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Influence of alkali concentration on the deproteinization and/or gelatinization of rice starch

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Abstract

Starch was extracted from BR-IRGA110 rice flour using solutions of NaOH at concentrations varying between 0.06% and 0.30% (w/v). The efficiency in protein removal was evaluated using intrinsic fluorescence. In parallel, the morphological changes in starch granules and their partial gelatinization were observed using scanning electron microscopy. The starch crystallinity was monitored by X-ray diffraction and the gelatinization properties were analyzed using differential scanning calorimetry. It was found that the granular organization was significantly altered when the alkaline extraction was conducted with NaOH solutions at concentrations higher than 0.24% (w/v).

Keywords: Starch; Alkaline extraction; Protein detection

1. Introduction

Starch occurs as semicrystalline granules mainly composed of two homopolymers of α-D-glucose, namely amylose and amylopectin. Amylose is a mostly linear polymer where α-D-glycosyl units are connected by α(1,4) glycosidic linkages. Amylopectin is a branched macromolecule containing α(1,4)-linked linear segments connected by α(1,6) branching points (Takeda, Tomooka, & Hizukuri, 1993). A wide range of starchy products are used as ingredients in processed foods (Miyazaki, Van Hung, Maeda, & Morita, 2006). Some of them have been developed to take up water and produce viscous fluids, pastes and gels with desired textural qualities (Singh, Kaur, & McCarthy, 2007).

In the processing of Brazilian rice, during husk and polishing, a significant amount of rice grains is broken (Elias, Dias, & Fagundes, 2005). This broken rice becomes a cheap product. It is abundant and can thus be used to produce starch. Due to its properties, rice starch can be used as a substitute of corn starch in food applications (Juliano, 1984).

The procedure to isolate starch from rice is different from that used to extract starch from corn, wheat and potato. This is due to differences in protein content and starch properties in each case. The rice grain contains four types of proteins present in the endosperm. They are tightly associated with the surface of the starch granule making their detection and removal difficult (Tanaka, Sugimoto, Ogawa, & Kasai, 1980). These proteins have been fractioned by selective solubility. The rice flour is first rinsed with water to remove albumin. Then, sequential treatments with dilute brine, dilute alkali and 70% ethanol solutions are performed to extract globulin, glutelin and prolamin, respectively (Agboola, Ng, & Mills, 2005).

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The alkaline method has commonly been used to extract starch (Chiou, Martin, & Fitzgerald, 2002; Lim, Lee, Shin, & Lim, 1999; Lumdubwong & Seib, 2000; Sodhi & Singh, 2003; Yang, Lai, & Lii, 1984), in spite of the fact that this treatment can alter the structure of starch granules (Cardoso, Samios, & Silveira, 2006; Chiou et al., 2002; Lim et al., 1999). Due to the variety of conditions (alkali concentration and contact time with rice flour), it is important to investigate the effect of alkali during the extraction of starch.

In a previous article, Cardoso et al. (2006) reported that repeated treatments with 0.25% (w/v) NaOH solutions induced variations in small-angle X-ray scattering (SAXS) spectra of treated starch. In the present work, we studied the influence of NaOH concentration on protein removal and degradation of starch granules during the alkaline extraction. On the one hand, we monitored the variation in protein content during the extraction procedure by measuring the intrinsic fluorescence of residual proteins. On the other hand, the morphology of starch granules was observed using scanning electron microscopy (SEM) and structural changes were followed by wide-angle X-ray scattering (WAXS) and differential scanning calorimetry (DSC).

2. Materials and methods

2.1. Materials

Industrial *indica* rice (BR-IRGA 410) of Brazilian origin was supplied by "Cooperativa Arrozeira Extremo Sul" (Pelotas, Brazil). It contained 8% protein, 32% amylose, 58% amylopectin and 2% other components. The rice was milled to achieve a granulometry between 0.10 and 0.42 mm. Milli-Q water was used in the chemical tests. All reagents were purchased from Merck.

2.2. Starch isolation

Starch was isolated by alkaline extraction of the proteins as described by Sodhi and Singh (2003). Six NaOH concentrations were tested: 0.06%, 0.12%, 0.15%, 0.18%, 0.24% and 0.30% (w/v) for treatments 1–6, respectively. For all treatments, in order to soften the endosperm, 1.8 g of milled rice was steeped in 18 mL of NaOH and allowed to settle for 24 h, at 20 °C. The supernatant liquor (12 mL) was discarded and the remaining slurry was diluted to the original volume (18 mL) with NaOH. The mixture was shaken for 10 min and centrifuged at 1250 rpm for 5 min in an ALC model PK 120 analytical centrifuge. Then, the supernatants and slurry parts were once more separated. This procedure was repeated eight times and eight supernatants were produced during each treatment. The slurry starch was suspended in distilled water, passed through a 0.125 mm nylon cloth and centrifuged at 2000 rpm for 5 min. The sample was washed until no pink color in the presence of phenolphthalein could be observed.

2.3. Fluorescence spectroscopy

Fluorescence spectroscopy was used in order to detect proteins in the supernatant fractions. The analyses were performed in a Hitachi F-4500 spectrofluorometer operating in the 300–600 nm range and using an excitation wavelength of 350 nm. The different supernatants obtained during starch isolation were analyzed without any dilution using a quartz optical cell. A multi-peak fit was performed in order to determine the area of emission spectra.

2.4. Scanning electron microscopy

Drops of granule suspensions were allowed to dry onto copper stubs. The specimens were coated with Au/Pd and observed in secondary electron imaging mode with a JEOL JSM-6100 microscope operating at 8 kV.

2.5. Wide-angle X-ray diffraction

WAXS data were collected at room temperature using a diffractometer powered by a Philips PW3830 generator providing a Ni-filtered CuKα radiation ($\lambda = 1.542$ Å) and operating at 30 kV and 20 mA. X-ray diffraction patterns were recorded after equilibration of the water content in the washed starch at 90% R.H. (relative humidity) for 9 days. The hydrated powders were sealed in borosilicate capillaries in order to prevent any significant change in water content during measurement. Diffraction patterns were recorded during 2 h exposures on Fujifilm imaging plates and read using a Fujifilm BAS 1800 II Phospo-imager. Calibration was achieved using calcite powder. Diffraction spectra were obtained by radial averaging of the powder patterns and normalized to the same total integrated area between $2\theta = 3°$ and 35°.

2.6. Differential scanning calorimetry

DSC measurements were performed with a Perkin–Elmer DSC-4 calorimeter using heating scan rates of 5 °C/min from 25 to 100 °C. Before analysis, the water content in the specimens was adjusted by water desorption at 90% R.H. for 9 days. The calibration was performed using indium and an empty aluminum pan was used as reference. The transition temperatures and enthalpies were determined automatically by the DSC equipment.

3. Results and discussion

3.1. Protein removal

Fig. 1a shows the fluorescence spectra of the supernatants obtained during treatment 3. All spectra have the same general appearance although the maximal intensity and peak area vary depending on the supernatant number. The first peak is located at 397 nm for all supernatants and it gets more clearly defined for supernatant 3 and onward.

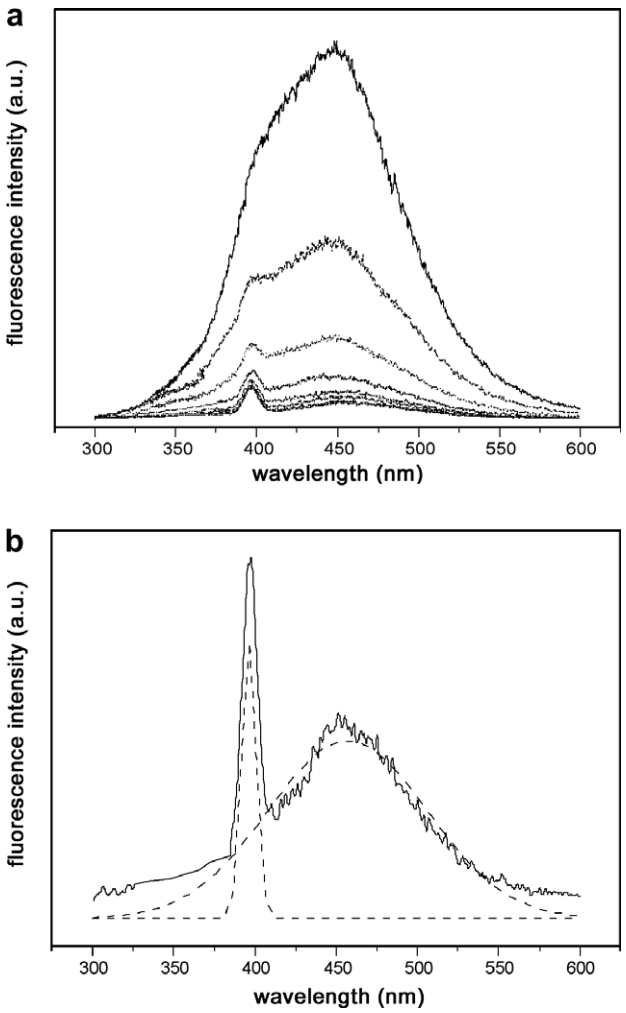

Fig. 1. (a) Fluorescence spectra of the supernatants obtained after treatment 3, from supernatant 1 (top) to supernatant 8 (bottom); (b) multi-peak fit (dashed lines) of the fluorescence emission spectrum of supernatant 8 (solid line).

The second peak is located at 450 nm for supernatants 1 to 4 whereas it is shifted to approximately 455 nm for supernatant 5 and onward. Fluorescence quenching was not observed during the procedure. Fig. 1b presents the fluorescence emission (solid line) and a multi-peak fit (dashed lines) for supernatant 8. The good agreement between the experimental emission and the fitted peaks validates this procedure to reliably define the area of fluorescence emission.

The calculated areas of the fluorescence emission from different supernatants are presented in Table 1. The alkaline extraction during the same treatment led to a decrease in peak area due to the reduction in protein content during the treatment. Hence, the sum of the peak areas in each treatment is related to the total amount of proteins removed during the procedure. In treatments 1 and 2, the total amount of proteins extracted was smaller when compared with the other ones. In treatments 3, 4, 5 and 6, no significant difference could be observed, indicating that, quantitatively, the protein removal was practically the same.

Supernatants 7 and 8 obtained in all treatments presented a positive and constant area. The positive area in supernatant analyses indicated the presence of proteins even after eight washing steps with different NaOH concentrations. This result is in agreement with the literature that reports that the starch obtained by alkaline extraction presents a small amount of residual proteins (Cardoso, Samios, Silveira, Rodembusch, & Stefani, 2007; Lumdubwong & Seib, 2000; Yang et al., 1984).

The peak area in the beginning of each treatment is probably related to the contributions of all proteins. As the treatment proceeds, the proteins are extracted by the alkaline solution and the fluorescence area is reduced. In the last two extractions (supernatants 7 and 8), the peaks are constant. The high solubility of albumin, globulin and glutelin in NaOH solutions suggests that these proteins were totally extracted in supernatant 6 and the presence of proteins in supernatant 7 and 8 can be assigned to prolamins (Cardoso et al., 2007). Moreover, the fluorescence emitted by supernatants 7 and 8 coincides with that of prolamin measured in alkaline medium using an excitation wavelength of 350 nm (data not shown). The presence of proteins at this stage is directly related to the poor solubility of prolamin in NaOH solutions.

3.2. Morphological and structural modifications of starch granules

Fig. 2 shows SEM micrographs of six alkali-treated rice starches. In the picture corresponding to treatment 1 (Fig. 2a), polyhedral starch granules are still connected to each other by proteins, making the observation of isolated granules difficult. Moreover, small protein particles, which have not been degraded by the alkaline treatment and that are also observed in native rice flour, can be seen. After treatment 2 (Fig. 2b), no protein particle was observed. However, starch granules are still generally connected. The presence of residual proteins was confirmed by fluorescence results. The images of the residues obtained after treatments 3 (Fig. 2c) and 4 (Fig. 2d) show more individualized granules. Their morphology and size (between 3 and 8 μm) are in agreement with the literature data (Dang & Copeland, 2003; Qi, Tester, Snape, & Ansell, 2003; Sodhi & Singh, 2003; Tester & Morrison, 1990; Wang & Wang, 2004). After treatment 5 (Fig. 2e), isolated granules were rarely observed and the sample exhibited a gel-like aspect. The formation of a continuous film was even clearer after treatment 6 (Fig. 2f). Thus, it appears that treatments with NaOH concentrations higher than 0.24% (w/v) result in a progressive loss of granular morphology, likely due to an alkaline gelatinization phenomenon (Cardoso et al., 2006).

The WAXS spectra of starch slurries obtained using different concentrations of NaOH are presented in Fig. 3. The profile shown in Fig. 3a was the same for the residues obtained after treatments 1, 2, 3 and 4. It corresponds to the diffraction pattern of A-starch, with reflections at $2\theta = 15.3°$, 19.7° and 23.4°, and a doublet at 17.0° and

Table 1
Peak area of the fluorescence emission spectra of the supernatants for different treatments, calculated by multi-peak fitting

Supernatant fraction	Supernatant area $\times 10^{-3}$ (a.u.)					
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
1	21.7	31.4	33.4	33.9	33.2	33.4
2	12.9	15.6	15.7	15.6	16.4	16.5
3	5.0	6.9	7.6	7.0	7.0	6.9
4	2.7	2.8	3.2	3.3	3.2	3.3
5	1.7	1.9	2.3	2.3	2.1	2.2
6	1.1	1.2	1.3	1.3	1.4	1.3
7	0.9	1.0	1.2	1.2	1.2	1.2
8	0.9	1.0	1.2	1.2	1.2	1.2
Total area	47.2	62.2	66.3	66.1	66.1	66.3

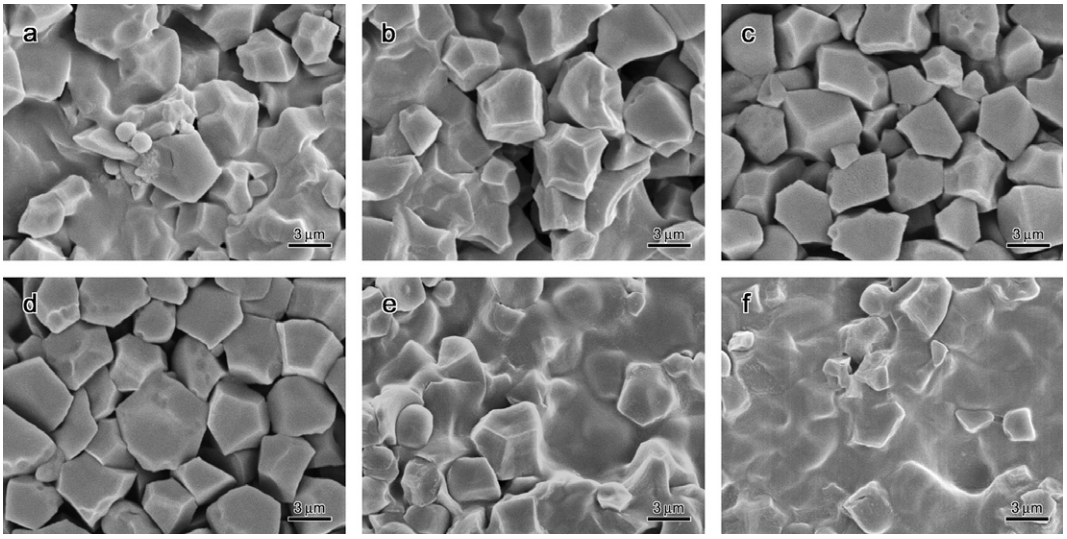

Fig. 2. Scanning electron microscopy images of starches obtained after different alkaline treatments. Images (a–f) correspond to treatments 1–6, respectively.

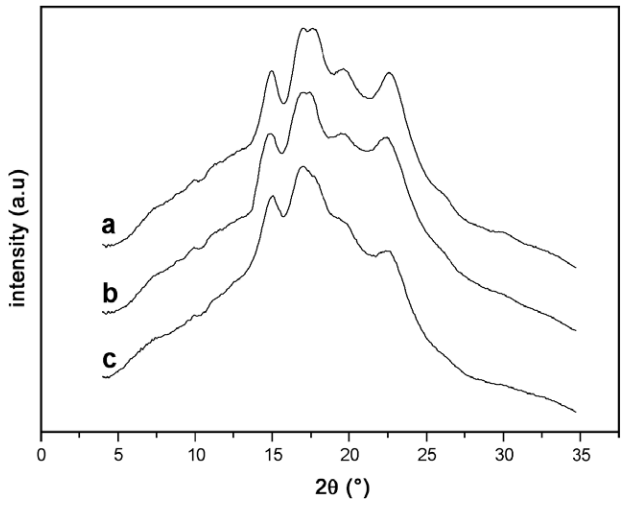

Fig. 3. X-ray diffraction profiles from starch slurries: (a) same profile for treatments 1–4; (b) treatment 5 and (c) treatment 6.

18.0° (Buléon, Colonna, Planchot, & Ball, 1998). However, the diffraction spectra of starches obtained after treatments 5 and 6 were different. After treatment 5 (Fig. 3b), the intensity of all peaks slightly decreased, although the A-starch pattern could still be recognized. The effect of alkali was even more evident after treatment 6 (Fig. 3c). The diffraction intensities decreased further and a single peak was now observed at 17.0–18.0°.

Sarko and Biloski (1980) reported the formation of a crystalline complex between starch and KOH which resulted in changes in the X-ray diffraction patterns. However, we did not observe any evidence of complex formation between starch and NaOH.

The intensity changes during treatments 5 and 6 are a direct consequence of the higher concentration of the alkaline solution. The swelling of the starch granules was drastically enhanced for NaOH concentrations higher than 0.20 mg/mL (Cardoso et al., 2006; Singh et al., 2007). Starch granules consist of alternating amorphous and crystalline regions, and gelatinization initially occurs in the amorphous regions. It was proposed that gelatinization was primarily driven by the swelling of the amorphous regions during water uptake (Donovan, 1979; French, 1984; Marchant & Blanshard, 1978). Thus, the changes in WAXS spectra for treatments 5 and 6 can be directly related to the swelling of starch and subsequent loss of crystallinity.

The evolution of WAXS spectra is particularly well correlated with the SEM observations. The decrease of crystallinity detected for treatments 5 and 6 is associated with the loss of the granular aspect of starch. However, the decrease of crystallinity deduced from the WAXS profiles (Fig. 3b and c) is not as high as would be expected from such a drastic change of morphology (Fig. 2e and f).

The modifications of starch properties were investigated using DSC. For all samples, a single endothermic transition was observed (Fig. 4). It corresponds to the phase separation of amylose and amylopectin within the starch granules, the amylose leaching out of the granules (Fujita, Lida, & Fujiyama, 1992; Liu, Lelièvre, & Ayoung, 1991). The characteristic temperatures and enthalpies deduced from the thermograms are given in Table 2. During gelatinization, all samples presented similar onset (T_{onset}) and final temperatures (T_{end}). T_{onset} and T_{end} varied between 51.6–53.1 and 66.2–68.4 °C, respectively. No significant difference in maximal peak temperature (T_{peak}) was found for the six treatments.

The enthalpy of gelatinization ΔH is an indicator of the loss of molecular order within the granule that occurs upon gelatinization (Cooke & Gidley, 1992; Hoover & Vasanthan, 1994; Tester & Morrison, 1990). ΔH has been reported to be influenced by the degree of crystallinity of starch (Thirathumthavorn & Charoenrein, 2006). In addition, it has been shown that starch granule size and shape, phosphorus content, amylopectin chain length and stability and/or size of crystalline regions also influenced the thermal properties of starch (Noda, Takahata, Sato, Ikoma, & Mochinda, 1996; Singh & Singh, 2001; Singh, Kaur, & Singh, 2004; Stevens & Elton, 1971; Wang, Gao, Chen, & Xiao, 2005; Yuan, Thompson, & Boyer, 1993). After treatments 5 and 6, we observed a gradual decrease of enthalpy that cannot be related to the presence of different amounts of water in the samples, since they were equilibrated in a controlled atmosphere before analysis. This variation should be attributed to the internal modification of starch granules due to the action of NaOH during the extraction process. The rheological properties and gelatinization behavior of starch treated with concentrated NaOH solutions are changed, due to the dissociation of intermolecular hydrogen bonding in the amylopectin (Tako & Hizukuri, 2002; Yamamoto, Makita, Oki, & Otani, 2006). The results of Donald, Kato, Perry, and Waigh (2001) suggested that amylose diffuses into the amylopectin crystalline lamellae and disturbs its lamellar organization. The alkaline solution probably reduces the rigidity as well as the stability of the molecular organization of starch. Therefore, the mobility of the amylose chains is increased, which contributes to the loss of granule architecture.

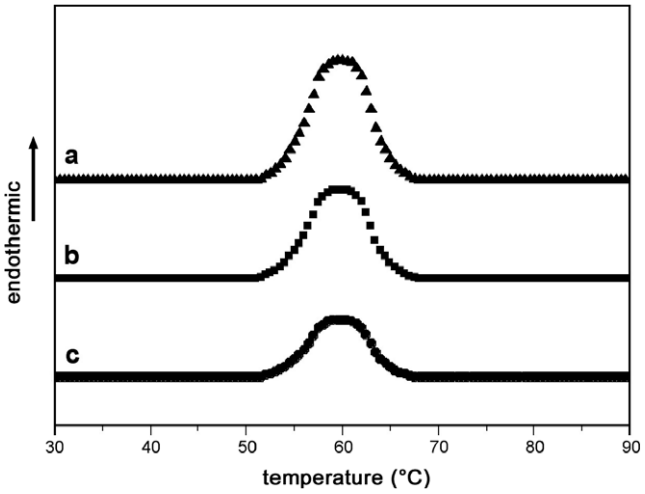

Fig. 4. Differential scanning calorimetry profiles of starch slurries: (a) same profile for treatments 1–4; (b) treatment 5 and (c) treatment 6.

Table 2
Parameters of the thermograms recorded by DSC for the starches extracted with different NaOH concentrations

Treatment	T_{onset} (°C)	T_{end} (°C)	T_{peak} (°C)	ΔH (cal/g)
1	51.6	68.0	58.9	1.2
2	52.1	66.2	59.1	1.1
3	53.1	67.1	59.6	1.2
4	52.9	68.2	59.2	1.1
5	52.2	66.3	58.6	0.8
6	52.4	68.4	59.4	0.6

T_{onset} and T_{end} are the initial and final temperatures of the endothermic transition. T_{peak} is the temperature at peak maximum and ΔH is the enthalpy of gelatinization. The values were obtained by triplicate average.

4. Conclusion

We studied the effect of NaOH concentration on the morphology and structure of Brazilian rice starch granules during the extraction of proteins. The intrinsic fluorescence was successfully used to detect small amounts of residual proteins in the supernatant of alkali-treated starch. Fluorescence results indicated that the proteins were not efficiently removed with NaOH concentrations lower than 0.15% (w/v). SEM images as well as fluorescence, WAXS and DSC data concurred to show that the alkaline extraction was optimal using NaOH concentrations between 0.15% and 0.18% (w/v). With NaOH concentrations higher than 0.24% (w/v), alkaline swelling of the granules occurred resulting in a significant disruption of the granular morphology associated with a decrease of crystallinity and gelatinization enthalpy.

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