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.

Highlights:

Amaranth-enriched rice-based pasta had improved nutrient content and digestibility

Extrusion-cooking of mixtures of rice/amaranth flours (75/25) gave good quality pasta

Simultaneous starch gelatinization/protein denaturation positively affected quality

Keywords: Gluten-free; celiac consumers; rice pasta; amaranth; extrusion-cooking.

Abbreviations used:

GF: Gluten-free; RF: Rice Flour; AF: Amaranth Flour; P1: Pasta sample 1; P2: Pasta sample 2; P3: Pasta sample 3; P4: Pasta sample 4; P5: Pasta sample 5; DTT: Dithiothreitol; DTNB: 5,5'-dithiobis-(2-nitrobenzoate); OCT: optimum cooking time; BU: Brabender Units

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

77 with other components of the systems at various stages in the process, and the ensuing
78 interactions among proteins or between proteins and other pasta components may improve
79 the textural properties of the product.

80 The goal of this work was to prepare high-quality amaranth-supplemented rice pasta
81 using extrusion-cooking of each or both the starting materials, followed by conventional
82 pasta-making. The effects of supplementation with amaranth and of processing conditions
83 on the pasta properties were assessed, along with the nature of the intermolecular
84 interactions ensuing from the various combinations of ingredients and processes.
85 Information provided from a number of diverse approaches was combined to define a
86 molecular-based rationale for the properties of the final product.

87

88 **2. Materials and methods**

89 *2.1. Flours and pasta samples.*

90 Parboiled milled rice (*Oryza sativa*, cultivar Indica; amylose, 25 g/100 g total starch; Riso
91 Viazzo s.r.l., Crova, Italy) was milled into flour (RF; total starch: 80.9 g; damaged starch:
92 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
93 seeds (*Amaranthus hypochondriacus)* were a mixture of organically grown commercial and
94 non-commercial varieties (Cooperativa Quali, Tehuacan, Mexico), milled just prior to use
95 into amaranth flour (AF; total starch: 61.1 g; damaged starch: 7.0 g; protein: 19.1 g; lipid:
96 9.7 g; ash: 3.0 g; fiber: 18.6 g, in 100 g dry matter). On the basis of previous unpublished
97 trials, 25 parts of AF were mixed with 75 parts of RF to prepare amaranth-enriched pasta.
98 This mixture of flours contained: 73.7 g total starch; 6 g damaged starch; 12.9 g protein; 2.9
99 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 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 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, Mariani, Nardi, Novaro, & Cubadda, 1990).

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

125

126 *2.3. Chemical analysis*

127 The composition of the different flours and pasta samples is reported in **Table 2.** Analyses
128 were performed according to AOAC (2005) for moisture (934.01), protein (960.52), ash
129 (942.05), and fat content (920.39). Total carbohydrates were calculated by difference. Zn,
130 Fe, and Ca were assessed by AOAC method 968.08 (2005). The total fiber content was
131 determined enzymatically (Prosky, Asp, Schweizer, DeVries, & Furda, 1988). All
132 analytical data are from triplicate determinations on two sets of materials.

133

134 *2.4. Protein digestibility*

135 *In vitro* protein digestibility was evaluated according to Hsu, Vavak, Satterlee, & Miller
136 (1977) by using a three-enzyme set (porcine trypsin (EC 3.4.21.4), chymotrypsin (EC
137 3.4.21.1), and intestinal peptidase (EC 3.4.14.5), Sigma-Aldrich, St Louis, MO). Percent
138 protein digestibility was calculated from the pH change after 10 min by using the equation
139 of Hsu, Vavak, Satterlee, & Miller (1977).

140

141 *2.5. Protein solubility and thiol accessibility*

142 The solubility of proteins in pasta samples was determined by suspending finely ground
143 samples in 0.05 mol L^{-1} mM sodium phosphate, 0.1 mol L^{-1} NaCl, pH 7.0, containing 8 mol
144 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 (~2,500 *x 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 (~2,500 x 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.

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, Seetharaman, & Pagani, 2010), on samples ground to particles smaller than 0.5 mm.

2.8. Statistical analysis

169 Analysis of variance was carried out to determine statistically significant differences
170 between samples ($P < 0.05$) by using Number Cruncher Statistical System software, version
171 2001.
172
173
174 **3. Results and discussion**
175
176 *3.1. Effects of ingredients and processing on pasta chemical composition and digestibility*
177 The proximate compositional data summarized in **Table 2** indicate that there are no
178 significant differences ($P<0.05$) among amaranth-enriched samples (P1, P2, and P3) or
179 between rice-only ones (P4 and P5). As expected, protein and fat contents were higher in
180 amaranth supplemented pasta than in rice-only samples. P1, P2 and P3 contain 30-40%
181 more protein than some commercial GF pasta (Mariotti, Iametti, Cappa, Rasmussen, &
182 Lucisano, 2011), to the expense of a significantly increased fat content. Addition of
183 amaranth increased the total fiber content (**Table 2**), which is of relevance for coeliac
184 individuals (Thompson, 2009), as is the increased content of Zn, Fe and Ca.
185 As also reported in **Table 2**, the overall protein digestibility was high in all
186 amaranth-enriched pasta samples but the extrusion-cooked AF/RF mixture. An increase in
187 protein accessibility to proteases was observed upon extrusion-cooking of amaranth alone
188 (Mendoza & Bressani, 1987). The opposite result reported here could be related to the
189 presence of the starch-rich rice matrix, that could have changed the pattern and outcome of
190 protein structural re-organization during extrusion-cooking or during the subsequent pasta-
191 making process.
192

193 *3.2. Role of ingredients and processes in the formation of an inter-protein network*

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

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

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

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

227 Mariotti, Iametti, Cappa, Rasmussen, & Lucisano (2011) have reported that urea-
228 soluble proteins extracted from GF rice and maize pasta participate in the formation of
229 disulfide-linked aggregates. Our data show that buffer-soluble proteins from amaranth may
230 form disulphide bonds (mainly during extrusion-cooking) that maintain a protein network
231 desirable in GF matrices.

232 The total thiol content was higher in amaranth-supplemented pasta than in rice-only
233 samples (**Figure 3**). The methodology used here does not distinguish between cysteine
234 thiols in soluble or insoluble proteins. There was no significant increase in cysteine thiol
235 reactivity in rice-based samples when urea was added, confirming that most cysteine
236 residues in rice proteins are involved in intermolecular disulfide bonds after processing.
237 Thiol accessibility in amaranth-enriched pasta showed a marked increase in the presence of
238 urea, indicating that amaranth proteins have a high free thiol contents, and - once processed
239 - must be denatured in order to make their thiols accessible (Iametti et al., 2006). Thus,

240 formation of inter-protein network in these systems relies on a combination of covalent
241 (disulfide) and of non-covalent, urea-sensitive, hydrophobic interactions. Less than half of
242 the total protein (given as nitrogen content in **Table 2)** could be solubilized even in the
243 presence of 8 M urea and DTT (see the colorimetric assay data in **Figure 1**), suggesting
244 that the formation of the intertwined starch-protein matrix hypothesized above may be
245 relevant to protein solubility issues.
246
247 *3.3. Effects of processing on starch properties*
248 Properties of starch in amaranth-enriched pasta are shown in **Figure 4**, where they are
249 compared with a AF/RF mixture. As reported in **Table 3**, conventional extrusion resulted in
250 higher starch accessibility to enzymatic action than equivalent AF/RF mixture that did not
251 undergo the room-temperature extrusion used for pasta making, indicating that minor
252 structural starch modifications may occur also during the pasta-making process. The lowest
253 starch accessibility was observed for P3, confirming previous reports on extrusion-cooking
254 leading "per se" to a high level of starch networking (Marti, Seetharaman, & Pagani, 2010).
255 The microviscoamilograph test also allowed to investigate process-related
256 molecular changes (Marti, Seetharaman, & Pagani, 2010). As shown in **Figure 4**, all
257 samples showed a type-C pasting profile (Schoch & Maywald, 1968), characterized by lack
258 of peak viscosity, breakdown, and low setback values. The pasting properties of P1 were
259 similar to those of the AF/RF mix, if not for the stability during prolonged heating at 95 °C.
260 Also, a maximum viscosity (154 BU) was reached in the case of P1, that remained almost
261 constant during the holding time. The AF/RF mix did not reach a maximum viscosity,
262 indicating that starch presents a highly compact and hydration-resistant structure, possibly
263 because of the contribution of native starch granules from AF.

264 Extrusion-cooking of RF (P2) or of AF/RF mixtures (P3) led to increased viscosity
265 during the heating step in the viscoamylograph. A peak at around 80 °C appeared in the
266 extrusion-cooked samples in place of a minor shoulder evident in separate runs on AF alone
267 (not shown). Viscosity then increased with temperature up to 95 °C, where a plateau was
268 reached. Compared to P1, P3 showed a lower pasting temperature and a higher viscosity
269 maximum, likely because of a different arrangement of starch molecules during the
270 extrusion step. The higher viscosity of P3 compared to P1 also could be due to higher
271 amylose release from P3, but cooking loss data in the next section speak against this
272 possibility.
273
274 *3.4. Cooking properties and textural features of amaranth-enriched rice pasta are related*
275 *to molecular interactions*
276 Previous extrusion-cooking of flours or flour mixtures reportedly increases the OCT, and
277 extrusion at high temperature creates in rice pasta a hydrophilic structure that absorbs high
278 water amounts (Marti, Seetharaman, & Pagani, 2010). However, the amount of water
279 absorbed during cooking showed no significant differences among the various samples
280 considered here, regardless of their formulation and processing conditions (not shown).
281 **Figure 5** presents cooking losses as a function of cooking time. The addition of
282 25% AF did not affect cooking losses in P1 and P4 at the OCT (10.5 and 11 min for P1 and
283 P4, respectively). However, the differences between P1 and P4 increased remarkably at
284 slightly longer cooking times, confirming literature reports on the possibility that a high
285 fiber content (**Table 2**) may interrupt the continuity of the pasta structure upon overcooking
286 (Tudorica, Kuri, & Brennan, 2002; Marti, Seetharaman, & Pagani, 2010). The leaching
287 behavior of P2 was similar to that of P1 and P4. Cooking losses were lowest for P3 and P5,

288 suggesting that extrusion-cooking of RF or of AF/RF mixture created an organized
289 structure able to withstand cooking stresses. However, in amaranth-enriched P3 cooking
290 losses also increased markedly upon overcooking, again because of the presence of fiber
291 (**Table 2**) that weakens the starch network, as observed in gluten-based (Tudorica, Kuri, &
292 Brennan, 2002) and GF (Marti, Seetharaman, & Pagani, 2010) matrices.

293 The extrusion-cooking step also increased firmness of rice-only pasta (from 7.4 N in
294 P4 to 8.2 N for P5). Addition of amaranth markedly decreased firmness, in particular when
295 AF did not undergo an extrusion-cooking step (**Table 3**). Only when RF and AF underwent
296 a concomitant extrusion-cooking treatment firmness (P3, 7.2 N) was comparable to that of
297 rice-only pasta. The high firmness of rice-only pasta is due to the elevated content of starch
298 in RF, and to starch retrogradation in the extrusion-cooking process. The addition of AF
299 increases the amount of proteins and fiber, that act synergistically in decreasing the extent
300 of retrograded starch. Extrusion-cooking of AF/RF mixtures affects the structure of
301 amaranth proteins, making them more able to interact with neighboring macromolecules
302 (Clark, Kavanagh, & Ross-Murphy, 2001) rather than with the solvent, as demonstrated by
303 our protein solubility data. Antagonistic and synergistic relationships have been reported
304 between fiber and other food components (mainly starch and protein) and have been related
305 to restricted water movement during the cooking of pasta products (Brennan, Kuri &
306 Tudorica, 2004). Thus, the decrease in firmness of fiber-enriched pasta may be associated
307 with a decrease in starch swelling and gelatinisation (Brennan & Tudorica, 2008).

308

309 *4. Conclusions*

310 Incorporation of amaranth flour in rice pasta combined with extrusion-cooking improves
311 the textural and nutritional quality of the final product. Addition of 25% amaranth flour

312 significantly improves the nutritional characteristics of rice-based pasta without much
313 dramatic worsening of cooking behavior. In this frame, introduction of the extrusion-
314 cooking step prior to pasta making is decisive, as pasta made from an extrusion-cooked
315 mixture of rice flour and amaranth flour (sample P3) had the best textural and nutritional
316 characteristics.

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

324

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330

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394

Table 1

Flours, flour mixtures, and treatments of flours and flour mixtures used for pasta making

Pasta sample	Flour content (g/100g)		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

403 **Table 2**

404 Proximate analysis (on a dry matter basis) and protein digestibility of the various pasta

405 samples

406

	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 [b]	80.38 [c]	79.97 [c]

407

408 Different superscripts in a given row indicate statistically significant differences ($P < 0.05$).

409 All data are from triplicate determinations on two sets of samples.

410

411 **Table 3**

412 Properties of ingredients and products

	RF	AF	AF/RF	P1	P2	P3
Damaged starch (g/100g)	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}

413

414 Different superscripts indicate statistically significant differences ($P < 0.05$).

415

FIGURE LEGENDS

Figure 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 ($P < 0.05$).

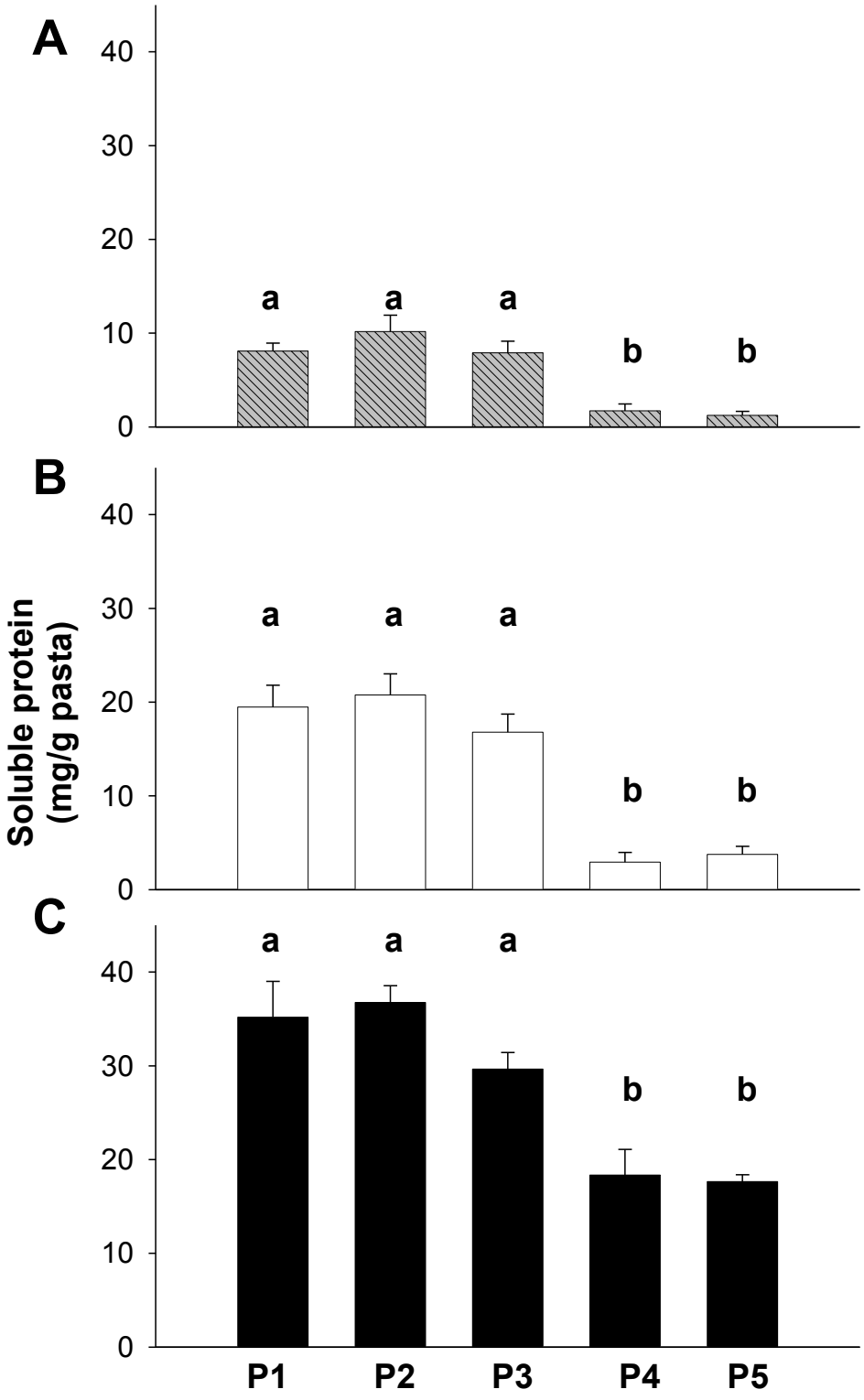
Figure 2. SDS-PAGE patterns of proteins solubilized in different media from the various pasta samples. Samples were denatured in the absence (A, B) or in the presence (C, D) of 2-mercaptoethanol, and diluted to allow loading the same amount of protein (0.01 mg) in each lane. M: molecular mass markers.

Figure 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 ($P < 0.05$).

Figure 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.

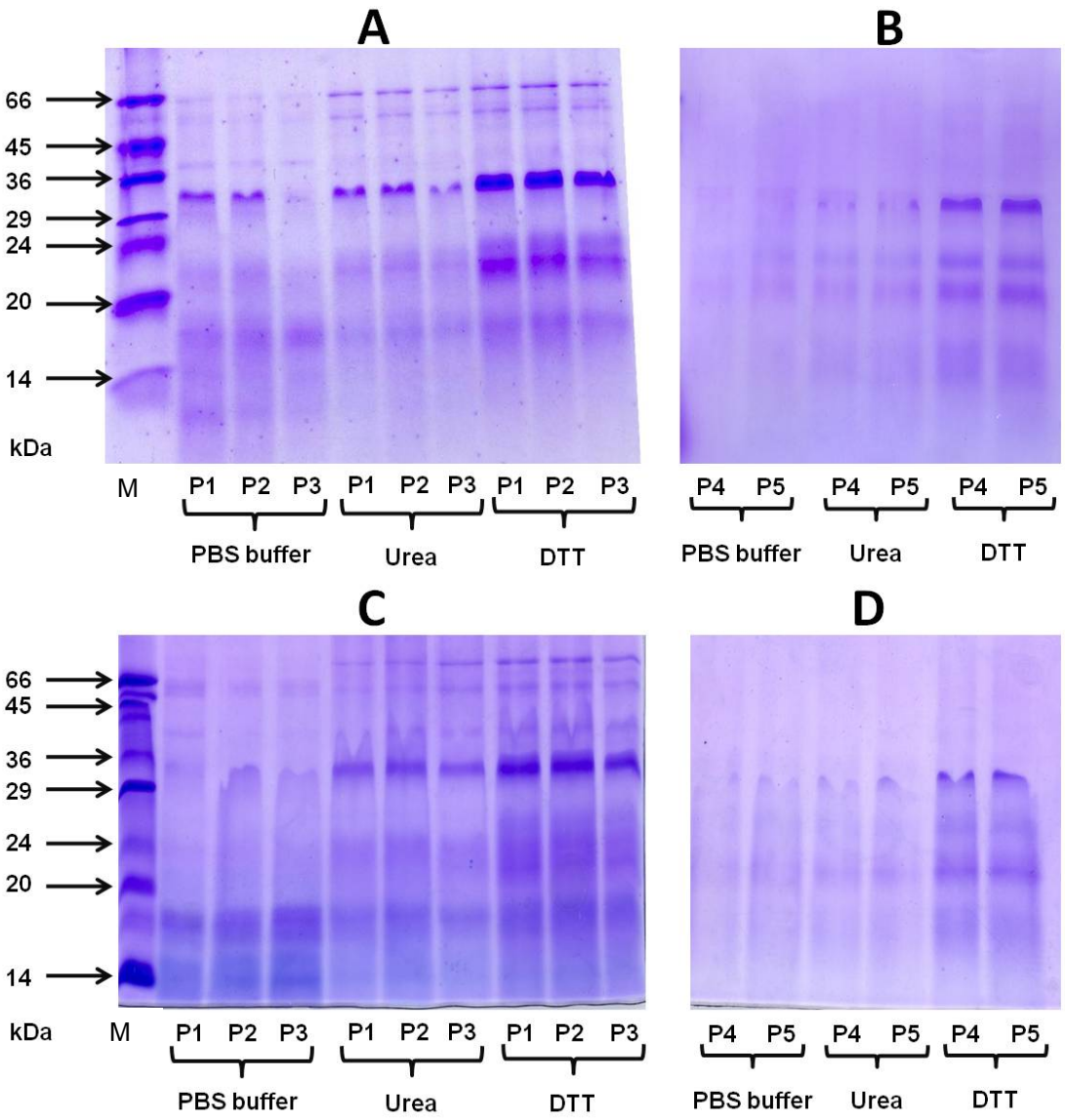
439 **Figure 5.** Time-dependence of cooking losses for the various pasta samples (P1, squares;
440 P2, full triangles; P3, open triangles; P4, full circles; P5, open circles). Standard deviation
441 is given for each experimental point (n=3).
442

443 **Figure 1**



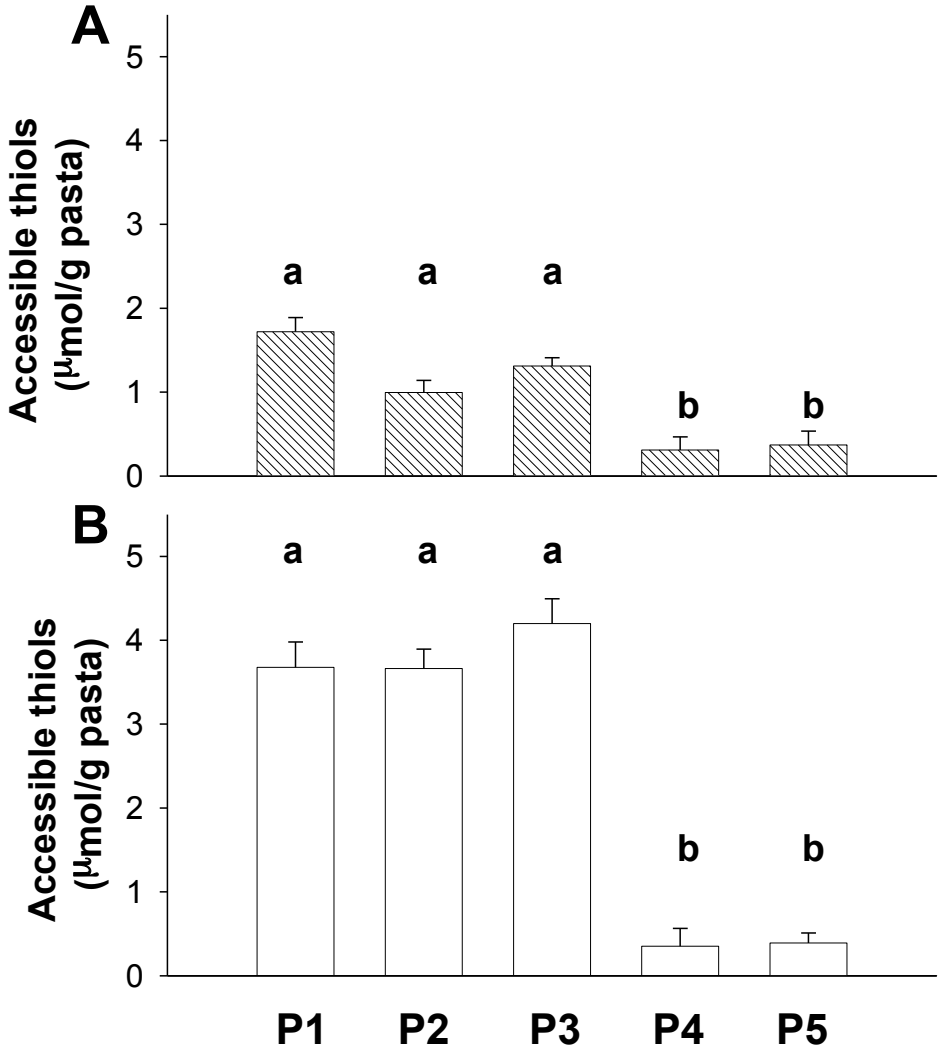
444

Figure 2


A
B
C
D
66
45
36
29
24
20
14
kDa
M
P1 P2 P3 P1 P2 P3 P1 P2 P3
P4 P5 P4 P5 P4 P5
PBS buffer
Urea
DTT

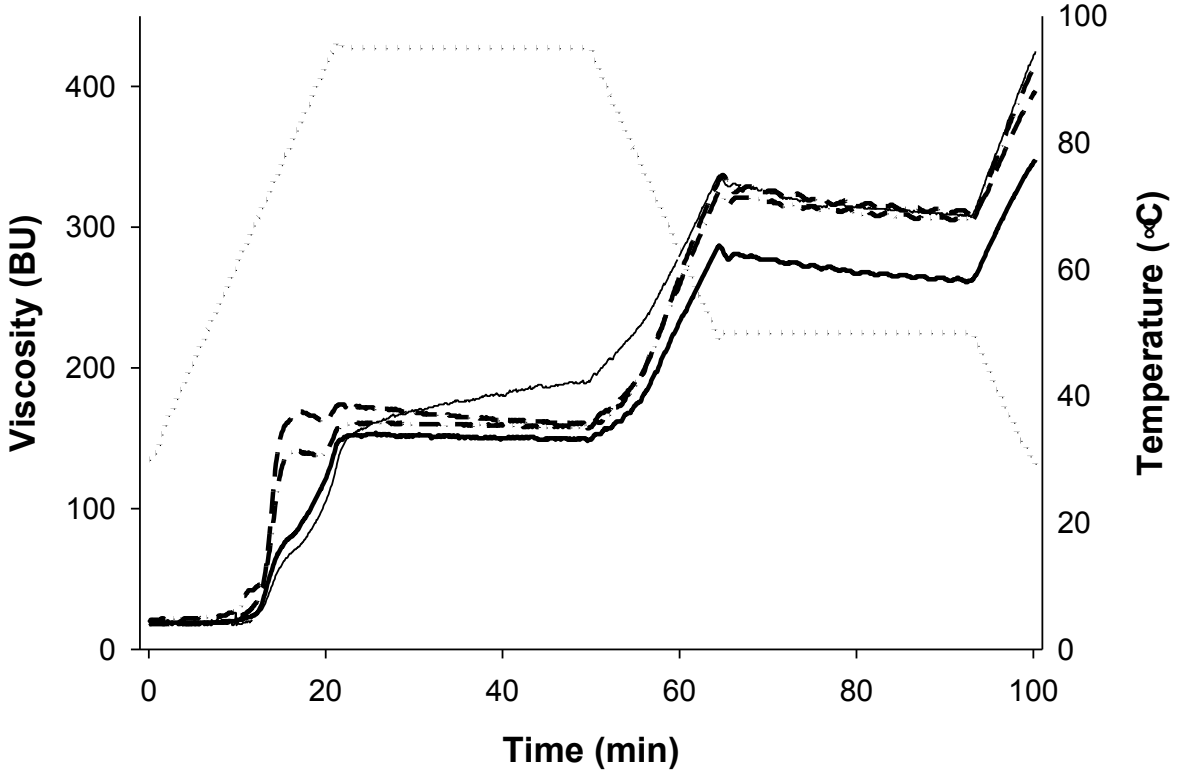
450 **Figure 3**

451
452



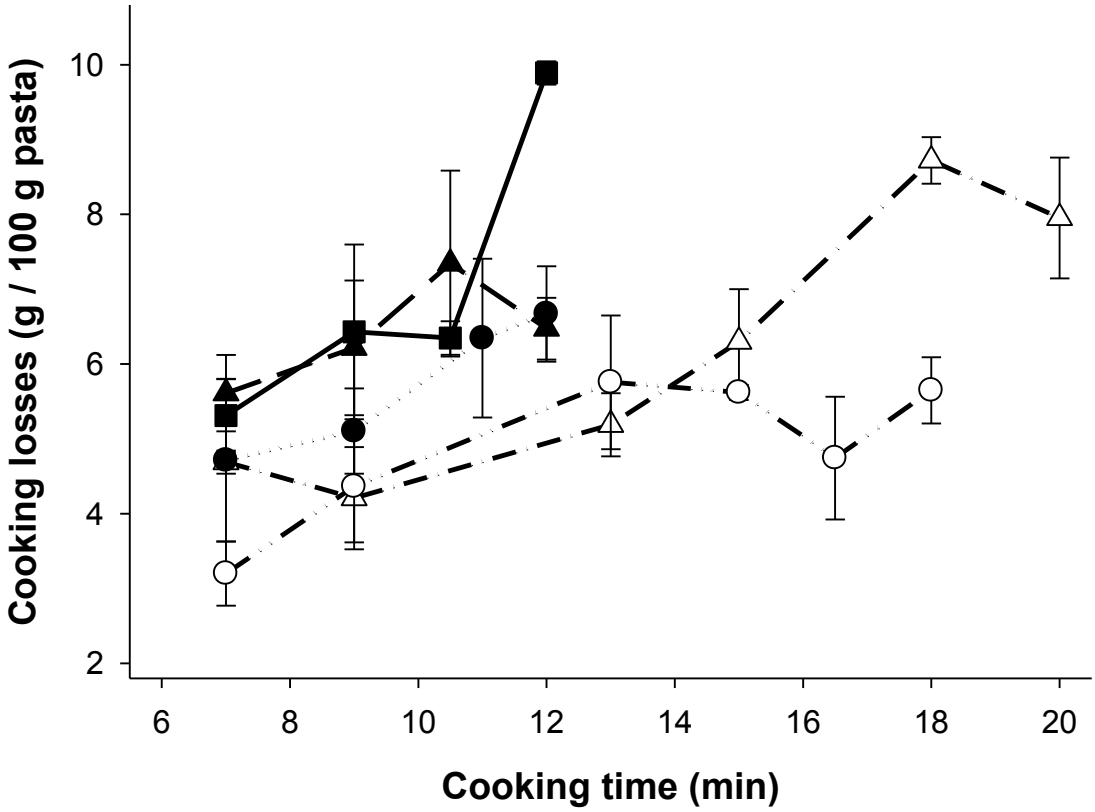
453

Figure 4


Viscosity (BU)
Temperature (°C)
Time (min)
0
100
200
300
400
0
20
40
60
80
100
0
20
40
60
80
100

460 **Figure 5**

461



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