Molecular rearrangements in extrusion processes for the production of amaranth-enriched, gluten-free rice pasta

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ABSTRACT

Gluten-free pasta represents a challenge for food technologists and nutritionists since gluten-free materials used in conventional formulations have poor functional and nutritional properties. A novel extrusion-cooking process was set up to improve the textural characteristics of rice-based pasta, and to enrich it with amaranth. Mineral and fiber content, and protein digestibility were improved by amaranth enrichment. Extrusion-cooking of a 75/25 mixture of rice flour and amaranth prior to pasta-making gave the best results as for the textural characteristics of the final product. The firmness of cooked pasta increased due to the extrusion-cooking process, that also decreased protein solubility in the amaranth-enriched pasta. The content in accessible thiols also decreased in amaranth-enriched pastas, indicating that amaranth proteins may be involved in forming disulphide bonds during the pasta-making process. Our results suggest that starch in rice flour interacts best with amaranth proteins when starch gelatinization occurs simultaneously to protein denaturation in the extrusion-cooking process.

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1. Introduction

Gluten-free (GF) foodstuffs – typically based on rice and maize – have a comparatively low content of poor-quality proteins, and are low in fiber, calcium, and iron. GF products also have a high fat and caloric content, to compensate for decreased sensorial acceptability (Thompson, 2009). Macronutrients content in amaranth flour is similar to wheat, and 2–3 times higher than other GF sources (Calderón de la Barca, Rojas-Martínez, Islas-Rubio, & Cabrera-Chávez, 2010). Proteins from amaranth have better amino acid nutritional balance than other vegetable proteins, including cereals, and the fiber and mineral content in amaranth is much higher than in other GF grains (Pedersen, Knudsen, & Eggum, 1990). Amaranth flour has already been used to enrich cereal-based foods, including GF pasta. However, noodles produced from amaranth alone had decreased firmness and increased cooking losses with respect to reference materials (Schoenlechner, Drausinger, Ottenschlaeger, Jurackova, & Berghofer, 2011).

When rice flour is used as the only ingredient for pasta production, it requires additives or particular processing techniques to modify in a suitable way the properties of macromolecular components (starch and proteins) relevant to the structure of the final product. Either gelatinization of the rice flour or steaming of the pasta may improve the textural properties of the final product (Lai, 2001; Pagani, 1986), and a process was developed for rice-based pasta, in which extrusion-cooking of the starting flour was followed by conventional pasta-making processes (Marti, Seetharaman, & Pagani, 2010). Extrusion-cooking causes starch gelatinization followed by retrogradation, forming a rigid starch network and improving the cooking quality of the product. Amaranth proteins in amaranth-enriched rice-based pasta could rearrange their organization or their interaction with other components of the systems at various stages in the process, and the ensuing interactions among proteins or between proteins and other pasta components may improve the textural properties of the product.

The goal of this work was to prepare high-quality amaranth-supplemented rice pasta using extrusion-cooking of each or both

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the starting materials, followed by conventional pasta-making. The effects of supplementation with amaranth and of processing conditions on the pasta properties were assessed, along with the nature of the intermolecular interactions ensuing from the various combinations of ingredients and processes. Information provided from a number of diverse approaches was combined to define a molecular-based rationale for the properties of the final product.

2. Materials and methods

2.1. Flours and pasta samples

Parboiled milled rice (*Oryza sativa*, cultivar Indica; amylose, 25 g/100 g total starch; Riso Viazzo s.r.l., Crova, Italy) was milled into flour (RF; total starch: 80.9 g; damaged starch: 5.9 g; protein: 10.7 g; lipid: 0.4 g; ash: 0.9 g; fiber: 4.2 g, in 100 g dry matter). Amaranth seeds (*Amaranthus hypochondriacus*) were a mixture of organically grown commercial and non-commercial varieties (Cooperativa Quali, Tehuacan, Mexico), milled just prior to use into amaranth flour (AF; total starch: 61.1 g; damaged starch: 7.0 g; protein: 19.1 g; lipid: 9.7 g; ash: 3.0 g; fiber: 18.6 g, in 100 g dry matter). On the basis of previous unpublished trials, 25 parts of AF were mixed with 75 parts of RF to prepare amaranth-enriched pasta. This mixture of flours contained: 73.7 g total starch; 6 g damaged starch; 12.9 g protein; 2.9 g lipids; 1.3 g ash; 5.3 g fiber, in 100 g dry matter.

As summarized in Table 1, pasta samples P1 and P4 were made by room-temperature extrusion from RF and AF in the absence of other treatments. In other cases, flours or flour mixtures were treated prior to pasta-making in a Progel two-zone extrusion-cooker (2 min, extruder zone temperature 120 °C; single screw; Braibanti, Milano, Italy). The process was applied to RF (samples P2 and P5), or to a 75/25 mixture of RF and AF (P3). Pasta was prepared using RF only (untreated, P4; extrusion-cooked, P5), or a mixture of 25/75 combination AF/RF (both untreated, P1; extrusion-cooked RF and untreated AF, P2). Sample P3 was prepared from pellets obtained from extrusion-cooking of a 75/25 mixture of RF and AF. Water content in dough prior to forming was always 400 g kg^{-1}. Pasta was formed into macaroni shape (7 mm outer diameter) in a laboratory-scale extruder (20 kg h^{-1}; MAC 30, Italpast, Parma, Italy; extrusion temperature 25 °C), and dried at low-temperature (50 °C max, 14 h).

2.2. Pasta quality indexes

Cooking losses were evaluated by determining the solids lost into cooking water (grams of matter lost for 100 g of dry pasta; D'Egidio, Mariani, Nardi, Novaro, & Cubadda, 1990), at a pasta:water ratio = 1:10 with no salt addition. Olive oil (10 mL L^{-1}) was added to limit leaching. After cooking, pasta was drained, water was brought back to the initial volume, and an aliquot was dried to

Table 1
Flours, flour mixtures, and treatments of flours and flour mixtures used for pasta-making.

Pasta sample	Flour content (g/100 g)		Flour treatment	
	Rice	Amaranth	Rice	Amaranth
P1	75	25	None	None
P2	75	25	Extrusion-cooked	None
P3	75	25	Extrusion-cooked[a]	Extrusion-cooked[a]
P4	100	0	None	–
P5	100	0	Extrusion-cooked	–

[a] A 75/25 (rice flour, RF/amaranth flour, AF) mixture was subjected to extrusion-cooking, and the resulting material used for pasta-making.

Table 2
Proximate analysis (on a dry matter basis) and protein digestibility of the various pasta samples.

	Pasta sample				
	P1	P2	P3	P4	P5
Ash (g kg^{-1})	12.8[a]	12.6[a]	12.9[a]	9.0[b]	9.6[b]
Protein (g kg^{-1})	128.8[a]	129.3[a]	126.5[a]	107.3[b]	100.1[b]
Total carbohydrates (g kg^{-1})	829.0[a]	827.7[a]	830.9[a]	879.8[b]	886.8[b]
Fat (g kg^{-1})	29.3[a]	30.1[a]	29.7[a]	3.9[b]	3.5[b]
Total fiber (g kg^{-1})	54.8[a]	59.7[a]	58.9[a]	31.9[b]	30.5[b]
Zn (g kg^{-1})	0.071[a]	0.073[a]	0.072[a]	0.007[b]	0.007[b]
Fe (g kg^{-1})	0.075[a]	0.076[a]	0.075[a]	0.016[b]	0.017[b]
Ca (g kg^{-1})	0.296[a]	0.299[a]	0.288[a]	0.036[b]	0.034[b]
Protein digestibility score	83.99[a]	84.74[a]	82.86[a]	80.38[c]	79.97[c]

Different superscripts in a given row indicate statistically significant differences ($P < 0.05$). All data are from triplicate determinations on two sets of samples.

constant weight at 105 °C. Weight increase of pasta due to water absorption during cooking was evaluated gravimetrically. For the purpose of recording leaching kinetics, pasta was also cooked longer than the optimum cooking time (OCT) (D'Egidio et al., 1990).

Texture measurements at OCT for each sample were carried out in a Texture Analyzer TA-HD (Stable Micro Systems, Surrey, UK). The maximum force assessed from the force−time diagram was used as an indicator of firmness.
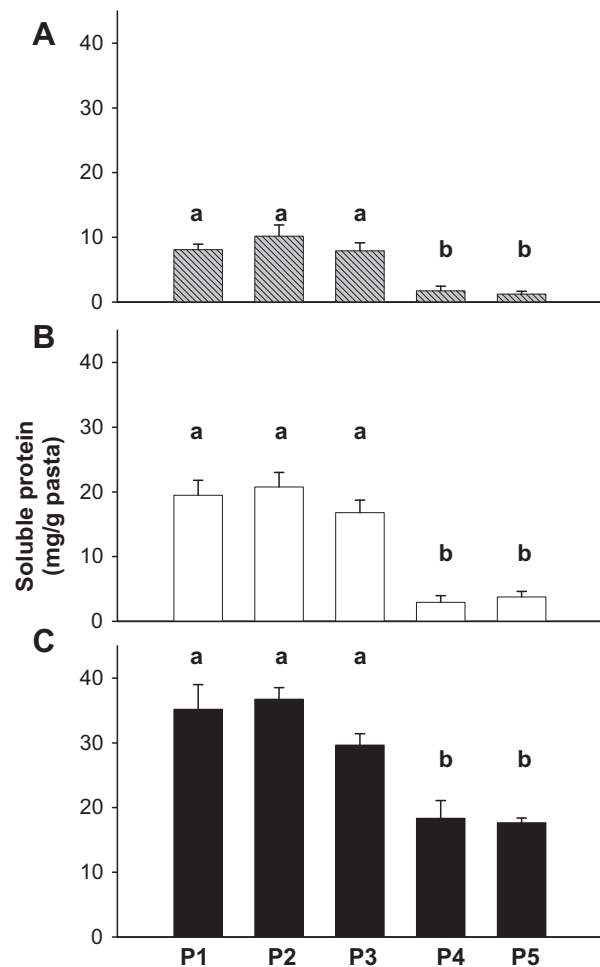

Fig. 1. Solubility of proteins from pasta samples in phosphate/saline buffer in the absence (A) or in the presence of urea (B) and of urea/DTT (C). Standard deviation is given for each sample ($n = 3$). Different letters within each panel indicate statistically significant differences ($P < 0.05$).

2.3. Chemical analysis

The composition of the different flours and pasta samples is reported in Table 2. Analyses were performed according to AOAC (2005) for moisture (934.01), protein (960.52), ash (942.05), and fat content (920.39). Total carbohydrates were calculated by difference. Zn, Fe, and Ca were assessed by AOAC method 968.08 (2005). The total fiber content was determined enzymatically (Prosky, Asp, Schweizer, DeVries, & Furda, 1988). All analytical data are from triplicate determinations on two sets of materials.

2.4. Protein digestibility

In vitro protein digestibility was evaluated according to Hsu, Vavak, Satterlee, and Miller (1977) by using a three-enzyme set (porcine trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1), and intestinal peptidase (EC 3.4.14.5), Sigma–Aldrich, St Louis, MO). Percent protein digestibility was calculated from the pH change after 10 min by using the equation of Hsu et al. (1977).

2.5. Protein solubility and thiol accessibility

The solubility of proteins in pasta samples was determined by suspending finely ground samples in 0.05 mol L^{-1} mM sodium phosphate, 0.1 mol L^{-1} NaCl, pH 7.0, containing 8 mol L^{-1} urea or 8 mol L^{-1} urea and 0.01 mol L^{-1} dithiothreitol (DTT) where indicated (Iametti et al., 2006). After 1 h stirring at 25 °C, the suspensions were centrifuged ($\sim$2500 $\times$ g, 30 min, 25 °C) and the protein concentration in the supernatant was determined by a dye-binding method (Bradford, 1976).

Accessible thiols (expressed as micromol thiols/g pasta) were determined by suspending finely ground pasta samples in 0.05 mol L^{-1} mM sodium phosphate, 0.1 mol L^{-1} NaCl, pH 7.0, containing 0.0002 mol L^{-1} 5,5′-dithiobis-(2-nitrobenzoate) (DTNB), in the presence/absence of 8 mol L^{-1} urea. After 1 h stirring at 25 °C and centrifugation ($\sim$2500 $\times$ g, 30 min, 25 °C), the supernatant absorbance was read at 412 nm (Iametti et al., 2006).

2.6. SDS-PAGE

Proteins solubilized from pasta as described above were diluted with denaturing buffer (0.125 mol L^{-1} Tris–HCl, pH 6.8, 500 ml glycerol L^{-1}, 17 g L^{-1} SDS; 0.1 g L^{-1} Bromophenol Blue), containing 10 ml L^{-1} of 2-mercaptoethanol when indicated, and heated at 100 °C for 10 min. SDS-PAGE was carried out in a MiniProtein apparatus (BioRad, Richmond, VA, USA). Gels were stained with Coomassie Blue. Sample volumes were adjusted to load 0.01 mg of protein per lane.
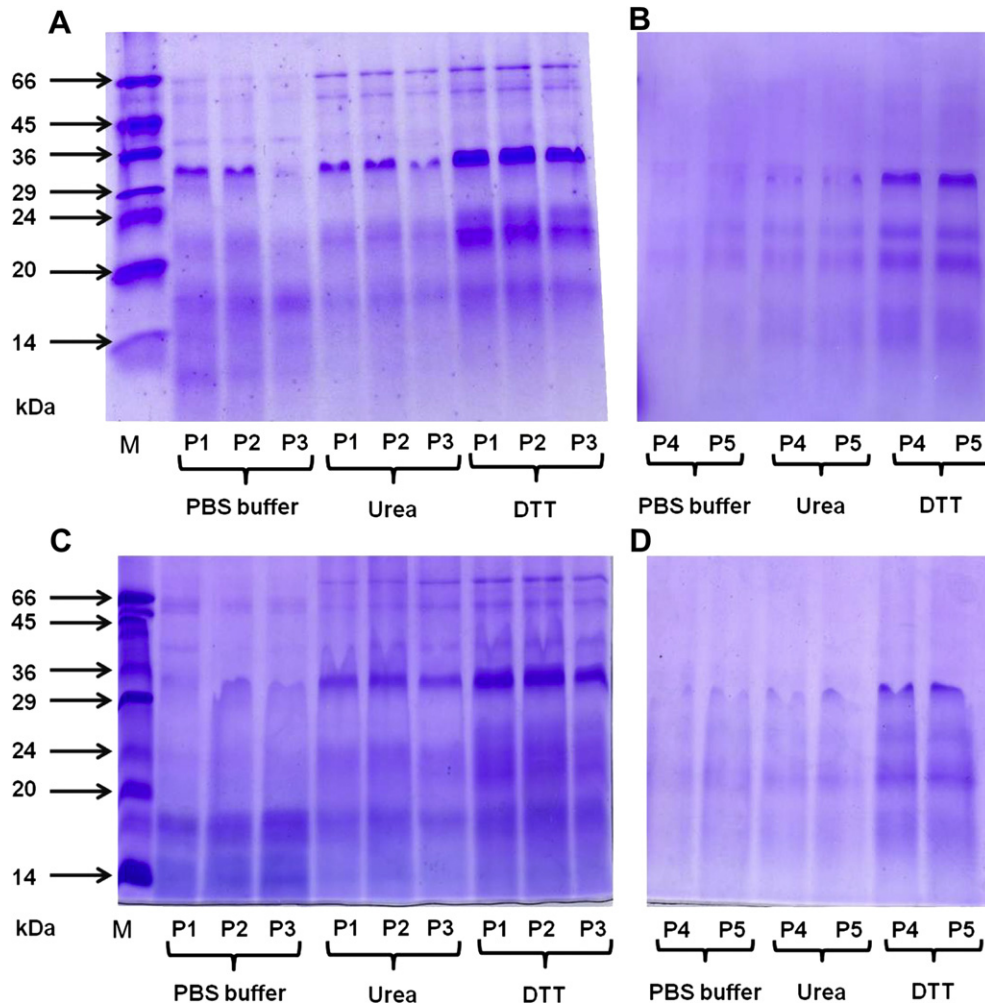

Fig. 2. SDS-PAGE patterns of proteins solubilized in different media from the various pasta samples. Samples were denatured in the absence (A, B) or in the presence (C, D) of 2-mercaptoethanol, and diluted to allow loading the same amount of protein (0.01 mg) in each lane. M: molecular mass markers.

2.7. Damaged starch and starch pasting properties

Damaged starch ($n = 4$) was assessed as for AACC method 76-31 (2001). Pasting properties were measured in triplicate in a Brabender Micro-Visco-AmyloGraph (Brabender, Duisburg, Germany) (Marti et al., 2010), on samples ground to particles smaller than 0.5 mm.

2.8. Statistical analysis

Analysis of variance was carried out to determine statistically significant differences between samples ($P < 0.05$) by using Number Cruncher Statistical System software, version 2001.

3. Results and discussion

3.1. Effects of ingredients and processing on pasta chemical composition and digestibility

The proximate compositional data summarized in Table 2 indicate that there are no significant differences ($P < 0.05$) among amaranth-enriched samples (P1, P2, and P3) or between rice-only ones (P4 and P5). As expected, protein and fat contents were higher in amaranth-supplemented pasta than in rice-only samples. P1, P2 and P3 contain 30–40% more protein than some commercial GF pasta (Mariotti, Iametti, Cappa, Rasmussen, & Lucisano, 2011), to the expense of a significantly increased fat content. Addition of amaranth increased the total fiber content (Table 2), which is of relevance for celiac individuals (Thompson, 2009), as is the increased content of Zn, Fe and Ca.

As also reported in Table 2, the overall protein digestibility was high in all amaranth-enriched pasta samples but the extrusion-cooked AF/RF mixture. An increase in protein accessibility to proteases was observed upon extrusion-cooking of amaranth alone (Mendoza & Bressani, 1987). The opposite result reported here could be related to the presence of the starch-rich rice matrix, that could have changed the pattern and outcome of protein structural reorganization during extrusion-cooking or during the subsequent pasta-making process.

3.2. Role of ingredients and processes in the formation of an inter-protein network

The use of chaotropes and disulfide-reducing agents in protein solubility studies allows to address the nature of the inter-protein interactions in the original materials and of their modification in technological processes (Iametti et al., 2006). Extrusion-cooking of flours or flour mixtures results in structural rearrangement of both protein and starch, and further structural reorganization of these macromolecules (proteins, in particular) may occur in the extrusion or in the drying step.

Protein solubility data are shown in Fig. 1. The solubility of proteins in rice-only pasta in the absence of denaturant and DTT is very low in comparison with that of proteins in amaranth-enriched pasta. Extrusion-cooking had no major effect on rice proteins, but decreased the solubility of amaranth proteins (mainly buffer-soluble albumins), confirming previous reports (Silva-Sánchez, González-Castañeda, De León-Rodríguez, & Barba de la Rosa, 2004). Extrusion-cooking of AF/RF mixtures (P3) caused a decreased protein solubility with respect to control (P5), regardless of the presence of urea and of urea/DTT. This is consistent with some of the proteins becoming inaccessible to the combined action of urea and DTT if cross-linked upon thermal treatment (Avanza, Puppo, & Añón, 2005).

These observations may represent the combined effects of different phenomena. One is the presence of the starch-rich rice matrix, that may affect temperature-related structural rearrangements of proteins, as discussed above. On the other hand, gelatinization and retrogradation of amaranth starch could lower the protein solubility because the water-insoluble crystallized starch granules can entrap proteins, independently of whether they are forming or not supra-macromolecular aggregates. Interchain disulfide exchange also may play an independent role in network formation, as demonstrated by the electrophoretic evidence in a forthcoming section.

SDS-PAGE was used to characterize the extracts obtained form various samples by different solubilizing agents, and to identify specific proteins involved in the events outlined above. Samples were prepared in the absence and in the presence of 2-mercaptoethanol (upper and lower half of Fig. 2, respectively), to verify whether disulfide-linked soluble aggregates had formed. The proteins extracted with urea/DTT from all pasta samples included the entire pattern of proteins from the flours used in this study (not shown). Patterns from rice-only pasta confirmed solubility data, indicating that the same proteins were solubilized from all samples regardless of previous treatments. A buffer-soluble amaranth protein (Mr ∼ 30 kDa) was present in P1 and P2, but not in P3. This species is no longer present after disulfide reduction (Fig. 2, panel C).

Mariotti et al. (2011) have reported that urea-soluble proteins extracted from GF rice and maize pasta participate in the formation of disulfide-linked aggregates. Our data show that buffer-soluble proteins from amaranth may form disulphide bonds (mainly during extrusion-cooking) that maintain a protein network desirable in GF matrices.

The total thiol content was higher in amaranth-supplemented pasta than in rice-only samples (Fig. 3). The methodology used
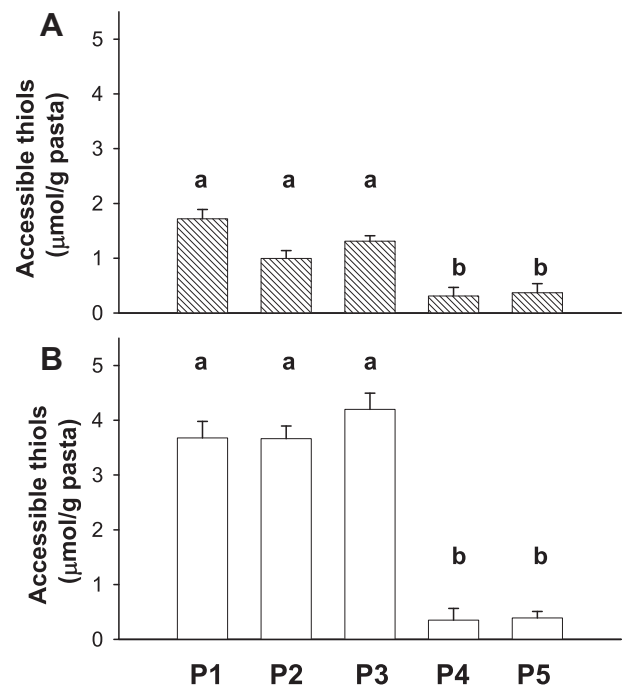
Fig. 3. Thiol content of proteins in the various pasta samples. Thiols were assessed spectrophotometrically on finely ground pasta samples suspended in phosphate/saline buffer in the absence (A) or in the presence of urea (B). Standard deviation is given for each sample ($n = 3$). Different letters within each panel indicate statistically significant differences ($P < 0.05$).
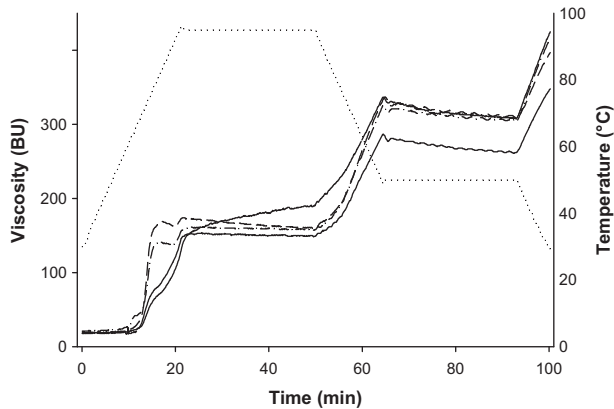
Fig. 4. Starch properties in amaranth-enriched pasta samples (P1, full thick line; P2 thick dashed line; P3, thick dashes and dots) and in a 75/25 (w/w) mixture of rice flour and amaranth flour (AF/RF, thin full line). The thick dotted line indicates the time/temperature profile used for these experiments.

here does not distinguish between cysteine thiols in soluble or insoluble proteins. There was no significant increase in cysteine thiol reactivity in rice-based samples when urea was added, confirming that most cysteine residues in rice proteins are involved in intermolecular disulfide bonds after processing. Thiol accessibility in amaranth-enriched pasta showed a marked increase in the presence of urea, indicating that amaranth proteins have a high free thiol contents, and — once processed — must be denatured in order to make their thiols accessible (Iametti et al., 2006). Thus, formation of inter-protein network in these systems relies on a combination of covalent (disulfide) and of non-covalent, urea-sensitive, hydrophobic interactions. Less than half of the total protein (given as nitrogen content in Table 2) could be solubilized even in the presence of 8 M urea and DTT (see the colorimetric assay data in Fig. 1), suggesting that the formation of the intertwined starch–protein matrix hypothesized above may be relevant to protein solubility issues.

3.3. Effects of processing on starch properties

Properties of starch in amaranth-enriched pasta are shown in Fig. 4, where they are compared with a AF/RF mixture. As reported in Table 3, conventional extrusion resulted in higher starch accessibility to enzymatic action than equivalent AF/RF mixture that did not undergo the room-temperature extrusion used for pasta-making, indicating that minor structural starch modifications may occur also during the pasta-making process. The lowest starch accessibility was observed for P3, confirming previous reports on extrusion-cooking leading "per se" to a high level of starch networking (Marti et al., 2010).

The microviscoamilograph test also allowed to investigate process-related molecular changes (Marti et al., 2010). As shown in Fig. 4, all samples showed a type-C pasting profile (Schoch

& Maywald, 1968), characterized by lack of peak viscosity, breakdown, and low setback values. The pasting properties of P1 were similar to those of the AF/RF mix, if not for the stability during prolonged heating at 95 °C. Also, a maximum viscosity (154 BU) was reached in the case of P1, that remained almost constant during the holding time. The AF/RF mix did not reach a maximum viscosity, indicating that starch presents a highly compact and hydration-resistant structure, possibly because of the contribution of native starch granules from AF.

Extrusion-cooking of RF (P2) or of AF/RF mixtures (P3) led to increased viscosity during the heating step in the viscoamylograph. A peak at around 80 °C appeared in the extrusion-cooked samples in place of a minor shoulder evident in separate runs on AF alone (not shown). Viscosity then increased with temperature up to 95 °C, where a plateau was reached. Compared to P1, P3 showed a lower pasting temperature and a higher viscosity maximum, likely because of a different arrangement of starch molecules during the extrusion step. The higher viscosity of P3 compared to P1 also could be due to higher amylose release from P3, but cooking loss data in the next section speak against this possibility.

3.4. Cooking properties and textural features of amaranth-enriched rice pasta are related to molecular interactions

Previous extrusion-cooking of flours or flour mixtures reportedly increases the OCT, and extrusion at high temperature creates in rice pasta a hydrophilic structure that absorbs high-water amounts (Marti et al., 2010). However, the amount of water absorbed during cooking showed no significant differences among the various samples considered here, regardless of their formulation and processing conditions (not shown).

Fig. 5 presents cooking losses as a function of cooking time. The addition of 25% AF did not affect cooking losses in P1 and P4 at the OCT (10.5 and 11 min for P1 and P4, respectively). However, the differences between P1 and P4 increased remarkably at slightly longer cooking times, confirming literature reports on the possibility that a high fiber content (Table 2) may interrupt the continuity of the pasta structure upon overcooking (Marti et al., 2010; Tudorica, Kuri, & Brennan, 2002). The leaching behavior of P2 was similar to that of P1 and P4. Cooking losses were lowest for P3 and P5, suggesting that extrusion-cooking of RF or of AF/RF mixture created an organized structure able to withstand cooking stresses. However, in amaranth-enriched P3 cooking losses also increased markedly upon overcooking, again because of the presence of fiber (Table 2) that weakens the starch network, as observed in gluten-based (Tudorica et al., 2002) and GF (Marti et al., 2010) matrices.

The extrusion-cooking step also increased firmness of rice-only pasta (from 7.4 N in P4 to 8.2 N for P5). Addition of amaranth markedly decreased firmness, in particular when AF did not undergo an extrusion-cooking step (Table 3). Only when RF and AF

Table 3
Properties of ingredients and products.

	RF	AF	AF/RF	P1	P2	P3
Damaged starch (g/100 g)	5.9 ± 0.5[d]	7.0 ± 0.1[c]	5.9 ± 0.2[d]	9.5 ± 0.4[a]	9.6 ± 0.5[a]	8.1 ± 0.3[b]
Pasting temperature (°C)	73.4 ± 2.7[a]	66.7 ± 0.3[b]	67.9 ± 2.7[b]	68.9 ± 0.1[b]	62.1 ± 0.1[c]	65.4 ± 0.2[bc]
Maximum viscosity (BU)	122 ± 2.8[d]	618.5 ± 30.4[a]	189.5 ± 2.1[b]	154.0 ± 0.1[c]	159.5 ± 3.5[c]	175.0 ± 1.4[bc]
Breakdown (BU)	0[b]	271.0 ± 15.5[a]	1.5 ± 0.7[b]	4.5 ± 0.7[b]	4.5 ± 0.7[b]	15.0 ± 0.1[b]
Setback (BU)	179.0 ± 5.6[d]	212.0 ± 18.4[c]	233.0 ± 2.8[b]	200.0 ± 1.4[c]	254.5 ± 4.9[a]	234.5 ± 4.9[ab]
Firmness (N)	–	–	–	3.1 ± 0.2[c]	5.3 ± 0.3[b]	7.2 ± 0.2[a]

Different superscripts indicate statistically significant differences (P < 0.05).
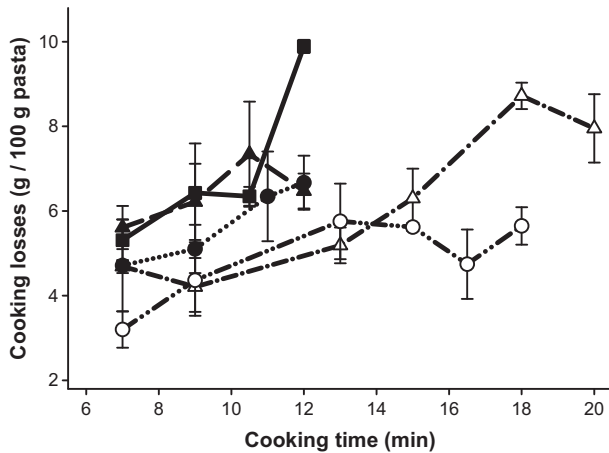
Fig. 5. Time-dependence of cooking losses for the various pasta samples (P1, squares; P2, full triangles; P3, open triangles; P4, full circles; P5, open circles). Standard deviation is given for each experimental point ($n = 3$).

underwent a concomitant extrusion-cooking treatment firmness (P3, 7.2 N) was comparable to that of rice-only pasta. The high firmness of rice-only pasta is due to the elevated content of starch in RF, and to starch retrogradation in the extrusion-cooking process. The addition of AF increases the amount of proteins and fiber, that act synergistically in decreasing the extent of retrograded starch. Extrusion-cooking of AF/RF mixtures affects the structure of amaranth proteins, making them more able to interact with neighboring macromolecules (Clark, Kavanagh, & Ross-Murphy, 2001) rather than with the solvent, as demonstrated by our protein solubility data. Antagonistic and synergistic relationships have been reported between fiber and other food components (mainly starch and protein) and have been related to restricted water movement during the cooking of pasta products (Brennan, Kuri, & Tudorica, 2004). Thus, the decrease in firmness of fiber-enriched pasta may be associated with a decrease in starch swelling and gelatinization (Brennan & Tudorica, 2008).

4. Conclusions

Incorporation of amaranth flour in rice pasta combined with extrusion-cooking improves the textural and nutritional quality of the final product. Addition of 25% amaranth flour significantly improves the nutritional characteristics of rice-based pasta without much dramatic worsening of cooking behavior. In this frame, introduction of the extrusion-cooking step prior to pasta-making is decisive, as pasta made from an extrusion-cooked mixture of rice flour and amaranth flour (sample P3) had the best textural and nutritional characteristics.

The physicochemical changes occurring in the pasta-making process affect the properties of the final product, and involve both the starch and the protein fractions, and their mutual interactions. Having the appropriate form of either macromolecule at a given step of the whole process seems of paramount relevance to the product quality. In other words, the best results — in terms of quality of the final product — are obtained when starch in rice flour is allowed to interact during gelatinization with amaranth proteins that are simultaneously undergoing thermal denaturation in the extrusion-cooking process.

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