Characterization of Whole Grain Pasta: Integrating Physical, Chemical, Molecular, and Instrumental Sensory Approaches

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ABSTRACT: The consumption of whole-grain food - including pasta - has been increasing steadily. In the case of whole-grain pasta, given the many different producers, it seems important to have some objective parameters to define its overall quality. In this study, commercial whole-grain pasta samples representative of the Italian market have been characterized from both molecular and electronic-senses (electronic nose and electronic tongue) standpoint in order to provide a survey of the properties of different commercial samples. Only one pasta product showed very low levels of heat damage markers (furosine and pyrraline), suggesting that this sample underwent to low temperature dry treatment. In all samples, the furosine content was directly correlated to protein structural indices, since protein structure compactness increased with increasing levels of heat damage markers. Electronic senses were able to discriminate among pasta samples according to the intensity of heat treatment during the drying step. Pasta sample with low furosine content was discriminated by umami taste and by sensors responding to aliphatic and inorganic compounds. Data obtained with this multidisciplinary approach are meant to provide hints for identifying useful indices for pasta quality.

Keywords: whole-grain pasta; heat damage; electronic nose; electronic tongue; solubilized proteins

Practical Application: As observed for semolina pasta, objective parameters based on heat-damage were best suited to define the overall quality of wholegrain pasta, almost independently of compositional differences among commercial samples. Drying treatments of different intensity also had an impact on instrumental sensory traits, that may provide a reliable alternative to analytical determination of chemical markers of heat damage in all cases where there is a need for avoiding time-consuming procedures.

Introduction

Given the well-known health benefits of whole grain consumption (Poutanen 2012), consumers are increasingly demanding whole-grain versions of many cereal-based products. Because of its simple formula and worldwide popularity, pasta has been a common product targeted by product developers for offering whole-grain formats. Indeed, a single serving of whole grain pasta – with its 7 g of fiber per 100 g of pasta (Casiraghi and others 2013) - could help in satisfying the recommended daily intake for this component (Fogliano and Vitaglione 2005).

Beside the nutritional benefits, there are a spate of sensory (Heiniö and others 2016) and technological (Foschia and others 2013; Rakhesh and others 2015) issues that may become problematic when dealing with whole wheat pasta. Whole-grain pasta appears dark in color, with a bitter taste and with the possibility of occurrence of off-flavors and off-odors during storage. Whole-grain pasta producers are therefore called to meet the demand for a product with high nutritional interest, while paying due attention to the acceptability of the product.

In this regard, particular attention should be paid to the choice of the raw material and the technological process. High levels of both damaged starch and amylase activity, typical of whole-meal flour, are responsible for the formation of reducing sugars and for intense Maillard reaction (MR) (De Noni and Pagani 2010). The drying step certainly represents the most critical phase in the pasta-making process, because of its impact on the texture, the color, the flavor, and the nutritional properties of the product. Therefore, the definition of the overall quality of pasta cannot neglect the intensity of the MR assessment that is carried out by the determination of appropriate molecular markers. Furosine (ε-N-furoylmethyl-l-lysine, FUR) is the most widely used index of heat damage in pasta and in cereal-based products (Resmini and Pellegrino 1994). Heat damage in dried pasta also involves protein glycation and formation of advanced glycation end products (AGEs, e.g. ε-pyrrole-lysine (pyrraline; ε-2-formyl-5-hydroxymethyl-pyrrolaldehyde; ε-PL) (Resmini and others 1993) - which may affect protein digestibility (Seiquer and others 2006; Stuknytė and others 2014) and be potentially involved in the onset of some non-communicable diseases (Uribarri and others 2010).

In the case of pasta, a relationship has been reported also between the intensity of the heat treatment and the association of proteins through covalent and non-covalent interactions (Bonomi and others 2012). This has been studied also in whole grain pasta (Bock and others 2015), but the system used in those studies was based on common wheat, and therefore different from the semolina-based products that are mandatory in Italy. It seems reasonable that the different availability of water and the different macromolecular organization in whole-grain systems may result – also in this case - in altered patterns for protein-related structural changes and for formation of protein-carbohydrate adducts.

Aside from their molecular traits, whole-grain products are worthless unless they have satisfactory sensory traits. Producers have their own panels, but their scores are not always accessible, and are far from being univocal. Therefore, there is a great interest in using electronic senses (electronic nose and electronic tongue) for objective analysis of sensory traits. Electronic nose and electronic tongue (e-nose and e-tongue) allow to evaluate the contribution of different chemical species in determining aroma and tastes in food products (Sliwinska and others 2014). Thus, the application of electronic senses (e-senses) to pasta could represent an attractive – and objective - alternative to sensory tests for quality control and process monitoring.

This study aimed at exploring the fundamental aspects of structure, flavor, taste, and cooking behavior of whole wheat pasta, and at relating these characteristics to the processing conditions as estimated by heat damage indices. Both chemical and structural indices were taken into account.

Materials and Methods

Materials

Packages of whole grain pasta manufactured in Italy by different companies were purchased in two Italian supermarkets. A total of 20 spaghetti samples (1.7 mm diameter) were used, from two different production batches of 10 different brands. The samples were labeled with separate codes for identification of the producer and batch, respectively (i.e., a1, a2, b1, b2, etc). Chemical composition of pasta samples was those

reported by the manufacturer (see Table 1). No information was available as for the specific materials and processes used by individual producers. the When appropriate, pasta samples were ground with a laboratory mill (IKA Universalmühle M20; Janke & Kunkel GmbH & CoKG, IKA Laborteknic, Staufen, Germany), fitted with a water cooling jacket in order to avoid overheating during grinding.

Cooking behavior

Cooking loss was evaluated according to the AACC official method 66-50.01 (AACC, 2001). An aliquot of pasta (10 g) was cooked in boiling water (100 mL) until the optimum cooking time suggested by the producer, as reported on the package with no salt added. After cooking, pasta was drained, cooking water was recovered and its level brought back to the starting volume. An aliquot of water (40 mL) was then dried to constant weight at 105 °C. The residue was weighed and the dry matter reported as percentage of the starting dry material. Results were expressed as grams of solid loss/100 g of dry pasta. Pasta weight increase due to cooking was evaluated by weighing pasta before and after cooking. Each measurement was determined in duplicate.

Evaluation of heat damage

The formation of chemical artefacts potentially capable of affecting protein digestibility was assessed by determining the following molecules arising from MR. The level of FUR and ε-PL was determined according to Resmini and others (1990) and Resmini and Pellegrino (1994) respectively. In the case of ε-PL, the measurement was carried out only on one batch of pasta, taking into account FUR data performed on all the samples. The analyses were performed in triplicate.

Properties of the protein network

The solubility of proteins in pasta samples was determined in triplicate by using buffers of different composition, as described by Iametti and others (2006). Typically, 1 g of finely ground sample was suspended in 20 mL of 50 mM sodium phosphate buffer pH 7.0 containing 0.1 M NaCl. After stirring at room temperature for

60 min and removal of insoluble materials by centrifugation (2500 x g for 30 min), the protein content in the supernatant was assessed by a dye-binding colorimetric method (Bradford, 1976). Where indicated, the buffer used for protein extraction also contained 8 M urea or 8 M urea and 10 mM dithiothreitol (DTT) as reported by Bonomi and others (2012). A given amount (10 mg) of the milled uncooked pasta was treated with 0.2 ml of SDS-PAGE denaturing buffer (0.125 M Tris-HCl, pH 6.8, 50 % glycerol, 1.7 % SDS; 0.01 % Bromophenol Blue) containing 1% 2-mercaptoethanol when indicated, and heated at 100 °C for 10 min. For comparison, SDS-PAGE analysis was also carried out on the proteins extracted by treating 10 mg of ground pasta samples directly with 0.2 ml of denaturing buffer containing 2-mercaptoethanol for 0 min at 100°C. SDS-PAGE was carried out on a fixed porosity gel (12% monomer), using a MiniProtein apparatus (BioRad, Richmond, VA, USA) and gels were Coomassie Blue-stained. Molecular Weight markers (14; 20.1; 30; 45; 67;96 kDa) were from BioRad (Richmond, VA, USA).

E-senses

The volatile profile of the various pasta samples was assessed by a portable e- nose (PEN2, Win Muster Airsense Analytics Inc., Schwerin, Germany). The device consisted of a sampling apparatus, a detecting unit with a sensor array, and an appropriate pattern-recognition software (Win Muster v.1.6) for data recording and elaboration. For e-nose measurements, the sample headspace was exposed to the sensors that provided a signal pattern related to the volatile compounds in the headspace (Benedetti and others 2008; Marengo and others 2017). The sensor array was made up of 10 metal oxide semiconductor–type sensors: W1C (aromatic), W5S (broad range), W3C (aromatics), W6S (hydrogen), W5C (aromatics–aliphatics), W1S (broad methane), W1W (sulfur-containing compounds), W2S (broad alcohol), W2W (sulfur-containing and chlorinated compounds), and W3S (methane-aliphatics).

E-nose measurements were performed on both cooked and uncooked pasta samples. For the determination of the aromatic profile, samples were placed in a 40 mL airtight glass vial fitted with a pierceable silicon/Teflon® disk in the cap. The uncooked pasta sample (3 g) was used as such, whereas the cooked pasta sample (5 g of

lyophilized material) was wetted with 2 ml of distilled water. After an hour equilibration at room temperature, headspace measurements were performed according to the following conditions: flow rate 300 ml/min, injection time 60 s, flush time 180 s (during which the surface of the sensors was cleaned with air filtered through active carbon). All samples were analyzed in triplicate and the average of sensor responses were used for the subsequent statistical analysis.

E-tongue measurements were carried out by a Taste-Sensing System SA 402B (Intelligent Sensor Technology Co. Ltd, Japan). For this study a total of 4 detecting sensors and 2 reference electrodes were used, separated in two arrays according to the membrane charge: hybrid (CT0; AAE) and positive (C00; AE1). Measurements are based on the capability of tasty compounds to modify sensors potential through electrostatic or hydrophobic interactions (Buratti and others 2011). Ten grams of cooked and lyophilized pasta were resuspended in 150 mL of distilled water. Solutions were vortexed for 5 min and centrifuged at 5000 rpm for 10 min at 20° C. After centrifugation, the supernatants were filtered, and placed in 40 mL vessels for e-tongue analysis. Before each measurement, the detecting sensors and the reference electrodes were dipped into a reference solution (30 mM potassium chloride, 0.3 mM tartaric acid) and the electric potential was measured for each sensor (Vr). The sensors were then dipped for 30 s into the sample and the potential (Vs) was measured. For each sensor the "relative value" (Rv) was calculated as the difference between the potential of the sample and that of the reference solution (Vs-Vr). Sensors were then rinsed with fresh reference solution for 6 s, and dipped again into the reference solution. The new potential of the reference solution was defined as Vr'. For each sensor, the difference between the potential of the reference solution before and after sample measurement (Vr'-Vr) is the CPAv (Change of Membrane Potential caused by Absorption value) and corresponds to the e-tongue "aftertastes" (Kobayashi and others 2010; Buratti and others 2013). Before a new measurement cycle started, sensors were rinsed for 90 s with washing solutions and then for 180 s with the reference solution. Each sample was analysed in triplicate and sensor outputs were converted to taste information. The "taste values" were calculated by multiplying sensor outputs for appropriate coefficients based on the Weber–Fechner law,

which gives the intensity of sensation considering the sensor property for tastes (Kobayashi and others 2010; Buratti and others 2013).

Statistical analysis

Data were elaborated by Principal Component Analysis (PCA) using the MINITAB 14 software package (Minitab Inc., State College, PA, USA, version 12.0). PCA was applied as an exploratory tool to uncover aroma and taste characteristics. Analysis of variance (ANOVA) was performed with Statgraphics XV version 15.1.02 (StatPoint Inc., Warrenton, VA, USA). Samples were used as factor. When the factor effect was significant ($p \leq 0.05$), differences among the respective means were determined using Fisher's Least Significant Difference (LSD) test.

Results and Discussion

Cooking behavior

The cooking behavior of commercial whole wheat pasta – evaluated as cooking loss and water absorption - is summarized in Table 2. The amount of material leached into cooking water is frequently used to define the cooking quality of semolina pasta (Marti and others 2016). Indeed, good pasta cooking quality is assured by the formation of a continuous and strengthened network of coagulated gluten proteins, which entraps the starch macromolecules, limiting their swelling and solubilization into the cooking water (Resmini and Pagani 1983). Cooking loss values ranged from 3.38 and 4.73 g/100g pasta for j1 and h2, respectively, but no statiscally significant difference was evident when considering this parameter. Although these samples presented the highest and the lowest protein content, no significant correlation ($p > 0.05$) was detected between protein content and cooking loss when all the samples were taken into account.

As regards water absorption, values ranged from 107.8 to 155.2 g/100g pasta for g2 and e1, respectively. Despite the contribution of fiber in increasing the amount of water absorbed during cooking, no significant ($p > 0.05$) correlation between fiber content and water absorption was detected. Results on cooking behavior suggest the key role of processing conditions (i.e. drying cycle) in affecting the cooking behavior of

175 whole grain pasta, as already shown in pasta from refined semolina (D'Egidio and others 1990; Marti and
176 others 2013).

177 *Heat Damage*

178 The extent of (MR) in commercial samples of whole wheat pasta, monitored by their FUR and ε-PL content, is
179 summarized in Table 2. The mean FUR content of the 10 brands is equal to 595 mg/100 g protein, ranging from
180 229 to 836 mg/100 g. The wide range of data herewith recorded suggests high variability in raw material
181 characteristics (i.e. α-amylase activity and reducing sugars content) and/or processing conditions (i.e. drying
182 diagram) among manufacturers. The lowest values of FUR, in the range of 229-262 mg/100 g protein, were
183 found for j1, and j2, respectively. This result suggests the use of medium temperature drying cycle, that results
184 in limited heat damage. Samples j1 and j2 also exhibited the lowest cooking losses (Table 2), likely because of
185 the high quality of the raw materials (Marti and others 2013).

186 FUR levels in whole grain pasta below 300 mg/100 g protein are indicative of a mild heat damage
187 (Casiraghi and others 2013). As expected, the whole grain pasta is characterized by higher average FUR values
188 compared to semolina pasta (De Noni and Pagani 2010; Bonomi and others 2012), likely due to the intense
189 amylase activity and the high content in reducing sugars and proteins of whole-meal semolina, that make these
190 products highly susceptible to MR (Resmini and others 1996). In general, no significant variability was observed
191 among pasta batches from the same producer, if not for the "d" brand. This result might be ascribed to the
192 differences in the expiration date between these two batches (5 months in the case of samples d1 and d2, vs 2
193 months in all other samples), and by the reported effect of storage time on the FUR content (Cattaneo and
194 others 2008).

195 In agreement with the FUR data, the lowest value of ε-PL was found for "j" brand samples (0.8
196 mg/100g proteins), whereas the highest values (>15 mg/100g proteins) were measured in the products of
197 brands "a" and "d". To the best of our knowledge, no ε-PL data have been reported for whole-grain pasta. In
198 the present work, we found a mean value of 8.5 mg/100 g protein, although this parameter spanned a quite

broad range in the various samples (from 0.8 to 15.8 mg/100g protein). Studies on pasta made from refined semolina found low levels of ε-PL (0.58 mg/100g protein) in products dried using a low-temperature cycle, whereas high level of ε-PL (3.39 mg/100g protein) were recorded after high temperature drying cycles (Stuknytė and others 2014). It is worth noting that "j" pasta samples exhibited much lower either FUR and ε-PL levels than "m" pasta, despite their similar high protein content (14%), suggesting that protein content could not be the only attribute chosen by producers or consumers to define product quality.

Indeed, in addition to semolina quality, processing conditions (i.e. drying cycle) play a key role in affecting heat damage and thus pasta nutritional quality. From a nutritional standpoint it is noteworthy that most of the samples from whole semolina here considered showed a level of ε-PL higher than values found in high temperature-dried semolina pasta (3.39 g/100 g of protein), for which a decrease in protein *in vitro* digestibility of the cooked product was measured (Stuknyte and others 2014).

Finally, the data in Table 2 indicate the absence of a possible connection between either FUR or PYR levels and cooking losses. This confirms that the application of high temperature drying cycles decreased the significance of semolina quality (i.e., protein content and gluten strength indices) in determining the pasta cooking behavior (D'Egidio and others 1990, Cubadda and others 2007, Marti and others 2013).

Overall protein organization

Structural features of proteins in the various pasta sample were investigated by detecting the amount of protein solubilized by buffers of different dissociating ability towards covalent (disulfide) and non-covalent inter-protein bonds. This approach has been shown to provide useful hints for understanding the nature and extent of protein-protein interactions in cereal-based foods including gluten- containing (Bock and others 2015; Bonomi and others 2012) and gluten-free (Cabrera-Chávez and others 2012; Marengo and others 2015) pasta, also in the presence of nutritionally relevant ingredients of non-grain origin (eggs/whey) (Marti and others 2014).

As shown in Table 3, protein solubility in plain saline buffer was low for all samples, confirming aggregation of otherwise soluble proteins in semolina (albumins and globulins) as a consequence of pasta processing. Addition of urea to the saline extraction buffer resulted in a significant increase in solubilized protein in all samples, suggesting that hydrophobic interactions were very relevant to the structure of whatever protein network is present in these pasta samples (Table 3). When both urea and DTT were present in the extraction medium, the amount of soluble proteins further increased markedly in all samples, suggesting that inter-protein disulfides also play a fundamental role in stabilizing the structure of protein network.

By taking into account both the protein solubility data in Table 3 and the indices of thermal damage in Table 2, it becomes evident that the sample with the lowest FUR content (i.e., j1 and j2) also had the highest amount of solubilized protein regardless of the composition of the solubilizing medium. It is worth noting that even a modest increase of thermal damage results in a markedly impaired protein solubility, as exemplified by samples g1 and g2. These findings confirm previous reports on commercial pasta from refined semolina (Bonomi and others 2012). The relationship between conditional proteins solubility and heat damage indices offers hints for a possible relationship between the nature of prevalent interactions among proteins and the type and intensity of processing used for pasta production also when other components are present, as is the case in whole-grain pasta.

The nature of the proteins solubilized from the various samples by buffers of different composition was investigated by SDS-PAGE. The resulting electrophoretic profiles were similar in all samples (Figure 1), suggesting that the differences in protein organization observed in the solubility studies may be due to the technological processing rather than to differences in the starting materials. In this frame, it is of interest to note that the intensity of SDS-PAGE traces for proteins in the sample with the lowest level of FUR (Figure 1) confirms the presence of high amount of solubilized proteins also upon treatment with detergents and disulfide reducing agents. In the same “j” samples, at difference with other pasta samples, there is also no evidence for formation of the large aggregates not capable of entering the separating electrophoretic gel (Figure 1).

E-senses

E-senses evaluation of the pasta samples was performed by e-nose before and after cooking, for assessment of the volatiles profile. E-nose data collected on uncooked pasta were elaborated by PCA in combination with heat damage (FUR) and protein solubility (in urea) data in correlation matrix where the first two principal components accounted for 84.8% of the total variance (Figure 2). From the score plot (Fig. 2A) it can be noticed that the pasta samples with the lowest furosine and the highest soluble protein levels (i.e., from the "j" brand, Tables 2 and 3) are discriminated from all the other pasta samples, and are located on the right side of the first principal component (PC1). As noted above, samples with an intermediate furosine content (g1 and g2) but with low protein solubility are not discriminated in Fig. 2A. By considering the loading plot (Fig. 2B) the "j" samples are characterized by the highest odor intensity perceived by WW and WS sensors (of broad-range sensitivity and specific for sulfur-containing compounds). In addition, the same "j" samples were characterized by the lowest odor intensity perceived by WC sensors (specific for aromatic compounds). All the other samples, which are located on the left side of the plot, showed high odor intensity perceived by WC sensors.

E-tongue measurements were performed on cooked and lyophilized pasta, after reconstitution in water as done for e-nose measurements on the same cooked pasta samples. E-tongue device is a liquid analytical approach that mimics the taste-sensing mechanism of gustatory system; in this work sensors specific for bitterness, umami and astringency evaluation were applied. The estimated taste values were elaborated together with e-nose data collected on the same pasta samples by PCA and the first two principal components (PC1 and PC2) accounted for 65% of the total variance. Considering the score plot (Fig. 3A) and the loading plot (Fig. 3B), it is possible to observe a clear discrimination of samples on the first two principal components. In particular, the "j1" and "j2" pasta samples, located on the bottom right side of the plot (Fig. 3A), were characterized by WW and WS e-nose sensors and by the umami taste detected by e-tongue (Fig. 3B). Samples "a", "b" and "d", located on the left of the plot (Fig. 3A), were discriminated by the aftertaste-A and by WC sensors; whereas samples "e", "f", and "g" were characterized mostly by astringency and by bitterness.

Conclusion

The results provided from this study indicate that the drying process is of paramount relevance to the structural and nutritional quality of whole-grain pasta. As reported for pasta made from refined semolina, wholegrain pasta dried at low temperature had low FUR and ε-PL content. Both these heat damage indices and indices of protein aggregation and structural compactness suggest that the involved events (i.e., protein glycation and structural rearrangement of gluten proteins) are relatively independent – in pasta – from the amount of available fiber and from water distribution among phases. Protein content in the original material had essentially no impact "per se" on the quality indices mentioned above, and no evidence was found for a selective involvement of specific protein classes or subclasses in these events. The e-senses results demonstrate that the low-temperature dried pasta can be easily discriminated from the high-temperature dried samples. In particular, samples dried at low temperature were characterized by e-tongue sensor specific for umami taste and by e-nose sensors of broad range sensitivity and specific for sulfur-containing compounds. The high temperature treated samples were perceived more bitter and were characterized by e-nose sensors specific for aromatic compounds. In conclusion, this combination of multidisciplinary approaches appears to provide some hints for identifying objective indices for the assessment of whole-grain pasta quality.

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Author Contributions

A. Marti evaluated pasta quality and drafted the manuscript. P. Abbasi-Parizad carried out protein-related studies, that were designed and supervised by S. Iametti, who also wrote the related parts of the manuscript. S. Benedetti carried out e-senses studies, that were designed and supervised by S. Buratti, who also interpreted

294 the results. F. Masotti and S. Cattaneo carried out and interpreted the heat-damage-related studies. S. Iametti
295 was responsible for the coordination of the experimental plan execution. M.A. Pagani conceived the study.

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370 Table 1. Chemical parameters for the various pasta samples, as indicated in the label

371

Brand	Ingredients	Proteins (g/100g)	Carbohydrates (g/100g)	Fiber (g/100g)	Lipids (g/100g)
a	Wholegrain organic durum wheat semolina	12.0	67.5	7.0	1.5
b	Wholegrain durum wheat semolina	12.0	65.5	6.0	2.2
c	Wholegrain organic durum wheat semolina	12.2	66.9	6.8	1.7
d	Wholegrain durum wheat semolina, 2% germ	13.0	66.0	7.0	2.5
e	Wholegrain organic durum wheat semolina	13.0	66.0	8.0	2.0
f	Wholegrain durum wheat semolina	13.0	66.7	6.5	2.5
g	Wholegrain organic durum wheat semolina	11.5	64.0	7.0	2.0
h	Wholegrain organic durum wheat semolina	11.0	66.0	7.8	1.9
j	Wholegrain durum wheat semolina	14.0	63.0	7.5	2.5
m	Wholegrain durum wheat semolina	14.0	62.5	7.5	2.5

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374 Table 2. Cooking behavior and heat damage of whole grain pasta.

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Sample	Cooking behaviour		Heat damage	
	Cooking loss (g/100g pasta)	Water absorption (g/100g)	Furosine (mg/100 g proteins)	Pyrraline (mg/100 g proteins)
a1	3.75 ± 0.1 [a]	138.2 ± 8.3 [a]	714 ± 9[b]	15.5 ± 0.6[a]
a2	4.23 ± 0.4 [a]	124.1 ± 7.4 [a]	742 ± 24 [b]	
b1	3.86 ± 0.4 [a]	129.2 ± 7.8 [a]	737 ± 7 [b]	6.5 ± 0.3 [c]
b2	3.70 ± 0.3 [a]	125.2 ± 7.5 [a]	738 ± 11 [b]	
c1	4.10 ± 0.1 [a]	133.0 ± 8.0 [a]	691 ± 11[b]	4.8 ± 0.3 [d]
c2	3.57 ± 0.1 [a]	131.9 ± 7.9 [a]	712± 4 [b]	
d1	4.25 ± 0.5 [a]	125.1 ± 7.5 [a]	836 ± 22[a]	15.8 ± 0.4 [a]
d2	3.87 ± 0.7 [a]	128.5 ± 7.7 [a]	618 ± 6[c]	
e1	4.37 ± 0.1 [a]	155.2 ± 9.3 [a]	742 ± 22[b]	7.0 ± 0.3 [c]
e2	4.31 ± 0.4 [a]	151.0 ± 9.1 [a]	699 ± 14 [b]	
f1	4.25 ± 0.4 [a]	145.8 ± 8.7 [a]	683 ± 6 [b]	4.6 ± 0.2[d]
f2	4.32 ± 0.3 [a]	151.2 ± 9.1 [a]	622 ± 19[c]	
g1	3.81 ± 0.2 [a]	112.2 ± 6.7 [b]	386 ± 16[e]	2.4 ± 0.2[e]
g2	3.88 ± 0.1 [a]	107.8 ± 6.5 [b]	350 ± 6 [e]	
h1	4.16 ± 0.8 [a]	144.3 ± 8.7 [a]	582 ± 11[d]	7.3 ± 0.3 [c]
h2	4.73 ± 0.2 [a]	135.9 ± 8.2 [a]	574 ± 3 [d]	
j1	3.38 ± 0.4[b]	135.7 ± 8.1 [a]	229 ± 8[f]	0.8 ± 0.1[f]
j2	3.63 ± 0.5 [a]	141.0 ± 8.5 [a]	262 ± 3 [f]	

m1	3.75 ± 0.1 [a]	139.3 ± 8.4 [a]	620 ± 12[c]	8.6 ± 0.4[b]
m2	4.01 ± 0.6 [a]	124.3 ± 7.5 [a]	677 ± 14[c]	

376 Values are means ± SD. Values with the same superscript letter in a given column are not significantly different

377 ($P \leq 0.05$).

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380 Table 3. Protein solubilized from different pasta samples in various media.

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Sample	50 mM phosphate buffer mg/g sample	+ 6 M urea mg/g sample	+ 6 M urea and 10 mM DTT mg/g sample
a1	1.78 ± 0.08 [d]	8.61 ± 0.54 [d]	28.35 ± 1.32 [b]
a2	1.97 ± 0.07 [d]	8.34 ± 0.53 [d]	32.04 ± 1.42 [b]
b1	3.34 ± 0.13 [c]	15.39 ± 0.95 [c]	33.31 ± 1.13 [b]
b2	3.54 ± 0.18 [c]	16.17 ± 0.98 [c]	32.42 ± 1.15 [b]
c1	6.79 ± 0.31 [b]	27.52 ± 1.50 [a]	39.39 ± 1.42 [a]
c2	6.08 ± 0.24 [b]	27.77 ± 1.04 [a]	37.45 ± 1.51 [a]
d1	2.43 ± 0.13 [d]	12.92 ± 0.97 [c]	25.12 ± 1.16 [c]
d2	3.01 ± 0.21 [c]	13.47 ± 0.98 [c]	23.08 ± 1.87 [c]
e1	4.25 ± 0.26 [c]	17.27 ± 1.41 [c]	34.09 ± 1.98 [b]
e2	5.26 ± 0.28 [c]	21.35 ± 1.82 [b]	38.94 ± 1.86 [a]
f1	5.79 ± 0.32 [b]	20.67 ± 1.12 [b]	40.48 ± 2.10 [a]
f2	5.11 ± 0.33 [b]	20.57 ± 0.99 [b]	37.24 ± 1.89 [a]
g1	3.52 ± 0.16 [c]	20.57 ± 1.05 [b]	32.41 ± 1.79 [b]
g2	4.64 ± 0.18 [c]	19.01 ± 1.11 [b]	31.14 ± 1.68 [b]
h1	3.01 ± 0.13 [c]	19.99 ± 1.23 [b]	35.60 ± 1.78 [a]
h2	3.85 ± 0.12 [c]	15.59 ± 1.14 [c]	39.60 ± 1.86 [a]
j1	7.56 ± 0.35 [a]	30.25 ± 1.71 [a]	43.98 ± 1.85 [a]
j2	6.75 ± 0.29 [a]	33.93 ± 1.87 [a]	42.27 ± 1.96 [a]
m1	4.85 ± 0.18 [c]	20.97 ± 1.23 [b]	33.60 ± 1.24

m2	4.54 ± 0.21 [c]	20.92 ± 1.25 [b]	36.66 ± 1.34 [a]

382 Values are means ± SD. Values with the same superscript letter in a given column are not significantly different

383 ($P \leq 0.05$).

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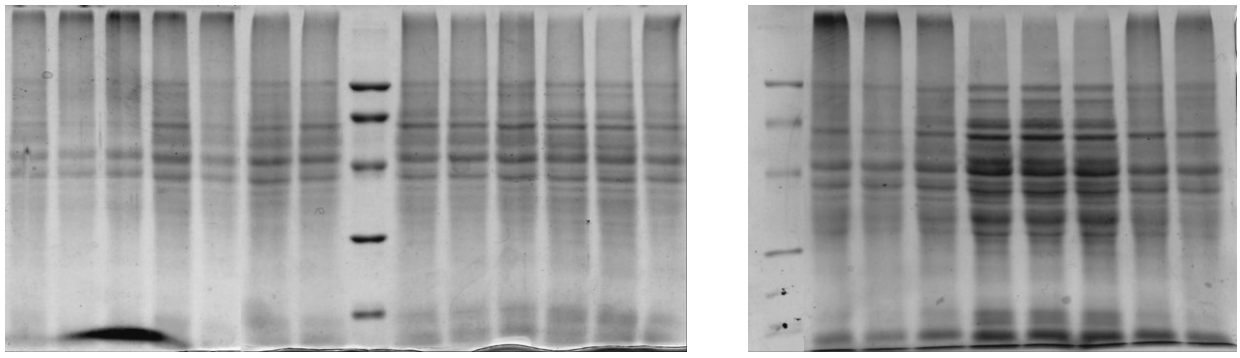

Figure 1 - SDS-PAGE of proteins in various whole grains pasta. Proper amounts of milled pasta were treated with denaturing buffer containing 1.7 % (w/w) SDS and 1% (w/w) 2-ME and denatured by boiling at 100°C. MW: molecular weight markers (range 14000-96000 Da).

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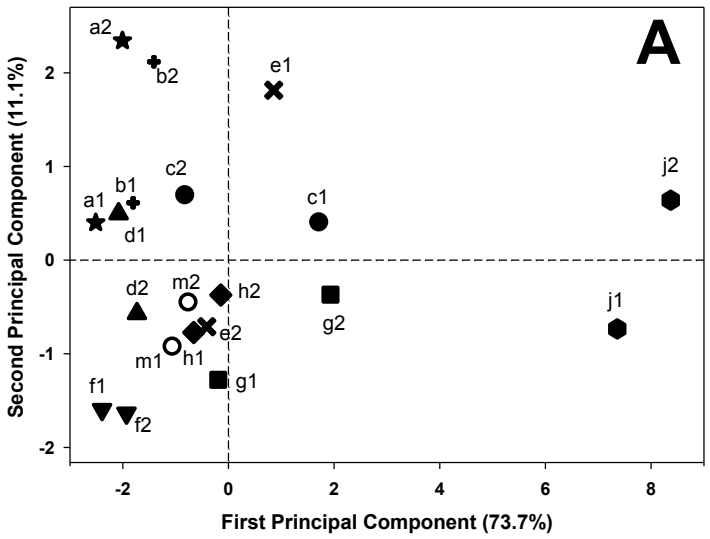

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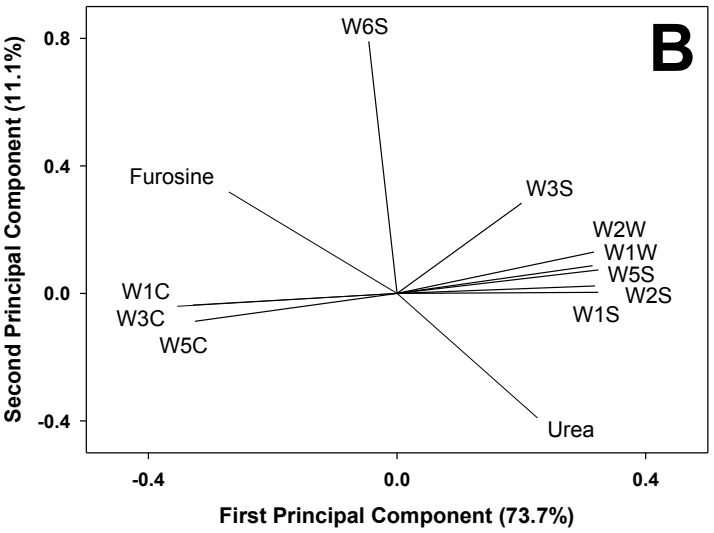

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407 Figure 2 - E-nose PCA score plot (Figure. 2A) and loading plot (Figure 2B) obtained by PCA elaboration of data
408 collected on uncooked pasta in combination with heat damage (FUR) and protein solubility in urea. The data
409 were elaborated in a correlation matrix where the first two principal components accounted for 84.8% of the
410 total variance.

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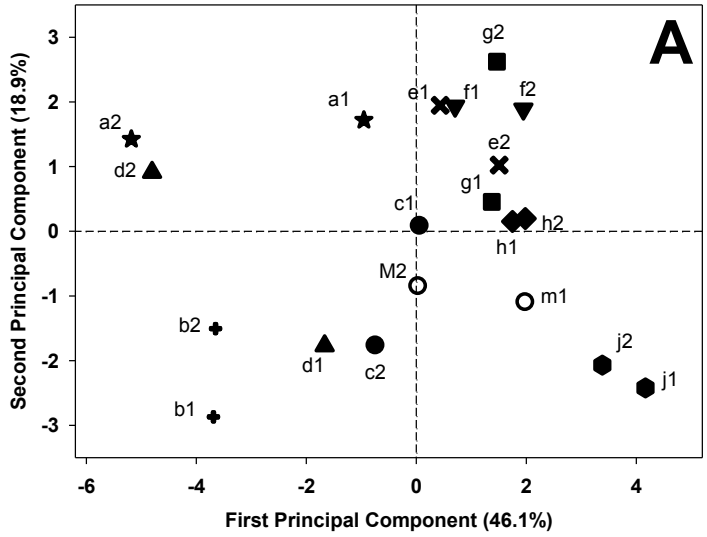
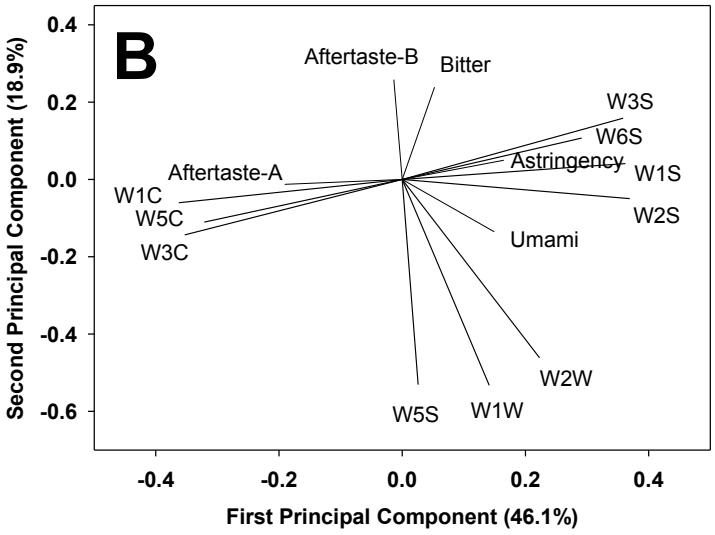


413



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416 Figure 3 - E-tongue PCA score plot (Figure. 2A) and loading plot (Figure 2B) obtained by PCA elaboration of data

417 collected on cooked pasta in combination with the e-nose data, accounting for 65% of the total variance.

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