1 Title: Characterizing starch structure in a gluten-free pasta by using iodine vapor as a tool

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Abstract

The suitability of starch-iodine complex to highlight differences in chain mobility and crystallinity of starch in rice pasta was investigated. Two pasta samples were produced starting from the same rice flour (RF) and using a conventional extrusion process without (Process A) and with (Process B) a preliminary extrusion-cooking step. Based on K/S spectra (obtained after equilibration above K_2SO_4 and exposure to iodine vapor), Pasta A showed a behavior similar to RF. Process B exhibited a greater iodine binding capacity suggesting greater starch chain mobility. Moreover, the extrusion-cooking conditions seem to favor the loss of starch crystallinity and the formation of larger amorphous regions. The starch organization observed in Pasta B could account for its higher capacity to water absorption during cooking.

Keywords

Iodine-polymer complex; rice pasta; conventional extrusion; extrusion-cooking

List of Abbreviations: K/S, absorption/scattering coefficient; NRF, native rice flour; NRF-I, NRF after exposure to iodine vapor; Pasta A, pasta obtained from conventional extrusion process; Pasta A-I, Pasta A after exposure to iodine vapor; Pasta B, pasta obtained from extrusion-cooking process; Pasta B-I, Pasta B after exposure to iodine vapor; RF, parboiled rice flour; RF-I, RF after exposure to iodine vapor.

1. INTRODUCTION

The ability of iodine to complex with linear glucan polymers is commonly used to identify and characterize starches and amylose from various botanical sources [1-3]. The amylose-iodine binding capacity has also been used to investigate the effects of heating and cooling on starch dispersions and gels [4-5] and both swelling capacity and amylose leaching from granules [6]. All these studies had involved the formation of starch-iodine complex in solution. More recently, the ability of iodine to form complex with starch molecules in the native granular arrangement has been investigated, highlighting the opportunity to use of iodine as a tool to probe structural and architectural differences among different starch types by using K/S spectra: the higher the K/S values, the higher the color intensity and, consequently, the greater the mobility linear chains in the amorphous regions [7].

Nevertheless the study of the molecular organization of granular starch by iodine binding capacity has been exclusively carried out on pure starch, in the absence of other food components and potential biopolymer interactions (starch-lipid, starch-protein, etc.) resulting from food processing. Moreover, the extraction of starch from a food matrix is a time-consuming, labour-intensive, and highly expensive approach that could promote modifications of the interactions between macromolecules actually present in the original matrix.

In the present research note, attention was focused on the possibility to investigate starch arrangements associated with rice-pasta processing, directly in the pasta matrix. The changes in starch structure, according to two different extrusion conditions, were evaluated in terms of glucan polymer chain mobility and iodine binding capacity following exposure to iodine vapor at low moisture content and in presence of other macromolecules (proteins, lipids, non-starch polysaccharides). While there is some evidence that starch-protein interactions interfere with glucan polymer-iodine complex formation [8], it is our hypothesis that, under these specific conditions and observational tools, only changes to starch are relevant.

2. MATERIALS AND METHODS

2.1 Materials

Rice flours (RF) from milled parboiled rice (Thai cultivar of commercial origin) was used. After industrial parboiling, RF was produced by removing hull and bran layers and grinding the dehulled parboiled rice in order to produce flour with particle size < 250 μm. Ground native dehulled rice (NRF) was used as reference.

2.2 Pasta preparation

Pasta samples were produced in the pilot-plant (50 kg/h) located at DiSTAM, University of Milan. RF and water were blended to produce a mixture with a final water content of 40%. After mixing, two different extrusion conditions were carried out: conventional extrusion (Process A) and extrusion-cooking (Process B), according to Marti et al [9]. During the Process A, commonly used for durum wheat semolina pasta, the dough temperature was kept at 50 °C and the extrusion pressure at 10-11 MPa. The dough was formed in macaroni-shape and dried using a low-temperature drying cycle (50 °C for 14 hours) (Pasta A). A patented process [10] was used to produce Pasta B. The mixture of flour and water was cooked at 115 °C for 2 min with steam in a single screw extruder. After this first extrusion step, the heat-treated dough (extruded as small cylinder of 3 mm of diameter) was formed in macaroni shape in the continuous extruder of Process A. Drying was carried out with the same cycle of Pasta A. Both pasta samples were stored at 25 °C and a_w less than 0.5 until analyzed and, before the analysis, they were ground (particle size < 500 μm).

2.3 Sample preparation

NRF, RF and ground pasta samples (2 g) were equilibrated above K_2SO_4 (EDM Chemicals Inc., Gibbstown, NY) saturated solution, to final values of 0.97 a_w for four weeks, in order to facilitate the mobility of linear glucan chains and highlight the differences in starch chain arrangement [7]. To determine iodine binding, 0.2 g of the equilibrated sample were exposed to iodine vapor

generated from 2 g of iodine crystals (J.T. Baker, Phillipsburg, NJ) for 24 h at room temperature, keeping the a_w equal to 0.97.

2.4 Wide Angle X-ray Powder Diffraction

Wide angle X-ray diffraction measurements were carried out on NRF, RF, and both pasta samples (0.1 g) after equilibration at 0.97 a_w, 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° 2 θ per second. Data were smoothed using Jade 6.5 software and were normalized to equal total scattering in 3-35° 2θ range.

2.5 Colorimetric analyses of granular samples exposed to iodine vapor

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 a CM 3500-d Spectrophotometer (Konika Minolta, Mahwah, NJ, USA). For each product (rice flours and pasta samples) the corresponding unstained sample was considered as the target color.

3. RESULTS AND DISCUSSION

3.1 Effect of pasta making-process on crystalline order

The X-ray diffractograms of rice flour and pasta samples equilibrated above K_2SO_4 are shown in Figure 1. NRF showed a typical A-type diffraction pattern with strong reflections at 15°, 17°, 18°,and 23° 2θ. The parboiling process applied on native rice kernels in order to produce RF resulted in the formation of complexes between starch and native lipids [11], accounting for the A+V structure characterized by 7°, 13°, and 20° 2θ peaks. Both pasta samples maintained the diffraction pattern detected in RF with only some changes in peak intensities. In fact, regardless to processing conditions, the intensity of the peak at 20° 2θ increased, suggesting that the lipid-

amylose complex was strengthened during pasta processing. This phenomenon could be related to the high pressure and shear stress developed during extrusion. A similar behavior was observed after semolina was processed into pasta [12]. Moreover, the pasta-making process seemed to be responsible for the slight change in peak at 15° 2θ that appeared sharper in both pasta samples compared to RF.

The different extrusion conditions affected the crystalline packing of the products. After Process B a general decrease in crystallinity was observed, highlighted by the decrease of the peaks at 7°, 13°, and 20 °2θ and suggesting a low crystallinity related to single helices [13]. Moreover, the peaks at 10° and 11° 2θ disappeared in this sample. Only the peak at 18° 2θ increased in intensity after extrusion-cooking. Similar differences between pasta samples were observed also at low a_w (<0.50) [9], but equilibration above K_2SO_4 allowed to detect a decrease in 13° 2θ peak intensity. At the same time, the increase in intensity of 15°, 17°, 18°, and 23° 2θ peaks confirmed the influence of water activity on crystalline order. In fact, the more favourable water distribution promoted by K_2SO_4 equilibration affected starch granule crystallinity, inducing changes in the amorphous regions [14]. A similar phenomenon was observed in maize starch after hydration [15].

3.2 Effect of pasta making-process on iodine absorption intensity

Absorption spectra of samples after iodine exposure are shown in Figure 2. The parboiling process decreased iodine binding and the mobility of polymer chains with higher DP values. The changes in absorption values were also used to evaluate the effect of pasta-making process on the arrangement of the amorphous regions of the starch structure. RF-I and Pasta A-I did not show any significant differences in K/S spectra (Figure 2), highlighting that the conventional pasta-making process did not affect starch organisation in the amorphous regions, such that there are any differences in the absorption spectra. On the other hand, the extrusion-cooking with steam caused an increase in the absorption values; in Pasta B, the glucan polymer bound more iodine suggesting the presence of more mobile polymers, probably as a consequence of higher temperature and shear stress of Process

B. Thus, the iodine-binding technique underlines the role of amorphous regions in starch architecture of Pasta B, confirming the organization that was also evidenced in the X-rays diffractograms. In fact, as reported by Saibene et al [16], the starch-iodine complexation is more likely to occur in the less organized and more mobile amorphous regions, than the crystalline ones. The sharper diffraction peaks in Pasta A confirmed a lower amount of amorphous regions in starch in comparison with Pasta B. In addition, the higher mobility of polymer chains detected in Pasta B could account for the higher water absorption during the equilibration period (17.1% and 14.2%, for Pasta B and Pasta A, respectively), confirming the formation of a more hydrophilic structure, able to absorb a higher amount of water during cooking [9].

3.3 Effect of starch-iodine complex on crystalline order

The X-ray diffractograms of NRF, RF, and both pasta samples equilibrated at a_w of 0.97 after exposure to iodine vapor are shown in Figure 1. After iodination, the intensity of the main diffraction peaks (13°, 15°, 17°, and 18° 2θ) slightly decreased. This behavior has been also detected in corn and potato starch and related to the scattered radiation by the iodine atoms [16]. The intensity of 20° 2θ peak increased with the formation of the complex between iodine and polymer chains. Compared to the corresponding unstained sample, the general change in X-ray diffraction intensity after iodine exposure suggested the disruption of the granule crystallinity as a consequence of the formation of the iodine-starch complex [7]. Even after iodine exposure, the X-ray diffractograms showed a change in crystalline packing induced by Process B, highlighted by the presence of higher peaks at 7° and 20° 2θ. The decrease in sharpness at 23° 2θ confirmed the less ordered structure of Pasta B.

5. CONCLUSIONS

The results highlight the possibility to explore the effects of processing conditions on starch arrangements directly in the food matrix and without the extraction of starch from the sample. This is possible by equilibrating the samples above K_2SO_4 (0.97 a_w), before the exposure to iodine vapor;

a strategy that facilitates the glucan chain mobility to complex with iodine. Moreover, the present study suggests that the presence of components other than starch (such as proteins, lipids and non-starch polysaccharides) did not interfere with the formation of starch-iodine complex. The data also demonstrated that the processing conditions including treatments at high temperature (as extrusion-cooking) seem to promote the formation of amorphous regions responsible for a more hydrophilic structure and water absorption capacity during pasta cooking. Further studies are in progress to better understand the localization of amorphous regions inside the starch matrix and how they can control starch solubilisation during cooking and pasta texture.

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211 **Figure 1.** X-ray powder diffraction spectra of flour and pasta samples after equilibration above
212 K_2SO_4 (0.97 a_w). The graphs are offset for clarity.
213 NRF = native rice flour; NRF-I = NRF after exposure to iodine vapor; RF = parboiled rice flour;
214 RF-I = RF after exposure to iodine vapor; Pasta A = pasta made from conventional extrusion; Pasta
215 A-I = Pasta A after exposure to iodine vapor; Pasta B = pasta made from extrusion-cooking; pasta
216 B-I = Pasta B after exposure to iodine vapor.

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Figure 2. K/S value of samples exposed to iodine vapor following equilibration over K_2SO_4 saturated solution. NRF-I = NRF after exposure to iodine vapor; RF-I = RF after exposure to iodine vapor; Pasta A-I = Pasta A after exposure to iodine vapor; pasta B-I = Pasta B after exposure to iodine vapor.