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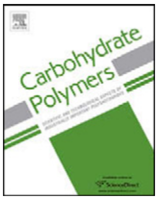

Understanding starch organisation in gluten-free pasta from rice flour

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ABSTRACT

Starches extracted from parboiled rice flour and pasta samples produced by two extrusion processes – a conventional method carried out at 50 °C and an extrusion-cooking process at 115 °C – were evaluated by differential scanning calorimetry (DSC) and size exclusion chromatography (SEC) analysis. Molecular changes induced by both pasta-making process and following cooking in boiling water were also investigated using iodine absorption properties of samples expressed as the ratio of absorption to scattering spectra (K/S) and X-ray diffraction. A decrease in polymer chain mobility and iodine binding capacity were observed after pasta-making process. While the characteristic A-type crystalline pattern remained, the exposure to iodine vapor changed the peak intensity of starch samples, especially at 0.97 a_w. The higher melting temperature of pasta samples in comparison with parboiled rice flour reflected the decrease in mobility of the amorphous regions detected by K/S spectral analysis. The pasta making-process also affected the molecular size distribution of starch samples. In particular, the elution peak shifted toward lower fraction numbers with increasing extrusion temperature, showing a higher molecular size for starch after the extrusion-cooking. All the differences detected between starch samples according to extrusion conditions were deleted during cooking. Compared to the uncooked samples, starch from cooked pasta showed higher K/S value at all wavelengths, highlighting the increase in mobility of the amorphous region. Moreover, beside the increase in melting temperature, a decrease in endothermic enthalpy was detected, confirming the loss of order observed by X-ray diffraction.

1. Introduction

Since milled rice consists of about 90% starch, the structure of this macromolecular fraction and its physicochemical properties are the primary characteristics used to select rice cultivar and rice starch for specific industrial applications (Bao, 2001). Several studies have been carried out on physicochemical, morphological, thermal, rheological properties of rice starch isolated from flour (Singh, Kaur, Singh Sandhu, Kaur, & Nishinari, 2006; Vandeputte, Derycke, Geeroms, & Delcour, 2003; Vandeputte, Vermeylen, Geeroms, & Delcour, 2003a; Vandeputte, Vermeylen, Geeroms, & Delcour, 2003b). However, no studies are present on rice starch properties in a complex matrix, such as pasta, where its properties and the extent of its modifications could be influenced by the presence and the interactions with other components.

Due to the absence of gluten, rice is recommended as safe for people affected by celiac disease and it is commonly used to produce gluten-free (GF) pasta, alone or in combination with other no-gluten cereals and/or additives (Marti, Seetharaman, & Pagani, 2010). In a preliminary study, the effect of pasta-making process on pasting, thermal, textural, and cooking properties have been evaluated in pasta from brown and milled rice flour, considering the whole product and, thus, the interactions among all the biopolymers (starch–starch and starch–protein interactions in sample from milled rice; starch–starch, starch–protein, starch–lipid, and starch–non-starch polysaccharides interactions in pasta from brown rice) (Marti et al., 2010). In order to complete and better understand the starch architecture in pasta-product it could be advisable to investigate the starch macromolecular characteristics after removing all the non-starch constituents from the samples. In fact, in a GF matrix, starch cannot be considered an inert filler, but it substantially contributes to the structure and the quality of the product. The textural and nutritional properties of GF pasta are related both to the organization of raw-material native starch and to starch modifications promoted by thermal and mechanical events occurring during the pasta-making process. In order to produce a GF pasta with similar appearance and texture as

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Abbreviations: Cooked C, starch from cooked pasta prepared by conventional extrusion; Cooked E, starch from cooked pasta prepared by extrusion-cooking; ΔH_{TS}, gelatinization enthalpy expressed on total starch content; DSC, Differential Scanning Calorimetry; GF, gluten-free; PRS, starch from parboiled rice flour; SEC, Size-Exclusion Chromatography; Starch C, starch from raw pasta prepared by conventional extrusion; Starch E, starch from raw pasta prepared by extrusion-cooking; T_c, gelatinization conclusion temperature; T_o, gelatinization onset temperature; T_p, gelatinization peak temperature.

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conventional products of *durum wheat* semolina, a new starch organization, effective in substituting the gluten network in the final product, can be formed applying one or more heating and cooling treatments to GF raw-material. The sequence of thermal events during gelatinization promotes a loss of native granular structure of starch and an extensive reticular and fibrillar network after cooling (Resmini & Pagani, 1983).

Combining high temperature with a large amount of mechanical energy input, extrusion causes an irreversible swelling or even disruption of the starch granules, depending upon the severity of the treatment applied (Karim, Norziah, & Seow, 2000). After its gelatinization, during the cooling phase starch undergoes retrogradation that involves the starch chains tend to reassociate in an ordered structure. Since retrogradation profoundly affects quality, acceptability, shelf-life, and nutritional properties of starch-based foods, the phenomenon is of great interest to food scientists and technologists (Biliaderis, 1991). Although starch retrogradation is considered a negative phenomenon in baking, it could positively affect the quality of extruded products as pasta (Karim et al., 2000). In fact, in order to decrease stickiness, prevent the dissolution of solid matter into the boiling water, and obtain a characteristic chewiness, the production of rice noodles involves cooling cycles (Tan, Li, & Tan, 2009). However, in pasta products starch retrogradation is often associated with an increase in firmness and springiness after cooking. The objective of this paper was to better understand starch modification that occurred during pasta-making process and the relationship between starch structure and processing conditions. Iodine was used as tool to highlight structural differences in the arrangement of pasta samples prepared by using different extrusion conditions. At the same time, the molecular changes promoted by cooking were investigated.

2. Materials and methods

2.1. Materials

Milled parboiled rice (Thai cultivar of commercial origin; total starch: 82.6% db; protein: 6.8% db; lipid: 1.0% db; amylose: 25% db) used in this study was provided by Riso Viazzo s.r.l. (Crova, Italy).

2.2. Pasta preparation

Two pasta samples were prepared in the pilot-plant of DiSTAM (University of Milan, Milan, Italy) by using a conventional extrusion (Process C) and an extrusion-cooking pasta-making process (Process E), as reported by Marti et al. (2010). Process C was carried out in a continuous press commonly used for the production of pasta from semolina (Braibanti, Milano, Italy). Parboiled rice flour (particle size less than 250 μm) and water were blended to produce a mixture with a final water content of 40%. After mixing, the dough was formed in Pasta C, maintaining the continuous press under vacuum and keeping the dough temperature below 50–55 °C, at an extrusion pressure of 10–11 MPa. A patented process was used to produce pasta by an extrusion-cooking process (Grugni, Mazzini, Viazzo, & Viazzo, 2009). The mixture of flour and water (with a final water content of 40%) was heat-treated for 2 min in a Progel® extruder (single screw; Braibanti, Milano, Italy) fed with steam at 115 °C. After the first extrusion step, the heat-treated dough was formed in the continuous extruder used for the conventional process (Braibanti, Milano, Italy), obtaining Pasta E. Both pasta samples (Pasta C and Pasta E) were dried in an experimental drying cell (Braibanti, Milano, Italy) using a low-temperature drying cycle (50 °C for 14 h) and stored at room temperature until analyzed. Before the analysis, rice pasta samples were ground in order to produce a product with less than 500 μm particle size.

2.3. Starch isolation

Starch samples were isolated from rice flour and rice pasta samples, according to Park, Bean, Wilson, & Schober (2006) and Goesaert, Leman, Bijttebier, & Delcour (2009) with some modifications. The samples were mixed with protein extraction buffer (12.5 mM sodium borate buffer, pH 10, containing 0.5% SDS and 0.5% $Na_2S_2O_5$) (solvent-to-flour ratios = 1:20). After stirring for 5 min and centrifugation (3000 rpm for 5 min at 10 °C), the supernatant was discarded and the mixing step with protein extraction buffer was repeated. The pellets obtained were dispersed and washed in deionized water for three times in order to remove the residual bran. After the buffer treatment, an aliquot of 100 mL of a protease solution containing 0.50 g of trypsin from porcine pancreas (13,000–20,000 BAEE units/mg protein; Sigma–Aldrich Chemie) and 0.25 g of papain from Carica papaya (≥3 units/mg; Sigma) was added to 10 g of sample. After overnight incubation at room temperature under magnetic stirring, the suspension was centrifuged (10 min, 3000 × *g*). The residues were washed three times with deionized water and twice with 10.0 mL of ethanol (95%). The enzymatic degradation, incubation (3 h, magnetic stirring, room temperature) and the washing step with water and ethanol (95%) were repeated. The isolated starch was washed with acetone and dried overnight. The dry starch was ground lightly with a mortar and pestle (with less than 500 μm particle size) for further analysis.

The same procedure was carried out to isolate starch from pasta samples after cooking in boiling water. Pasta C and Pasta E were cooked as reported by Marti et al. (2010); after draining, samples were dipped into liquid nitrogen and then freeze-dried before starch isolation. The rapid cooling contains retrogradation phenomenon which extent is at 4–6 °C.

2.4. Sepharose CL-2B chromatography

Granular starches were prepared according to Klucinec and Thompson (1998). Starch (0.5 g) was dispersed in 10 ml of 90% (v/v) dimethyl sulfoxide in water, by heating the mixture in a boiling water bath with constant stirring for 3 h. Following dispersion, 40 ml of ethanol was added and the mixture was centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was discarded and the pellets were washed by suspending them in 50 ml of ethanol, followed by centrifugation (10,000 rpm). The washing procedure was repeated once with ethanol and once with acetone. The acetone-washed precipitate was dried in a forced air oven at 50 °C for 24 h. The nongranular starches were stored under desiccation ($CaSO_4$) before the analysis. Nongranular starches (4 mg) were dispersed in 2 ml of NaOH 0.1 N for one night. The dispersed starches were loaded into a Sepharose CL-2B (Sigma Aldrich Inc.) SEC column (50 cm × 1 cm, Pharmacia Fine chemicals, Sweden) using gravity flow. The mobile phase in the system was NaOH, 0.1 N, containing 0.02% (v/v) sodium azide. For each sample, 1 ml of fraction was collected by using a fraction collector, at a flow rate of 0.3 ml/min and the analysis was repeated twice. Every two SEC fraction was examined for total carbohydrate and iodine binding λ_{max}. Total carbohydrate of the fractions was determined using the phenol–sulfuric acid assay of Dubois, Gilles, Hamilton, Rebers, & Smith (1956). The iodine binding λ_{max} was determined using the procedure of Klucinec and Thompson (1998). An aliquot of 0.4 ml of a SEC fraction was neutralized with 25 μl of HCl, 1.0 N and mixed with 2.5 ml of iodine solution. The iodine solution was prepared immediately before the analysis, diluting 0.5 ml of the stock iodine solution (0.026 g of I_2 and 0.26 g of KI/ml water) in 130 ml of deionized water. The absorbance of the solution (composed by SEC fraction, HCl, and iodine solution) was examined between 450 and 800 nm

(VARIAN Cary 1 Bio). The measurement was carried out in duplicate.

2.5. *Thermal properties*

The thermal properties of starch samples were determined using a Perkin Elmer Differential Scanning Calorimeter (DSC-7/DX; Perkin Elmer, Cambridge, United Kingdom). Each sample was weighed in a steel pan and an empty steel pan was used as reference. Distilled water was added to the sample to make a 1:3 (w/w) sample:water ratio. The pans were hermetically sealed and allowed to equilibrate 24 h at room temperature. Samples were heated from 25 to 180 °C at a rate of 10 °C/min. The onset temperature (T_o, °C), peak temperature (T_p, °C), conclusion temperature (T_c, °C), and gelatinization enthalpy (ΔH, J/g) were determined using the software provided with the equipment. All measurements were replicated at least twice.

2.6. *Colorimetric analyses of granular samples exposed to iodine vapor*

Samples of 2 g were equilibrated to the respective water activity (a_w), with final values of 0.33, 0.75, and 0.97 a_w using saturated solutions of $MgCl_2$ (EMD Chemicals Inc., Gibbstown, NY), NaCl (Fisher Chemicals Inc., Fair Lawn, NJ), and K_2SO_4 (EDM Chemicals Inc., Gibbstown, NY) (Greenspan, 1977), as described by Saibene and Seetharaman (2006). Following equilibration, the moisture content of samples was measured according to the AACC method 44-15A (2000). To determine the iodine binding, 0.2 g of the equilibrated sample were placed in the corresponding a_w desiccator, and exposed to iodine vapor generated from 2 g of iodine crystals (J.T. Baker, Phillipsburg, NJ) for 24 h at room temperature. Each sample was equilibrated and exposed to iodine vapor twice. A CM 3500-d Spectrophotometer (Konika Minolta, Mahwah, NJ, USA) was used to evaluate the color (expressed as luminosity) and the K/S value of stained samples. The K/S value of the samples after iodine exposure was measured at wavelength range from 400 to 700 nm, at 10 nm intervals, using unstained starch as the target color.

2.7. *Wide angle X-ray powder diffraction*

Wide angle X-ray diffraction measurements were carried out on starch samples (0.1 g) after equilibration, before and after iodine exposure (Rigaku Powder Diffractometer equipment – Rigaku Co., Tokyo, Japan). CuKα1 radiation (λ = 1.54 Å) was selected using a quartz monochromator and the operational settings for the diffractometer were 40 mA and 40 kV. For this instrument, the diffractometer had a 0.5° divergence slit, a 0.33 mm receiving slit and a 0.5° scattering slit. The samples were scanned in the range 3–35° 2θ at a rate of 1° 2θ per minute. Data were smoothed using Jade 6.5 software and were normalized to equal total scattering in 3–35° 2θ range. The analysis was done in duplicate.

3. Results and discussion

3.1. *Sepharose CL-2B chromatography*

The Sepharose CL-2B chromatograms of starch samples are shown in Fig. 1. Two main fractions can be distinguished for each sample: the amylopectin peak showing iodine absorption at about 550 nm, and the amylose fraction with high iodine binding absorption at more than 600 nm. In parboiled rice starch (PRS), the molecular weight of the peak corresponding to amylopectin was smaller than the corresponding peaks observed for the starch from pasta samples (Starch C and Starch E). Pasta samples exhibited a sharp transition from the low wavelength fraction to the high wavelength fraction (Fig. 1). Compared to PRS, the increase of the molecular weight of amylopectin peak after pasta-making process was accompanied, at the same time, by the formation of amylose fraction characterized by lower λ_{max}. The extrusion conditions affected the molecular size distribution in pasta samples; the elution peak shifted toward lower fraction numbers with increasing extrusion temperature, indicating an increase of the molecular size after extrusion-cooking (Fig. 1). The results suggest that the amylopectin matrix is likely a combination of amylose and amylopectin chains. The average molecular weight of amylose and amylopectin, as well as their molecular organization within the granule, affected starch functionality. During extrusion-cooking, the higher extrusion temperature and mechanical stress associated with the screw turning promoted starch gelatinization, involving the disruption of molecular order within the starch granule. After heating, retrogradation occurred and starch polymers showed a tendency to reassociate in an ordered structure, resulting in a new rearrangement (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988). Moreover, the amylopectin structure seems to explain the differences in texture among rice varieties. According to Reddy, Ali, & Bhattacharya (1993), the presence of a large number of long unbranched chains at the exterior region of the amylopectin molecule leads, by their interaction, to the formation of strong and elastic starch aggregates as a consequence of the numerous interactions arising among chains: the new organization could account for firm and non-sticky cooked rice. On the other hand, the lack of such chains makes starch material weak and fragile, and the cooked rice results to be soft and sticky (Reddy et al., 1993). In the present study, the structure of the amylopectin could account for the texture of the cooked product: starch E, characterized by high molecular weight distribution (Fig. 1) resulted in a product characterized by extreme firmness after cooking, as recently reported by Marti et al. (2010).

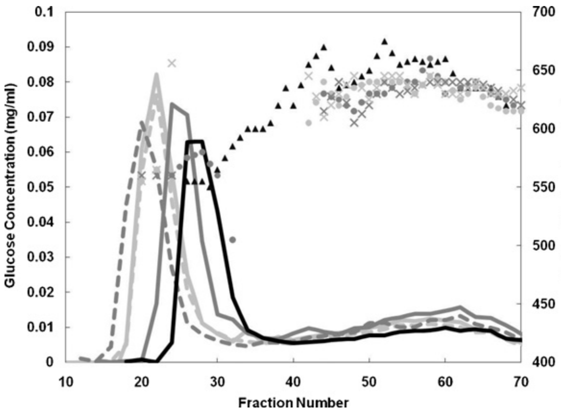

Fig. 1. Size-exclusion chromatograms of nongranular starch samples. Total carbohydrate: PRS (black solid line), Starch C (dark grey solid line), Starch E (light grey solid line), Cooked C (dark grey broken line), Cooked E (light grey broken line). Iodine binding λ_{max}: PRS (▲), Starch C (●), Starch E (●), Cooked C (✕), Cooked E (✕). PRS = starch from parboiled rice flour; Starch C = starch from raw pasta prepared by conventional extrusion; Starch E = starch from raw pasta prepared by extrusion-cooking; Cooked C = starch from cooked pasta prepared by conventional extrusion; Cooked E = starch from cooked pasta prepared by extrusion-cooking.

Further changes occurred in starch samples after cooking, according to the extrusion conditions (Fig. 1). Compared to the uncooked sample, the SEC chromatograms of cooked starch from conventional extrusion shifted toward lower fraction numbers, indicating that the cooking process resulted in an increase in molecular size, while the absorbance values were lower at values similar to rice flour starch. On the other hand, the profile related to the amylose fraction did not change after cooking, keeping the iodine binding λ_{max} values between 600 and 640 nm. In Starch E, cooking did not change the SEC profile compared to uncooked pasta, suggesting that the starchy structure created during the extrusion-cooking was still present in the product, but with even higher wavelength of absorption. It is likely that this structure provides the stability and lower cooking loss (4.2%) observed for this pasta compared to the conventionally processed pasta (15.9%), as reported in a previous publication (Marti et al., 2010).

3.2. Thermal properties

The thermal properties of starch samples are shown in Table 1. Starch from PRS exhibited a peak at 53.7 °C suggesting the presence of recrystallized amylopectin, and a peak at 95 °C indicating the presence of amylose-lipid complexes, as can be expected in parboiled rice (Lamberts, Gomand, Derycke, & Delcour, 2009). The endothermal peaks shifted to higher temperatures in pasta. The shift of T_p from 53.7 °C to 57.7 °C was accompanied by a slight decrease of gelatinization enthalpy from 3.8 to 3.1 J/g in pasta made using conventional process. A similar trend has been reported for pasta from semolina, implying starch molecules underwent conformational reorganization as a result of the extrusion step and the drying cycle (Yue, Rayas-Duarte, & Elias, 1999). However, no differences were observed for the amylose–lipid complex endotherm in pasta made using conventional process when compared to PRS.

Starch in pasta made by using extrusion process (Starch E) exhibited a different endothermal profile with a broad peak starting at 57.2 °C and ending at 96.4 °C. There were two unresolvable melting peaks at 68.7 °C and 83.7 °C; and thus, it was not possible to calculate the endothermal energy (Table 1). Nevertheless, this appears consistent with the data shown in the SEC profile, wherein the amylopectin matrix has a larger molecular size (Fig. 1). The higher melting temperature detected in Starch E could highlight a higher thermal stability in the product, confirming previous results (Marti et al., 2010). A similar trend was detected in pasta from semolina and related to the stabilization of amylopectin crystallites (Yue et al., 1999).

Following cooking, the endothermic profile only exhibited a peak at 89 °C and no peak was observed at the lower temperature, for pasta made by using both processes. However Cooked E sample still had a higher end temperature that is consistent with the SEC profile that shows the amylopectin fraction with higher wavelength of absorption.

3.3. Moisture content of equilibrated samples

The moisture content of starch samples equilibrated above saturated solutions of $MgCl_2$, NaCl, and K_2SO_4 is shown in Table 2. The moisture content of the samples was similar when equilibrated above $MgCl_2$ (0.33 a_w) and, as expected, it increased with increasing a_w. Starch C had a lower moisture content compared to PRS and Starch E both at 0.75 and 0.97 a_w. Moisture of the starch from cooked pasta increased; however, no differences were observed between the two cooked pasta samples (Cooked C and Cooked E).

3.4. Color development in iodine exposed starches

The color development (in terms of luminosity, L^*) of starch samples after iodine exposure following equilibration above $MgCl_2$ (0.33 a_w), NaCl (0.75 a_w), and K_2SO_4 (0.97 a_w) solutions is shown in Table 2. L^* values were not significantly different between the starch samples following equilibration above $MgCl_2$. With increasing moisture content, the L^* value decreased for each sample, indicating a darker color. These results are supported by previous studies (Saibene & Seetharaman, 2006, 2010; Saibene, Zobel, Thompson, & Seetharaman, 2008; Waduge, Xu, & Seetharaman, 2010). Moreover, at higher water activity, the effect of pasta-making process appeared more evident on the L^* values of samples. In particular, following equilibration above NaCl solution, L^* value increased slightly, reaching higher value in the extrusion-cooking process. An overall increase in L^* value was also observed in the cooked samples, regardless of process conditions. A different trend was observed when starch samples were equilibrated above K_2SO_4 (0.97 a_w). Only starch from process C exhibited a higher L^* value compared to PRS. A further decrease in L^* value was detected after cooking, regardless the extrusion conditions ($p < 0.05$).

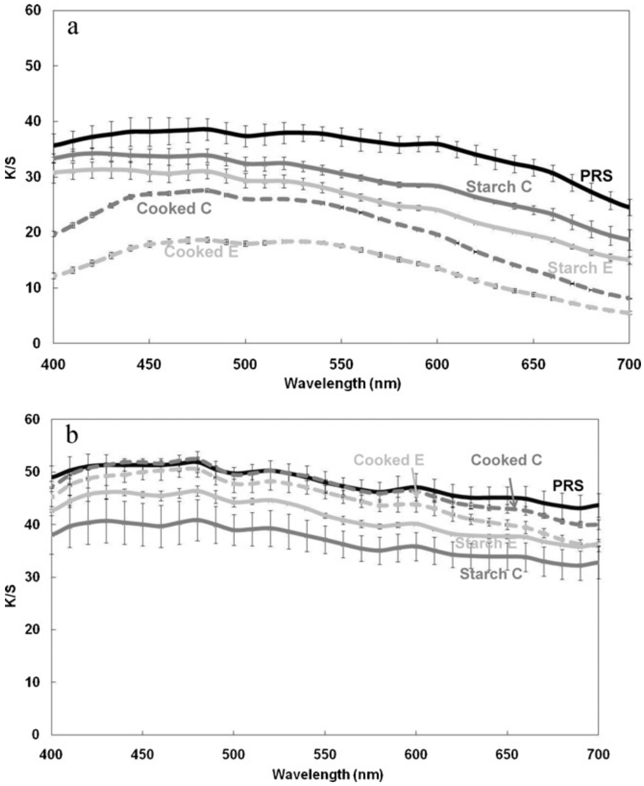

Fig. 2. K/S values of starch samples exposed to iodine vapor following equilibration at 0.75 a_w (a) and 0.97 a_w (b). PRS = starch from parboiled rice flour; Starch C = starch from raw pasta prepared by conventional extrusion; Starch E = starch from raw pasta prepared by extrusion-cooking; Cooked C = starch from cooked pasta prepared by conventional extrusion; Cooked E = starch from cooked pasta prepared by extrusion-cooking.

3.5. Iodine absorption spectra

Absorption spectra of starch samples after iodine exposure, presented as a ratio of absorption/scattering (K/S), are shown in Fig. 2. At the lowest a_w value (0.33 a_w), all the samples exhibited a low K/S value at all wavelengths (data not shown). Absorption intensities increased for all samples with increasing water activity, as expected (Fig. 2a and b). This is due to the greater mobility of longer chains with increasing moisture content (Saibene & Seetharaman, 2006). A similar effect was reported previously with potato, corn, and wheat starches (Saibene & Seetharaman, 2010; Saibene et al., 2008; Waduge et al., 2010).

After equilibration of samples above NaCl solution, PRS had the highest absorption values, although no distinct peaks are evident, followed by starches extracted from pasta; starches from cooked pasta had the lowest absorption values. This trend is similar to the L^* values reported in Table 2; higher the iodine absorption, darker the color and higher the K/S values. Therefore, with increasing the intensity of physical (both thermal and shear) stresses associated with processing, there was a decreasing amount of mobile polymers capable of binding iodine. Furthermore, differences were also observed between starches processed using the two extrusion con-

Table 1
Thermal properties of starch samples.

Sample	First peak				Second peak			
	T_o (° C)	T_p (° C)	T_c (° C)	ΔH (J/g)	T_o (° C)	T_p (° C)	T_c (° C)	ΔH (J/g)
PRS	46.3 ± 0.3	53.7 ± 0.0	62.9 ± 1.5	3.8 ± 0.1	76.8 ± 0.0	86.2 ± 0.7	95.0 ± 0.4	2.0 ± 0.2
Starch C	50.3 ± 0.2	57.7 ± 0.5	64.6 ± 0.2	3.1 ± 0.0	77.0 ± 0.5	86.0 ± 0.5	94.3 ± 1.1	2.0 ± 0.2
Starch E	57.2 ± 1.6	68.7 ± 1.4	–	–	–	83.7 ± 1.4	96.4 ± 0.9	–
Cooked C	–	–	–	–	81.0 ± 0.8	89.2 ± 0.2	95.5 ± 1.1	2.1 ± 0.2
Cooked E	–	–	–	–	80.1 ± 0.4	89.0 ± 0.0	108.4 ± 4.8	2.2 ± 0.2

T_o = gelatinization onset temperature; T_p = gelatinization peak temperature; T_c = gelatinization conclusion temperature; ΔH = gelatinization enthalpy; PRS = starch from parboiled rice flour; Starch C = starch from raw pasta prepared by conventional extrusion; Starch E = starch from raw pasta prepared by extrusion-cooking; Cooked C = starch from cooked pasta prepared by conventional extrusion; Cooked E = starch from cooked pasta prepared by extrusion-cooking.

Table 2
Moisture content and luminosity (L^*) of starch samples, following equilibration at 0.33 a_w ($MgCl_2$), 0.75 a_w (NaCl), and 0.97 a_w (K_2SO_4).

Starch samples	Moisture content (%)			Luminosity (L^*)		
	0.33 a_w ($MgCl_2$)	0.75 a_w (NaCl)	0.97 a_w (K_2SO_4)	0.33 a_w ($MgCl_2$)	0.75 a_w (NaCl)	0.97 a_w (K_2SO_4)
PRS	9.7 ± 0.4	11.6 ± 0.2	18.2 ± 0.7	46.0 ± 1.3	11.5 ± 0.4	9.2 ± 0.3
Starch C	9.4 ± 0.3	10.6 ± 0.1	17.5 ± 1.2	43.5 ± 1.5	13.4 ± 0.2	12.0 ± 0.1
Starch E	9.3 ± 0.3	12.8 ± 0.1	20.0 ± 0.5	45.6 ± 3.2	14.6 ± 0.2	10.4 ± 0.1
Cooked starch C	10.3 ± 0.1	13.4 ± 0.2	19.2 ± 1.2	44.9 ± 1.0	16.3 ± 0.3	9.2 ± 0.1
Cooked starch E	9.8 ± 0.2	13.2 ± 0.3	18.3 ± 1.3	41.1 ± 1.4	19.9 ± 0.3	9.5 ± 0.2

PRS = starch from parboiled rice flour; Starch C = starch from raw pasta prepared by conventional extrusion; Starch E = starch from raw pasta prepared by extrusion-cooking; Cooked C = starch from cooked pasta prepared by conventional extrusion; Cooked E = starch from cooked pasta prepared by extrusion-cooking.

ditions. Starch from raw pasta (Starch C) and cooked pasta (Cooked C) processed using the conventional process had higher iodine absorption values compared to the respective starches processed using extrusion cooking (Starch E and Cooked E). In particular, the wavelength maxima was 420 nm for starch from pasta made using conventional extrusion, while 480 nm for Starch E, suggesting the chain length of the mobile polymers in starch from conventionally processed pasta was smaller. This is also reflected in the SEC data (Fig. 1) wherein Cooked E sample had a higher wavelength of absorption. The different chain mobility of samples is likely related to molecular changes that occurred during pasta-making process.

As expected, all starch samples exhibited higher absorption values at 0.97 a_w compared to when equilibrated to 0.75 a_w (Fig. 2). This is due to increased plasticization of polymers at the higher moisture contents allowing more polymers to complex with iodine. However, two interesting differences were observed in iodine absorption following equilibration at 0.97 a_w (Fig. 2b) when compared to samples equilibrated over NaCl. First of all, starch from PRS had iodine absorption values similar to starches from both cooked pasta; while starches from the raw pasta had lower absorption values. Secondly, starch from conventionally processed pasta had lower absorption values compared to starch from extrusion cooked pasta, a switch from what was observed at 0.75 a_w. Cooking the pasta appeared to increase the mobility and amount of polymers available to complex with iodine, likely due to starch gelatinization and the consequent rearrangement of the starch polymers. However, the differences in raw pasta starch are interesting, because it seems that Starch E has higher amount of mobile polymers compared to Starch C. the cooking in boiling water promoted further change in chain mobility, which overcome the differences among uncooked samples.

3.6. X-ray powder diffraction

The X-ray diffractograms of starch samples before and after iodine exposure at 0.75 and 0.97 a_w are shown in Fig. 3. Sample PRS showed a typical A-type diffraction pattern with peaks at 15°, 17°, 18°, and 23° 2θ, as reported by previous study (Marti et al., 2010). Pasta samples also exhibited similar peaks, suggesting that the pasta-making process and the extrusion condition did

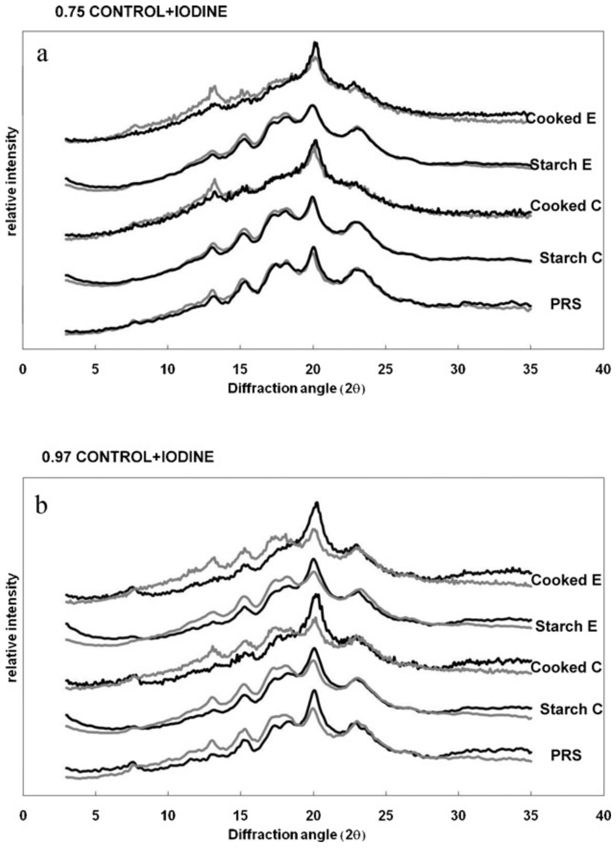

Fig. 3. X-ray powder diffraction spectra of starch samples following equilibration at 0.75 a_w (a) and 0.97 a_w (b). Light color represents the control starch, while dark color represents the iodine exposed starch. PRS = starch from parboiled rice flour; Starch C = starch from raw pasta prepared by conventional extrusion; Starch E = starch from raw pasta prepared by extrusion-cooking; Cooked C = starch from cooked pasta prepared by conventional extrusion; Cooked E = starch from cooked pasta prepared by extrusion-cooking.

not change the A-type organization of starch polymers. However, in starches from cooked pasta, the peaks at 17° and 18° 2θ disappeared; highlighting a loss in crystallinity as a consequence of starch gelatinization. At a_w value of 0.75, the effect of iodine exposure on crystalline order was evident only in cooked samples. In particular, Cooked E starch exhibited higher V-type peak at 20° 2θ compared to Cooked C sample. This is consistent with the high presence of longer chains complexing with iodine to form single helical complexes in Cooked E sample compared to Cooked C; as is evident in the SEC profile (Fig. 1).

Greater differences among the iodine exposed samples were observed at high a_w values (0.97) (Fig. 3b). Iodine did not change the polymorphic pattern of PRS and pasta samples. The same behavior has been previously observed in corn (Cheetham & Tao, 1998) and wheat starches (Waduge et al., 2010). However, an overall change in X-ray diffraction intensity after iodine exposure was observed for all starches, suggesting an alteration and disruption of crystallinity of the granules as a consequence of the formation of the iodine–starch complex. After iodination, starch samples exhibited a slight increase in intensity at 7° and 20° 2θ peaks – both related to single helical complexes; and a decrease in peak intensity at 13°, 15°, and 17–18° 2θ (Fig. 3b).

4. Conclusions

The molecular size distribution, the DSC profile, the iodine binding, and the X-ray diffraction analysis elucidated differences in starch organization/architecture in rice flour and pasta samples according to the extrusion conditions adopted for pasta-making. In particular, the extrusion-cooking promoted the formation of a starchy structure characterized by amylopectin fraction with higher wavelength of absorption, broad melting temperature, and high mobility and V-type peak at 20° 2θ of longer chains complexing with iodine.

During cooking two main phenomena occurred:

- starch gelatinization with loss of crystalline order and increase of both mobility and amount of polymers available to complex with iodine;
- starch polymers rearrangement resulting in high molecular size fractions, with high melting temperature.

Further studies are underway to investigate if the differences in starch structure among the pasta samples could influence starch digestibility and blood glucose response.

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