equal intensities. It calculates how often two pixels, in the matrix element $P_{\delta}(i, j)$, with intensity values i and j at a particular displacement distance δ from along a given direction θ (horizontally, vertically, or diagonally) occurs in the image (Barathi et al., 2004). Before building the matrix, two parameters θ (direction of the pixel pairs) and d (distance between the pixel pairs) need to be chosen: The direction θ can be selected from four different values (0, 45, 90, and 135°), while d depends on the resolution of texture (Bharati et al., 2004; Haralick et al., 1973). In most cases in the food industry, d is usually selected according to the properties of the foodstuff being investigated. When there is limited knowledge for selecting the optimal distance, the value of 1 or a group of different values is usually used (Zheng, Sun, & Zheng, 2006). In this study, the choices were: 1, 2, 3, 4, 5, 7 and 10.

Because of the high dimensionality of the resulting matrix, the individual elements of the co-occurrence matrix are rarely used for means of texture analysis. Instead, a large number of textual features can be derived from the matrix The following five features were used in this study: angular second moment (ASM shows the uniformity of an image; it is 1 for a constant image); inverse difference moment (IDM; illustrates the homogeneity of an image); contrast (CNT; shows the amount of local variations present in an image; it is 0 for a constant image); entropy (ENT; indicates the amount

of the order in an image; correlation (COR; for measuring the pixel linear dependencies (Zheng et al., 2006). For each image in the datasets, these five features were calculated for the seven chosen distances.
Images were processed with a specific plug in of the ImageJ v. 1.44c software (Rasband, 1997–2012, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/), named *GLCM_Texture* (http://rsb.info.nih.gov/ij/plugins/texture.html; Cabrera, 2005; Fongaro & Kvaal, 2013), revised in order to run on image stacks.

Angle Measure Technique
Applications of AMT for the surface texture characterization of different materials are still limited (Kucheryavski & Belyaev, 2009), in particular in the food area (Kvaal et al., 1998; Johansen et al., 2008; Fongaro & Kvaal, 2013). This AMT algorithm transforms the image into a one-dimensional spectrum without losses of the most important structure information. It is based on a three steps procedure: *i)* unfolding process (Johansen et al., 2008); *ii)* mean angle (MA) calculation and graphical representation (AMT spectra); *iii)* chemometric techniques application to elaborate the AMT spectra. A complete description of the AMT algorithm, and its implementation and optimization, is described in detail by Esbensen et al. (1996), Kucheryavski et al. (2008), and Kvaal et al. (2008).
In this work, a maximum scale of 500 pixels and a sampling of 500 points were used as AMT algorithm set up. To apply the AMT algorithm and obtain the AMT spectral data, the eAMTexplorer v.0.51 Software (Kvaal, 2006) was used. On the AMT spectra obtained from each image data sets, the principal component analysis (PCA) was applied, in order to find the sample class modeling only as a function of the surface texture characteristics. The models found were tested with cross-validation method, and the results have been expressed as sensitivity and specificity (Ortiz et al., 2006).

3.2.1.3 Bread making process

The various mixtures previously reported, as weel as the ‘reference’ sample (WF) underwent the same breadmaking process, defined on the basis of previous trials. In particular, a small scale breadmaking process (due to the lack of big amounts of GSPs) was setup during the research.
Doughs were prepared in the Farinograph bowl (Brabender OHG, Duisburg, Germany; 300 g), setting the temperature at 30°C. Powders (300g WF or mixture) were pre-mixed for 1 min, and the other ingredients were added within the next 1min: 18g sugar (previously suspended in an aliquot of water), 9.0g fresh yeast (previously suspended in an aliquot of water), 6.0g extra virgin olive oil, 6.0g salt (previously suspended in an aliquot of water). The remaining amount of water (to a total of 187 mL) was carefully

added, and kneading was carried out for 15 min. The optimized recipe and procedure come from preliminary trials. The optimum water amount and the optimum mixing time were determined by preliminary farinographic tests carried out on the control (WF) and on the mixtures.
At the end of the kneading period, 6 aliquots (50g each) of the dough were placed into small baking moulds. The doughs were leavened at 30°Cx80% RHx40min (Haereus Vötsch, mod. HC0020; Frommern, Germany), then shaped and leavened again at 30°C and 80% RH for 60min. At the end of the proofing period, samples were baked in an oven (IGNIS- Whirpool Europe s.r.l, Comerio VA, Italy) for 14min at 180°C.
After baking, the samples were allowed to cool down to room temperature for 1h, before being removed from their mould for the analyses.

3.2.1.4 Bread: physicochemical properties

a) Height, weight and specific volume

After baking, the loaves were removed from the pan and cooled for 60min at room temperature before being characterized for weight (g; n=6), height (mm; n=6), and volume (mL; n=6; rapeseed displacement methos). The specific volume (mL g^{-1}) was calculated.

Bread samples were then packaged in paper bags (as for a domestic shelf-life) and stored at controlled conditions (20°C, 60%RH) for 3 days. At prefixed times during storage (3h, 22), the stored samples were further investigated. Two bread loaves for each formulation, at each storage time, were weighed (to evaluate weight loss during storage, %), then transversely sliced to obtain uniform 25mm slices, and further characterized as follows.

b) Moisture and water activity

The moisture of the central slice (HuSL, %; n=3) and of the crumb core (HuCC, %; n=3) were determined using a single-stage drying process for 15h at 105°C, in accordance with previous studies (Mariotti et al. 2013). Bread weight loss (%; difference between the fresh bread weight and the weight of the bread stored for prefixed times, divided by the initial weight), was evaluated, too. The crumb core water activity (aw; n=2) was measured by the Octagon Aqualab Series 3 (Decagon Devices Inc., Pullman, USA).

c) Color

Crumb and crust color were measured using a chroma meter (Minolta CR-100, Osaka, Japan; 8 mm measuring area and standard illuminant C conditions, 6774K), recording the L*a*b* values (n=6). Lightness, chroma, and hue were determined.

d) Texture

Variations in breadcrumb softness were assessed by means of a compression test performed with a TA-HD plus Texture Analyzer (Stable Micro Systems, Surrey, UK), equipped with a 500N load cell. The Texture Exponent TEE32V 3.0.4.0 Software (Stable Micro System, UK) was used to control the instrument and for data elaboration. Bread slices (3 from each sample) were compressed up to 40% deformation, using a 36mm diameter cylindrical probe, at a compression speed of 1.7mm/s. The following parameters were evaluated: crumb hardness (N; load at 25% deformation) and Young's Modulus (N/mm^2; slope of the first linear trait of the stress *vs.* strain compression curve). At least ten replicates ($n \geq 10$) were performed for each bread recipe, at each storage time.

e) Image analysis

As already mentioned for the doughs, various Image Analysis techniques were adopted for investigating bread features, too. Images of 4 slices (15 mm thick) coming from 3 breads, for each formulation, were acquired. Therefore, 12 images were acquired for each bread mixture, and a dataset of 84 bread crumb images, in total, was analyzed.

Acquisition process

As previously mentioned for the acquisition of dough images, during the acquisition process, performed with a flatbed scanner (Epson Perfection 3170 Photo, Seiko Epson Corp., Japan), samples were covered with a black box in order to prevent loss of light, and images were acquired at a resolution of 300 dpi (dots for inch) and at a color depth of 24 bits. The captured images were then saved in uncompressed. TIFF format. To create the final dataset of images shown in Fig. 38, a region of interest (ROI) of 254 * 161 pixel was extracted from each single image. The ROI images were converted to 8 bit grey scale and subjected to spatial calibration before the image analysis processing.

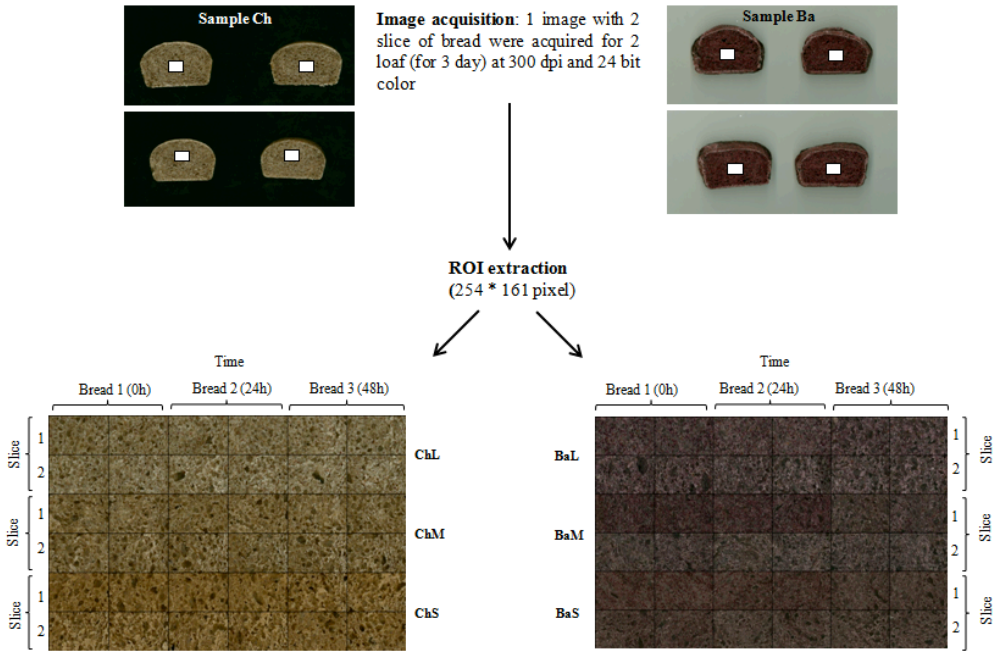


Figure 38. Construction of the whole image data set used for bread sample image texture analysis. On the left: images of samples from Chardonnay variety, with the tree different particle sizes: ChL, ChM and ChS; on the right, images of samples from Barbera variety, with the tree different particle sizes: BaL, BaM and BaS

Surface texture analysis

The assessment of bread surface texture features was carried out by means of two different image texture analysis methods (Gray level co-occurrence matrix; Angle Measure Technique), as already reported for the doughs, at paragraph 3.2.1.2.

3.2.1.5 Bread: liking test

Breads were also submitted to the sensory analysis in order to assess the consumers acceptability of breads enriched with fibeous fractions Chardonnay and Barbera skins; investigate the consumers' willingness to buy the enriched samples.

The samples were prepared at UNIMI laboratories. After production, the loaves were frozen and carefully transported, as frozen samples, to the Laboratory of Sensory Analysis UNISG, where they were stored in the freezer (-18 ° C) till their evaluation. Three hours before the sensory evaluation, the loaves were removed from the freezer, thawed for 2h, and cut into 1cm thick slices. Bread slices were placed in an electric

oven at 180°Cx50s, and served in plastic containers, hermetically closed (volume: 236 mL; and conical form; upper diameter: 11.5cm; bottom diameter: 10cm; height: 3cm). Each sample was identified by a random three-digit numeric code. The samples were presented sequentially and in random order and monadic balanced between subjects. An example of a set of samples submitted to the taster is shown in Fig. 39.

Figure 39. Samples of breads (a), viewing Booth (b), participants (c).

The participants (n=98, 37 men and 61 women; average age:25 years) were recruited from the students and the staff of UNISG. They were required to perform a test, consisting of two parts, according to the structure shown in Tab. 21.

Table 21. Test structure of the bread liking test with the consumers

Part	Response	Condition
1	• Rating: – Odor – Taste – Flavour – Texture – Overall liking	Appearance of the samples masked (BLUE LIGHT)
2	• Overall liking • Availability to buy	WHITE LIGHT

The instructions required first the subjects to rinse their mouth with water before starting the test, then tasting half of each sample, storing the remaining half in the container by closing the lid. This phase was conducted with blue light, and tasters were asked to express an opinion of liking for each sample with respect to: odor, taste, flavor, texture and overall liking. After the evaluation of all the samples, tasters were askedto turn off the blue light, switch on the white light, and test again the samples, consuming the remaining half. During the second part of the test, they were asked to express an opinion on their overall liking and willingness to buy the tasted samples.

The liking of all considered items was rated on a 9 points category scale (1 = extremely unpleasant, 2 = very unpleasant, 3 = unpleasant, 4 = little unpleasant, 5 = neither pleasant nor unpleasant, 6 = little pleasant, 7 = pleasant, 8 = very pleasant, 9 = extremely pleasant) and for the willingness to buy the sample (*"Would you buy this product?"*) a 7 points rating scale was used (1 = definitely no, 2 = no, 3 = probably not, 4 = do not know, 5 = probably yes, 6 = yes, 7 = definitely yes).

A 20s break was observed when moving from one sample to the next one, in which the subjects were asked to rinse their mouth with water, while a 2min break was introduced between the first and the second part of the test.

At the end of the test consumers were asked to answer the following questions using a 7 point scale (1 = not at all attractive, 7 = very interesting):

- Do you think that baked goods containing pomace and seeds may be of interest?
- Do you think that baked products containing antioxidants from grapes may be of interest?

Data were acquired automatically with software version 2.47B Acquisition FIZZ (Biosystèmes, France).

3.2.1.6 Statistical analysis

Analysis of variance (ANOVA) and significant correlations were performed on the

data adopting the least significant difference (LSD) and Pearson correlation analysis procedure, at a P<0.05 confidence level. Data were processed using Statgraphics®Plus v. 5.01 (StatPoint, Inc., Herndon, Virginia, USA). The UnscramblerX software (v. 10.1, Camo, Inondhcim, Norway) was used for Principal Component Analysis (PCA) analysis. To model the kinetics of the dough leavening, Table Curve 2D v.4 (Systat Software Inc., San José, California, USA), and a negative exponential function, were used. For the sensory evaluation, fixed two-way ANOVA models (tasters and samples as fixed factors) were separately applied to the analysis of the mean values obtained for the liking of appearance, odor, taste, flavor, texture and overall liking (Part 1) and for the satisfaction and willingness to buy (part 2). The ANOVA was followed by Fisher's LSD test (p <0.05) (FIZZ Calculations version 2.47B, Biosystèmes, France). On average scores refer to the overall enjoyment assessed under white light was made an ascending hierarchical cluster analysis (Euclidean distance, Ward method binding) to detect the presence of groups of consumers with similar preferences (XLSTAT version 2011.3.02, Addinsoft) To evaluate the influence on the appearance of the cluster index, was compared to the average rating of each sample evaluated in a position to look masked (light blue) and visible appearance (white light) with a paired t-test (XLSTAT version 2011.3 .02, Addinsoft) for each cluster.

3.2.2 RESULTS AND DISCUSSION

3.2.2.1 Bread mixtures: rheological properties

a) Pasting properties

The differences observed in the chemical and physical properties among WF and the various grape skins powders (GSPs) included in the bread mixtures were highlighted by the broad variability in the viscoamylograph properties. Results have been reported in Tab. 22 (viscoamylographic indices) and Fig. 41 (viscoamylographic profiles). When the temperature exceeded 60°C, the viscosity of the system began to increase rapidly, and to a greater extent in the presence of GSPs. The fiber present in the mixtures, therefore, seemed to be able to act in a synergistic way with the swelling of starch granules. Similarly, fibers appeared to interfere with the reorganization of the starchy matrix during the cooling of the systems (retrogradation), since the increase in viscosity of the blends was smaller if compared to that of WF (thus suggesting a lower tendency to retrogradation). Generally, the presence of Ba GSPs determined higher peak viscosities and lower tendencies to retrogradation, in comparison to Ch. In addition, the effect of particle size was very evident within each grape variety: the lower the particle size, in fact, the higher the peak viscosity and the lower the tendency to retrogradation.

Figure 40. Example of the slurries obtained the end of the viscoamylographic test

Table 22. Bread mixtures viscoamylographic parameters

	100 WF			90WF-10BaL			90WF-10BaM			90WF-10BaS			90WF-10ChL			90WF-10ChM			90WF-10ChS		
Pasting temperature (°C)	61.4	±	0.5	63.3	±	0.3	63.6	±	0.4	63.2	±	1.4	63.8	±	0.4	63.6	±	0.1	64.0	±	0.1
Peak viscosity (BU)	567	±	15	735	±	12	770	±	7	779	±	31	622	±	14	655	±	6	710	±	9
Viscosity 95°C (BU)	388	±	2	560	±	5	547	±	9	546	±	12	481	±	4	486	±	5	517	±	5
Viscosity 30x95°C (BU)	331	±	5	349	±	8	281	±	3	268	±	9	355	±	7	327	±	2	321	±	4
Viscosity 50°C (BU)	797	±	11	722	±	23	614	±	4	578	±	6	766	±	15	708	±	1	696	±	9
Viscosity 30x50°C (BU)	779	±	11	691	±	26	581	±	13	553	±	2	717	±	21	661	±	4	637	±	14
Viscosity 30°C (BU)	1250	±	22	1078	±	22	928	±	1	876	±	1	1079	±	60	1071	±	7	1032	±	14
Breakdown (BU)	1274.3	±	53.6	1108.3	±	43.4	982.5	±	0.7	926.0	±	1.4	1148.5	±	74.2	1096.5	±	19	1094	±	14.1
Setback 50°C (BU)	236.3	±	11.9	386.3	±	10.8	489.0	±	4.2	511.5	±	21.9	266.5	±	6.4	328.5	±	3.5	388.5	±	4.9
Setback 30°C (BU)	448.0	±	7.0	341.7	±	19.3	300.0	±	9.9	285.5	±	12.0	362.0	±	14.1	334.5	±	2.1	315.5	±	9.2

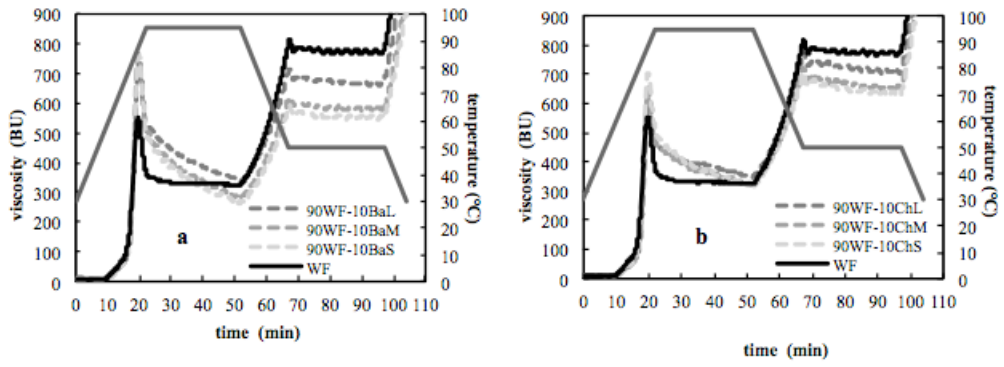


Figure 41. Viscoamylographic profiles of the bread mixtures made of WF and Ba grape skins powders (a) or Ch grape skins powders (b).

b) Farinographic properties

Results related to bread mixtures behavior during mixing have been reported in Tab. 23 (farinographic indices) and Fig. 42 (farinographic profiles). The replacement of the wheat flour with 10% GSPs was generally associated with an increase in the farinographic water absorption, mainly due to the high fiber content of GSPs (Tab. 23). Grape skins contain 51-57% of dietary fiber, mostly as cellulose, which has recently been shown to have a unique organizational structure (Lu et al., 2012). Grape skins cellulose is made up of small spherical microcrystals, not fibers as found in wood or cotton. This structure provides great potential for texture options and lends itself to better incorporation and coating in mixtures. GSPs also can be used to introduce more acids, mostly tartaric and malic, to help in conditioning a rapid-rise of the doughs. Another effect of GSPs presence in the mixtures was related to doughs stability: this index decreased from 19.0min for WF to 8.0-9.9min for the composite bread mixtures. Despite this variation, the composite doughs still exhibited a good tolerance to mixing. The addition of GSPs to wheat flour, therefore, did not appear detrimental in relation to the bread making properties of the mixture. This could be probably due to the presence of soluble fiber, which has the ability to increase the viscosity of the system, while still providing the formation of a gel that "stabilizes" the dough during the ‘process’.

Table 23. Bread mixtures farinographic indices

	100WF	90WF-10BaL	90WF-10BaM	90WF-10BaS	90WF-10ChL	90WF-10ChM	90WF-10ChS
Hu (%)	14.3	13.3	13.2	13.1	13.4	13.3	13.3
Water Absorption (%)	60.4	63.1	63.1	62.8	62.6	62.5	62.5
Consistency (FU)	503.7	513	528	498	511	516	510
Development time (min)	7.8	8	7.2	7.2	1.9	7.4	7.3
Stability (min)	19.0	8	8.7	9.9	8.6	8.5	9.6
Tolerance Index (MTI)	14.7	70	69	53	6	82	68
Time to breakdown (min)	19.3	9.4	9.1	10.5	9.2	8.9	10
Farinograph quality number	192.7	94	91	105	92	89	100

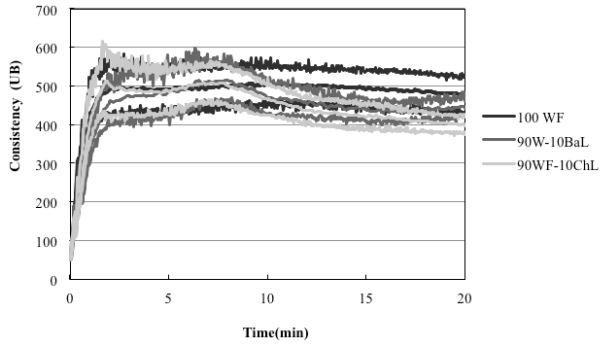


Figure 42. Examples of bread mixtures farinograms:90WF-10BaL and 90WF-ChL, in comparison to WF.

c) Rheofermentographic properties

In order to evaluate the ability of the composite doughs in retaining the CO_2 produced during the leavening phase, a specific rheofermentographic test was developed, as reported at paragraph 3.2.1.2. Results have been reported in Tab. 24 (dough development) and Tab. 25 (CO_2 production and retention). In general, the presence of GSPs determined a lower development of the dough during proofing and an earlier time of dough porosity appearance, as expected. This was particularly true for Ba composite bread mixtures, while better results were obtained when Ch was present in the mix. Generally, indications on short-term fermentation times were thus obtained, in order to avoid both a breakdown of the dough structure and an excessive CO_2 release.

Table 24. Bread mixtures rheofermentographic indices: dough development.

Sample	H_m (mm)	h (mm)	$(H_m/h)/Hm$ (%)	T_1 (min)
100 WF	18	16.7	8.15	142.25
90WF-10BaL	12.2	9	11.1	114
90WF-10BaM	11.3	11.2	0.9	145.5
90WF-10BaS	13.9	13.9	0	180
90WF-10ChL	15	13.5	10	162
90WF-10ChM	16.7	16.7	0	180
90WF-10ChS	15	15	0	180

Note: H_m, dough maximum height; h, dough development at the end of the test; $(H_m/h)/Hm$, decrease of the development of the dough at the end of test than maximum developing recorded; T_1, time at which the dough reaches the maximum height.

Table 25. Bread mixtures rheofermentographic indices: CO2 production and retention

Sample	H'm (mm)	T'1 (min)	Tx (min)	CO_2 tot (ml)	CO_{2PR} (ml)	CO_{2RL} (ml)	R_c (%)
100 WF	25.4	134.25	114	597	7.5	589.5	98.8
90WF-10BaL	24.6	144	126	557	12	545	97.8
90WF-10BaM	26.4	172.5	94.5	614	20	594	96.7
90WF-10BaS	27.8	170.5	105	615	19	596	97
90WF-10ChL	21.2	178.5	142.5	418	5	414	98.9
90WF-10ChM	21.2	180	172.5	412	4	409	99.1
90WF-10ChS	21.7	180	153	417	5	412	98.9

Note: H'm, maximum height of the curve; T'1, time required to obtain H'm; Tx, time of dough porosity appearance; CO_2tot, total volume of gas produced; CO_2PR, volume of gas released during leavening; CO_2RL, volume of gas still present in the dough at the end of test; R_c, gas retention coefficient.

d) Image analysis

Dough morphological features: variations during leavening

By means of Image Analysis, accurate measurements of dough development during leavening at 30°C into Petri dishes (Fig. 37), in terms of geometrical indices (perimeter, diameter, area, aspect), were performed at prefixed times. Results have been graphically reported in Fig. 43 as a function of time. In addition, ANOVA was performed on the data obtained, in order to verify if significant differences ($P<0.05$) could be evidenced between the red and white GSPs and, within each grape variety, among the various batches having different particle sizes (*data not reported*). Statistically significant differences ($P<0.05$) were highlighted among the formulations at the different leavening times (0, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, and 180 min) in terms of dough area, perimeter and diameter. The greater variability was observed at the beginning of the leavening phase; the morphology of

the various doughs, in fact, changed quickly throughout the first proofing period and then reached a *plateau*.

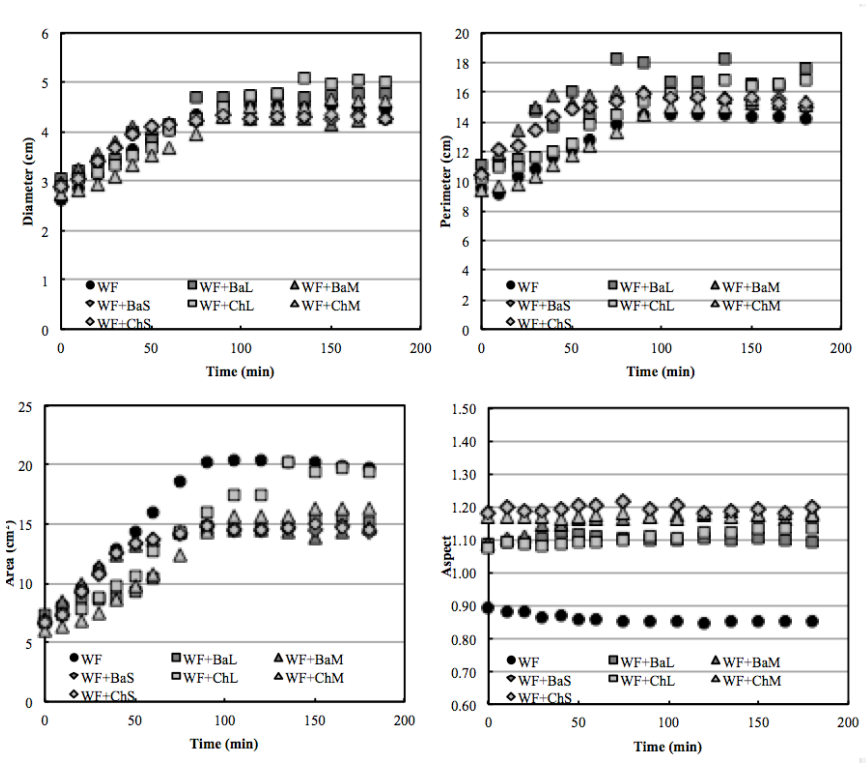


Figure 43. Area, diameter, perimeter, and aspect values of the dough, asmeasured at the different leavening times.

In particular, WF morphological parameters reached the stability after 105min leavening, while the doughs containing Ba GSP reached a *plateau* after 60min. Leavening behavior similar to WF were exhibited by the doughs containing Ch GSP, except for WF-ChS, whose changes in morphology stopped after 40min leavening. In terms of aspect, a significant difference ($P<0.05$) was evident between WF and the enriched doughs: values lower than 0.90 characterized WF, while values between 1.05-1.20 were obtained for the enriched doughs. Despite this difference, a common behavior was observed for all the doughs: they all changed their dimensions, during leavening, but not their shapes (no significant differences, in relation to leavening times, within each sample).

Dough leavening kinetics: fitting of the curves

In addition to the morphological features, the percentage of dough increasing area (IA, %), at each fixed leavening time $IA_{(t)}$, was calculated. In order to better describe the

leavening kinetics related to the various doughs, values were plotted as a function of time (Fig. 44a) and the resulting curves were studied (Fig. 44b). An exponential function was found to be the best curve fitting the experimental $IA_{(t)}$ data points:

$$y=a\ (1-e^{-bt}) \qquad \textit{Equation 1}$$

where: "*y*" is the experimental IA*(t)* obtained at the different checked leavening times; "*a*" is a coefficient obtained from the fitting of the curves, related to IA at an infinite leavening time (i.e. the asymptotic value); "b" is a coefficient obtained from the fitting of the curve, related to the speed of the phenomenon; "t" is the leavening time.
As the final estimation of the doughs increasing area is performed after 180 min leavening, the *a* index could be substituted by IA_{∞} (assuming $IA_{180min} \approx IA_{\infty}$). Eq. 1 could thus be written as follows:

$$IA_{180min}=IA_{\infty}\ (1-e^{-kt}) \qquad \textit{Equation 2}$$

The IA_{180min} obtained after the fitting of the experimental data points (FIA_{180min}) and the related regression coefficients (r^2) have been reported in Tab. 26. The high values of the regression coefficients indicate that the association of the dough leavening kinetics to an exponential equation could be a good way of interpreting the phenomena.
Moreover, being the k coefficients related to the speed of the phenomena, it was possible to record how quickly the increase of dough area took place. Lower k coefficients (ranging from 0.0045 to 0.0071) were evidenced by the WF, WF-BaL, WF-ChL, and WF-ChM doughs, indicating slower progressions of the leavening phase, in comparison to those of the other enriched doughs. It is interesting to notice that the fastest increases were observed for those doughs containing GSP characterized by the smaller particle size. GSP particle size could therefore influence dough-leavening kinetics. To attest this observation, the $IA_{(t)}$ kinetics of the doughs were also studied applying the first derivatives, getting the maximum speed of their growth. For the doughs obtained from WF, WF-BaL, WF-ChL, and WF-ChM the maximum speed development was obtained at 45min, while for the doughs obtained from WF-BaM, WF-BaS and WF-ChS the highest growth rate was evidenced at 30min, 32min and 33min, respectively. In addition, the first derivative of the dough obtained from WF, WF-BaL, WF-ChL and WF-ChM never reached the zero, indicating that these doughs are still growing even after 180min.

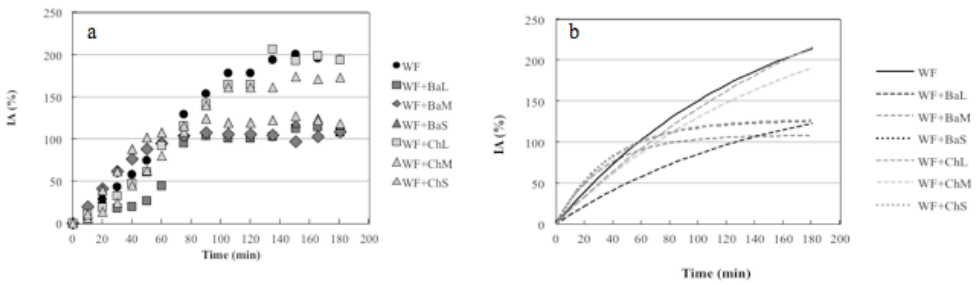


Figure 44. Experimental IA(%) values of the different doughs integrated with Barbera and Chardonnay grape skin powders (a) and fitting of the experimental data using the exponential kinetic $y=a(1-e^{-bx})$ (b)

Table 26. Coefficients obtained after fitting the exponential points of the percentage of the increasing area (IA, %).

Sample	**IA(%)$_{180min}$**	**r^2**	**k** (min)
WF	297.9	0.98	0.0071
WF-BaL	174.1	0.94	0.0067
WF-BaM	108.4	0.99	0.0302
WF-BaS	126.8	0.98	0.0264
WF-ChL	388.0	0.98	0.0045
WF-ChM	286.3	0.97	0.0061
WF-ChS	128.12	0.98	0.0258

Dough and bread surface texture: evaluation by gray level co-occurrence matrix

Texture features of images describe the textural patterns or properties including coarseness, fineness, granulation, randomness, lineation, and hummocky of the surface of the food products (Haralick et al., 1973). These textural properties, which explain details of how the surfaces are composed of and structured by the dependency of pixels from each other or by the intensity variation across pixels, correspond to the ways of human perception of food surface. Meanwhile, texture can also be used to determine chemical properties or physical properties of food products. Various methods developed for characterizing texture can be categorized into four main types as statistical texture, structural texture, model-based texture, and transform based texture

(Zheng, Sun, & Zheng, 2006a). Statistical texture is extracted by applying statistical approaches on a matrix that is obtained according to the orders of the intensity of pixels across images. The gray level co-occurrency matrix (GLCM) belongs to the statistical methods. In this study, GLCM was applied both to the doughs, to investigate their surface texture variation during leavening, and to the breads, to evaluate the textural features of their crumb.

The images of the various doughs during leavening were processed. The parameters values coming from the GLCM algorithm (ASM, CON, COR, ENT, and IDM) were calculated taking into account the four directions (0, 45, 90, and 135°) and the seven *d* (1, 2, 3, 4, 5, 7, and 10). Therefore, the mean values reported in Fig. 45, for each leavening time, represent the mean value of the 4 directions and 7 distances.

Higher values of ASM, COR and IDM, and lower values of CON and ENT, generally indicate a highly uniform and homogeneous surface, as the level of local variation of the intensity values of the pixel in the image is low and there is an elevated state of order. When the leavening is starting, the bubbles on the surface samples are still limited; for this reason the value of the parameters, in particular of contrast and entropy, depends only on the presence of the particles fibers that are dark respect to the background (the dough). While on the contrary, when the samples are characterized by high value of CON and ENT and low values of ASM, COR and IDM, the surfaces are more heterogeneous. In this case the number of bubbles on the surface of the sample is increased and this causes an increase of the values of contrast and entropy (and consequently reduction of the values of the other parameters). This is explained by the fact that are increased the dark spots on the dough surface making their aspect more heterogeneous.

At the very beginning of the leavening phase, the presence of bubbles at the surface of the doughs was still limited, and the surface texture of the various doughs, therefore, was quite uniform. ASM, COR and IDM were high (index of an homogeneous surface). CON and ENT values, for this reason, were only related to the presence of GSP in the doughs, that appeared as dark spots with respect to the background (the dough). For the same reason, higher CON and ENT values characterized those samples containing red grape skin powders. However, as the process went on, the surface of the samples changed quickly, due to the appearance of bubbles from yeast activity, determining a more heterogeneous aspect. This was attested by a decrease in ASM, COR and IDM (indicating a less homogeneous surface) and an increase in CON and ENT values. As can be appreciated from Fig. 45, these changes were highly evident in all the samples up to 50min leavening. This means that the main structural changes, as evidenced by the GSCM, took place during the very first period of the leavening phase, that is actually the period in which the development of a dough usually takes place at the highest rate. In addition, interesting differences were highlighted among the various enriched doughs. Those samples containing Ch GSP generally exhibited larger and

quicker structural changes than the samples enriched with Ba GSP, during the first 50min leavening. When the GSP particle size was considered, the major changes in the surface features were evidenced for those sample enriched with the S fraction. When considering the GSP variety, larger differences due to particle size were recorded among the doughs containing Ch GSP than among those enriched with Ba GSP. After the first 50min leavening, changes were less evident, as underlined by the linear trend assumed by all the GLCM parameters for all the samples. Despite the presence of a large amount of fiber in the matrices, the various doughs were able to sustain the mechanical stress induced by a prolonged leavening time, and their structure, once changed, remained stable up to the end of the proofing time. After 50min leavening, larger differences were evident among those doughs containing Ba GSP than among those enriched with Ch, particularly in terms of CON and ENT.

Overall, the results achieved by means of the GLCM allowed following dough physical structural changes during leavening, highlighting differences among and within the various samples.

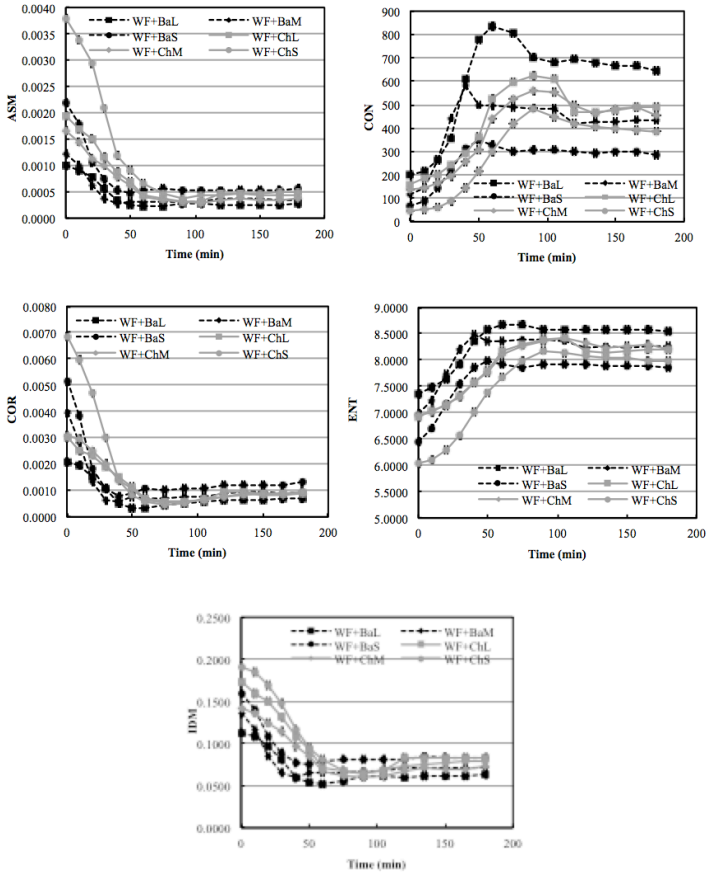


Figure 45. GLCM algorithm parameters: variation during dough leavening.

Dough and bread surface texture: evaluation by angle measure technique

First of all, qualitative results were given by the evaluation of the surface texture of the samples by means of the angle measure technique (AMT) method. The principal component analysis was applied on the matrix of the mean angle spectral data obtained by the application of the AMT algorithm on the image samples, as proposed by Fongaro & Kvaal (2013). The AMT method was applied, as well as GLCM, both to the doughs and to the breads.

Two different PCA, one for the doughs enriched with Ba GSP and one for the doughs containing Ch GSP, were carried out as a function of the leavening time. Results are reported in Fig. 46.

The first two PC were enough to well explain doughs distribution only as a function of their surface textural features (PC1=99% and PC2=1% for Ba GSP enriched breads; PC1=97% and PC2=3% for Ch GSP enriched breads). In particular, the majority of the variation (PC1) was linked to the dough leavening time. Looking at the *score plots*, in fact, a clear distribution of the sample was evident along PC1: from the left to the right, doughs were well separated, following a leavening kinetic. In addition, the majority of the changes took place during the first period of proofing. If PC2 was considered, samples appeared well separated as a function of the GSP particle size: the lower the particle size, the higher the PC2 value.

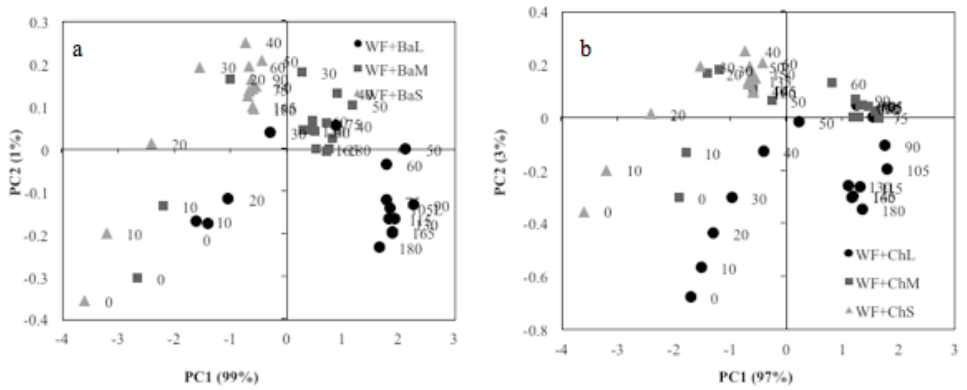


Figure 46. PCA score plot for the Chardonnay (a) and Barbera (b) enriched doughs, in relation to their surface textural features.

Thanks to a specific in house algorithm written for MatLab, the dots of all the samples in the score plot were replaced by the relative images used to perform image analysis; in this way a visual representation of the samples distribution in function of their surface aspect was obtained and as shown in Fig. 47. This makes the surface texture changes along the first two principal components simpler to understand.

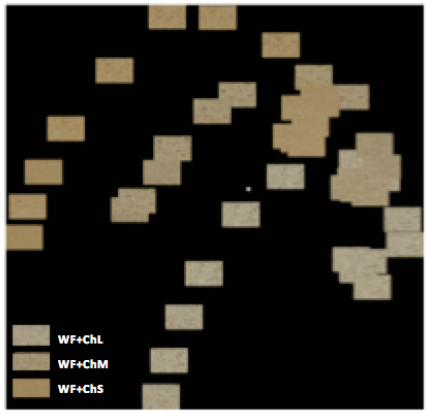


Figure 47 Visual representation of sample distribution inside the score plot in function of their surface texture characteristics obtained from PCA elaboration of AMT spectral data.

3.2.2.2 Bread: physicochemical properties

a) Moisture and water activity

Results related to bread moisture and water activity have been reported in Tab. 27. Differences were detected among the samples. This may be attributed to the use of the optimal hydration for each formulation, instead of using the same hydration across all the samples. The optimal hydration accounted for the different characteristics of the RWGSPs and WWGSPs and their effect on the moisture of the samples.

Table 27. Bread moisture content and water activity

Bread	Moisture content (%)		Water activity (Aw)
	Core crumb	Slice	
100WF	43.13±0.22	36.02±0.46	0.960±0.006
90WF-10BaL	43.51±0.06	38.92±0.44	0.958±0.003
90WF-10BaM	44.22±0.19	39.93±0.01	0.985±0.001
90WF-10BaS	43.59±0.07	38.13±0.07	0.981±0.004
90WF-10ChL	43.30±0.01	38.69±0.38	0.967±0.001
90WF-10ChM	42.93±0.08	37.25±0.59	0.961±0.004
90WF-10ChS	42.76±0.10	38.45±0.31	0.964±0.001

b) Height, weight and specific volume

Data related to the geometrical features of the composite breads have been reported in Tab. 28. Positive and encouraging results were obtained, in spite of the high GSPs

supplementation level adopted, in particular in terms of specific volumes (2.46-2.87mL/g *vs.* 3.65±0.25mL/g for 100% WF bread). Loaf volume is one of the most important characteristics of baked goods for indicating the baking performance (Kohajdová et al., 2012). As expected, the composite breads had lower loaf volumes than the control (WF). Probably, fibers coming from GSPs interfere with gluten during dough networking, damaging its structure, as evidenced also by the decrease in CO_2 gas retention abilities of the doughs(Kohajdová et al., 2012). Kohajdová et al. (2012) also suggested that fibers compete for water in the system, thus resulting in less water available to develop the starch- gluten network. Particle size did not seem to affect bread geometrical features, while a influence was exerted by the variety: the presence of Ch GSPs, in fact, led to higher bread performance.

Table 28. Bread geometrical features

Sample	Weight (g)	Volume (mL)	Height (cm)
100 WF	5.35 ± 0.46	3.37 ± 0.25	4.41 ± 0.28
90WF-10BaL	4.48 ± 0.84	2.35 ± 0.07	2.86 ± 0.05
90WF-10BaM	4.08 ± 0.22	2.46 ± 0.11	3.05 ± 0.22
90WF-10BaS	4.45 ± 0.64	2.55 ± 0.08	2.90 ± 0.45
90WF-10ChL	4.27 ± 0.50	2.63 ± 0.14	3.39 ± 0.06
90WF-10ChM	4.87 ± 0.28	2.87 ± 0.14	3.59 ± 0.13
90WF-10ChS	4.47 ± 0.32	2.80 ± 0.13	3.36 ± 0.07

c) Color

Results related to bread crust color have been reported in Tab. 29. The RWGSPs used in this study varied from a dark purple to a red colour. The WWGSPs had a yellow to brown colour. In general, all the fortified breads were significantly darker than the control (WF). In particular, both for RWGSPs and the WWGSPs fortified breads, the colour became darker with the decreasing of the GSPs particle size. As regards the other colorimetric indices, they depended on the color of the GSPs added to the mixture: a* (redness)was higher in Ba enriched breads, while b* (yellowness) was higher in Ch enriched breads.

Table 29. Bread crust color

Sample	Color Crust		
	L*	a*	b*
100 WF	71.78 ± 1.80	2.11 ± 1.40	27.96 ± 2.23
90WF-10BaL	32.25 ± 1.01	40.60 ± 4.00	-256.22 ± 4.08
90WF-10BaM	41.36 ± 3.37	36.77 ± 5.40	-259.00 ± 1.24
90WF-10BaS	41.07 ± 4.10	40.94 ± 3.29	-254.67 ± 1.57
90WF-10ChL	52.08 ± 1.48	2.79 ± 0.37	15.84 ± 0.85
90WF-10ChM	59.06 ± 2.13	4.15 ± 0.68	29.44 ± 1.64
90WF-10ChS	52.27 ± 2.29	5.30 ± 0.61	17.21 ± 1.61

d) Texture

Results related to bread crumb softness have been reported in Tab. 30. Breads were characterized immediately after the production (t=0) and during a storage period, at controlled conditions (25 C, 60%RH), for up to 48h (t=2). In particular, the crumb softness of the fresh products (0.32-0.80N *vs.* 1.01±0.24N for 100% WF bread, at 25% deformation level) is here underlined. The greater crumb softness exhibited by the fresh products containing GSPs was maintained also during the whole investigated storage period. These positive and encouraging results highlighted the potential use of wine industry grape skins (in which large amounts of dietary fiber and antioxidant are present) for the production of innovative and nutritionally convincing baked goods.

Table 30. Bread crumb softness variation during storage.

Sample	Shel life (day)	Crumb core moisture (g/100g)	Young's Modulus	Total Energy (kJ)	Total energie (KJ)
100WF	0	43.04 ± 0.31	0.02 ± 0.01	0.79 ± 0.17	3.18 ± 0.59
	1	42.97 ± 0.00	0.01 ± 0.00	3.83 ± 0.93	1.49 ± 0.37
	2	40.39 ± 0.01	0.01 ± 0.00	4.00 ± 0.60	7.41 ± 2.41
90WF-10BaL	0	43.51 ± 0.06	0.01 ± 0.00	0.49 ± 0.05	0.62 ± 0.11
	1	42.70 ± 0.34	0.03 ± 0.01	0.93 ± 0.31	2.17 ± 0.84
	2	40.02 ± 0.49	0.06 ± 0.01	2.19 ± 0.01	4.35 ± 0.25
90WF-10BaM	0	44.22 ± 0.19	0.02 ± 0.00	0.80 ± 0.14	1.43 ± 0.29
	1	42.62 ± 0.09	0.03 ± 0.00	1.54 ± 0.41	3.14 ± 0.89
	2	40.87 ± 0.27	0.05 ± 0.01	2.69 ± 0.24	5.33 ± 0.55
90WF-10BaS	0	43.59 ± 0.07	0.01 ± 0.00	0.55 ± 0.02	0.93 ± 0.11
	1	42.48 ± 0.36	0.03 ± 0.01	1.03 ± 0.19	1.03 ± 0.19
	2	41.89 ± 0.01	0.05 ± 0.01	1.62 ± 0.29	3.61 ± 0.58
90WF-10ChL	0	43.30 ± 0.00	0.01 ± 0.00	0.50 ± 0.03	1.00 ± 0.08
	1	42.56 ± 0.19	0.02 ± 0.01	0.81 ± 0.27	1.81 ± 0.69
	2	40.01 ± 2.67	0.03 ± 0.00	1.41 ± 0.24	2.87 ± 0.44
90WF-10ChM	0	42.93 ± 0.08	0.01 ± 0.00	0.34 ± 0.07	0.63 ± 0.15
	1	42.79 ± 0.73	0.01 ± 0.00	0.72 ± 0.16	1.57 ± 0.33
	2	39.23 ± 1.03	0.01 ± 0.01	0.70 ± 0.22	1.54 ± 0.43
90WF-10ChS	0	42.70 ± 0.10	0.01 ± 0.00	0.32 ± 0.06	0.59 ± 0.12
	1	41.84 ± 0.10	0.02 ± 0.01	0.83 ± 0.26	1.82 ± 0.52
	2	40.47 ± 0.47	0.02 ± 0.00	1.07 ± 0.09	2.15 ± 0.24

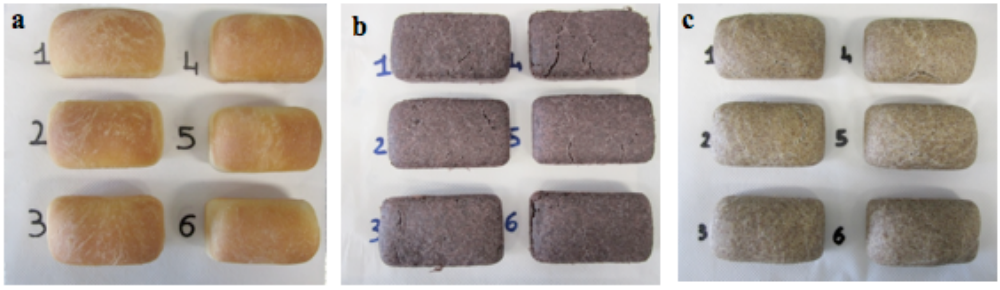


Figure 47. Example of bread: wheat flour (a) flour supplemented with 10% Barbera (b) and flour supplemented with 10% Chardonnay (c)

e) Image analysis

Bread surface texture: evaluation by gray level co-occurrence matrix

Data related to bread crumb surface features, as determined by GLCM, are reported in Tab. 31. Within breads enriched with the same GSP variety, the influence of the particle size is significantly evident: the larger the particle size, the lower ASM, IDM and COR, and the higher CON and ENT. This means that the particles size of grape skins powder can affect the dimension and distribution of the bubbles and then the final aspect of the bread crumb.

It is also possible to observe that the parameters values of Barbera samples are always higher than those of Chardonnay samples. This means that the surface aspect of the Chardonnay slices is more homogeneous of the surface aspect of the Barbera slices samples.

Table 31 Comparison of energy (ASM), inverse difference moment (IDM), contrast (CON), entropy (ENT) and correlation (COR) value for the bread integrated with different particle size of grape skins powder of Barbera (Ba) and Chardonnay (Ch). For each sample the value represent the mean of the 4 angles (0, 45, 90, 180) e seven step (1,2,3,4,5,7,10).

Sample	ASM	IDM	CON	ENT	COR
WF+BaL	0.0145 ± 0.0061^{a}	0.0528 ± 0.0135^{a}	863.509 ± 256.1763^{c}	8.7769 ± 0.1553^{c}	0.00043 ± 0.0003^{ab}
WF+BaM	0.0166 ± 0.0078^{b}	0.0586 ± 0.0143^{b}	652.344 ± 200.7605^{b}	8.5097 ± 0.2021^{b}	0.00051 ± 0.0005^{b}
WF+BaS	0.0175 ± 0.0067^{c}	0.0629 ± 0.0143^{c}	535.316 ± 137.1214^{a}	8.3851 ± 0.1282^{a}	0.00062 ± 0.0005^{c}
WF+ChL	0.0117 ± 0.0054^{a}	0.0429 ± 0.0127^{a}	1251.247 ± 406.6636^{c}	9.1545 ± 0.1754^{c}	0.00034 ± 0.0003^{b}
WF+ChM	0.0120 ± 0.0048^{b}	0.0447 ± 0.0120^{b}	1175.213 ± 351.4733^{b}	9.1070 ± 0.1330^{b}	0.00031 ± 0.0002^{a}
WF+ChS	0.0126 ± 0.0064^{c}	0.0495 ± 0.0153^{c}	1064.920 ± 392.7712^{a}	9.0279 ± 0.2141^{a}	0.00044 ± 0.0003^{b}

*within each GSP variety, values followed by different letters in a column are significantly different at $P<0.05$.

Bread surface texture: evaluation by angle measure technique

One PCA was carried out for bread samples, too. Results are reported in Fig. 48. Looking at the *score plot*, samples were well distributed along PC1 (PC1=99%), and both the influence of the GSP variety and of the GSP particle size on the surface texture features of breads crumb was well evident: the samples integrated with Ba GSP were clearly positioned in the left part of the score plot, while the samples integrated with Ch GSP were positioned in the right part of the score plot. Moreover, the AMT algorithm also allowed also to split the samples according to the different particle size of grape skins powder used, in particular for those containing Ba GSP. Thanks to a specific in house algorithm written for MatLab, the dots of all the samples in the score plot were replaced by the relative images used to perform image analysis; in this way a visual representation of the samples distribution in function of their surface aspect was obtained and as shown in Fig. 48. This makes the surface texture changes along the first two principal components simpler to understand.

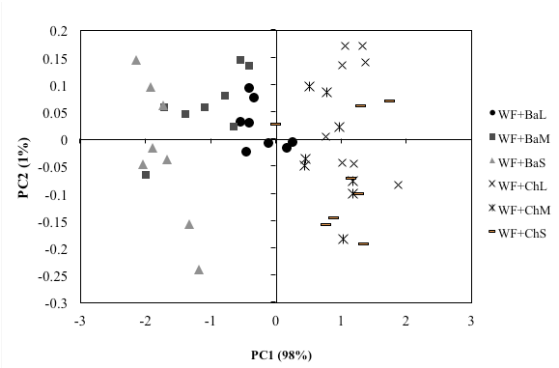


Figure 48. PCA score plot of Chardonnay and Barbera enriched breads, in relation to their crumb surface teztural features.

3.2.2.3 Bread: liking test

Data related to the sensorial evaluation of the composite breads, as well as of the reference bread, have been reported in Tab. 32 and Tab. 33.

Table 32. Composite breads sensorial evaluation: average scores (n 98) obtained under blue light, in relation to smell, taste, flavor, texture and overall enjoyment.

Rating	ChS	ChM	ChL	BaS	BaM	BaL	WF	F	p
Smell	5.18	5.28	5.35	5.45	5.34	5.21	6.89	24.43	<0.0001
	B	B	B	B	B	B	A		***
Taste	5.48	5.6	5.72	4.87	4.69	5.41	6.17	14.59	<0.0001
	B	B	B	C	C	B	A		***
Flavour	5.32	5.56	5.65	4.7	4.52	5.16	6.11	17.74	<0.0001
	BC	B	B	D	D	C	A		***
Texture	5.58	5.55	5.53	5.41	5.28	5.39	5.78	1.48	0.1812
	ns	ns	ns	ns	ns	ns	ns		
Overall	5.36	5.55	5.59	4.83	4.66	5.27	6.16	15.03	<0.0001
	B	B	B	C	C	B	A		***

* Significant at 5%, ** significant at 1%, *** significant at 0.1%, ns = not significant

In general, during the assessment under blue light (Tab. 32), all the samples get acceptable results, with scores greater than 5. Consistency was not significant for the discrimination of the samples, and the new breads enriched with fiber did not differ in terms of smell. The reference sample (WF) was the favorite, as expected, with the highest average scores for overall acceptability, smell, taste and flavor. Both for taste and flavor, the samples enriched with Ch fiber fractions (ChS, ChM, ChL) and the sample BaL did not significantly differ from each other, while the samples and BaS BaM breads were the less welcome, and achieved significantly lower scores in

comparison to the others.

During the assessment under white light (Table 33), color and appearance influenced the approval of the samples. In addition, the particle sizes was associated with acceptability: ChM and ChL enriched bread were more appreciated than ChS, getting a score almost comparable to that observed for the reference. In the same way, BaL enriched bread was significantly more acceptable in comparison to those containing BaS and BaM). Considering the consumers' willingness to buy (Tab. 33), rated on a 7 point scale, the reference sample got a significantly higher score. In general, Ch enriched breads reported higher scores in comparison to Ba added samples. In particular, ChM and ChL reported the higher scores, while BaS and BaM showed the worst results.

Table 33. Bread sensorial evaluation: average scores (n=98) obtained under white light, in relation to the consumers' overall enjoyment and willingness to buy.

Rating	**ChS**	**ChM**	**ChL**	**BaS**	**BaM**	**BaL**	**WF**	**F**	**p**
	5.2	5.59	5.64	4.32	4.4	5	5.97	18.79	<0.0001
Overall liking	BC	AB	A	D	D	C	A		***
	3.66	4	3.96	3.02	3.02	3.58	4.41	18.83	<0.0001
Availability to buy	CD	B	BC	E	E	D	A		***

* Significant at 5%, ** significant at 1%, *** significant at 0.1%, ns = not significant

Segmentation of the subjects according to the liking

By means of the cluster analysis conducted on the 'satisfaction' scores expressed by the total 98 subjects, two clusters of consumers were identified: cluster 1 (n=53) and cluster 2 (n=45). The composition of the two groups was homogeneous in terms of age and gender (Tab. 34). The mean scores in terms of smell, taste, flavor, texture and overall liking related to cluster 1 and cluster 2 have been reported in Fig. 49.

Table 34. Composition of the two clusters of consumers, identified on the basis of the 'satisfaction' scores expressed on the enriched breads.

			cl 1 (n=53)	**cl 2 (n=45)**
gender	n	M	19	18
		F	34	27
	%	M	35.8	40
		F	64.2	60
age		mean	24.7	25.2
		min	19	19
		max	38	54

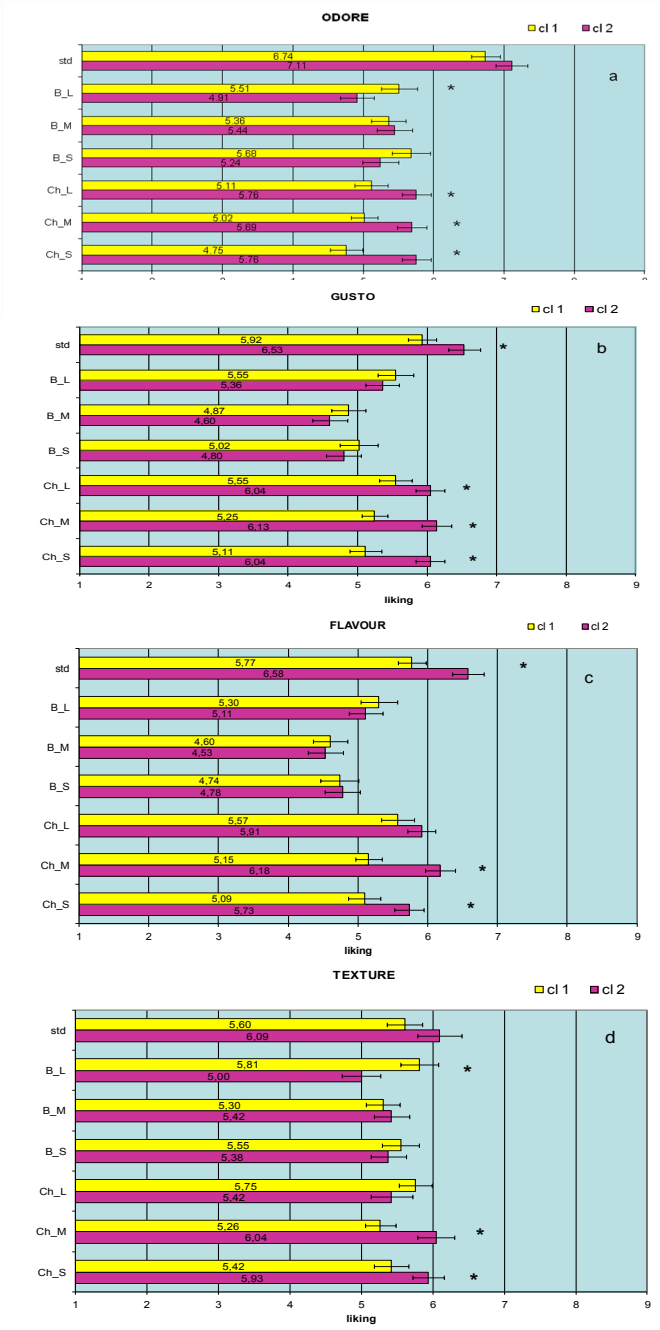


Figure 49a. Comparison between cluster 1 and cluster 2 for liking odor (a), taste (b), flavor (c), and consistency (d).. The graphs with blue background refer to the first part of the test (conducted under blue light). The asterisk on the charts indicates a statistically significant difference at 95%.

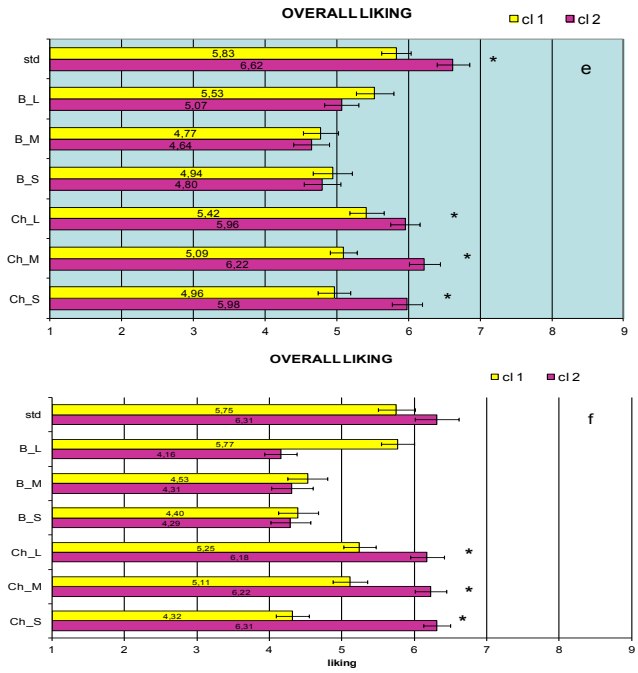


Figure 49b. Comparison between cluster 1 and cluster 2 for the overall enjoyment under blue light (s) and the overall enjoyment under white light (f). The asterisk on the charts indicates a statistically significant difference at 95%.

In Tab.35 the results of the ANOVA performed separately for each cluster have been reported.

Table 35a Results of ANOVA for cluster 1 (a) and cluster 2 (b) for all the judgments required.

Cluster 1 (a)	**ChS**	**ChM**	**ChL**	**BaS**	**BaM**	**BaL**	**WF**	**F**	**p**
Odor	4.75	5.02	5.11	5.68	5.36	5.51	6.74	16.44	<0.0001
	E	DE	CDE	B	BCD	BC	A		***
Taste	5.11	5.25	5.55	5.02	4.87	5.55	5.92	3.87	0.001
	BC	BC	AB	C	C	AB	A		***
flavour	5.09	5.15	5.57	4.74	4.6	5.3	5.77	5.37	<0.0001
	BCD	BC	AB	CD	D	AB	A		***
texture	5.42	5.26	5.75	5.55	5.3	5.81	5.6	1.34	0.2407
	ns	ns	ns	ns	ns	ns	ns		
Overall blu	4.96	5.09	5.42	4.94	4.77	5.53	5.83	4.46	0.0002
	CD	BCD	ABC	CD	D	AB	A		***
Overall white	4.32	5.11	5.25	4.4	4.53	5.77	5.75	10.92	<0.0001
	D	C	BC	D	D	A	AB		***
Availability to buy	2.96	3.6	3.62	2.85	3.04	3.98	4.25	11.85	<0.0001
	C	B	B	C	C	AB	A		***

* Significant at 5%, ** significant at 1%, *** significant at 0.1%, ns = not significant

Cluster 2 (b)	ChS	ChM	ChL	BaS	BaM	BaL	std	F	p
Odor	5.76	5.69	5.76	5.24	5.44	4.91	7.11	13.75	<0.0001
	B	B	B	BC	B	C	A		***
Taste	6.04	6.13	6.04	4.8	4.6	5.36	6.53	15.81	<0.0001
	A	A	A	C	C	B	A		***
flavour	5.73	6.18	5.91	4.78	4.53	5.11	6.58	15.29	<0.0001
	B	AB	B	CD	D	C	A		***
texture	5.93	6.04	5.42	5.38	5.42	5	6.09	4.66	0.0002
	AB	A	BC	C	BC	C	A		***
Overall blu	5.98	6.22	5.96	4.8	4.64	5.07	6.62	18.03	<0.0001
	B	AB	B	C	C	C	A		***
Overall white	6.31	6.22	6.18	4.29	4.31	4.16	6.31	30.76	<0.0001
	A	A	A	B	B	B	A		***
Availability to buy	4.53	4.53	4.42	3.27	3.04	3.16	4.69	19.83	<0.0001
	A	A	A	B	B	B	A		***

* Significant at 5%, ** significant at 1%, *** significant at 0.1%, ns = not significant

The preferences recognized in the two clusters were opposed. Cluster 2 significantly preferred the samples prepared with fiber fractions obtained from the pomace of Chardonnay, with no significant differences between the prototype ChS, ChM and ChL. For these samples, the appreciation within cluster 2, was comparable to that observed for the reference samples, and the judgments were highly acceptable (>5). In contrast, the samples prepared with fiber fractions obtained from Barbera obtained significantly lower scores that did not reach the acceptability (with no significant differences among the three different particle sizes). For this cluster, there has been an effect due to the type of fiber fraction used (hence the variety), but not of the grain size.

In contrast, cluster 1 showed in general a lower appreciation of all the samples and a different attitude with respect to cluster 2. In fact, in cluster 1 an effect of the particle size has been observed, for which the subjects of this group tended to prefer the larger granulometry. The preferred specimen was BaL, with an overall liking not significantly different, on average, from the reference (WF) and with liking scores of taste and flavor higher than BaM and BaL. In contrast, samples ChS, BaS and BaM did not reach the acceptability. Therefore, within this group, an effect of particle size on the acceptability of the samples was evident, while the grape variety used for the extraction of the fiber fractions did not affect the index.

For both the clusters, the consumers' willingness to buy was highly correlated with the liking. In cluster 1, this parameter was higher for BaL, accordingly to the liking obtained by this sample, and it did not differ significantly from that declared for the reference. In cluster 2, the three samples ChS, ChM and ChL reported higher scores,

significantly different from the standard. Looking at the results of the two questions posed at the end of the test (Tab. 36), both the groups declared a high interest in the use of products containing derivatives of the wine industry.

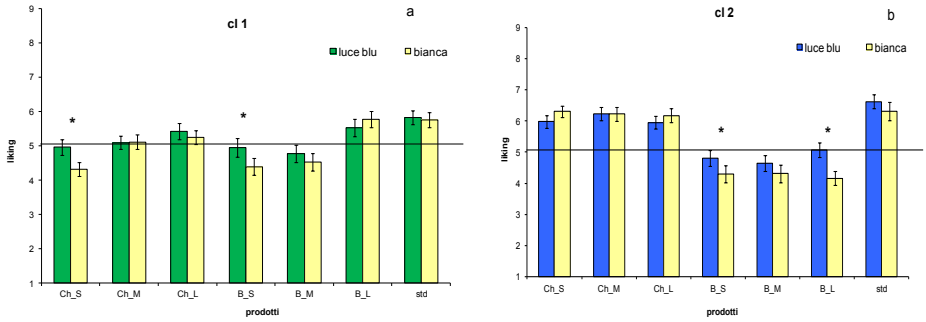
Table 36. Results related to the questions on *'enrichment of baked goods with fiber fractions obtained from the wine industry'* for cluster 1 and cluster 2.

Question		cl 1	cl 2
1 Grape pomaces and seeds	not interested	20.8	26.7
	neutral	18.9	13.3
	interested	60.4	60
2 Antioxidants from grapes	not interested	24.5	22.2
	neutral	20.8	13.3
	interested	54.7	64.4

Note: the values are expressed in %. The questions were: *"Do you think baked goods containing pomace and grape seed / grape antioxidants can be interesting?"*

Influence of the appearance of the samples on the clusters' liking

Results related to the influence of the sample appearance in the clusters have been reported in Fig. 50. In cluster 1, the passage from the condition of a masked appearance (blue light) to a visible appearance (white light) caused a significant effect only on the samples enriched with GSPs of a lower particle size (ChS and BaS), inducing a significant decrease of acceptability. For the other samples, it was not found any significant effect. In cluster 2, the same effect was found for the samples BaS and BaL.



* Significant at 5%

Figure 50. Effect of measuring the appearance in cluster 1 (a) and in cluster 2 (b).

In general, the experimentation related tothe use of fibrous fractions deriving from grape pomaces in the development of enriched breads has shown positive results. In

particular, it was possible to identify a cluster of consumers (cluster 2) for which all the three bread prototypes derived from Chardonnay GSPs developed in the study (ChS, ChM and ChL) were pleasant, like the reference sample.
In contrast, in those subjects for which the acceptability of breads enriched with fibrous fractions was lower (cluster 1), a greater predisposition to differentiate between products prepared with flour having different particle sizes and a preference for larger particle sizes, in particular for the BaL sample, has been observed.

3.2.3 CONCLUSIONS

In the current research, breads enriched with 10% of grape skins powders were developed. Despite the high level of substitution, interesting results were obtained, both from a technological and sensorial point of view. In relation to the different parameters tested, the influence of the grapes variety and/or of the grape skins powders particle size came out to be relevant for the final features of the products. This means that both the variety and the granulometry of the powders have to be carefully taken into consideration when developing an innovative baked good. These positive and encouraging results highlighted the potential use of wine industry grape skins (in which large amounts of dietary fiber and antioxidant are present) for the production of innovative and nutritionally convincing baked goods. Overall, the satisfactory results obtained provide very positive for future applications of 'novel ingredients' from wine industry by-products in bakery goods, and also allow to hypothesize the developments of new products, such as biscuits.

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3.3 BREAD: SOURDOUGH PROCESS

Sourdough is a mixture of flour and water fermented with lactic acid bacteria and yeasts, which determine its characteristics in terms of acid production, aroma and leavening (Moroni et al., 2009). The use of the sourdough process as a form leavening is one of the oldest biotechnological process in food production, and it has a long tradition in the production of wheat and rye breads. Early dough fermentation would probably have relied on a mixture of natural yeasts and lactic acid bacteria (LAB) (Arendt et al., 2007). When used in optimised proportions, sourdough can improve volume, texture, flavour, nutritional value of bread and increase the shelf life by retarding the staling process and by protecting bread from mould and bacterial spoilage (Clarke, et al., 2002; Crowley et al., 2002; Thiele et al., 2002; Dal Bello, et al., 2006; Moroni et al., 2009). In some cases, the utilization of sourdough is necessary for reducing the pH, to achieve the suitable values for baking (rye bread making) (Hammes & Ganzle, 1998; Salovaara, 1998). In wheat breads, sourdough is manly used to improve flavour (Hansen & Hansen, 1996), however it also has a major effect on the dough and final bread structure. The pH of a ripe sourdough varies with the nature of the process and starter culture used, but for wheat sourdoughs it usually ranges from 3.5 to 4.3 (Thiele et al., 2002). Depending on the rate of addition, therefore, the pH of the bread dough will also vary.

In this study, the sourdough process was taken into account for a further exploitation of the various grape skins powders previously described (see paragraph 1.3.1). The study was carried out both at the VTT Technical Research Centre of Finland, during a visiting research period under the supervision of Prof. Kaisa Poutanen, and at the DeFENS. .

3.3.1 MATERIALS AND METHODS

3.3.1.1 Grape skins, wheat flour, and bread mixtures

The grape skin powders (GSPs) obtained as reported at § 3.2.1.1 were used in this study. Their chemical-physical features have already been reported in Ch.1. For bread production, a commercial wheat flour was used(Melia Ltd Raisio, Finland). It was characterized by the following features: 12.1g/100g moisture, 1.8g/100g fat, 67g/100g carbohydrates, 3g/100g dietary fiber, 13g/100 proteins, and 0.004g/100g sodium.
In addition to WF (reference), the following 6 mixtures were used in the sourdough process:

1) BaS: 90% WF + 10% BaS;
2) BaM: 90% WF + 10% BaM;
3) BaL: 90% WF + 10% BaL;
4) ChS: 90%WF + 10% ChS;

5) ChM: 90% WF + 10% ChM;
6) ChL: 90%WF + 10%ChL.

3.3.1.2 Microbial strains

As regards the fermentation tests with lactic acid bacteria (LAB), six different microbial strains have been tested (Tab. 37) three wereobtained from cherry (Cill), and three came from blackberry (M/PR). The selection was done according to the features (included those critical) to which they had to adapt to start the fermentation process (e.g. pH<4).
LAB) were cultivated in modified MRS (mMRS), prepared with 1% (wt/v) maltose and 5% (v/v) fresh yeast extract, final pH equal to5.6

Table 37. Lactic acid bacteria strains used for the fermentation tests.

Code	Strain
CilB1	*Pediococcus Acidolactici*
CilSWE5	*Pediococcus Pentosaceus*
CilFF3	*Lactobacillus Plantarum*
M6	*Lactobacillus Plantarum*
PR1	*Lactobacillus Plantarum*
M1	*Weissolia Cibaria*

3.3.1.3 Sourdough

'Sourdough' was prepared by mixing 220g of tap water, 100g of GSP, 100g of wheat flour, and the inoculum of LAB or yeasts (10^6–10^7 cfu/g), in a large beaker (2000mL), then covered with analuminum foil. The size of the inoculum for LAB was 10^7 cfu/g of sourdough, and for yeasts 10^6cfu/g of sourdough. The mixture thus obtained was fermented for 16h at 25°C before being used

3.3.1.4 Bread-making process

Sourdoughs were prepared as reported at § 3.3.1.3 and used in the subsequent bread-making process to produce sourdough breads, according to the recipe reported in Tab. 38. GSP were used at the 10% integration level. The optimum water amount and the optimum mixing time were determined by preliminary farinographic tests carried out on the control (WF) and on the mixtures. Seven different thesis were submitted to the sourdough breadmaking process: STD, BaL, BaM, BaS; ChL, ChM, ChS (as reported at § 3.3.1.1).

Table 38 Recipe for the sourdough bread

INGREDIENTS	**Amounts** (g)
Sourdough	420
Flour [1]	800
Oil[2]	20
Instant yeast[3]	30
Sugar[4]	60
NaCl[5]	30

[1] Melia LtD., Raisio, Finland [2] Olivi Oijy, Rainbow, Finland; [3] Tuore Hiiva, Suomen Hilva Oy, Lahti, Finland; [4] Kidesokeri, Rainbow, Finland; [5] Soltion mineraalisuola, Cederroth, Finland.

For the control breads (without sourdough) the same recipe used for with wheat flour and GSP was adopted, but without the LAB *inoculum*. Thus, the amount of flour and water was the same in the control breads and in the sourdough breads.

Breads were prepared by mixing (sourdough), flour, water, sugar, salt, yeast, and fat. After a resting time of 15min at 30°C and 70%RH, the dough was divided into 300g loaves and moulded mechanically. The loaves were proofed in pans (80minx 30°Cx60%RH) and baked at 180°C for 20min. After 1h of cooling at room temperature, bread characteristics were determined.

The baking process and the experimental plan adopted have been reported in Fig. 51.

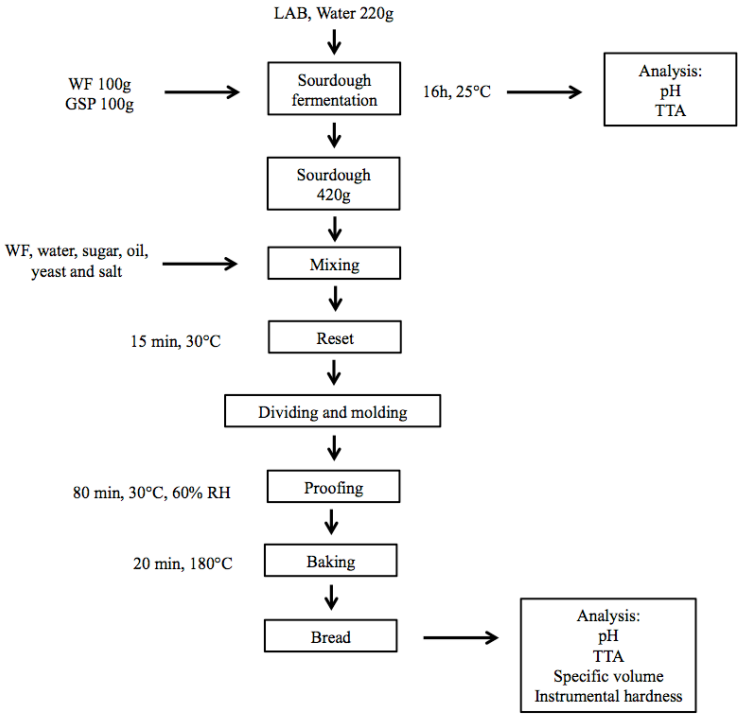

Figure 51. Schematization of the sourdough breadmaking process and of the related analytical experimental plan.

3.3.1.5 Sourdough and bread characterization

a) pH and TTA

The pH value was measured from an aliquot of 10 g of sourdough blended with 100mL distilled water in a TitroLine Alpha471217 (Schott, Germany). For the total titratable acidity (TTA) determination, the suspension was titrated against 0.1 mol/L NaOH to a final pH value of 8.5 using the TitroLine Alpha. Total titratable acidity was expressed as the amount of NaOH consumed (mL; n=4).

b) Bread features

Bread loaves were cooled for 1h at room temperature, before being weighed (g; n=6). The moisture content of each sample slice (g/100g; n=3) was evaluated by determining the loss in weight of an exactly weighed 5g sample after 15h at 105°C. Bread volume (mL) was determined by the rape seed displacement method (n=6). Specific volume (mL/g) was calculated. Crumb hardness was measured on fresh bread by two methods. In the first one, bread crumb hardness was determined as maximum compression force (40% compression; AACC 1998, Modified Method 74-09) using the Texture Profile Analysis (TPA) (Texture Analyser, Stable Micro Systems, Godalming, England). Eight bread slices (originating from 3 loaves) were measured. The height of each bread slice was 2.5cm and edges were cut off before measurement. Mean values of volume and hardness were calculated and used in modelling. In the second method, bread slices (8 for each sample) were compressed up to 60% deformation, using a 50mm diameter cylindrical probe, at a compression speed of 0.83mm/s. The following parameters were evaluated: crumb hardness (N).

3.3.2 RESULTS AND DISCUSSION

Fourteen breadmaking trials (with/without sourdough) were performed, and each bread loaf was characterized. Results have been reported in the following paragraphs.

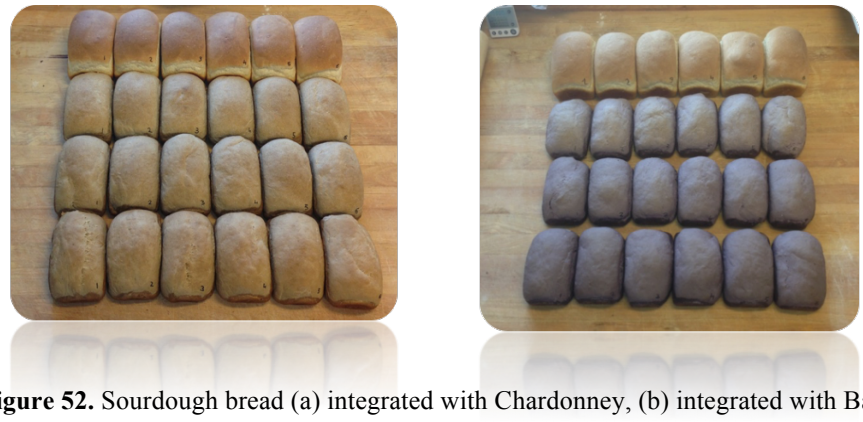

Figure 52. Sourdough bread (a) integrated with Chardonney, (b) integrated with Barbera

3.3.2.1 Microbial strain selection

The LAB fermentation tests were conducted on the two grape skins varieties (Chardonnay and Barbera). Six different microbial strains were tested (Tab. 39), in order to find out the most suitable both to the purposes of the study and to the experimental environment.. The choice was restricted to these strains for the difficult conditions of adaptation to the investigated matrix (pH<4). Due to the lack of raw materials (GSPs), these explorative experiments were carried out on the GSP fraction available in higher amounts: the "S" one. Results have been reported in Tab. 39.

Table 39 Results of the fermentation tests conducted on BaS and ChS

Sample	pH (t0)	pH (t4)	pH (t24)	Δ
BaS FF3	3.38	3.34	3.14	0.24
BaS CilSWE5	3.38	3.3	3.3	0.08
BaS CilB1	3.41	3.45	3.24	0.17
BaS M1	3,55	3.41	3.25	0.3
BaS PR1	3.47	3.32	3.15	0.32
BaS M6	3.61	3.3	3.27	0.34

Sample	pH (t0)	pH (t4)	pH (t24)	Δ
ChS FF3	4.21	4	3.87	0.34
ChS CilSWE5	4.27	3.85	3.81	0.46
ChS CilB1	4.18	4.12	3.84	0.34
ChS M1	4.27	4.18	3.7	0.57
ChS PR1	4.24	3.97	3.88	0.36
ChS M6	4.22	4.18	3.75	0.47

The strain that has gave the best result in terms of fermentation (ΔpH) and flavour, with both the grape varieties, was *Lactobacillus Plantarum* (M6). The fermented matrix thus obtained was then used as a "sourdough" starter for the production of composite breads (10% GSP integration level).

3.3.2.2 Sourdough and bread features

a) Sourdough features

Results related to sourdough features have been reported in Tab. 40. In general, when Ba was present, lower pH values were exhibited by the samples. After 24h, however, similar pH values were reached, independently from the grape variety present in the dough: the pH of the sourdough varied from 3.52 (t_0) to 3.29 (t_{24}) for Barbera, and from 4.11 to 3.94 for Chardonnay, taking into account the various particle sizes. As expected, TTA values were higher when Ba was present in the system, and generally they increased when the particle size of the GSPs decreased. As regards the reference sample (WF+M6), the pH varied from 5.99 (t_0) to 3.44 (t_{24h}), while TTA was equal to 14.5 mL NaOH: a lower lactic acid accumulation was detected with respect to the doughs enriched with GSPs.

Table 40. pH and TTA values of the GSP sourdough.

Sample	pH (t0)	pH (t24)	TTA (mL NaOH)
WF-BaL+M6	3.52±0.01	3.49±0.01	35.35±4.07
WF-BaM+M6	3.53±0.01	3.29±0.01	44.43±2.83
WF-BaS+M6	3.64±0.03	3.39±0.03	53.09±2.16
WF-ChL+M6	4.22±0.02	3.43±0.01	23.06±1.59
WF-ChM+M6	4.18±0.02	3.43±0.01	23.69±2.27
WF-ChS+M6	4.11±0.01	3.94±0.01	28.27±4.82
WF+M6	5.99±0.01	3.44±0.01	14.5±4.95

b) Fresh bread features

Bread loaves were analyzed in terms of weight, height, specific volume, moisture at the product core, moisture of the crust, and texture (TPA and compression test). Results have been reported in Tab. 41.

In general, an increase of the volume of Ba enriched breads was observed when the sourdough process was adopted, while the opposite was evidenced for Ch enriched breads. (Tab. 41). The reference bread, with or without LAB (WF+M6 and WF, respectively), as expected, exhibited the highest specific volume.

Generally, the application of sourdough has been reported to either improve (Corsetti et al., 2000; Crowley et al., 2002) or decrease bread volume (Barber, Ortola, Barber, & Fernandez, 1992; Salovaara, 1998), being the type of influence dependent on the acidification rate obtained and on the microbial strains utilized. Positive influence of sourdough on volume can be due to various factors: (1) heterofermentative lactic acid bacteria have been reported to increase metabolic activity of yeast (Gobbetti, Corsetti, & Rossi, 1995) , thus producing more $CO_2$for leavening; (2) appropriate acidity might enhance the ability of gluten to retain CO_2 (Gobbetti et al., 1995); (3) accumulation of water- soluble pentosans may increase volume as a result of altered water distribution (Corsetti et al., 2000).

Table 41. Physical properties of breads

Sample	Weight (g)	Specific volume (mL/g)	Height (cm)	Moisture core (g/100g)	Moisture Slice (g/100g)
WF-BaL+M6	263.68±2.06	2.51±0.05	6.10±0.13	40.69±0.01	36.25±1.02
WF-BaM+M6	261.43±4.25	2.45±0.12	6.72±0.39	42.28±0.26	34.35±1.69
WF-BaS+M6	255.78±4.94	2.36±0.07	6.62±0.18	44.71±0.12	36.67±1.97
WF-ChL+M6	260.55±3.65	2.34±0.06	5.88±0.17	44.33±0.22	35.26±3.00
WF-ChM+M6	257.82±3.20	2.31±0.04	5.97±0.34	44.77±0.38	37.35±2.71

WF-ChS+M6	260.81±3.60	2.41±0.05	6.92±0.18	44.72±0.11	37.58±0.93
WF+M6	253.57±2.47	3.62±0.07	9.35±0.37	40.81±0.37	34.15±0.91
WF-BaL	259.92±3.54	2.23±0.04	6.30±0.38	42.23±0.02	36.41±0.07
WF-BaM	258.07±1.25	2.13±0.03	6.13±0.28	42.65±0.21	33.52±1.34
WF-BaS	258.58±2.76	2.32±0.03	6.72±0.31	48.48±0.39	46.16±1.95
WF-ChL	257.2±2.71	2.66±0.08	7.22±0.31	42.49±0.01	33.99±1.48
WF-ChM	258.37±1.97	2.54±0.05	7.37±0.24	42.97±0.03	33.20±1.36
WF-ChS	259.1±1.72	2.38±0.06	7.10±0.37	42.53±0.11	34.69±1.31
WF	251.73±3.18	3.64±0.11	8.55±0.60	43.35±0.03	34.44±0.16

The GSP fortified breads, with and without sourdough, were evaluated for their crumb features, in terms of hardness, springiness, and chewiness (Tab. 42). Hardness is a measurement of the force required to compress the sample. Both the red and white GSPs enriched breads were significantly harder than the control samples. This increase in crumb hardness is consistent with the findings of Mildner-Szkudlarz et al. (2011) who evaluated the effect of the presence of grape byproducts on sourdough rye bread. The increase in hardness may be a result of the increased fiber absorption of water in the system. Springiness is described as ability of a product to physically spring back after deformation from the first compression (Bourne, 2002). No significant differences were detected in terms of springiness among all the samples (Tab. 42). Chewiness describes the amount of time needed to chew a food in order to reduce it to a suitable consistency for swallowing (Bourne, 2002). GSPs enriched breads had a significantly higher level of chewiness when compared to the control bread (WF;Fig. 42), while no significant differences were evidenced among the samples obtained with and without the sourdough.

Better results in terms of texture, due to the use of sourdough in the mix, were detected through the use of a simple compression test (Tab. 43). In fact, for instance, as regards the bread enriched with BaL, its crumb hardness changed from a value of 32.47 ± 3.14 (N), in the case of a traditional mixture, to 20.40 ± 5:24 (N), in the case of the dough integrated with sourdough. This trend was observedfor all the samples.

It has also been suggested that the phenols in the GSPs may affect the yeast activity, as a result of achanged enzyme activity in the system of the bread dough (Mildner-Szkudlarz et al., 2011). Phenols may decrease the amylase activity, resulting in decreased maltose availability for the yeast during the proofing process (Mildner-Szkudlarz et al., 2011). Catechins have also been proved to inhibit yeast activity, leading to a decreased gas production (Mildner-Szkudlarz et al., 2011).

The improvement of the loaves obtained with sourdough was not very clear in terms of texture, since no differences between the breads obtained with or without sourdough were evidenced. The highest changes were related to breads smell and taste, as the

samples loose their particular tannic taste and acquired a fruity flavor.

Table 42 Results of the TPA Test

Sample	Hardness (g)	Springiness	Chewiness (g)
WF-BaL+M6	887.17±32.77	0.77±0.03	238.02±65.92
WF-BaM+M6	823.54±26.22	0.80±0.06	393.72±10.80
WF-BaS+M6	959.67±55.23	0.91±0.05	635.62±13.95
WF-ChL+M6	887.92±14.11	0.90±0.01	553.37±40.81
WF-ChM+M6	807.99±72.15	0.91±0.02	778.09±75.04
WF-ChS+M6	817.91±74.80	0.92±0.01	546.85±53.97
WF+M6	262.74±32.80	0.97±0.01	199.25±27.10
WF-BaL	641.90±71.10	0.88±0.02	369.27±27.39
WF-BaM	762.57±58.76	0.85±0.02	418.07±40.44
WF-BaS	557.88±77.31	0.88±0.07	342.04±44.72
WF-ChL	443.10±40.40	0.94±0.01	301.53±18.30
WF-ChM	518.25±57.68	0.92±0.02	334.00±38.66
WF-ChS	730.4±90.52	0.91±0.02	455.98±51.16
WF	238.9±36.91	0.99±0.01	191.93±27.36

Table 43. Result of the compression test.Sample

Sample	Force (N)
WF-BaL+M6	20.40±5.24
WF-BaM+M6	23.77±7.43
WF-BaS+M6	27.51±6.45
WF-ChL+M6	31.03±4.69
WF-ChM+M6	28.96±3.05
WF-ChS+M6	23.43±1.52
WF+M6	9.07±0.90
WF-BaL	32.47±3.14
WF-BaM	33.51±4.43
WF-BaS	29.06±2.72
WF-ChL	20.02±2.83
WF-ChM	21.85±3.23
WF-ChS	32.83±3.71
WF	9.25±2.70

3.3.3 CONCLUSIONS

This study validated Barbera and Chardonnay wine grape pomace as a valid source of antioxidant and dietary fiber to be used in fortified sourdough bread. Generally, the combination of sourdough and yeast mixture improves the volume, texture and shelf life of bread. As well known, sourdough fermentation is based on lactic acid and alcoholic fermentation, depending on the composition of microflora and fermentation conditions. Typical pH and TTA values of acidic wheat sourdough are 3.6-3.8 and 8-13, respectively (Brummer & Lorenz, 1991). Similar results, in terms of pH, were obtained for the variety Barbera, while TTA values were significantly higher (44mL NaOH for red GSPs; 28mL NaOH for white GSPs).

This proteolysis produces free amino acids, which may act as flavour precursors (Spicher and Nierle 1984, Gobbetti et al. 1995). Gluten proteins determine, to a great extent, the rheological properties of wheat doughs and texture of wheat breads. The proteolytic degradation of gluten proteins also alters the formation of the gluten network (Kawamura and Yonezawa 1982), which can result in weak and sticky dough. Even minor changes in the gluten structure can cause considerable changes in dough properties (Pizzinatto and Hoseney 1980).

Significant improvements in terms of crumb softness were not always obtained. The greater improvement, as reported in bibliography, was undoubtedly obtained in terms of taste, as it changed from bitter and astringent in fruity. Another positive aspect, but not evaluated instrumentally, was the extension of the shelf-life, as with the use of sourdough bread staling is generally slowed down. Other studies should be conducted to enlarge the experimental part and to evaluate the behaviour of the loaves during a prolonged shelf-life, to objectify the improvements obtained from a sourdough technology

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3.4 BISCUITS

The overall satisfactory results obtained from breads enriched with grape skins powders provided a good basis for thinking about other potential applications of the new ingredients obtained from wine-industry by-products. In particular, the final part of the current research was focused on the development of GSPs enriched biscuits.

3.4.1 MATERIALS AND METHODS

3.4.1.1 Grape skins and biscuits

The grape skin powders (GSPs) obtained as reported at § 3.2.1.1 were used in this research: BaL, BaM, BaS, ChL, ChM, and ChS. Their chemical-physical features have already been reported in Ch.1.
For biscuits production, a commercial wheat flour (WF) was used (Barilla G. e R. F.lli S.p.A., Parma, Italy). .
WF/GSP blends, containing 30% (w/w) GSP were prepared, mixing them for 20min at speed 1 in a Hobart N-50 Mixer (Troy, Ohio) before being used, in order to guarantee their homogeneous distribution in the final mass.

Biscuits were then prepared according to the recipe described in the AACC Official Standard Method 10.52 (AACC, 1997), and reported in Tab. 44. The control sample was prepared just using WF, without any addition of GSPs. In total, 7 different formulations were prepared: 70WF+30BaL; 70WF+30BaM; 70WF+30BaS; 70WF+30ChL; 70WF+30ChM; 70WF+30ChS; WF).
The biscuit doughs were prepared in an electric mixer (Hobart C-100, Manufacturing Co., Troy, OH), adding the various ingredients following a standardized procedure and going on with mixing until a homogeneous mixture was obtained (15min).

According to the different mixture types, the optimal amount of water was determined, so as to obtain an optimal consistency of the dough and not a sticky mass. The dough was sheeted to a thickness of 7mm, cut into square shapes using a 60mmx60mm cookies cutter, placed on an aluminium tray, baked at 205°C for 11min and then allowed to cool at room temperature for 30min. Sixteen cookies were obtained for each formulation.

Table 38 Recipe for WGS Biscuit

INGREDIENTS	Amounts in the recipe
Wheat flour/GSP (70:30)[1]	320g
Sugar [2]	192g
Shortening[3]	96g
Nonfat dry milk[4]	9.6g
Sodium bicarbonate ($NaHCO_3$)[5]	3.2g
Solution A*	30mL
Solution B**	16mL
Deionized water	Variable

[1] Barilla G. e R. F.lli S.p.A., Parma, Italy; [2] Carrefour da MAXI s.r.l., Nizza Monferrato, Italy; [3] Carrefour da De Paoli Luigi e Figli, Bolzano, Milano, Italy; [4] Ristora, Montichiari, Italy; [5] Carrefour da Formec Biffi S.p.A. Milano, Italy; * Sodium Bicarbonate; **Ammonium Cloride + NaCl.

3.4.1.2 Biscuits characterization

a) Moisture, water activity and geometrical features

After 1h cooling at room temperature, biscuits were weighed and the average weight (g; n=16) and weight loss during baking were determined for each formulation. Size (W; mm; n=16) and thickness (T; mm; n=16) of each sample was measured, and specific volume (cm^3g^{-1}) was calculated.
The moisture content was evaluated by determining the loss in weight of an exactly weighed 5g sample after 15h at 105°C (n=3). The core of the sample was used for the evaluation of water activity (aw; n=3), by means of the Octagon Aqualab Series 3 (Decagon Devices Inc., Pullman, USA).

b) Color

The lightness, chroma, and hue of the various biscuits were measured using a chroma meter (Minolta CR-100, Osaka, Japan; 8 mm measuring area and standard illuminant C conditions, 6774K), on the day of baking, recording the L*a*b* values. Measurements were taken on the top crust, at the center of three biscuits..

c) Texture

Textural properties were measured in a TA-HD*plus* Texture Analyzer (Stable Micro Systems, Surrey, UK), using the triple-beam snap (three point break) technique (Gaines, 1991), a crosshead speed of 2mm/s, and a load cell of 50kg. The force required to break the biscuits was recorded. Results, expressed as Force (N), are the average of 10 replicates (n=10).

d) Water absorption capacity

The water absorption capacity of the various samples was determined by dipping the biscuits in distilled water for 15, 30, and 45s). Two biscuits were used at this purpose, for each formulation (n=2). Results were expressed as percentage weight increase (%), after dipping for the specified times, in comparison to the initial weight of the biscuit.

e) Surface textural features (Image Analysis)

Image Analysis was performed the on top surface of the biscuits, in order to evaluate their surface structural features. Biscuits images were collected by means of a flatbed scanner (Epson Perfection 3170 Photo, Seiko Epson Corp., Japan), covering the samples with a black box in order to prevent loss of light. Images were acquired at a resolution of 300dpi (dots for inch) and at a color depth of 24bits. The captured images were then saved in uncompressed. TIFF format. To create the final dataset of images shown in Fig. 53, a region of interest (ROI) of 511*421 pixel was extracted from each single image. The ROI images were converted to 8bit grey scale and subjected to spatial calibration before image processing.
The images of 6 biscuits for each formulation were acquired, and a data set of 42 images in total was analyzed. The parameter taken into account for the evaluations of surface texture the heterogeneity (fraction of pixels that deviates more than 10% from the value of the average intensity of the object), using the software Image Pro-Plus (4.5.1.29, Media Cybernetics Inc,MD, USA). Gray level co-occurrence matrix and Angle measure technique (explained in chapter 3.2.1.2) were performed, too, using the Image J v. 1.44c software (Rasband, 1997–2012, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/).

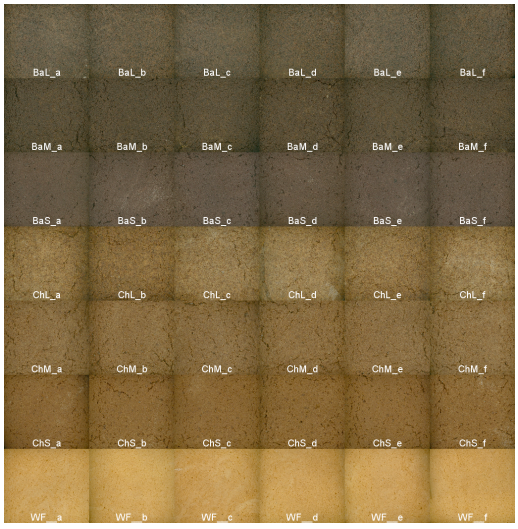

Figure 53. Final data set of biscuits images, composed of the ROI

3.4.2 RESULTS AND DISCUSSION

3.4.2.1

a) Moisture, water activity and geometrical features

Results related to biscuits weight, specific volume, weight losses, moisture and water activity have been reported in Tab. 39. The incorporation of GSPs in the biscuit recipe generally determined a weight increase, in comparison to the reference sample, probably because of the higher amount of water required by the mixtures containing to fully hydrate the mass. On the other hand, weight losses were very similar to those of the reference samples, suggesting a higher ability of the GSPs enriched mixtures in linking water. This hypothesis was confirmed by the higher moisture content of the composite biscuits (the larger the GSP particle size the lower the ability in retaining water) and water activity. The specific volume generally decreased in biscuits supplemented with GSPs, and the decrease was higher for those biscuits enriched with Ba.

Table 39. Geometrical features, moisture and water activity of the GSPs enriched biscuits.

Sample	Weight (g)	Weight loss (g)	Specific Volume (cm^3/g)	Moisture (g/100g H2O)	Aw
WF-BaL	27.15±2.41	6.13±0.27	2.05±0.14	5.24±1.76	0.388±0.01
WF-BaM	28.36±1.63	5.53±0.34	2.17±0.14	8.27±0.72	0.420±0.01
WF-BaS	28.61±3.32	5.15±0.68	2.01±0.12	8.80±1.00	0.580±0.07
WF-ChL	28.50±1.81	5.50±0.91	2.38±0.17	6.66±0.39	0.550±0.01
WF-ChM	30.77±1.25	4.94±0.56	2.25±0.19	8.83±0.14	0.579±0.03
WF-ChS	31.97±2.52	4.85±0.19	1.89±0.11	8.65±0.33	0.599±0.03
WF	26.18±1.01	6.63±0.26	3.12±0.10	3.04±0.40	0.398±0.01

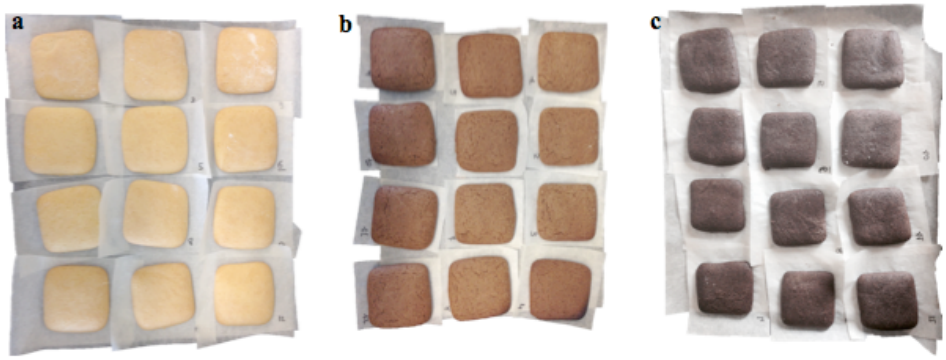

Figure 54. Example of biscuits obtained from:(a) WF, (b) WF integrated with Chardonnay GSPs and (c) with Barbera GSPs.

b) Color

Biscuits colorimetric indices have been reported in Tab 40. Biscuits with added GSPS were characterised by higher L* values in comparison to the control, and by a higher portion of yellowness for white GSPs. GSPs are rich in polyphenols, which are substrates for polyphenoloxidases. These enzymes, in the presence of oxygen, catalyse first the hydroxylation of monophenols to diphenols, and then the subsequent oxidation of diphenols to corresponding quinine intermediates that polymerise to form brown pigments (Rapeanu et al., 2006). On the other hand, GSPs contain more sugar, which favours browning reactions. Therefore, besides the original colour of each GSP, that could have some impact on the colour of the biscuits, the decreased brightness and yellowness are mainly due to Maillard and caramelisation reactions or enzymatic browning.

Table 40. Biscuits colorimetric indices

Campioni	L*	a*	b*
WF-BaL	33.92±1.60	3.94±0.32	6.36±0.90
WF-BaM	30.36±1.07	3.84±0.24	5.65±0.59
WF-BaS	32.30±1.00	6.04±0.23	3.84±0.74
WF-ChL	45.52±3.18	4.49±0.67	18.52±0.79
WF-ChM	42.73±0.82	5.40±0.38	17.38±0.63
WF-ChS	41.30±1.10	6.63±0.41	18.88±0.93
WF	7.70±0.40	5.01±0.05	7.20±0.04

c) Texture

Results related to biscuits hadrness (force, N) have been reported in Fig. 55. Generally, the presence of GSPs reduced the hardness of all the enriched samples. In particular, the smaller the GSP particle size, the lower the resistance to the applied force during the analysis. Recently, Uysal et al., (2007) reported a decrease in biscuit hardness upon the addition of apple fibre. However, an opposite trend was noted by Sudha et al. (2007) and by Ajila et al. (2008), that showed that the crisp texture of biscuits derives from slight starch gelatinisation, almost certainly due to the limited water content coupled with the low baking temperature. Also, proteins do not aggregate and hydrate enough to form a gluten network (Chevaller et al., 2002). Changes in the textural characteristics of the investigated biscuits might be due to the decreased water absorption of the dough, to the lower amount of gluten forming proteins in the formulations enriched with GSPs, as well as to the higher amount of fiber that catches and retains more water in the sample, thus leading to a softer structure.

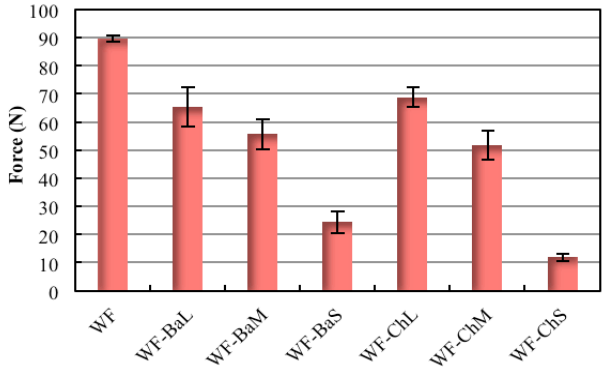

Figure 55. Force (N) required causing the biscuits rupture, in relation to the different formulations.

d) Water absorption capacity

In Fig. 56 the values of the percentage weight increase of the samples, with respect to the initial weight, after their dipping in water for the established times. In general, all the samples exhibited a high increase in weight after immersion in water for 15s: WF-BaL evidenced the lowest water absorption capacity (22:57%), while WF-ChM WF-ChL exhibited the highest ones (94.22 - 94.78%, respectively). After 30s of imbibitions, however, biscuits enriched with GSPs were characterized by fixuration with a consequent loss of material into the water and therefore a decrease in weight. A higher structural resistance was showed by the reference sample up to 30s imbibitions. After that time, all the samples evidenced similar behaviours..

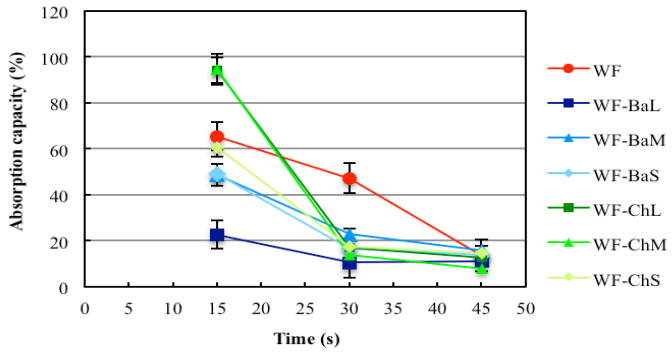

Figure 56. Water absorption capacity of the various GSPs enriched biscuits.

e) Surface textural features (Image Analysis)

The surface textural features of the various biscuits was evaluated. In foods, surface texture is a parameter that is first perceived by the view, anticipating a specific tactile perception (Jianshe, 2007). In this study, the surface textural features have been described by measuring the surface heterogeneity index (HGT; 0 = homogeneity, 1 = heterogeneity), which provides information on the degree of roughness of the sample.
HTG values related to the textural features of the seven different types of biscuits investigated have been reported in Fig. 57. In general, independently from the grape variety adopted or from the particle size of the grape skin powders, biscuits enriched with GSPS appeared more heterogeneous than the control. This suggests that these samples had a rougher and more irregular surface, characterized by cracks formed during cooking.

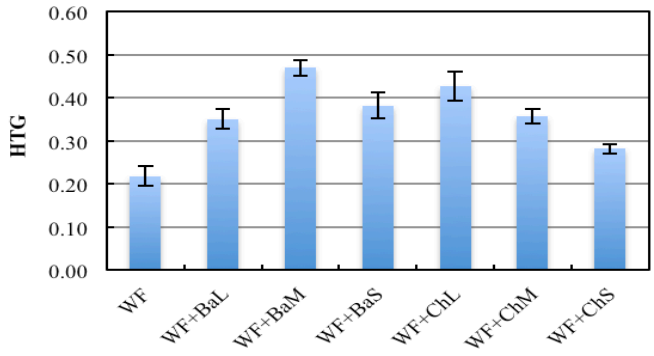

Figure 57. Heterogeneity values, in relation to the textural features of the various biscuits.

Table 41. Comparison of angular second moment (ASM), contrast (CON), entropy (ENT) and correlation (COR) values obtained for the biscuits integrated with grape skins powders, of different particle size, from Barbera (Ba) and Chardonnay (Ch).

Sample	ASM	CON	ENT	COR
70WF+30BaL	0.60±0.07	205617.59±46751.23	7771.25±97.40	0.028±0.005
70WF+30BaM	0.75±0.07	191896.06±41754.50	7619.08±90.66	0.060±0.009
70WF+30BaS	0.25±0.20	138627.29±33330.70	7331.38±188.78	0.003±0.001
70WF+30ChL	0.37±0.08	271574.81±83388.03	8249.34±183.76	0.020±0.001
70WF+30ChM	0.52±0.07	234687.30±6654.51	7958.43±126.40	0.045±0.001
70WF+30ChS	0.73±0.18	169311.76±51152.73	7629.01±147.08	0.002±0.001
WF	0.70±0.28	120913.30±34232.09	7522.38±156.54	0.003±0.001

Data related to biscuits crumb surface features, as determined by GLCM, have been reported in Tab. 41. The influence of the GSPs particle size was evident. In fact, observing the PCA Bi-Plot (Fig. 58), samples containing BaS and ChS were positioned along the positive side of PC1, together with WF. Their positioning was mainly due to GLCM parameters ASM and CON. ASM shows the uniformity of the image (1= homogeneity), while CON shows the amount of local variation in a image (1= heterogeneity). Samples containing the small GSPs fractions were characterized by a more homogeneous surface. Along the negative side of PC1, samples BaL and BaM appeared well separated from samples ChL and ChM.

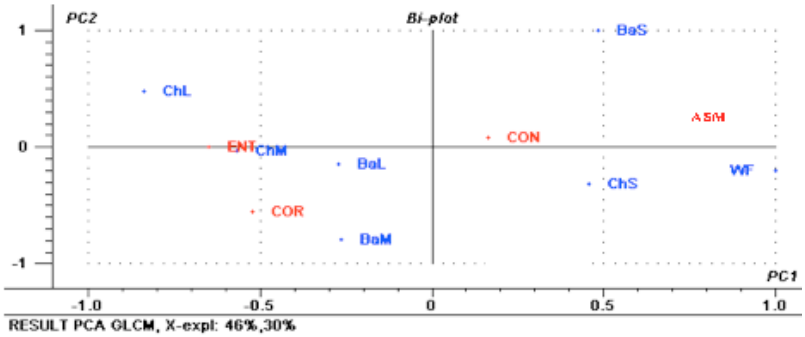

Figure 59. Bi-Plot obtained from PCA performed on GLMC parameters.

Qualitative results were obtained from the evaluation of the surface texture of the samples by means of the angle measure technique (AMT) method. Results of PCA have been reported in Fig. 60. Looking at the score plot, samples were well distributed along PC1 (PC1=91%), and grouped both in relation to the GSPs variety and to the GSPs particle size. Along PC2 (6%), a clear separation between the enriched biscuits and the reference one was also evident.

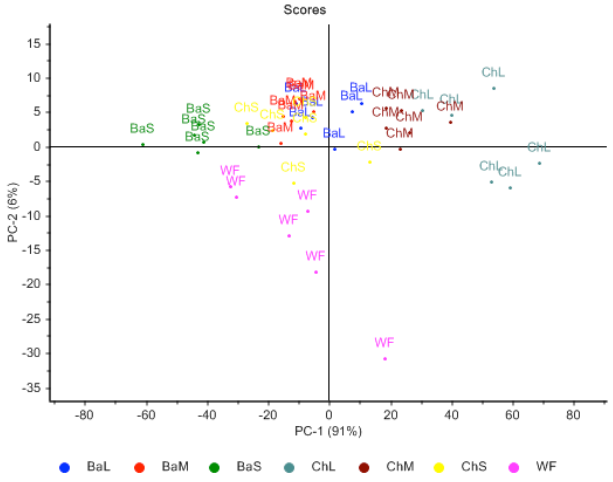

Figure 60. Results of PCA on AMT data related to the GSPs enriched biscuits.

3.4.3 CONCLUSIONS

Grape skins powders were used, in this step of the research, for the production of non-conventional biscuits. A high level of integration (30%) was adopted, in order to keep at the maximum the health benefits coming from the presence of an 'ingredient' rich in dietary fiber and antioxidants. Biscuits came out to be highly acceptable, from a technological point of view; too Fibres coming from wine-industry by-products, therefore, can play a very important role, and could be used for enriching the fibre content of biscuits. These studies have shown the feasibility of developing fibre rich biscuits able to increase the dietary fibre intake

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4. General conclusions and future perspectives

Grape seeds and skins represent about the 20% of the total fresh fruit weight that enters a winery. Most pomace is sent to landfills, and the remaining is returned to farms as livestock feed or composted fertilizer. Although these residues are not per se dangerous, their organic nature and the fact that their mass is in some cases concentrated in certain periods of the year, give rise to problems of great environmental impact. Unfortunately, it is not possible to define a general way of by-products recovering, but the optimum technology has to be carefully established from time to time on the basis of the waste type and the food application, in order to keep at the maximum the interesting health benefits of these materials.

An interesting way of recovering wine-industry by-products to develop functional powdered ingredients usable in food matrices have been developed in this study. In particular, grape skin powders having different particle sizes and grape seeds flours, have been produced. Grape skins contain 24-36% dietary fiber, mostly as cellulose, which has recently been shown to possess a unique organizational structure: grape skin cellulose is made up of small spherical microcrystal, not fibers, as it is found in wood or cotton. This structure provides great potential for texture options, and leads itself to better incorporation and coating in mixtures. Grape skins flours were also obtained. They can be used to introduce more acid, mostly tartaric and malic, to help in conditioning a rapid-rise of a dough. Grape seed flours are made from press cake after defatting. They are higher in dietary fiber (31-50%) than grape skins flours and containing more proteins. Fat is also present in grape seed flour, depending on seed moisture, variety, and temperature, and fat extraction, and some amount of polyunsatured fat is left in the flour.

Besides the optimization of processing technologies to convert by-products into multi-functional ingredients, a complementary research area involved the strategy for adding value to these ingredients through the development of novel foods. To this regard, bread was first selected, representing an interesting model food to study the rheological, macro- and micro-structural effect of fiber fortification. In addition, due to bread popularity, fortification strategies would be convenient to deliver beneficial compounds for human health to a large number of people. When using wine-industry by-products in breadmaking, many aspects were taken into account. Hydration of grape powders, for instance, is different than that of typical whole-wheat flours. In most formulation using grape skins or seed flours, more water is needed to achieve the desired texture. The principal effect of their presence on baking performance is evident on gas cell formation: cells tend to be more irregular and randomized, most likely due to the interaction of grape tannins with gluten. Grape skin flours most commonly contribute purple and rose colors that vary in depth and hue depending on the grape variety used and the pH of the dough mixture. These products have high concentration of anthocyanins, a complex of natural plant color similar in form to flavones, that acts

as natural oxygen radical scavengers. Among the dark colored species of grapes, curcial is themalvidin, which is red under acidic condition and shifts to purple at neutral pH. The use of these by-products is very challenging within the food industry. However, interesting results were obtained when including grape skins powders, from Barbera and Chardonnay, as functional ingredients in a baked goods. Conventional breads (straight-dough process), sourdough breads and biscuits, all enriched with high amounts of grape skins powders (10% w/w for breads; 30% w/w for biscuits) were developed in this project research, and good results were obtained both from a technological and sensorial point of view. These positive results were very encouraging, and pointed out the consumers' willingness to buy these 'unconventional' products. In order to develop quick and solid analytical methods able to evaluate the main nutritional traits of red and white grape marcs, NIR and MIR spectroscopy were adopted in the current research, collecting spectra from a large dataset of samples. Good PLS regression models have been obtained to quantifying those parameters related to the antioxidant activity of wine marcs (total solids, total polyphenols, total anthocyanins, proanthocyanidins and antioxidant activity). To strengthen the validity of these models, a higher number of samples should have to be used. Future perspectives could involve the use of these 'new ingredients' in the development of other foods of large consumption or of dedicated foods, such as gluten-free formulations. Furthermore, recovered products need to have standard characteristics in order to be successful marketed. The properties of wine-making by-products, for instance, can be greatly influenced by grape cultivars, vinification processes and climate conditions. It comes that appropriate technologies able to produce standard high-added products starting from multiple and variable raw materials are required. In addition, economical analysis and process optimization are required for the production of functional ingredients from food by-products at a low price, in order to enhance the production and diffusion of low-cost healthy foods also to low-income populations.