



# Process conditions affect starch structure and its interactions with proteins in rice pasta

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## ABSTRACT

Structural changes of starch and proteins in rice pasta were investigated as a function of raw-materials and pasta-making conditions, and their impact on cooking behaviour and glycaemic index was assessed. Rice pasta was prepared from untreated or parboiled rice flour by conventional extrusion or by extrusion-cooking. Starch structure was studied by assessing starch accessibility to specific enzymes ( $\alpha$ -amylase and pullulanase), and by evaluating the molecular properties of fragments from enzymatic action. Protein solubility in presence/absence of chaotropes and accessibility of protein cysteine thiols allowed to evaluate the intensity and nature of inter-protein interactions. Parboiling stiffens the protein network in rice flour and makes starch more accessible to hydrolysis. Pasta-making induced further changes in the starch structure, that were most evident in pasta made from untreated rice and were mainly related to the amylopectin fraction. Thus, the interplay among structural modifications on starch and/or proteins affects the features of products.

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## 1. Introduction

Consumption of rice pasta is increasing also outside the traditional Asian markets, mostly because of health-related issues. Rice-based products are low in allergens and fat, easily digestible, and suited for gluten-free diets (Rosell & Marco, 2008). Pasta with appropriate cooking behaviour can be obtained from rice flour either by adding additives (Marconi & Carcea, 2001), or by applying appropriate heating/cooling treatments during pasta processing (Pagani, 1986). However, development of these processes to improve the sensory features of products and to satisfy market requirements has been based almost invariably on semi-empiric approaches (Parker & Ring, 2001). The ability to relate process-dependent modifications in some macromolecular properties or in the interactions among different or similar macromolecules to product features may represent the starting point for engineering rice pasta with suitable features in terms of texture and nutritional properties on a somewhat more scientific basis. Various

approaches have been developed to predict the macromolecular behaviour during the process (Cabrera-Chávez et al., 2012; De Noni & Pagani, 2010; Petitot, Abecassis, & Micard, 2009), and some studies have tried to address non-gluten matrices, where the main structural backbone is provided by a three-dimensional starch network (Marti, Pagani, & Seetharaman, 2011; Marti, Seetharaman, & Pagani, 2010).

The aim of this study was to investigate in detail the organization of macropolymers in native and heat-treated rice flours used as starting ingredients for rice pasta, and the modifications caused by pasta-making itself. The structural and nutritional effects ensuing from the different organization of proteins and polysaccharides as induced by the various processes were also investigated on the cooked pasta.

## 2. Materials and methods

### 2.1. Flour and pasta samples

Rice flour (RF; total carbohydrate: 84% db; protein: 7% db; particle size < 250  $\mu$ m) was produced in an industrial plant by directly grinding native dehulled rice (*Oryza sativa*, cultivar Indica; amylose 25 g/100 g total starch; Riso Viazzo s.r.l., Crova, Italy). Parboiled rice was produced from the same paddy rice in an industrial plant (Riso Viazzo s.r.l., Crova, Italy) by steeping at 70 °C, followed by steaming at 100 °C, and by a final drying step at 50 °C for 5 h. The parboiled rice

**Abbreviations:** DTNB, 5,5-dithiobis(2-nitrobenzoate); DTT, dithiothreitol; GI, glycaemic index; M, protein markers; P1, pasta made from native rice flour by extrusion-cooking; P2, pasta made from parboiled rice flour by conventional extrusion; P3, pasta made from parboiled rice flour by extrusion-cooking; PRF, parboiled rice flour; RF, untreated rice flour; RS, resistant starch; TS, total starch.

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was ground into flour (PRF; total carbohydrate: 83% db; protein: 8% db; particle size <250  $\mu\text{m}$ ).

Pasta from RF (P1) was prepared by an extrusion-cooking process (Marti et al., 2010). Rice flour (RF) and water at 60 °C were blended to a final moisture of 40%. The mixture was heated by steam at 2.5 atm (>120 °C) for 10 min in a gelatinization tank. The pre-gelatinized dough was then subjected to a first extrusion at 120 °C in a Progel extruder (single-screw type, Braibanti, Milano, Italy) to generate pellets (small cylinders, 2–3 mm diameter). The wet pellets were immediately transferred to a continuous press for semolina pasta (Braibanti, Milan, Italy), in which a second and final extrusion step was carried out at 50 °C.

Pasta from parboiled flour was produced using two different extrusion conditions. Pasta P2 was produced by applying to a PRF/water mixture (40% final moisture) the conventional extrusion process used for preparing durum wheat semolina pasta, at an extrusion temperature of 50 °C. Pasta P3 was produced from a PRF–water mix (40% final moisture) that underwent a double extrusion step, as described for sample P1.

All the samples were formed into macaroni shape (7 mm o.d.) and dried in a pilot-scale plant using a low-temperature drying cycle (50 °C max, 14 h) (Marti et al., 2010). When appropriate, pasta was ground (particle size <250  $\mu\text{m}$ ) in a laboratory mill (IKA Universalmühle M20, Janke and Kunkel GmbH & Co KG, IKA Labortechnik, Staufen, Germany).

## 2.2. Chemical analysis

Analysis of the various rice flours was performed according to AOAC (1999) for moisture (934.01), protein (960.52) and to AACC (2001) for carbohydrates (AACC 76-13). Data are from triplicate determinations.

## 2.3. Properties of the polysaccharide network

### 2.3.1. Enzymatic susceptibility to $\alpha$ -amylase

Susceptibility to  $\alpha$ -amylase was determined by using the Starch Damage Assay Kit (AACC 76-31, 2001; Megazyme International Ireland Ltd., Bray Business Park, Bray, Co., Wicklow, Ireland) with some modifications. The sample (100 mg) was dispersed in 3 ml of 0.1 M sodium phosphate buffer, pH 6.9.  $\alpha$ -Amylase (1.1 U) from *Bacillus subtilis* (EC 3.2.1.1, 449 U/mg) was added, followed by incubation at 20 °C. The amount of amylase was increased to 11.1 U when treating RF samples, as lower amounts of enzyme did not produce measurable hydrolysis. At appropriate times, samples were centrifuged (12,000  $\times$  g, 10 min, 20 °C), and amyloglucosidase was used to convert into glucose all soluble starch material from the previous hydrolytic step. An 1-ml aliquot of the supernatant was diluted with 4 ml of 0.2 M sodium acetate buffer pH 4.5, prior to adding 0.1 ml of amyloglucosidase (E.C. 3.2.1.3, 3300 U/ml, from *Aspergillus niger*, Megazyme International Ireland Ltd.). After incubation for 30 min at 50 °C, volume was adjusted to 100 ml with water. A 0.1 ml aliquot of the diluted sample was added to 3 ml of the GOPOD reagent solution (Megazyme International Ireland Ltd.), and the absorbance at 510 nm was read against a reagent blank after incubation at 50 °C for 20 min, using glucose for calibration. Results are the average of four replicates, and are expressed as mg glucose/g sample.

### 2.3.2. Susceptibility to pullulanase

A sample aliquot (100 mg) was dispersed in 3 ml of 50 mM sodium acetate buffer, pH 6.0, to which 200 U of pullulanase (EC 3.2.1.41,  $\geq$ 400 U/mL; from *Bacillus acidopullulyticus*; Sigma P2986) were added. After 1 and 24 h incubation at 37 °C, the amount of

soluble hydrolysis products was quantified as glucose according to the procedure presented above.

## 2.4. Characterization of starch fragments from enzymatic hydrolysis by SE-HPLC/Light Scattering

Supernatants from enzymatic treatments (prior to the treatment with amyloglucosidase for glucose quantitation) were filtered through a 0.22  $\mu\text{m}$  filter, and 0.2 ml of the filtrate were loaded into a HPLC system (515 pump, Waters Co., Milford, MA, USA); UV detector (Dual Absorbance detector 2487, Waters Co., Milford, MA, USA), connected in series to a differential refractometer (Optilab T-rEX, Wyatt Co., Santa Barbara, CA, USA) and to a Multi Angle Light Scattering instrument (DAWN HELEOS, Wyatt Co., Santa Barbara, CA, USA). Polysaccharides were fractionated on a size-exclusion column (Ultrahydrogel™ Linear 7.8  $\times$  300 mm, Waters Co., Milford, MA, USA), using as eluant either 0.1 M sodium phosphate buffer, pH 6.9 (for samples deriving from  $\alpha$ -amylase hydrolysis) or 0.05 M sodium acetate, pH 6 (for samples deriving from pullulanase hydrolysis), at a flow rate of 0.4 ml/min. The Astra software (ASTRA V 5.1.9.1, Wyatt Technology Co., Santa Barbara, CA, USA) was used for data analysis.

## 2.5. Pasting properties of starch

Pasting properties were measured in a Brabender Micro-Visco-AmyloGraph (MVAG) (Brabender OHG, Duisburg, Germany). Each sample was finely ground, and 15 g of the resulting powder were dispersed in 100 ml of distilled water. The pasting properties were evaluated, in triplicate, under constant conditions (speed: 250 rpm; sensitivity: 300 cm<sub>g</sub>), according to Marti et al. (2010).

## 2.6. Properties of the protein network

### 2.6.1. Protein solubility

Protein solubility in native and denaturing conditions was determined by suspending 0.5 g of finely ground sample in 10 ml of 50 mM phosphate, 0.1 M NaCl, pH 7.0 containing 8 M urea or 8 M urea and 10 mM dithiothreitol (DTT) when indicated. Suspensions were stirred for 30 and 60 min at 25 °C. After centrifugation (10,000  $\times$  g for 20 min, 20 °C) the amount of protein in the supernatant was determined by a dye-binding method (Bradford, 1976) using bovine serum albumin as a standard. Results are expressed as mg proteins/g sample.

### 2.6.2. SDS-PAGE

The protein profile in various samples and extraction conditions mentioned above was analysed by SDS-PAGE after denaturation in the presence of 2-mercaptoethanol on a 12% gel using a Miniprotein apparatus (Biorad, Richmond, VA) as described by Cabrera-Chávez et al. (2012). Low molecular weight markers (Amersham Biosciences, Amersham, UK) were used for calibration.

### 2.6.3. Protein thiols

Accessible –SH groups were measured by suspending 0.5 g of finely ground sample in 10 ml of 50 mM sodium phosphate buffer, pH 6.8, containing 0.1 M NaCl and 0.2 mM 5,5'-dithiobis(2-nitrobenzoate) (DTNB; Ellman, 1959). After 15 min at room temperature, insoluble material was removed by centrifugation at 12,000  $\times$  g for 10 min at 15 °C, and the absorbance at 412 nm of the supernatant was read against a DTNB blank. Total accessible thiols were measured according to the same protocol outlined above, but adding 8 M urea to the DTNB-containing buffer.

## 2.7. Cooked pasta characterization

Pasta was cooked in natural spring water (pasta/water ratio 1:10, no salt added) until the optimal cooking time, evaluated according to D'Egidio, Mariani, Nardi, Novaro, & Cubadda, 1990. Optimal cooking times were 9, 10, and 9 min for P1, P2, and P3, respectively. The amount of material leached into cooking water (cooking loss) was measured according to Marti et al. (2010) and expressed as grams of matter lost/100 g of dry pasta. Weight increase of pasta due to water absorption during cooking was evaluated gravimetrically. Textural characteristics of cooked pasta were determined by using a TA.HD-plus Texture Analyzer (Stable Micro System Ltd., Godalming, UK), equipped with a Kramer cell, according to Marti et al. (2010). The following indices were considered: compression energy, as the area under the part of the curve related to the compression phase; firmness, as the maximum strength necessary to pack the sample; shear force, as the force necessary for blades to pass through the sample. Total (TS) and resistant (RS) starch were determined by using the enzymatic procedure proposed by Brighenti, Casiraghi, and Baggio (1998).

## 2.8. In vivo studies

This study was approved by the Research Ethics Committee of the University of Milan. Ten (five male and five female) healthy volunteers aged 21–25 years, with normal body mass index ( $22.7 \pm 2.6 \text{ kg/m}^2$ ) and basal glycemia ( $4.2 \pm 0.1 \text{ mmol/l}$ ) participated in this study, after signing informed consent. Each volunteer took part in the experiment on two non-consecutive days per week. Each subject consumed, in separate meals, each of the pasta samples (40 g of carbohydrates portions), or glucose solutions (40 g/500 ml water). Foods were eaten within 10–12 min and 500 ml of water were drunk with the meals. Capillary blood samples were taken using a softclix plung (Glucolet® 2, Bayer Diagnostics) in the fasting state and 15, 30, 45, 60, 90, and 120 min after each meal. Blood samples were taken in capillary tubes (Microvette® CB 300 Sarstedt, Germany) and stored at  $-18^\circ\text{C}$  until analysis. Blood samples were analysed with a semi-automatic analyzer for glucose (YSI 2373, Yellow Spring Instruments, OH, USA), results been given as mmol glucose/L. The glycemic index (GI) was estimated from the 120 min incremental areas under post-prandial glucose responses, calculated geometrically, ignoring the area beneath the baseline (ISO, 2010), and using the mean IAUC of the three glucose trials as the reference for calculating GI.

## 2.9. Statistical analysis

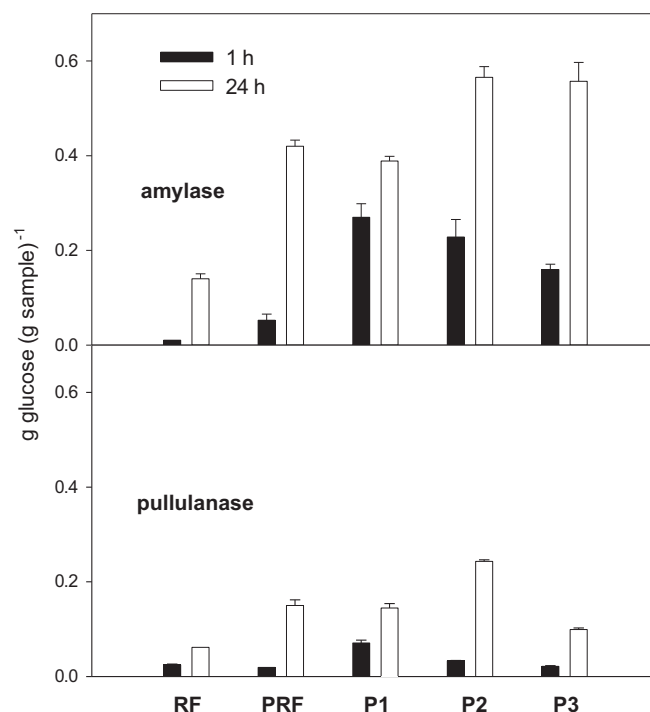
Results are expressed as mean values and standard deviation of their mean. Data were analysed using one-way analysis of variance (ANOVA). In vivo results (expressed as mean  $\pm$  SEM) were submitted to Repeated Measures Analysis of Variance (RM-ANOVA). The significance of differences between products and/or time were checked by Tukey's Honest Significant Differences post hoc test. The analyses were performed by using the StatSoft Statistica Package (release 5, Statsoft Inc., Tulsa, OK, USA).

## 3. Results and discussion

### 3.1. Properties of the polysaccharide network in the starting materials and in uncooked rice pasta

#### 3.1.1. Accessibility to hydrolytic enzymes and characterization of hydrolytic fragments

Investigating the accessibility of starch to various and specific enzymatic activities can provide insights into the possible structural differences among samples. The accessibility of rice flour



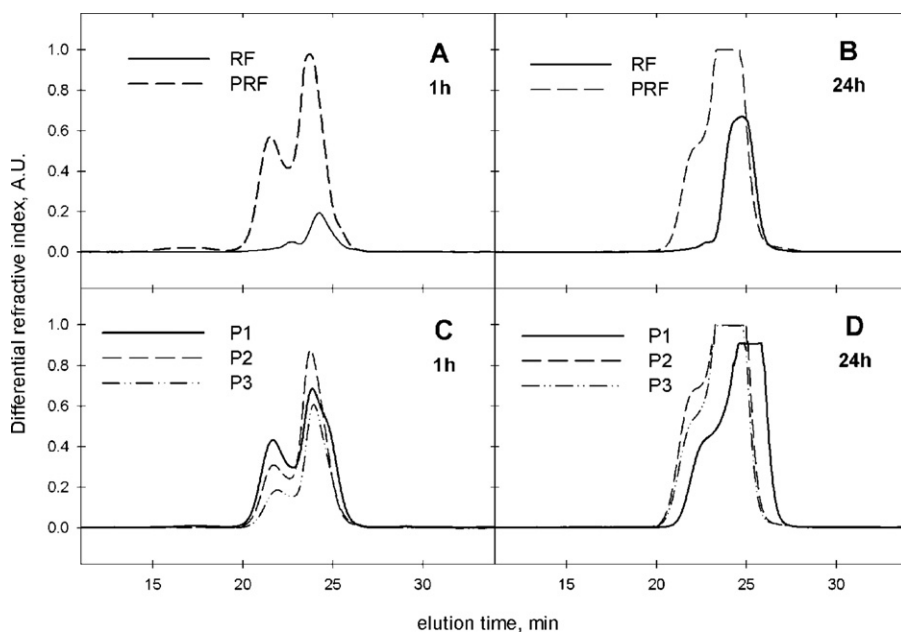
**Fig. 1.** Susceptibility to  $\alpha$ -amylase (a) and pullulanase (b) hydrolysis. RF, native rice flour; PRF, parboiled rice flour; P1, pasta from RF; P2, pasta from PRF by conventional extrusion; P3, pasta from PRF by extrusion-cooking.

samples to  $\alpha$ -amylase action is shown in Fig. 1. RF showed the lowest susceptibility to  $\alpha$ -amylase, even if the amount of enzyme used here was ten times higher than that used for all other samples, and low susceptibility to  $\alpha$ -amylase persisted after 24 h of exposure to the enzyme, underscoring the starch structural compactness in RF.

Parboiling promoted starch granule gelatinization (Bhattacharya, 2004) making the structure more susceptible to  $\alpha$ -amylase action, as shown for PRF in Fig. 1A. However, the initial steps (1 h) of glucose release from PRF upon hydrolysis were slow, suggesting conservation of starch organization in PRF, as gelatinized starch granules reassociate in the parboiled kernels after the cooling and drying steps (Ong & Blanshard, 1994).

The  $\alpha$ -amylase susceptibility increased markedly after processing into pasta, also as a function of the absence/presence of a pre-treatment step on the raw material (Fig. 1A). In all pasta samples, glucose release after 1 h was higher than in the corresponding starting materials. The most marked effects were observed on P1, where most of the starch solubilized by  $\alpha$ -amylase action was released within 1 h. The starch solubilized from P1 after 24 h was lower than from P2 and P3, both obtained from PRF. This suggests that extrusion-cooking of untreated flour do not allow complete accessibility of starch to  $\alpha$ -amylase. Colonna, Tayeb, and Mercier (1989) mentioned that extrusion-cooking causes macromolecular degradation of starch leading to different digestibility profiles and to increased starch accessibility in extrudates. This was related to the severity of the extrusion-cooking, as the rupture of individual granules made the starch more accessible and facilitated the amylolytic hydrolysis in vitro (Hagenimana, Ding, & Fang, 2006).

On the other hand, the fraction of glucose released after 1 h treatment with  $\alpha$ -amylase decreased in pasta obtained from parboiled flour, and was lowest in P3, where an extrusion-cooking step was involved also in pasta-making. This implies that parboiling (carried out on paddy rice) and extrusion-cooking of rice flour had different and non-synergistic effects on starch structure. The interplay of intrinsic factors such as the degree of retrogradation or recrystallization, the starch–protein interactions, and



**Fig. 2.** SE-HPLC-Light Scattering chromatograms after  $\alpha$ -amylase hydrolysis. (A) Rice flours after 1 h of hydrolysis; (B) rice flours after 24 h of hydrolysis; (C) pasta samples after 1 h of hydrolysis; and (D) pasta samples after 24 h of hydrolysis. Samples are identified as in the legend to Fig. 1.

the degree of crystallinity are known to play a significant role in the rate of enzymatic starch hydrolysis (Hagenimana et al., 2006). Impaired enzyme degradation of extrusion-cooked pasta (as evident here by comparing the glucose release at 1 h from P2 and P3) has been attributed to the formation of amylose–lipid complexes, to starch–protein interactions, and to limited water availability, as observed in starchy systems by Guha, Ali, and Bhattacharya (1997). However, our observations can be explained also by the changes in starch organization after its gelatinization during the extrusion-cooking process, that leads to irreversible loss of the crystalline regions with concomitant transition to an amorphous state. Cooling of the product results in starch recrystallisation (Haralampu, 2000), forming a network with novel crystalline properties. Both the crystallite structure and the packing of the amorphous phase reportedly affect enzymatic susceptibility (Colonna, Leloup, & Buleon, 1992).

The susceptibility to pullulanase of the same samples is reported in Fig. 1B. Pullulanase is a debranching enzyme with the ability to cleave  $\alpha$ -1,6 linkages in amylopectin molecules (Lin & Chang, 2006). The amount of material made soluble by pullulanase hydrolysis could give information of the amount and organization of branches in amylopectin and on possible effects of the processing conditions on specific regions of its structure.

RF showed the lowest susceptibility to pullulanase, suggesting dense packing of amylopectin branches. The parboiling process increased sensibly the amount of hydrolyzable  $\alpha$ -1,6 linkages. In the pasta sample made from RF (P1), amylopectin debranching within 1 h of pullulanase action increased >10-fold, suggesting the presence of a high portion of  $\alpha$ -1,6 linkages in the external regions of the amylopectin structure. Such an increase was much less evident in pasta samples made from PRF (P2 and P3). The amount of soluble material released by pullulanase was higher in P2 than in P3, suggesting that extrusion-cooking of previously parboiled flour could generate a starch structure characterized by less accessible regions. This also suggests that the pullulanase-accessible amylopectin fraction may be responsible for a good deal of the differences in starch arrangement in the various pasta samples.

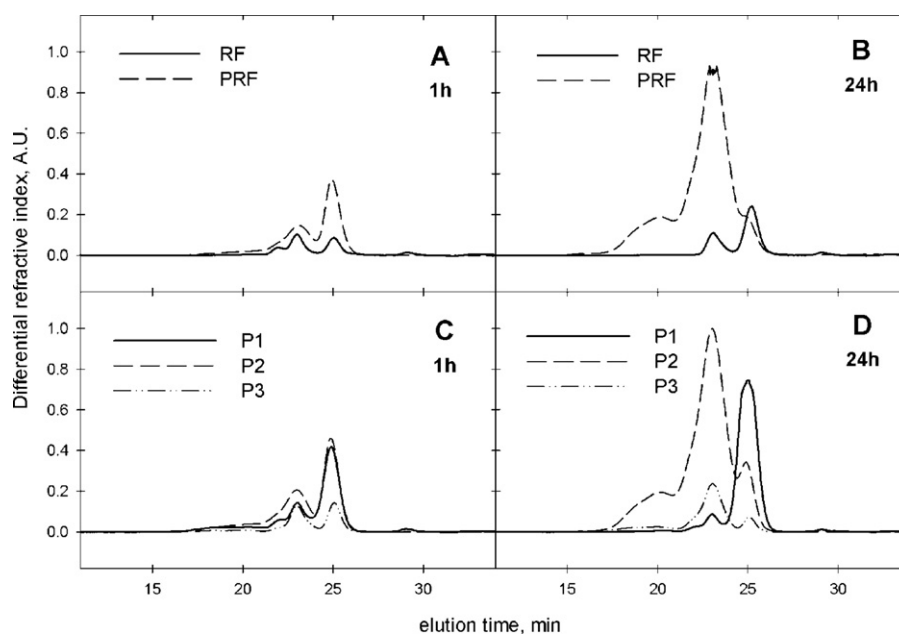
SE-HPLC was used in this study in combination with Static Light Scattering to obtain information about the nature and size of soluble products of enzymatic hydrolysis. The separation of fragments

released by  $\alpha$ -amylase is shown in Fig. 2.  $\alpha$ -Amylase hydrolysis of RF (used on this substrate at concentrations ten-fold higher than those used for other materials) gave a small peak at 23 min and a larger one at 24 min, likely related to di- and trisaccharides (Fig. 2A). Hydrolysis of PRF gave a peak at 22 min (10–40 kDa, 60–250 glucose molecules, as assessed by Static Light Scattering) along with a large peak at 24 min. The intensity of the peak at 22 min remained unchanged at 24 h hydrolysis of PRF (Fig. 2B), suggesting that these soluble species represent a hydrolytic intermediate. These hydrolytic intermediates were also observed after  $\alpha$ -amylase action on pasta samples, regardless of the starting material and/or the pasta-making process. After 1 h hydrolysis, their abundance in the various samples increased in the order  $P3 < P2 < P1$  (Fig. 2C), whereas at 24 h the abundance of these intermediates followed the order  $P2 > P3 \gg P1$  (Fig. 2D). The lower amount of hydrolytic fragments in P3 with respect to P2 at either incubation times may be a consequence of the higher extent of starch retrogradation in P3.

The size distribution of molecules released in solution by pullulanase is shown in the various panels of Fig. 3. Pullulanase action on RF for 1 h gave three different peaks (Fig. 3A). The one corresponding to largest fragments (at 22 min) was no longer present after 24 h (Fig. 3B). Conversely, pullulanase action on PRF gave a time-dependent accumulation of large soluble polymers, with a constant content in di- and trisaccharides (Fig. 3B). The effects of parboiling on starch susceptibility to pullulanase seem more evident when comparing the area of chromatographic peaks in Fig. 3B than when measuring the amount of carbohydrates present as glucose after complete hydrolysis of the released fragments (Fig. 1B).

The pasta-making process promoted important changes in the distribution of soluble polysaccharides produced by pullulanase action, in particular as a function of the hydrolysis time (Fig. 3C and D). Starch in P1 – made from RF by extrusion-cooking – showed a greater release of di- and trisaccharides than the corresponding starting flour at both 1 h and 24 h. No major effects of the pasta-making process were evident when considering the larger polymeric species in pasta made from PRF or RF after 1 h exposure to pullulanase. However, release of di- and trisaccharides from P3 was very low, suggesting once again a significant impact of the extrusion-cooking step on amylopectin, and the absence of





**Fig. 3.** SE-HPLC-Light Scattering chromatograms after pullulanase hydrolysis. (A) Rice flour after 1 h; (B) rice flours after 24 h; (C) pasta samples after 1 h; and (D) pasta samples after 24 h. Samples are identified as in the legend to Fig. 1.

a synergistic effect between parboiling and extrusion-cooking. This is confirmed by analysis of the products released after 24 h exposure to pullulanase. The chromatographic profile from pasta P2 again closely resembled that of PRF, whereas the products of prolonged pullulanase action on pasta P3 indicated a modest release of hydrolytic intermediates and the presence of minimal amounts of di- and trisaccharides.

This may stem from the occurrence of organized clusters within the starch structure, proposed to stem from extrusion-cooking of a pre-treated rice flour (Marti et al., 2010). These organized regions, characterized by a poorly packed external region and by a compact core, may be responsible for the higher RS content in extrusion-cooked pasta in comparison to pasta made by conventional extrusion, as discussed in what follows and in agreement with reports indicating that starch containing both amorphous and partially ordered regions is digested slowly (Guraya, James & Champagne, 2001; Miao, Jiang, & Zhang, 2009).

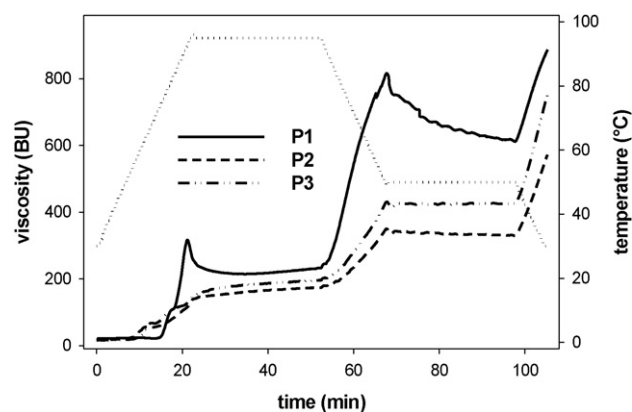
### 3.1.2. Pasting properties of starch in rice pasta

The pasting profiles in Fig. 4 may provide information on macromolecule arrangement in the products considered here. Samples P2 and P3 had the lowest pasting temperature and P1 the highest, a result that could be related to the strength of bonds in the starchy network (Eliason & Karlson, 1983). Only sample P1 showed a peak viscosity (at  $\sim 90^\circ\text{C}$ ), suggesting the presence of starch granules with a high swelling capacity, as previously observed in rice flours (Marti et al., 2010).

During the holding period at  $95^\circ\text{C}$ , the product slurries were subjected to high temperatures and mechanical shear, causing starch granule disruption and amylose leaching, which led in turn to a slight decrease in viscosity in P1, whereas viscosity in P2 and P3 continued to increase while holding these samples at  $95^\circ\text{C}$ . Upon cooling, P1 gave the highest setback values, suggesting the highest retrogradation tendency among all the samples, followed by P3 and P2 in the order. Also noteworthy is the absence of viscosity changes in the starch paste formed by samples P2 and P3 while being stirred at  $50^\circ\text{C}$ , in contrast with the decreased viscosity observed for P1, that stems from physical breakdown of the starch gel formed upon cooling of this particular sample.

### 3.2. Properties of protein network in the starting materials and in uncooked pasta

Structural features of proteins in rice pasta were evaluated by extraction in buffers with different dissociating ability towards covalent and non-covalent interprotein bonds, and by accessibility of specific residues under the same conditions. As shown in Fig. 5A, protein solubility in buffer was low in P1 ( $\leq 3.2$  mg/g pasta), and almost nil in P2 and P3, confirming aggregation of soluble protein components (albumins and globulins) upon parboiling (Bhattacharya, 2004). Addition of urea to the buffer/saline extractant resulted in a significant increase in solubilized protein from all samples, suggesting that hydrophobic interactions are relevant to the structure of whatever protein matrix in rice pasta. A similar behaviour was also detected in commercial rice and corn pasta (Mariotti, Iametti, Cappa, Rasmussen, & Lucisano, 2011) and in amaranth-enriched pasta (Cabrera-Chávez et al., 2012). When both urea and DTT were present in the extraction medium, the amount of soluble proteins increased further, most notably in samples (P2 and P3) prepared from PRF. This suggests that inter-protein disulphides play a fundamental role in the structure of the protein network in



**Fig. 4.** Pasting properties of rice pasta. Samples are identified as in the legend to Fig. 1.

**Table 1**  
Cooking behaviour of rice pasta.

	Cooking loss (g/100 g)	Water absorption (g/100 g)	Compression energy (N mm)	Firmness (N)	Shear force (N)
P1	9.8 ± 0.2 <sup>b</sup>	90.7 ± 4.2 <sup>b</sup>	328.4 ± 6.9 <sup>a</sup>	190.6 ± 6.9 <sup>a</sup>	150.4 ± 4.6 <sup>a</sup>
P2	12.6 ± 0.7 <sup>c</sup>	79.5 ± 3.8 <sup>a</sup>	553.3 ± 30.9 <sup>b</sup>	275.3 ± 8.2 <sup>b</sup>	259.1 ± 15.1 <sup>b</sup>
P3	5.6 ± 0.1 <sup>a</sup>	77.3 ± 3.5 <sup>a</sup>	1914.8 ± 364.3 <sup>c</sup>	901.6 ± 119.3 <sup>c</sup>	524.7 ± 70.6 <sup>c</sup>

Means and standard deviation followed by different superscript letters in a column are significantly different at  $p < 0.05$ .

pasta made from parboiled flour, almost regardless of the pasta-making process. The minor but significant differences observed between P2 and P3 may be related to the different starch structure in these samples. It has been suggested that sequential starch gelatinization/retrogradation cycles may result in protein entrapment in an organized starch structure (Cabrera-Chávez et al., 2012).

The nature of the proteins solubilized in the different media from the various pasta samples was investigated by SDS-PAGE. As shown in Figure 1S, buffer-soluble albumins (25, 18, and 16 kDa; Shih, 2004) were present in P1 but absent in P2 and P3, confirming the data in Fig. 5A. Some of the same low-molecular weight species were solubilized by urea in P2 and P3, along with another polypeptide at 36 kDa, also present in urea extracts of P1 together with bands at high molecular weight (60 and 65 kDa). These bands were absent in urea extracts of P2 and P3, but the one at 60 kDa was present in both samples when extraction with urea was carried out in the presence of disulfide-reducing agents. These latter conditions also allowed solubilization of the 18 kDa species, but not of the one at 65 kDa, possibly as a consequence of easier physical entrapment of the latter and larger species into process-modified starch structures, as discussed above.

Supplementary material related to this article found, in the online version, at <http://dx.doi.org/10.1016/j.carbpol.2012.11.047>.

Thus, the pasta-making process results in almost all proteins being linked through hydrophobic interactions. A previous parboiling step on rice flour provides further stabilization of these interactions by intramolecular disulfide bonds, whose formation

seems insensitive to the conditions used for pasta making and apparently involving specific proteins in a preferential way.

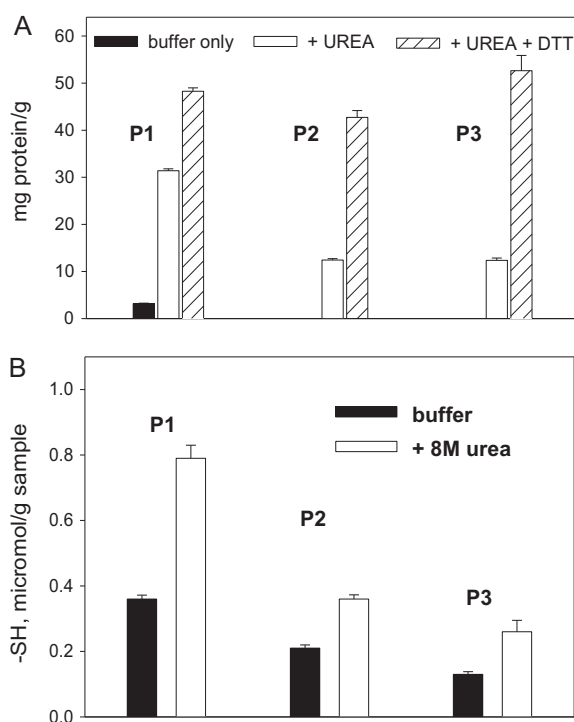
Quantification of accessible –SH groups, that may be carried out independently of protein solubility, has been applied to understanding the nature and evaluating the extent of modification induced in cereal-based foods by processing (Cabrera-Chávez et al., 2012; Elkhailifa et al., 2006; Mariotti et al., 2011). As shown in Fig. 5B, the number of accessible thiols decreased in the order  $P1 > P2 > P3$  both in the absence and in the presence of urea, confirming the impact of parboiling on the compactness of the protein matrix. The lower content of accessible thiols in P3 vs P2 indicates that the effects of extrusion-cooking on protein structure rearrangements are more dramatic than those of conventional extrusion. The relevance of hydrophobic interactions to the compactness of the protein matrix is made evident by the increase in accessible thiols upon addition of urea. Altogether, thiol accessibility data confirm the fundamental role of the pasta-making process in establishing the compactness of the protein aggregates, as pointed out by the solubility approaches described above.

### 3.3. Properties of cooked pasta as related to the starting material and the pasta-making process

#### 3.3.1. Physical and chemical properties of cooked pasta

Data summarizing the cooking performance of the various samples of rice pasta are presented in Table 1. Pasta P3, made by extrusion-cooking of flour from previously parboiled rice (PRF), gave the lowest cooking losses and the lowest water absorption, and was by large the most resilient product. The impact of extrusion-cooking is evident when considering that P2, made from the same starting material by conventional extrusion, had the highest cooking losses, although it retained some of the texture characteristics of the much harder P3. On the other hand, extrusion-cooking of an otherwise untreated rice flour gave pasta P1, that upon cooking gave the highest water absorption, with cooking losses only slightly lower than those measured for P2, and showing by far the lowest values for all textural indices.

As reported in Table 2, retention of protein components and of total starch upon cooking increased in the order  $P3 > P2 > P1$ . The differences in protein and starch content among the samples can be related solely to the pasta-making process, as the parboiling process had been shown to leave starch (Bhattacharya, 2004) and protein content (Bhattacharya & Ali, 1985) totally unaffected. Conversely, the lower sugar content measured in pasta made from parboiled flour (P2 and P3, in comparison to P1) seem to relate to the parboiling process, where sugars from the bran layers leach into the soaking water (Ali & Bhattacharya, 1980; Lamberts, Brijis,



**Fig. 5.** Amount of solubilized protein (A) and accessible protein thiols (B) in different buffer systems. Samples are identified as in the legend to Fig. 1.

**Table 2**  
Chemical composition of cooked rice pasta.

	Protein (%)	Sugars (%)	TS (%)	RS (%)
P1	3.01 ± 0.05 <sup>a</sup>	0.80 ± 0.0 <sup>b</sup>	37.21 ± 1.99 <sup>a</sup>	2.73 ± 0.18 <sup>a</sup>
P2	3.51 ± 0.05 <sup>b</sup>	0.10 ± 0.0 <sup>a</sup>	40.11 ± 0.22 <sup>b</sup>	3.34 ± 0.10 <sup>b</sup>
P3	4.49 ± 0.02 <sup>c</sup>	0.20 ± 0.0 <sup>a</sup>	48.78 ± 1.15 <sup>c</sup>	4.62 ± 0.22 <sup>c</sup>

Means and standard deviation followed by different superscript letters in a column are significantly different at  $p < 0.05$ .

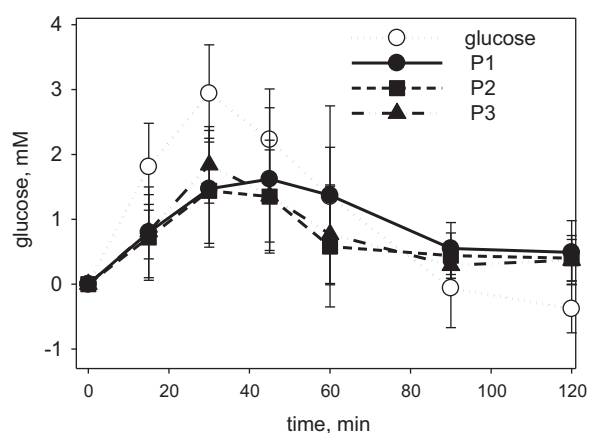


Fig. 6. Mean blood glucose concentrations in healthy subjects after intake of rice pasta. Samples are identified as in the legend to Fig. 1.

Mohamed, Verhelst, & Delcour, 2006; Lamberts, Rombouts, Brijis, Gebruers, & Delcour, 2008).

P3 showed the highest content of RS among the pasta samples, suggesting that RS content was affected from both the parboiling process and the extrusion conditions, in agreement with previous reports (Singh, Dartois, & Kaur, 2010). Higher amounts of RS starch were found in parboiled rice with respect to raw rice (Casiraghi, Brighenti, Pellegrini, Leopardi, & Testolin, 1993; Eggum, Juliano, Perez, & Acedo, 1993). Extrusion-cooking was conducive to a compact product structure (Marti et al., 2010), also due to reorganization of the crystalline structure of resistant starch. This behaviour is consistent with the viscoamlographic data in Fig. 3, suggesting the presence of starch granules with a high swelling capacity in P1. Indeed, sample P1 absorbed the greatest amount of water (Table 1), consistent with high swelling capacity of starch in this pasta, and with reports relating the granules swelling to the tendency to leach contents into cooking water (Sisson & Bately, 2003).

### 3.3.2. In vivo glycemic response

Incremental post-prandial blood glucose curves after consumption of the various rice-based pasta samples by healthy volunteers are shown in Fig. 6. No significant differences were detected among all the samples at any time of these tests. However, in the case of P1, the glycemic peak shifted towards a longer time (45 min) than in pasta prepared from parboiled flour (P2 and P3). This suggests that parboiling affected the starch digestion rate and glucose intestinal absorption. Changes in accessibility to degradation of starch in samples that underwent one or more steps of thermal treatment (see Fig. 1 and related comments) translate into a lower glycemic index for P2 and P3 (61 and 65, respectively) in comparison with P1 (71), although these differences are not statistically significant. The similar trend of the glycemic response in P2 and P3, that have been shown to display quite different starch structures (see Fig. 3 and related comments), may in turn be related to the fact that the different protein organization in these two samples may counteract – at least to some extent – the effects of extrusion-cooking on specific starch fractions.

## 4. Conclusions

This study shows that individual treatments (or their combination) had a markedly different impact on the structural features of macromolecules in rice-based pasta. Pasta made from parboiled rice through an extrusion-cooking process (P3) was extremely firm after cooking. This seems to be consequent to the presence

in the uncooked pasta of a peculiar amylopectin organization, as made evident by the limited accessibility of this polysaccharide to pullulanase, that hydrolyzes  $\alpha$ -1-6 glycosidic bonds. At the opposite end of the sensory spectrum was the pasta made through extrusion-cooking of untreated rice flour (P1), that was extremely soft. This stems from limited protein reticulation, and from part of the starch being present in a compact native-like conformation, as evidenced by its limited accessibility to  $\alpha$ -amylase action in the uncooked pasta and by the delayed increase in blood glucose when this pasta is eaten.

Pasta made from parboiled rice flour through conventional extrusion (P2) had satisfactory sensory parameters. Amylopectin in the uncooked material was loosely structured, as indicated by its sensitivity to the action of pullulanase, whereas starch as a whole had a structure comparable to that of pasta P3. These structural considerations may explain the observation that the time course of post-prandial glucose levels were almost overlapping in subjects consuming P2 or P3, where treatments increased the content of resistant starch.

From a methodological standpoint, the approaches reported here seem able to provide useful insights as for improving our current molecular-level understanding of the effects of treatment conditions (and of their combination and temporal sequence) on overall product quality.

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