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Structuring and texturing gluten-free pasta: egg albumen or whey proteins?

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Abstract

The effects of adding egg albumen or whey proteins to pasta made from parboiled rice flour were investigated. Pasta quality was evaluated in terms of color, of furosine content, and of cooking properties (water absorption, cooking loss, and consistency at the optimal cooking time). The surface heterogeneity of the cooked and uncooked materials was studied, and some starch properties (pasting properties, starch susceptibility to alpha-amylase hydrolysis) were assessed, along with the features of the protein network as determined by conditional solubility studies and with ultrastructural features of the cooked products. Egg albumen improved pasta appearance, and gave a product with low cooking loss, firmer and nutritionally more valuable than the other ones. In albumen enriched pasta, small starch granules appear homogeneously surrounded by a protein network. In the uncooked product, the protein network is stabilized mostly by hydrophobic interactions, but additional disulfide interprotein bonds form upon cooking. Thus, addition of 15% liquid albumen to parboiled rice flour results in significant improvement of the textural and structural features of rice-based gluten-free pasta.

Keywords: gluten free pasta, proteins, cooking behaviour, ultrastructure

Abbreviations: BU, Brabender units; DTT, dithiothreitol; EA, egg albumen; FV, final viscosity; HV, hot viscosity; MVAG, micro-viscoamyloghraph; PaEA, rice pasta with egg albumen; PaPR, rice pasta; PaWP, rice pasta with whey proteins; PR, parboiled rice; PT, pasting temperature; SB, setback; WP, whey proteins.

Introduction

The replacement of gluten functionality in gluten-free products represents a major technological challenge. Gluten-free products are seeing a growing demand, due to the increase in the incidence of pathologies of various type, all linked to some form of intolerance to gluten (1). At a prevalence of the coeliac disease approaching 1/200 in some European countries (2), the potential market for gluten-free products is of interest also for non-specialized industries.

Up-to-now, two main approaches have been proposed and recently reviewed by Marti & Pagani [3]. One is focused on choosing appropriate processing conditions able to create a new and efficient arrangement of starch components in the final product [4-6]. The other approach is based on the choice of appropriate ingredients and/or additives (mainly hydrocolloids and emulsifiers) suitable for inducing a cohesive structure that overcomes the absence of gluten. Generally, the additives are obtained through chemical synthesis or are extracted from sources other than cereals. Despite the amply reported positive effects of the addition of emulsifiers and hydrocolloids [7-12], the consumers often associate their presence in gluten-free pasta to a “non-natural” food.

Thus, the use of proteins as structuring building ingredients could be an interesting approach for producing gluten-free pasta, also because of their positive role in improving the nutritional value of the product [13]. The effects of whey proteins were recently investigated on rheological and mechanical properties of fresh handmade tagliatelle from pseudocereal flours [14]. Moreover, the addition of whey proteins to sweet potato gave high quality pasta with strong starch-protein network formation leading to slow starch digestibility (15,16).

The addition of egg white (0.25%) and casein (0.25%) to a rice dough was associated with improved handling and processing [12]. Recent reports deal with the effectiveness of egg white powder (6%) and emulsifiers (1.2%) in improving texture and cooking quality of gluten-free pasta prepared from buckwheat, amaranth, and quinoa flour blends [17].

In the present work, we investigated the role of texturing proteins (egg albumen and whey proteins) in defining the overall quality of rice pasta in the absence of other additives. The effects of these proteins on the starch/protein interactions and on the overall pasta structure and cooking performance was also investigated.

Materials and Methods

Materials

Parboiled milled rice (PR; Indica type cultivar of commercial origin; total starch: 85.9% db; protein: 7.1% db; lipid: 1.0% db; ash: 0.89% db; amylose: 25% db) used in this study was provided by Riso Viazzo s.r.l. (Crova, Italy). Kernels were ground in an industrial plant (Riso Viazzo s.r.l., Crova, Italy) to produce flour with particles smaller than 250 μm.

Liquid egg albumen (EA) (11% protein, 0.8% carbohydrates, 0.03% fat) was purchased from Ovopel s.p.a. (San Giovanni in Croce, Cremona, Italy). Ultrafiltered spray-dried whey proteins (WP) (80% protein, 6% lactose, 6% fat) were provided by Tosi & G. s.r.l. (Vimercate, MB, Italy).

Pasta production

Experimental pasta samples were produced using the pilot-plant at DeFENS, University of Milan. Three different pasta samples were prepared starting from the same parboiled rice flour (PR) with or without the addition of texturing proteins: 1) PaPR, pasta from rice; 2) PaEA, rice pasta with egg albumen; 3) PaWP, rice pasta with whey proteins. The amount of EA (15g/100g flour) and WP (3g/100g flour) were chosen to produce pasta samples with a comparable protein content. A conventional pasta-making process was applied according to Marti et al. [4]. Rice flour and water were blended in order to produce a mixture with a final moisture of 40%. When WP were used, a

previous suspension in water was prepared and mixed until complete solubilisation of proteins. Liquid albumen was added to the flour-water mixture during mixing.

For all the pasta samples, the flour-water mixture was formed into pasta by conventional extrusion, carried out in a lab scale extruder for semolina pasta (20 kg/h; MAC 30, Italpast, Parma, Italy), keeping the extrusion temperature at 50 °C. Samples were formed into macaroni shape (7 mm external diameter) and dried in an experimental drying cell, by using a low-temperature drying cycle (50 °C for 14 hours) [4]. All the samples were stored at room temperature until analysed; when appropriate, pasta samples were ground to 500 μm with a laboratory mill (IKA Universalmühle M20, Staufen, Germany), fitted with a water cooling jacket in order to avoid overheating during grinding.

Ultrastructural observations

Ultrastructural observations were carried out on cooked and lyophilized pasta. Samples were mounted on aluminum stubs, and sputter-coated with gold. Pasta ultrastructure was imaged in the Scanning Electron Microscope (SEM) LEO438 VP (LEO Electron Microscopy Ltd., Cambridge, UK), under high vacuum conditions (10^{-4} Pa) at an accelerating voltage of 15 kV.

Color analysis

A reflectance color meter (CR 210, Minolta Co., Osaka, Japan) was used to measure the lightness and saturation of the color intensity of rice flours by utilizing the CIE-LAB uniform color space procedure. CIE-LAB-System color values L*, a*, and b* as measures of lightness, redness-greenness, and yellowness–blueness, respectively, were recorded for each sample. Each measurement was replicated five times and the average value was used.

Furosine determination

Furosine was determined by HPLC after acid hydrolysis according to Resmini et al. [18], and expressed as mg/100 g of protein. Protein content was determined according to the AACC 46-11.02 official method [19].

Cooking behaviour

Cooking loss was evaluated by determining the amount of solids lost into cooking water according to the AACC 66-50.01 official method [19]. An aliquot of pasta (20 g) was cooked at the optimal cooking time for each sample in boiling natural water (pasta:water ratio = 1:10) with no salt added. The optimum cooking time of rice pasta was evaluated as the time required for disappearance of the central core when gently squeezed between two glass plates, according to the AACC 66-50.01 official method [19].

After cooking, pasta was drained, water was recovered, and its level brought back to the initial volume. Twenty-five ml of cooking water were then collected and 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 matter loss/100 g of dry pasta. Weight increase of pasta during cooking was evaluated by weighing pasta before and after cooking. The results were expressed as the ratio between the weight increase and the weight of uncooked pasta.

Textural characteristics of the cooked pasta were determined by using a Texture Analyzer TA.HD-plus (Stable Micro System Ltd., Godalming, United Kingdom), calibrated for a load cell of 2.5 kN. The analysis was repeated at least five times: for each replicate, 6 pieces of pasta were cooked at the optimal cooking time and analyzed using a Kramer cell (test speed of 0.67 mm/s). Firmness (expressed in Newton) was calculated by Texture Exponent TEE32 software (v. 3.0.4.0).

Image analysis and surface heterogeneity

The lengthwise and cross section images of 20 macaroni for each sample were taken before and after cooking at the optimum cooking time, using a flatbed scanner (Epson Perfection 3170 Photo, Seiko Epson Corp., Japan), at 300 dpi (dots per inch) of resolution and a color depth of 24 bits in standard conditions. During the acquisition, samples were covered with a black box to avoid reflections during acquisitions. The images were saved as TIFF format and then processed using a dedicated software (Image Pro-Plus 4.5.1.29, Media Cybernetics Inc, UK). The assessment of surface texture of uncooked and cooked products was performed on a surface of 50pxl * 30pxl extracted from the images of the macaroni [20]. After conversion in 8-bit grayscale, the surface texture of each image was evaluated and expressed in terms of heterogeneity (HTG). This parameter is defined as the fraction of pixels whose intensity value deviates more than 10% compared to the average intensity of the entire image: a value equal to 0 corresponds to a homogeneous (smooth) surface, whereas a value equal to 1 corresponds to a heterogeneous (rough) surface.

Starch properties: α-amylase susceptibility and pasting properties

The starch susceptibility to α-amylase hydrolysis was determined by evaluating the damaged starch content (AACC 76-31, [19]; “Starch Damage Assay Kit” by Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). Results are the average of at least four replicates. Pasting properties of rice flours were measured according to Marti et al. [4] using a Brabender Micro-Visco-AmyloGraph (MVAG) (Brabender OHG, Duisburg, Germany). Fifteen grams of sample were dispersed in 100 mL of distilled water, scaling both sample and water weight on a 14% flour moisture basis.

The suspensions were subjected to the following temperature profile: heating from 30 up to 95 °C, holding at 95 °C for 30 min, cooling from 95 to 50 °C, holding at 50 °C for 30 min and cooling to 30 °C. A heating/cooling rate of 3.0 °C/min was applied. The following indices were considered: pasting temperature (PT, °C; temperature at which an initial increase in viscosity occurs), hot viscosity (HV, Brabender Units, BU; maximum paste viscosity achieved during the heating cycle), final viscosity (FV, BU; paste viscosity achieved at the end of the cooling cycle), and setback (SB, BU; increase in viscosity during cooling and corresponding to the difference between the final viscosity and the viscosity reached after the first holding period). Measurements were performed at least in duplicate and the average value was used.

Properties of the protein network: protein solubility

Protein solubility in native and denaturing conditions was determined by suspending 0.5 g of finely ground uncooked or cooked sample in 10 mL of 50 mM phosphate, 0.1 M NaCl, pH 7.0, containing 6 M urea or 6 M urea and 10 mM dithiothreitol (DTT) when indicated. Suspensions were stirred for 60 minutes at 25 °C. After centrifugation (10000 × *g* for 20 min, 20 °C) the amount of protein in the supernatant was determined by a dye-binding method [21] using bovine serum albumin as a standard. Results were expressed as mg proteins/g pasta.

Statistical analysis

Analysis of variance (ANOVA) was performed on the data adopting the least significant difference (LSD). Data were processed by Statgraphic Plus for Windows v. 5.1. (StatPoint Inc., Warrenton, VA, USA).

Results and Discussion

Color and furosine content

The color is the first quality attribute the consumer takes into consideration to accept or refuse pasta-products. Luminosity and chromatic indices are affected by both processing conditions and formulation, in terms of raw materials characteristics and/or presence of specific ingredients [22]. The color indices of experimental rice pasta are shown in Table 1.

PaPR - made solely by rice flour and used as control - showed a similar luminosity as commercial sample from semolina [22]. Adding egg albumen did not affect the colour of rice pasta, whereas a significant ($p<0.05$) decrease in luminosity and an increase in both redness and yellowness was highlighted by the addition of whey proteins, as a likely consequence of non enzymatic browning phenomena [22]. Indeed, furosine levels were almost twice as high in PaWP with respect to PaEA (Table 1).

Cooking behaviour

The cooking behaviour of pasta samples is shown in Table 2. In GF pasta, because of the lack of gluten, starch polymers are less efficiently entrapped in the matrix, giving a product with high cooking loss and low firmness [23]. The use of PR improved the texture of the product, but it seemed quite efficient as for limiting the leaching of solid matter into cooking water, that was four times higher than in semolina pasta [23]. Adding soluble proteins to the formulation did not promote significant ($p>0.05$) changes in the amount of absorbed water during cooking. However, the use of proteins decreased the cooking loss, and EA was by far more efficient than WP in lowering the cooking loss and in increasing the firmness of pasta (Table 2).

Thus, a protein network suitable for retaining starch and the other constituents was formed as a consequence of protein coagulation upon cooking the protein-enriched samples [24]. The high solubility and hydration properties of the proteins used in this study also favoured a homogeneous distribution of albumen and whey proteins inside the matrix during the mixing phase. The proteins used here were hypothesized to contribute emulsifying effects as well [25]. The positive effect of egg addition was observed also in pasta from pseudocereals [17], but only a few studies have been carried out on the use of whey proteins [24].

Surface textural characteristics

The surface texture of pasta samples before and after cooking, as described by the heterogeneity (HTG) parameter, is shown in Fig. 1. Roughness is relevant here in what it affects the ability to retain condiments. The addition of proteins significantly ($p<0.05$) decreased roughness of the uncooked product, increasing the surface homogeneity. In particular, whey protein-enriched pasta showed the highest homogeneity (low HTG). In all samples, a decrease in HTG was detected after cooking. A similar phenomenon was observed in durum wheat pasta enriched with buckwheat flour [20].

Although cooking lowered the differences in surface heterogeneity among the samples, PaPR still showed the highest HTG, accounting for the high cooking loss (Table 2). Samples with the highest HTG (and therefore with the roughest surface) expose a greater area to water action during cooking and, consequently, a high amount of material can be released into the cooking water [20]. This combination of evidences suggest that the high homogeneity in protein-enriched rice pasta may be due to starch-protein interactions that occurred during pasta-making.

Ultrastructure of cooked pasta

SEM images of cross-sections of cooked pasta are shown in Fig. 2. For each sample an image at low magnification is proposed in order to appreciate at the same time the organization of both the external and the central region. Apparently, PaPR sample showed a more compact internal area (Fig. 2a). The addition of soluble proteins was associated with a wider porous structure in the external area. In other words, in PaEA (Fig. 2b) and PaWP (Fig. 2c) macaroni the absorption of boiling water during cooking promoted the formation of a highly hydrated region as shown by the extension and the size of the honeycomb cells that form during the freezing step of highly hydrated gels as required by SEM sample preparation [26].

Interesting structures can be distinguished when looking at the core of cooked macaroni at high magnification. PaPR presents an undifferentiated structure (Fig. 2d), in which starch material is organized in a very thin honeycomb network made of numerous small cells. The resulting high surface exposure could contribute to the high cooking loss in PaPR (Table 2). PaEA has a very different structure, as starch material is easily identified as separated by a thin protein layer, and no porous starch organization was distinguishable (Fig. 2d). In PaEA, protein and starch granules appear densely packed, accounting for the high firmness of this sample (Table 2). In PaWP, large agglomerates of gelatinized starch material were separated by long protein fibrils (Fig. 2e). Some discontinuities are present among protein and starch, providing a rational for the higher cooking loss observed in PaWP with respect to PaEA (Table 2).

Starch properties

Changes in viscosity of pasta samples (ground into flours of uniform size before cooking) was evaluated by the microviscoamylograph test (MVAG), and the results are shown in Fig. 3. Although this approach is conventionally adopted for evaluating the pasting properties of starch and flours, it was also performed on dry pasta, giving information on potential starch behavior during cooking [4]. The MVAG curves of PR and PaPR confirmed previous studies [23]. The low viscosity of PR

could be related to the presence of a matrix with a low hydration capacity, as a consequence of both starch gelatinisation and retrogradation phenomena occurring during the parboiling process [27]. PaPR showed a lower pasting temperature and a higher hot viscosity compared to PR (Table 3). These results suggested that pasta-making process promoted structural changes, resulting in a product with altered starch properties and accounting for the higher amount of starch quickly accessible to enzymatic hydrolysis, as already discussed by Marti et al. [4]. Starch susceptibility to α-amylase hydrolysis (expressed as damaged starch, see Table 3) as promoted by pasta-processing can complete the information about the starch organisation: the higher the enzymatic susceptibility index, the lower the pasting temperature, and the higher the maximum viscosity [4].

PaEA showed a viscosity profile similar to that of PaPR (Fig. 3), and only a slight but not significant ($p>0.05$) increase in the maximum hot viscosity was observed (Table 2). On the contrary, the presence of whey protein significantly ($p<0.05$) decreased the peak viscosity in PaWP, that showed a higher pasting temperature and a lower hot viscosity than PaPR. Results suggest that the whey proteins might have slowed down water uptake by individual starch granule (and, consequently, their gelatinisation) as a consequence of the possible competition of the different biopolymers for available water. A decrease in viscosity was also observed by Marco and Rosell [25] when whey proteins were added to rice flour, likely due to the dilution effect on starch concentration. Indeed, in previous reports, a negative correlation had been established between the protein content and the peak viscosity in rice flour [28,29]. However, the dilution effect could not be solely responsible for the decrease in the peak viscosity promoted by whey proteins, because no significant changes were observed when the amount of whey proteins in rice pasta was increased (data not shown). The presence of whey proteins significantly decreased the final viscosity with respect to PaPR, confirming that the increase of viscosity during cooling, usually related to the crystallization of the amylose chains, could be affected also by the reorganization of the denatured proteins [25]. Finally, the presence of large agglomerates of gelatinized starch material in PaWP

(see Fig. 2e) could account for the lower susceptibility of PaWP to α-amylase hydrolysis in comparison with PaPR, as also reported in Table 2.

Protein network

The structural role of covalent and non-covalent interactions among proteins in different pasta samples - before and after cooking - was assessed by detecting the amount of protein solubilized in media with a different ability in dissociating protein-protein complexes (Fig. 4). Albumins and globulins are soluble in plain saline buffer, while proteins involved in aggregates stabilized by non-covalent and/or covalent interactions (that is, interprotein disulfide bonds) are soluble in buffer containing urea or urea/DTT, respectively [30,31]. In the case of uncooked pasta, solubility of PaPR proteins was negligible in saline buffer, and remained very low in the presence of urea unless DTT was added (Fig. 4a). These results indicate that rice proteins were involved in a network stabilized by disulphide bonds.

The amount of proteins soluble in saline buffer also was very low in PaEA and PaWP, but it increased significantly after addition of urea, and increased much further when both urea and DTT were present in the extraction buffer. In the presence of urea, about 20 mg proteins per gram of pasta sample can be extracted, suggesting that both albumen and whey proteins form a non-covalently bound protein network in these materials. Indeed, the amount of protein solubilized by the chaotrope is comparable to the quantity of structuring proteins (EA or WP) added to these samples.

Presence of a disulfide-reducing agent was necessary to allow solubilization of appreciable amount of proteins from cooked pasta samples (Fig. 4b). Thus, cooking resulted in the formation of a protein network in which the contribution of interprotein disulfide bonds was much greater than in the uncooked product.

Information about the compactness of the protein network can be obtained by comparing the amount of protein extracted from each pasta sample before and after cooking. Whereas figures for PaPR and PaWP did not change after cooking, solubility of PaEA protein decreased markedly after cooking. This suggests that aggregates formed by egg albumen proteins in the cooked pasta are so compact to be inaccessible to the disulfide-reducing agents. This compactness provides a rationale for minimizing solid loss and for maintaining good firmness in cooked PaEA (Table 2).

Conclusions

The addition of egg albumen and whey proteins as texturing ingredients is an interesting approach for producing GF pasta with improved cooking quality without using chemical additives. On a similar protein enrichment level, the best results were obtained by using egg albumen. Egg albumen gave pasta of better appearance, with lower cooking loss, and firmer and nutritionally more valuable than the one made by using whey proteins. In pasta made with a 15% addition of liquid albumen to parboiled rice flour, starch molecules are homogeneously surrounded by a protein network stabilized mostly by hydrophobic interactions and by disulfide bonds. Further disulfide interprotein bonds form upon cooking, resulting in significant improvement of the textural and structural features of the product without substantial interference with the pasting behavior of starch or its accessibility to amylolytic enzymes.

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Table 1 Characteristics of uncooked pasta.

	PaPR	PaEA	PaWP
Luminosity (L*)	89.8[a]	90.2 [a]	88.6[b]
Redness (a*)	0.92[a]	0.82[a]	1.41[b]
Yellowness (b*)	20.4[a]	20.3[a]	23.7[b]
Furosine (mg/ 100 g protein)	148[a]	284[b]	458[c]

Means with different superscripts in each line are significantly different (LSD; $p<0.05$)

PaPR, rice pasta; PaEA, rice pasta with egg albumen; PaWP, rice pasta with whey proteins

Table 2 Characteristics of cooked pasta.

	PaPR	PaEA	PaWP
Cooking loss (g/100g)	12.6[c]	8.1[a]	11.1[b]
Water absorption (g/100g)	88.6[a]	88.9[a]	90.1[a]
Firmness (N)	275[ab]	308[b]	245[a]

Means with a different superscript in each line are significantly different (LSD; $p<0.05$)

PaPR, rice pasta; PaEA, rice pasta with egg albumen; PaWP, rice pasta with whey proteins

Table 3 Effect of texturing ingredients on starch properties of rice pasta.

	PR	PaPR	PaEA	PaWP
Starch susceptibility (damaged starch; % db)	8.42[a]	14.48[c]	13.92[c]	10.35[b]
PT (°C)	76.4[c]	56.3[a]	56.8[a]	60.9[b]
HV (BU)	114.0[a]	174.0[b]	199.0[b]	130.5[a]
FV (BU)	272.5[a]	572.0[c]	638.5[c]	375.0[b]
SB (BU)	158.5[a]	398.0[c]	448.0[c]	245.0[b]

Means with a different superscript in each line are significantly different (LSD; $p<0.05$)

PT = pasting temperature; HV = hot viscosity; FV = final viscosity; SB = setback.

PR = parboiled rice; PaPR, rice pasta; PaEA, rice pasta with egg albumen; PaWP, rice pasta with whey proteins

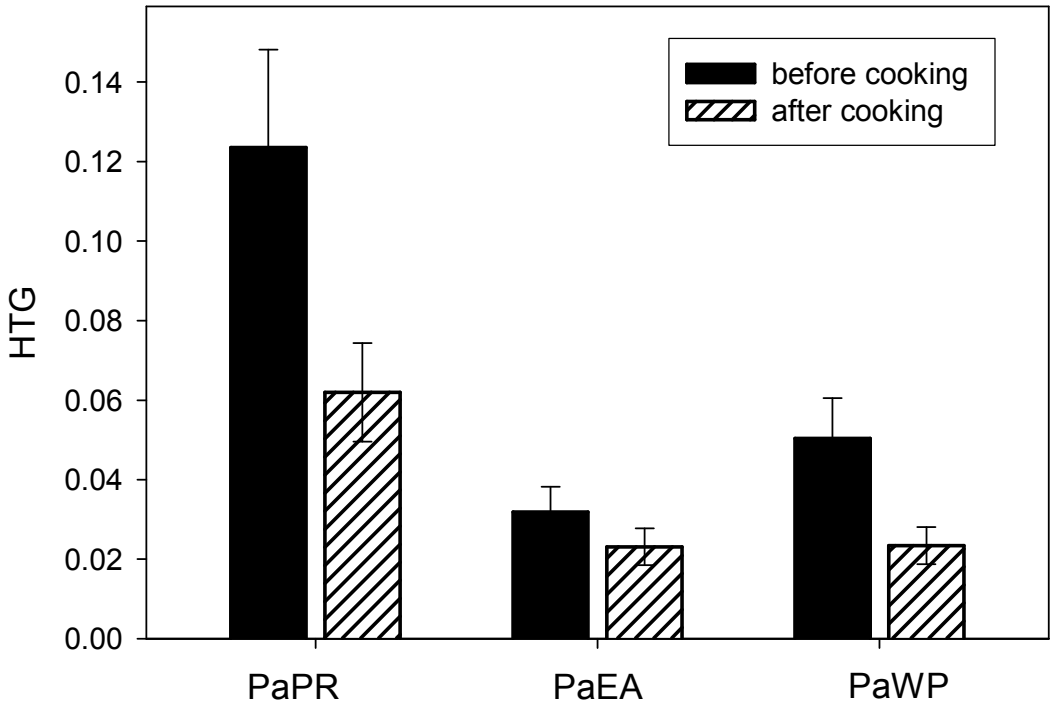
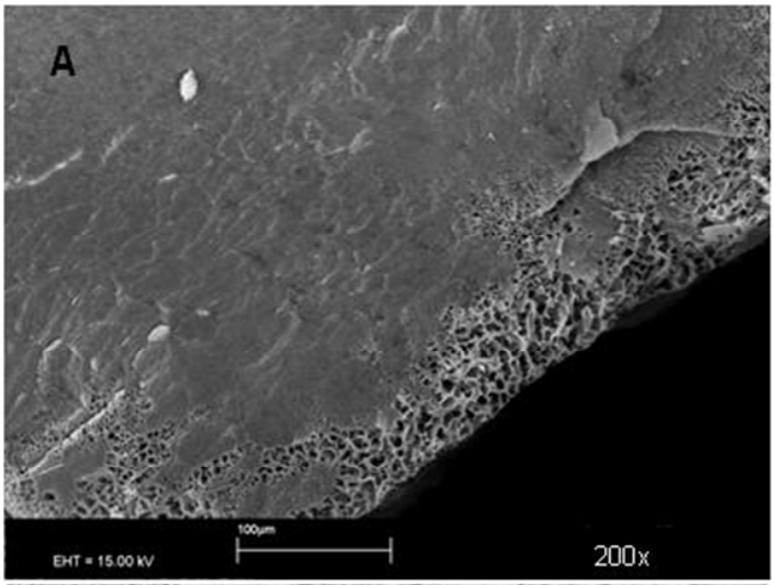


Fig.1 Surface heterogeneity of pasta before and after cooking

PaPR, rice pasta; PaEA, rice pasta with egg albumen; PaWP, rice pasta with whey proteins



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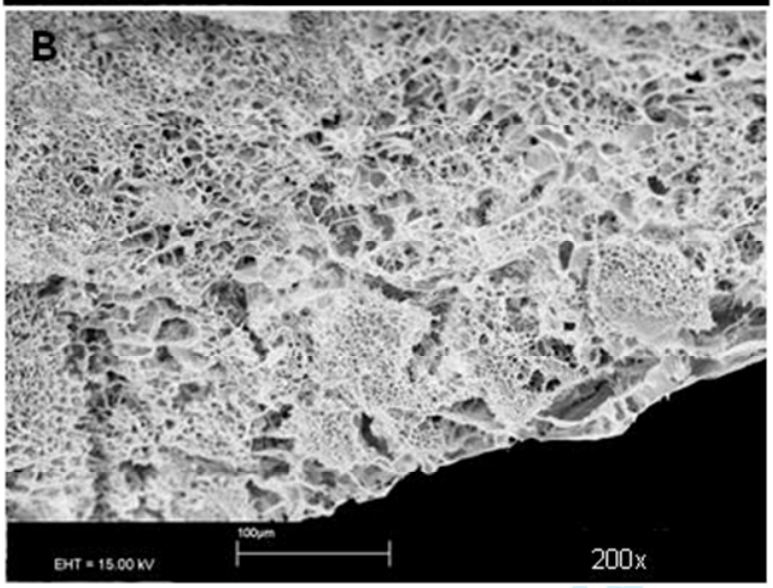

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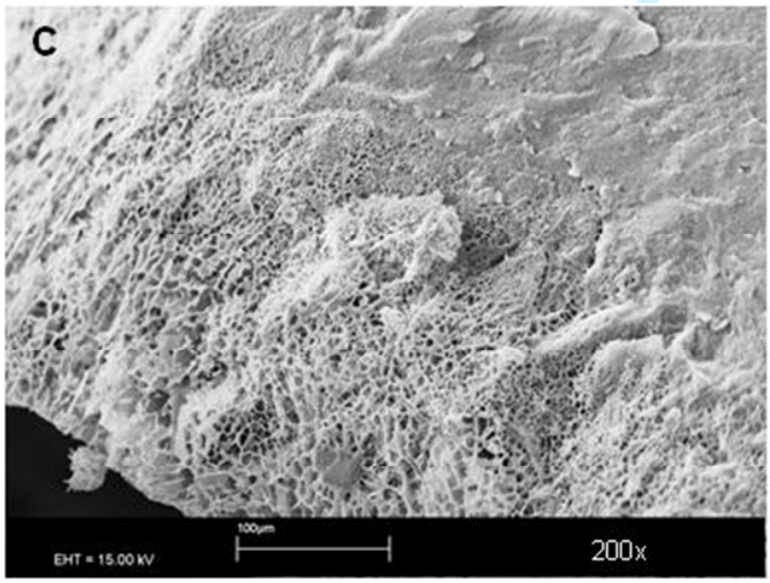

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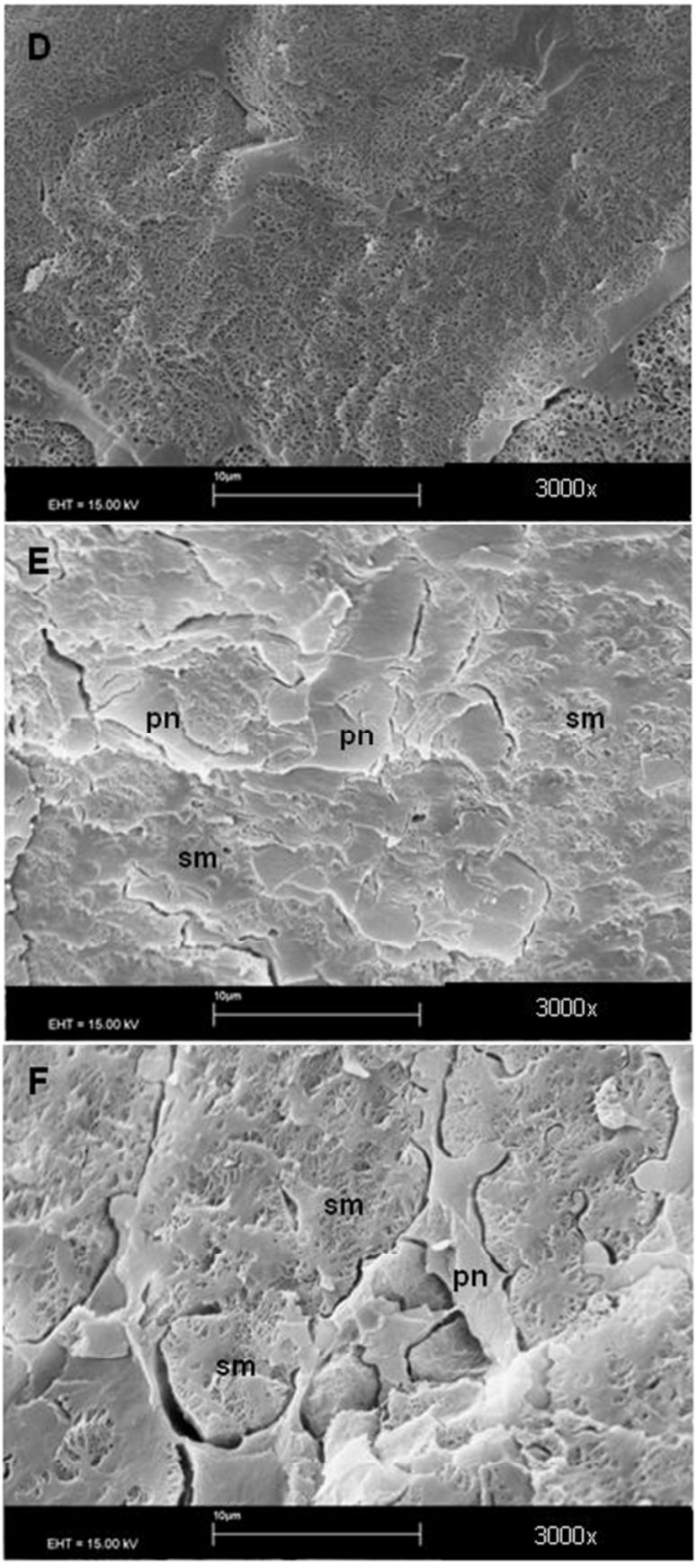
D
EHT = 15.00 kV
10µm
3000x
E
pn
pn
sm
sm
EHT = 15.00 kV
10µm
3000x
F
sm
pn
sm
EHT = 15.00 kV
10µm
3000x

Fig. 2 SEM images of cross-sections of cooked pasta.

PaPR at low (A) and high (D) magnification; PaEA at low (B) and high (E) magnification; PaWP at low (C) and high (F) magnification. pn, protein network; sm, starch material.

PaPR, rice pasta; PaEA, rice pasta with egg albumen; PaWP, rice pasta with whey proteins

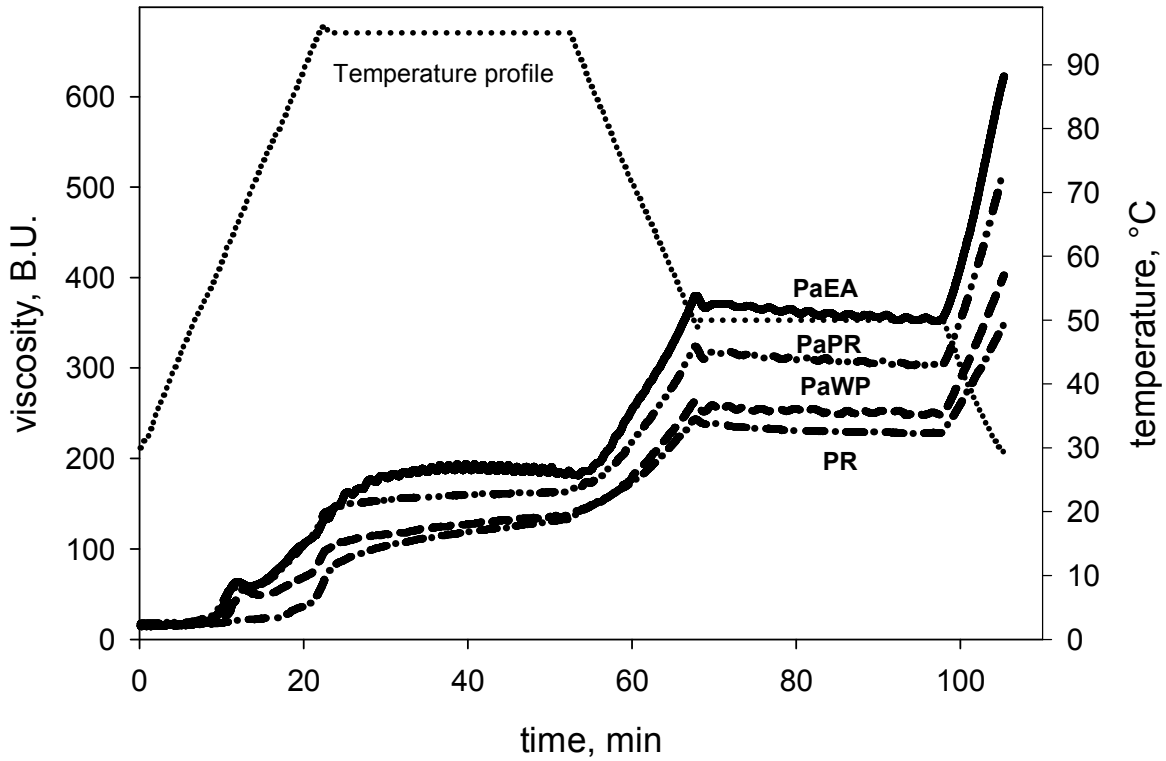

Fig. 3 Pasting properties of rice pasta.

PR = parboiled rice; PaPR, rice pasta; PaEA, rice pasta with egg albumen; PaWP, rice pasta with whey proteins

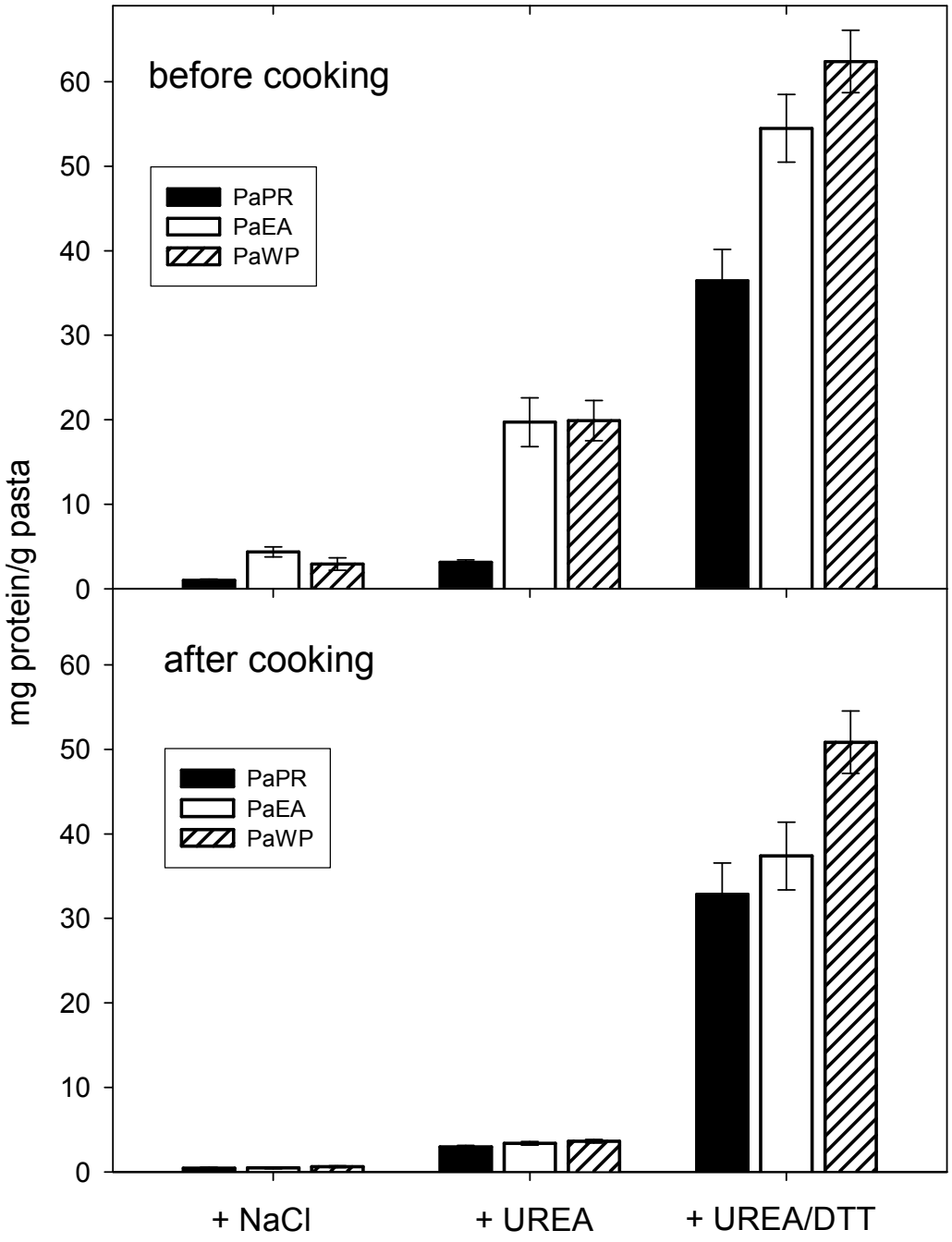

Fig 4 Protein solubility in uncooked (upper panel) and cooked (lower panel) rice pasta. PaPR, rice pasta; PaEA, rice pasta with egg albumen; PaWP, rice pasta with whey proteins