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Polymeric Proteins Formation During Pasta-making with Einkorn (*Triticum monococcum*) and Semolina Mixtures and Effects on Cooking Behaviour and Acceptability

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Abstract

Pasta was produced in a pilot plant from semolina and semolina blended with increasing amounts of einkorn flour. According to size exclusion high-performance liquid chromatography (SE-HPLC), einkorn proteins interact with semolina proteins during pasta making, forming polymers of high molecular weight. Of these, the unextractable polymeric proteins (UPP) of pasta replaced with 50% einkorn were at significantly higher concentrations than in pasta made from semolina. The increase of S-S bonds and the decrease of –SH free groups in 50% einkorn pasta, with respect to that made of semolina, suggested that polymerization among the different class of proteins happen mainly through disulphide bonds. A decrease in stickiness and an increase in firmness in 50% einkorn pasta corresponded to the formation of large and insoluble protein aggregates.

Keywords: *Triticum monococcum* pasta; Polymeric proteins; SE-HPLC; Cooking quality

Practical Application

Results shown in this work and obtained on a pilot plant represent an interesting starting point for producers aiming to launch on the market new pasta products combining health benefits and consumer liking.

Introduction

Einkorn wheat (*Triticum monoccoccum* ssp. *monococcum*) is ancient diploid (AA) wheat, which originated about 10,000 years ago and represents one of the ancestors of the modern polyploid wheat's [1]. Presently einkorn wheat is cultivated only marginally in the Mediterranean area [1] but there is a renewed interest in this crop due to growing sensibility of the public opinion for dietetic-nutritional aspects, probably influenced by preliminary results which provided that an excellent flour composition may have a significant role in the prevention of pathologies such as cancer, diabetes and chronic inflammatory diseases. Indeed, einkorn wheat has high protein, carotenoid, tocol [2], micro-element [3], resistant starch and fiber [4] content. Therefore it has been proposed as a promising candidate for the development of new foods such as bakery products, baby food or enriched foods [5]. Furthermore, due to the simplicity of its genome einkorn wheat has attracted the interest of the scientific community on nutritional and health aspects in relation to celiac disease. If we consider in fact, that for each genome (AA, BB, DD) there are dozens of genes coding for prolamins in wheat caryopses, it is evident that in einkorn flour the mere presence of the AA genome encodes for a reduced variety of gluten proteins (and of potential immune toxic peptides). Recent evidence shows that prolamins of some einkorn flour cause a reduced inflammatory effect in celiac patients [6-9], articularly for the inability to activate the branch of immune cells [6], or to induce apoptosis of enterocytes [10]. The search of naturally detoxified, or less toxic, ancient grains is of great interest for their potential use in the general diet to prevent disease in those individual at high risk to develop gluten intolerance.

In contrast to nutritional and health advantages, the dough, bread making and pasta making quality of einkorn has been described to be poor in comparison to common wheat [11,12].

From a technological point of view, the exclusive use of semolina durum wheat ensures some desirable parameters in cooked pasta, such as good texture, resistant to surface disintegration, and retention of a firm structure [13]. Thus the replacements of semolina present a major technological challenge.

Compared to common wheat, einkorn flours are characterized, by an extreme imbalance of gliadins and glutenins and by very low amounts of HMW-GS [14]. With respect to single gluten protein types, einkorn is deficient of an entire group of γ-gliadins, which are present in all other wheat species [15]. In contrast to common wheat, the bread quality of einkorn flour is not influenced by the content of total gluten proteins, whereas other parameters such as glutenin content and the ratio of gliadins to glutenins are as important as for common wheat [14].

From a commercial point of view and despite the growing interest in the health aspects of einkorn food products, good sensory properties still remain a key priority as a consumer choice criterion. The challenge is to find einkorn-semolina formulations and processing suitable to give pasta having pleasant sensory properties, in order to meet the consumer's acceptance though it is to be considered that durum wheat's differ in the quantity and quality of gluten and thus various durum wheat semolinas may tolerate the addition of einkorn to varying degrees.

The aim of the paper was to study the effect of the replacement of semolina with increasing amount of einkorn flour on pasta protein polymers, by size-exclusion high performance liquid chromatography

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(SE-HPLC), in order to gain a better understanding of possibly interactions at the molecular level. In addition, similarities and differences in changes in protein structure between pasta replaced with increasing amount of einkorn flour and their relationship to cooking behavior were also evaluated.

Experimental

Pasta samples

Blends of durum wheat semolina and blends of einkorn (*Triticum monococcum*) whole flour were supplied by De Vita mill (Casalnuovo Monterotaro, Foggia, Italy) and Sobrino mill (La Morra, Cuneo, Italy), respectively.

The durum wheat semolina was of the type commonly employed for pasta production (particle size distribution: 15% <200 µm; 200 µm <30% <300 µm; 300 µm <50% <400 µm; 5% >400 µm). The einkorn flour had a granulometry <250 µm. Macaroni were produced at Belladauna pasta factory, (Candela, Foggia, Italy) using a 10-Kg pilot plant (Italpast, Parma) equipped with a mixer and an extruder, by mixing the flours with tap water, at 41°C, in order to obtain a final water content of 28-31% in each dough. This water content was obviously obtained by adding different amounts of water as a function of the percentage of semolina replaced by einkorn. Increasing einkorn flour levels just determined a slightly decrease of dough firmness and an increase of stickiness but these changes did not significantly affect the pasta-making procedure that was performed in the same conditions (temperature 50 ± 5°C; kneading time 15 min; vacuum degree 700 mmHg) independently upon formulation with the exception of the pressure that ranged between 60 and 125 atm as a function of the specific dough composition. A Bronze die-plate was used. Macaroni were dried at 55°C for 12 h. This temperature was applied from the beginning to the end of the drying cycle and then pasta was equilibrated to room conditions. The relative humidity of the hot air was in the 68% range. Four kinds of macaroni were produced: a control made of 100% durum wheat semolina and pasta where 30, 50 and 100% of durum wheat semolina was replaced with einkorn flour (30% einkorn pasta; 50% einkorn pasta; 100% einkorn pasta). The pasta-making procedure was repeated three times.

Chemical composition of flours

Total protein, gluten and fiber content of raw material samples were determined by using the standard AACC methods (AACC, 2000). Total starch content was determined with an enzymatic assay kit (Megazyme, Co. Wicklow, Ireland). Quality of gluten was determined by Glutomatic according to the standard method AACC (AACC, 2000). Analyses were conducted in triplicate.

SDS-PAGE (sodium dodecyl sulphate-polyacrilamide gel electrophoresis) analysis

Total proteins from samples (1g) were extracted using 10 ml of an extraction buffer containing Tris-HCl 0.0625 M pH 6.8, SDS 2%, Glycerol 10% (v/v), Dithiotreitol (DTT) 1.5% (w/v). Flour samples were left in contact with the extraction buffer for 2 h, and then they were centrifuged at 9500 rpm for 15 min at 10°C. The supernatants, containing proteins, were carefully removed and stored at -20°C until the use. Single protein fractions (albumins/globulins, gliadins and glutenins) from flours (1g) were extracted using the Osborne sequential extraction [16]. To separate the extracted proteins, SDS-PAGE was performed on a 12.5% gel under reducing conditions, using a horizontal electrophoresis system Hoefer SE 600, (GE Healthcare, Milan, Italy). SDS-PAGE analysis was carried out at 25 mA for 3 h at room temperature. The gels were stained with 0.25% w/v Coomassie Brilliant Blue (CBB) overnight.

Extraction and fractionation of proteins

Proteins from semolina, einkorn flour and milled macaroni samples were extracted following the method of Gupta and others [17]. The macaroni were milled at a granulometry included within 200 and 350 µm. Soluble proteins from 10 mg of samples were extracted with 1 ml 0.5% SDS-phosphate buffer (pH 6.9); the suspension was shaken for 30 minutes and the solubilised protein ("soluble" protein) was recovered by centrifugation for 10 minutes.

The resulting residues were extracted with 1ml 0.5% SDS-phosphate buffer (pH 6.9) by sonication for 15s (Microson Ultrasonic cell distributor), ensuring that the samples were completely dispersed within the first 5s and then heated to 35°C for 30 minutes. The supernatants after centrifugation (10 min at 17,000 g) were termed "unextractable" proteins ("insoluble" protein). Total proteins (10 mg) were extracted in 1 ml of the same buffer, vortexed, sonicated for 30 seconds and supernatants ("total" protein) were recovered for SE-HPLC analysis. All extracts were filtered through a 0.45 µm PVDF filter prior to be injected on column.

SE-HPLC analysis

SE-HPLC was performed using a LC 10 AD Shimadzu HPLC system (Shimadzu corporation, Kyoto, Japan) and Phenomenex Biosep SEC S-4000 column (300 × 7.8 mm, Phenomenex, Torrence, CA, USA).

Each sample (20 µl) was injected on the column and the eluted proteins were monitored at 214 nm. Three replicates of each samples were used for the investigation of protein composition. The mobile phase was 50% acetonitrile containing 0.05% trifluoroacetic acid with a flow rate of 0.7 ml/min. The SE-HPLC column was calibrated using protein standards with a range of molecular weights (KDa) as follows: ribonuclease A (13.7), chymotripsinogen (25.0), ovalbumin (43.0), bovine serum albumin (67.0), aldolase (158), catalase (232), ferritin (440) and thyroglobulin (669).

The percentage of unextractable polymeric protein (UPP) was calculated as described by Gupta and others [17]. Briefly, the percentage of total UPP was calculated as [peak 1 + 2 area (unextractable)/peak 1 + 2 area (total)] × 100. Peak 1 + 2 area (total) refers to the total of peak 1 + 2 (extractable) and peak 1 + 2 (unextractable) [18].

Determination of SH and S-S groups

The protein disulfide and sulphydryl content in the flours and pasta samples was estimated by a colorimetric determination of free SH groups, using a solid phase assay $NTSB^{2-}$, according to the method of Chan and Wasserman [19].

Statistical analysis

The results were compared by one-way variance analysis (ANOVA). A Duncan's multiple range test, with the option of homogeneous groups ($p<0.05$), was used to determine significance between samples. STATISTICA 7.1 for Windows (StatSoft, Inc, Tulsa, OK, USA) was used for this purpose.

Results

Chemical composition and technological properties of durum wheat semolina and einkorn flour

Durum wheat semolina contains two major components, starch (67%) and proteins (14.30%), and minor components such as lipids and fibers (Table 1). Gliadins (alcohol-soluble) and glutenins (acid/alkali-soluble) represent 87% of total proteins vs. 13% for albumins (water-soluble) and globulins (salt-soluble). Compared with durum wheat semolina, einkorn flour contains higher amount of proteins (15.66%), fiber (8.2%) and lipids (2.5%) and lower amounts of starch (60.2%) and gluten proteins (5.57%).

Durum wheat semolina gluten proteins were characterized by a high gluten index value indicating very good attitude of these proteins to form a strong network in dough and consequently in pasta. In comparison, einkorn flour shows a lower value of gluten index in accordance with its low amount of gluten proteins (35% of total proteins vs. 66% of albumins and globulins) (Table 1).

Effect of the replacement of semolina with einkorn (Triticum monococcum) flour on pasta protein fractions

To study differences in protein composition and for a better understanding of the effect of different formulation on pasta protein polymers, durum wheat semolina, einkorn flour and different types of pasta protein fractions were detected as CBB-stained bands on SDS-PAGE obtained under reducing conditions. Figure 1 shows protein fractions of durum wheat semolina and einkorn flour (Figure 1A) and composite pasta (Figure 1B). Semolina and einkorn flour show several protein bands with a molecular weight included between 100 e 10 kDa. High molecular weight bands (included between 90 and 116 kDa) are represented by High Molecular Weight Glutenin Subunits (HMW-GS), but also by high molecular weight globulin and ω-gliadins. Bands with molecular weight included between 67 and 10 kDa contain mainly Low Molecular Weight Glutenin Subunits (LMW-GS), gliadins (α, β e γ) and low molecular weight globulin and albumins. Differences between the two *Triticum* species can be noticed observing gliadin and glutenin profiles. In particular, considering, the glutenin electrophoretic pattern, a classification of these polymeric prolamins, in four groups (A, B, C, D,), on the basis of their electrophoretic mobility, after disulphide bonds (S-S) reduction, was possible. The A group (with a molecular weight included between 80,000-120,000 Da) corresponds to HMW-GS [20]. Within this group is possible make a further distinction in x type-HMW (with a higher molecular weight) and y type-HMW (lower molecular weight) glutenin subunits. The B group (42,000-51,000 Da) corresponds to B-type Low Molecular Weight termed typical LMW-GS, and the C group (30,000-40,000 Da) to the LMW-GS whose amino-acid sequences is similar to those of γ- and α-gliadins [20].

Finally, D group, (about 80,000-60,000 Da) also belongs to LMW-GS, with highly acidic amino acid sequence very similar to ω-gliadins ones [21]. It is possible to observe that in the A group (HMW-GS) both einkorn flour (lane 5) and semolina (lane 6) show a band whose molecular weight is included between 200 and 97 KDa, and that the einkorn HMW-GS band has a molecular weight greater than that present in semolina. The x-type subunits typically have a slower electrophoretic mobility in SDS-PAGE and, hence, a molecular weight higher than the y-type subunits.

For comparison with data in the literature [22] is thus apparent

Samples	Water content %	Ashes %	Proteins %	Dry gluten %	Total fiber %	Starch %	Gluten Index 1-100
Durum wheat semolina	14.95[a]	0.84[a]	14.30[a]	12.50[A]	1.5[A]	67[a]	80A
Einkorn flour	11.26b	2.08b	15.66b	5.57B	8.2B	60.2b	60B

A, B = ρ < 0.001

a, b = ρ < 0.05

Table 1: Chemical composition and gluten strength of einkorn flour and durum wheat semolina.

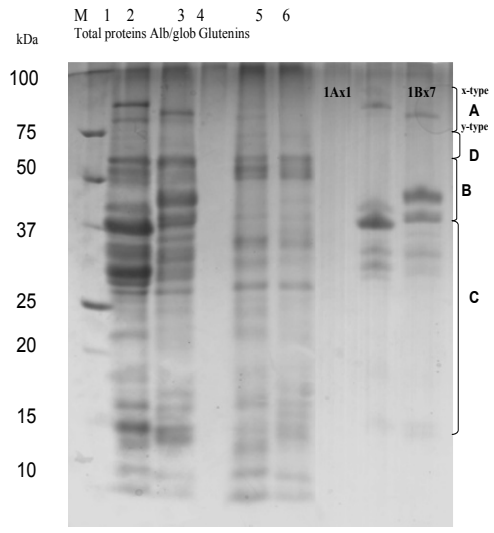

Figure 1a: SDS-PAGE separation of durum wheat semolina and einkorn flour proteins under reducing conditions. Lane 1:einkornflour total proteins; lane 2: durum wheat semolina total proteins; lane 3: einkorn flour albumin and globulin proteins; lane 4: durum wheat semolina albumin and globulins proteins; lane 5: einkorn flour glutenin proteins; lane 6: durum wheat semolina glutenin proteins; lane 7: einkorn flour gliadin protein; lane 8: durum wheat semolina gliadin proteins; M: molecular marker.

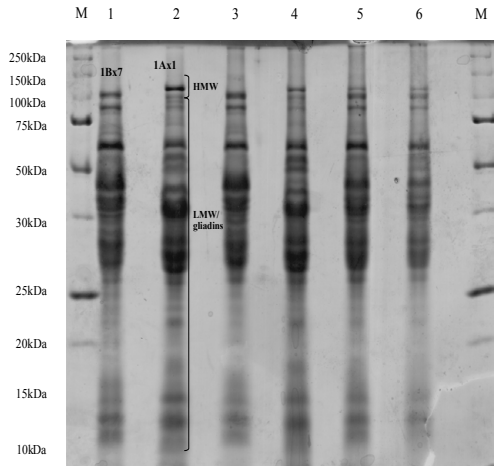

Figure 1b: SDS-PAGE separation of semolina-einkorn pasta proteins under reducing conditions. Lanes 1: durum wheat semolina total proteins; lanes 2: einkorn flour total proteins; lanes 3: 100%-semolina pasta total proteins; lanes 4: 100%-einkorn pasta total proteins; lanes 5-6: pasta proteins with increasing amount of einkorn flour (30% and 50%, respectively); M: molecular marker.

that einkorn flour used in this study contains a 1A × 1 glutenin subunit and semolina a 1B × 7 one [23]. The semolina HMW-GS 1Bx7 is correlated to good technological performance [23] and this would be in agreement with its gluten index value (Table 1).

Even for what concerns the einkorn flour, the Payne and others [24] classification, shows that the 1Ax1 subunits good for the quality of the final product. Therefore, the low Gluten Index value (60) reported in Table 1 would be more likely due to the low amount of gluten proteins present in einkorn flour that to the HMW-GS quality. In addition to the HMW-GS, an important role has also determined by LMW-GS and gliadins.

Glutenin electrophoretic profile evidences higher amount of B-type LMW-GS, with respect to the other types (LMW-D and -C), in semolina than einkorn that, instead, show a higher intensity of the C-type LMW-GS bands. The B-type LMW-GS are related to the good performance of wheat flour as they act as chain extenders of the polymers that are formed [25], thanks to their ability to form two intermolecular disulfide bonds. It is, however, well known that the C-type LMW-GS, are the terminator s of the polymer chain that are form in gand this is due to the presence of a single cysteine residue available to form intermolecular disulfide bridges [26].

Considering, instead, the gliadin pattern, on the basis of their electrophoretic mobility are recognizable: cysteine (S-) poor monomeric prolamins termed ω-gliadins (molecular weight of about 80 KDa), followed by S-rich monomeric prolamins, γ-gliadins (45-30 KDa), β-gliadins (30-14 KDa) and α-gliadins (14-10 KDa). Within the four groups of gliadins it is possible to observe significant differences between semolina (lane 8) and einkorn flour (lane 7). In particular, einkorn flour ω-gliadins show numerous bands with respect to that of semolina ones and also a higher molecular weight in accordance with Wieser and others [14]; γ-gliadins are more numerous in semolina than einkorn, which is deficient of an entire group of this type of proteins [14] and dominated instead by β-gliadins. With regard to the α-gliadins, it is interesting to note that the einkorn flour present a single bands than the four shown in the semolina profile.

Very interesting it is also the albumin/globulin einkorn flour profile (lane 3) which shows protein bands with an higher intensity with respect to durum wheat semolina (lane 4) in accordance with its chemical composition (section 6.1). In addition, the einkorn flour albumin/globulin pattern shows high molecular weight protein bands (between 200 and 80 KDa) which are not all detectable in durum wheat semolina profile.

In Figure 1B the electrophoretic profile of the different types of pasta are represented. Pasta made with 100% semolina (lane 3) and 100% einkorn flour (lane 4) show a protein pattern similar to that of the respective flours (lane 1, semolina and lane 2, einkorn) both in terms of amount and intensity of bands. Although, differences in the ω-gliadins pattern can be inferred passing from 100% einkorn flour to its respective pasta that shows the absence of one of these protein bands. Composite pasta produced replacing semolina with einkorn flour (lane 5, 30% einkorn pasta; and lane 6, 50% einkorn pasta) show hybrid protein profiles between that of semolina and einkorn flour and the presence of both1Bx7 and the 1Ax1 HMW-GS. Furthermore, it is possible to note that the protein bands intensity of composite pasta vary concurrently to the percentage and type of flour added and as for the 100% einkorn pasta, the composite one do not show the presence of the einkorn flour ω-gliadin bands, suggesting the possibility that these proteins may undergo to a self-assembling by hydrophobic bonds at the temperature of 50-60°C, forming structure with molecular weight higher than 200 KDa. It is note of worthy that the protein profile of pasta containing 50% of einkorn flour (lane 6) show a total decrease of bands intensity compared to the other typologies of pasta, suggesting the formation, in this pasta, of larger protein aggregates (with a molecular weight realistically higher than 250 KDa) involving different class of proteins as previously demonstrated by Lamacchia and others [27-28].

Effect of the replacement of semolina with einkorn (Triticum monococcum) flour on pasta protein polymers

Since not only the presence or absence of specific proteins but also the quantities of gluten proteins and the ratios of gliadins to glutenins or of gluten proteins types determine the dough properties and baking performance of common wheat flour [14], extractable and unextractable protein profiles from semolina and einkornflour obtained by fractionation through SE-HPLC (Figure 2) were analyzed. Semolina profile was as described by Lamacchia and others [27,28,29], with a polymeric protein peak (peak 1) of high molecular weight glutenin at the extreme left of the profile (large polymeric proteins, LPP), followed by a small peak (peak 2) of glutenin with a lower molecular weight (small polymeric proteins, SPP), a large peak (peak 3) of monomeric gliadins proteins (large monomeric proteins, LMP) and finally small peaks (peaks 4 and 5) of albumins and globulins, known as small monomeric proteins (SMP).

The SE-HPLC elution profile of einkorn flour was quite similar to that of durum wheat semolina both for the quality and number of peaks. The marked similarity of the two flour elution profiles can be explained considering the fact that einkorn is a cereal belonging to the *Triticum* species and although it is a diploid (AA), contains all classes of proteins (albumins, globulins, gliadins and glutenins) present in the tetraploid (AABB, durum) and hexaploid (AABBDD, soft) wheats. Although, it is noteworthy that einkorn differently to semolina has less

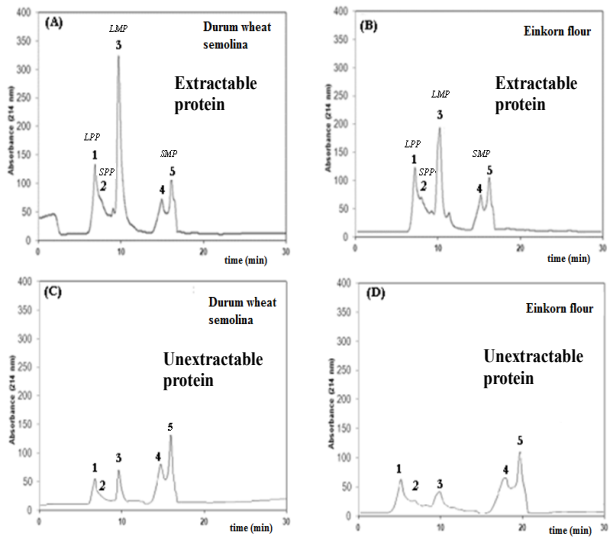

Figure 2: SE-HPLC profiles of extractable (A; B) (obtained without sonication) and unextractable proteins (C; D) (obtained by sonicating the residue after removal of the extractable protein fraction in SDS-phospate buffer) of durum wheat semolina and einkorn flour. Panel A and B show peaks 1 and 2 corresponding to large (LPP) and small (SPP) polymeric proteins, respectively; peaks 3 and 4 and 5 corresponding to large (LMP) monomeric proteins and small (SMP) monomeric proteins, respectively

LMP (peak 3; gliadins) and consequently a lower ratio value of gliadins (LMP, peak 3) to glutenins (High Molecular Weight, LPP, peak 1+ Low Molecular Weight, SPP, peak 2).

SE-HPLC profiles for total proteins in pasta made with durum wheat semolina and increasing amount (30, 50, 100%) of einkorn flour, contain three similar main peaks to high, medium and low molecular weight that differ in peak size (data not shown). Table 2 summarises this information in percentage terms of total proteins. Glutenins (LPP and SPP) and non gluten proteins (SMP) peak area increased significantly ($p<0.001$) as einkorn flour percentage increased (Table 2) in the composite pasta, while the gliadin (LMP)peak area tend to decrease. This in accordance with the fact that the einkorn wheat flour is particularly rich in high and low molecular weight albumins and globulins with respect to durum wheat semolina (Figure 1A), present in the LPP + SPP and SMP peaks, respectively and poor in gliadins (Table 2).

It is worth to note a significant decreases of the total area of the composite pasta with 50% of einkorn flour with respect to other types of pasta, in accordance with what was seen in Fig. 1B suggesting the formation of larger insolubleprotein aggregates. The formation of polymeric aggregates in this formulation of pasta is confirmed by the percentage of UPP. The unextractability of 50% einkorn pasta was significantly ($p<0.001$) higher with respect to those of pasta made with 100% (23.99%) and 30% (19.48%) einkorn flour, respectively and significantly higher ($p<0.001$) with respect to that of 100% semolina pasta, up to a value of about 53.41%. An explanation for these results could come considering the formation of different polymeric structures based on the percentage of the flours used.

In particular, polymeric structures formed in the 30% einkorn pasta could have been particularly affected by the high content in albumins, globulins and ω-gliadins of einkorn flour, and by an excess among the Low Molecular Weight subunits of C-type ones which tend to prevent the elongation of the polymer due to the availability of only a cysteine residue to form interchain disulphide bonds. Therefore a decrease of UPP in 30% einkorn pasta could be due by both an interaction, due to drying temperature, of the chain terminators C- type LMW and HMW-GS, forming polymers of small size and to the self-assembling of ω-gliadins which are not available to form interchain disulphide bonds due to the absence of cysteine residues. Moreover, high molecular weight albumins and globulins, only present in einkorn flour (Figure 1A), coagulate very easily at low temperature forming specific ultrastructure such as fibril like pattern as found by Shomer and others [30]. Thus, the aggregates formed in this type of pasta, because of their chemical nature, consisting mainly of C-type LMW, ω-gliadins, albumins and globulins, and structure, composed of gluten polymers of small size and fibril like–patterns of non-gluten proteins [30] and ω-gliadins, remain for the majority extractable in SDS-phosphate buffer without sonication. This would be supported by the fact that the amount of S-S bonds (Table 3), in this type of pasta, is the lowest one together with that of 100% einkorn pasta and did not reflect the percentage of semolina added, in fact an S-S- bond value closer to that of 100% durum wheat pasta would have been expected.

Polymeric structures of 50% einkorn pasta, instead, could have been influenced more by the increase of HMW-GS with a higher molecular weight (1A × 1) than by albumins, globulins, ω-gliadins and C- type LMW einkorn content. In fact, it is possible that the 1Ax1 HMW-GS amount present in 50% einkorn pasta represents the minimum amount (quantitative effect [31,32]), enough to give rise, together with the 1B × 7 HMW-GS present in semolina, to a growing large polymer, with the two HMW-GS forming the backbone by end-to-end or head-to-tail linkages whose branches are represented by B-type LMW-GS present in durum wheat semolina. Also in this case the result is supported by the amount of S-S bonds of this type of pasta (Table 3). 50% einkorn pasta shows the highest S-S value among the different type of pasta but also with respect to the control pasta made of 100% semolina. The protein polymers in the 50% einkorn pasta, which result by interactions of HMW and LMW proteins, are very large polymers mainly unextractable in SDS-phoshate buffer without sonication that generally lead to an increase of UPP [32]. Furthermore, sometimes a part of these polymers, due to the size, become also unextractable in SDS-phoshate buffer applying sonication [29,27,28]. The formation of

	Durum wheat semolina	Einkorn flour	100%-semolina pasta	30%-einkorn pasta	50%-einkorn pasta	100%-einkorn pasta
LPP+SPP	28.7% A (5469.2μV.s)	33.6% B (6047.95μV.s)	31.1% C (6939.35μV.s)	33.5% B (7641.7μV.s)	35% D (5762.45μV.s)	36% D (6746.6μV.s)
LMP	48.55% A (8898.25μV.s)	38.03% B (6553.45 μV.s)	49.35% A (10603.9μV.s)	46.39% C (9990.3 μV.s)	42.5% D (7632.55μV.s)	40.74% D (7119.85μV.s)
SMP	22.75% A (4214.75μV.s)	28.37% B (4993.05μV.s)	19.55% C (4260.05μV.s)	20.11% C (4449.8 μV.s)	22.5% A (4294.95μV.s)	23.26% D (4166.5 μV.s)
Total area	100% (18582.2 μV.s) A	100% (17594.45μV.s) A	100% (21803.3μV.s) B	100% (22081.8 μV.s) B	100% (14641.2 μV.s) C	100% (18032.9 μV.s) A

A,B,C,D=ρ<0.001

Table 2: Percentage values of peak areas computed from SE-HPLC profiles of extractable proteins.

	Durum wheat semolina	Einkorn flour	100%-semolina pasta	30%-einkorn pasta	50%-einkorn pasta	100%-einkorn pasta
%UPP	35.35A	39.78B	39.56B	19.48C	53.41D	23.99E
S-S μmol/g di prot	80.06Aa	73.38Bb	77.38Aa	68.94Bc	90.90D	68.18Bc
S-H μmol/g di prot	90.68Aa	74.63Bb	87.80Aa	88.89Aa	68.25C	78.32Bb
Total Cysteine μmol/g di prot	250.81Aa	221.39Bb	242.56Aa	226.77Bb	250.05Aa	241.68Dd

A,B,C,D,E=ρ<0.001

a,b,c,d=ρ<0.05

Table 3: Effect of the replacement of semolina with increasing amount of einkorn flour on percentage of total UPP and concentration of SH and S-S groups expressed as μm/g of pasta proteins.

	100%-semolina pasta	30%-einkorn pasta	50%-einkorn pasta	100%-einkornpasta
Stickiness	76	36	81	27
Firmness	64	40	78	33
Overall judgment	70	68	88	40

Table 4: Results of the panel test on a 0 (very bud)-100 (very good) scale.

such polymeric structures in the 50% einkorn pasta would explain the significant decrease of both electrophoretic bands and total peak area in this type of pasta with respect to the other ones.

Effect of the replacement of semolina with einkorn (*Triticum monococcum*) flour on pasta sensory properties

The results of the panel test, performed at the optimum cooking time are reported in Table 4. A decrease in stickiness and an increase in firmness were detected in 50% einkorn pasta. Stickiness is affected by the amount of unabsorbed water associated with drained cooked pasta and is related to the amount of amylose leached from the gelatinized starch granules. Firmness, on the other hand, is primarily affected by protein level [33]. Therefore, a possible explanation for these results is that the replacements of semolina with 50% einkorn flour induce deep changes in the structure of pasta protein polymers. In particular, this pasta formulation may cause the formation of larger and insoluble polymers (as evidenced from the gel electrophoresis and SE-HPLC). The formation of very large and insoluble proteins aggregates may allow starch to absorb water in minor amount preventing starch leaching and therefore stickiness. In addition, the larger aggregates of polymeric proteins, formed during 50% einkorn pasta-making, may contribute to a stronger gluten network which results in a firmer pasta after cooking, thus improving pasta overall quality (Table 4).

Conclusion

In the current investigation, pasta produced with different mixture of semolina and einkorn flour exposed difference in the amount of UPP and in cooking quality. In particular, pasta replaced with 50% einkorn flour showed an increase of the unextractable polymeric proteins with respect to the other type of pasta and also to the control pasta, prepared using 100% durum wheat semolina. According to the panel test, stickiness decreased in 50% einkorn pasta. This type of pasta was less sticky and more firm than the other type of pasta and of the control one. The formation of large and insoluble aggregates at the end affects pasta cooking quality. The replacement of 50% einkorn flour induce deep changes in the structure of the protein polymers that increase in size and strength allowing starch to absorb less water, preventing pasta stickiness and increasing firmness and affecting in a positive way cooking behavior and acceptability. The present study highlighted that the reactions responsible for the better sensory properties of composite pasta with respect to semolina pasta took place at drying temperatures lower than those usually applied in industrial plants. All these results needs to be transferred from pilot to large scale but could represent an interesting starting point for producers aiming to launch on the market new pasta products combining health benefits and consumer liking.

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