

## Effect of enzymatic hydrolysis on some physicochemical properties of root and tuber granular starches

*Efeito da hidrólise enzimática sobre algumas propriedades físico-químicas de amidos de raízes e tubérculos*

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### Abstract

Enzymatic hydrolysis of granular starch is an important tool to provide information about granule structure. Cassava, sweet potato, Peruvian carrot, and potato starches were hydrolyzed by bacterial  $\alpha$ -amylase at 37 °C for 48 hours, and the physicochemical properties of the residues from hydrolysis were determined. Cassava starch was the most susceptible to enzyme displaying 20.9% of hydrolysis, whereas potato starch was the most resistant with 5.9%. The granule average size varied from 10.8 to 23.4  $\mu\text{m}$  for Peruvian carrot and potato starches, respectively. With the use of SEM, a smooth granule surface was observed for all native starches. Cassava and sweet potato starches displayed an A-type X-ray diffraction pattern, while Peruvian carrot and potato starches showed a B-type pattern. After hydrolysis, cassava, sweet potato, and Peruvian carrot starches showed some well degraded granules, whereas potato starch presented a slight sign of degradation. The amylose content of the starches decreased with hydrolysis for cassava, sweet potato, and Peruvian carrot starches and was kept unchanged for the potato starch. As expected, intrinsic viscosity and pasting properties decreased for all hydrolyzed starches. There is no difference between thermal properties of native and hydrolyzed starches. These results suggested that hydrolysis occurred in amorphous and crystalline areas of the granules. The B type diffraction pattern in conjunction with the big granule size of the potato starch may have contributed to the greatest resistance of this starch to hydrolysis.

**Keywords:**  $\alpha$ -amylase; granular starch; structure.

### Resumo

A hidrólise enzimática do amido pode fornecer informações importantes sobre sua estrutura granular. Amidos de mandioca, batata-doce, mandioquinha-salsa e batata foram hidrolisados por  $\alpha$ -amilase bacteriana a 37 °C durante 48 horas, e algumas propriedades físico-químicas dos resíduos da hidrólise foram determinadas. O amido de mandioca foi o mais suscetível à enzima com 20,9% de hidrólise, enquanto o amido de batata foi o mais resistente com 5,9%. O tamanho médio dos grânulos variou de 10,8 a 23,4  $\mu\text{m}$  para os amidos de mandioquinha-salsa e batata, respectivamente. Amidos de mandioca e batata-doce apresentaram um padrão de difração de raio-X tipo A, enquanto os amidos de mandioquinha-salsa e batata mostraram padrão tipo B. Todos os amidos nativos mostraram superfície granular lisa e, após hidrólise, os amidos de mandioca, batata-doce e mandioquinha-salsa mostraram alguns grânulos bastante degradados, enquanto o amido de batata apresentou sutil sinal de degradação. O teor de amilose dos amidos diminuiu com a hidrólise para os amidos de mandioca, batata-doce e mandioquinha-salsa, permanecendo inalterado para o amido de batata. Como esperado, a viscosidade intrínseca e as propriedades de pasta diminuíram para todos os amidos hidrolisados. Não houve diferença significativa entre as propriedades térmicas dos amidos nativos e hidrolisados. Estes resultados sugeriram que a hidrólise ocorreu nas áreas cristalinas e amorfas dos grânulos. O padrão de difração do tipo B e o grande tamanho dos grânulos do amido de batata podem ter contribuído para a maior resistência deste amido à hidrólise.

**Palavras-chave:**  $\alpha$ -amilase; amido granular; estrutura.

## 1 Introduction

Enzymatic hydrolysis of granular starch has been used as an effective tool to better understand the starch granule structure.

Starch granule is organized in amorphous areas and in regions of higher and lower crystallinity, and the transition between them is gradual. The crystalline area is constituted of linear fractions of amylopectin, whereas branch points and amylose are the main components of amorphous areas (OATES, 1997; JANE, 2006).

Differences in the enzymatic susceptibilities of starches have been attributed to the interactions of many factors

such as starch source, granule size, extension of association between starch components, rate of amylose and amylopectin, crystallinity, polymorphic type (A, B, C), amylose-lipid complex, type of enzyme, and hydrolysis conditions (concentration, pH, temperature) (OATES, 1997; COLONNA; BULÉON; LEMANE, 1988; HOOVER; ZHOU, 2003; LI et al., 2004; TESTER; QI; KARKALAS, 2006).

Starches that naturally present a porous surface, such as corn starch, are degraded easier than those with a smooth surface such as cassava starch (FRANCO; CIACCO; TAVARES, 1988, FRANCO; CIACCO, 1992). Granules with lower diameter are more susceptible to enzymes than those of higher

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diameter (FRANCO; CIACCO, 1992; FRANCO; CIACCO; TAVARES, 1998; YONEMOTO; CALORI-DOMINGUES; FRANCO, 2007) due to their higher surface area (TESTER; QI; KARKALAS, 2006). Franco and Ciacco (1992) observed that the enzymatic attack on the large granules of cassava and corn starches ( $> 16 \mu\text{m}$ ) was characterized by considerable corrosion of granule surface, mainly in the radial direction. For small granules, the enzymatic action was characterized, mainly by surface erosion with partial or total solubilisation of the granules.

Starches with different polymorphic patterns (A, B, or C) have shown different susceptibilities to enzymatic hydrolysis. Hoover and Zhou (2003) reported that legume granular starches (C-type) are more susceptible to pancreatic  $\alpha$ -amylase than granular starches of potato or corn with high amylose (B-type), but less susceptible than cereal or cassava granular starches (A-type).

During enzymatic hydrolysis, some regions of granules are more attacked than others. Those more susceptible areas are the less organized amorphous rings, whereas the crystalline lamella offers higher resistance to enzymatic erosion (OATES, 1997). However, Colonna, Buléon and Lemane (1988) suggested that  $\alpha$ -amylase can simultaneously attack the crystalline and amorphous areas of starch granule, whereas Lauro et al. (1999) reported that at the initial stage of hydrolysis of barley starch, both amorphous and crystalline areas were equally attacked by  $\alpha$ -amylase. Nevertheless, the crystallinity and gelatinization enthalpy decreased after a long period of hydrolysis suggesting that an extensive hydrolysis effectively destroys and dissolves the crystalline areas of the granule (HOOVER; ZHOU, 2003).

The enzymatic hydrolysis of granular starch also causes significant changes in the functional properties of starches such as gelatinization temperatures, gel formation, and paste viscosity which are very important to define the industrial uses of starches. Starches hydrolyzed by enzymes have their molecular weight reduced and show reduction of paste viscosities and swelling power (NODA et al, 2004).

Commercial starches are obtained from seeds (corn, waxy corn, high amylose corn, wheat, and rice) and from tubers and roots, particularly potato, sweet potato, and cassava (WHISTLER; BEMILLER, 1997). Other starches such as that of Peruvian carrot have less commercial applications, and publications about this starch are fewer in relation to those of corn starch. Although extensive studies have been carried out in recent years, the available information on root and tuber starch granule structure and the action of amylases on these starches still deserves attention.

The purpose of this work was to investigate the enzymatic susceptibility of different root and tuber starches (cassava, sweet potato, Peruvian carrot, and potato starches) to bacterial  $\alpha$ -amylase action and to evaluate some physicochemical characteristics of hydrolyzed residues to obtain more information about starch structure and functionality.

## 2 Materials and methods

### 2.1 Material

Cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), Peruvian carrot (*Arracacia xanthorrhiza*), and potato (*Solanum tuberosum*) roots and tubers were cultivated in São José do Rio Preto - SP, Brazil. Bacterial  $\alpha$ -amylase (A6380), with declared activity of 2150 units.mg<sup>-1</sup> solid was obtained from Sigma Chemical CO., USA.

### 2.2 Isolation of starches

Starches were isolated as described by Peroni, Rocha and Franco (2006).

### 2.3 Chemical composition

The starches were analyzed for moisture, ash, protein, and lipid contents according to the American Association of Cereal Chemists, (AMERICAN ASSOCIATION OF CEREAL CHEMISTS, 2000).

### 2.4 Granule size distribution

The granule size distribution of the different starches was performed using an image analyzer system "Image-pro-plus" (Media Cybernetics) attached to a light microscope as described by Peroni, Rocha and Franco (2006). The parameters evaluated were shape and the largest diameter ( $\mu\text{m}$ ). Three slides for each sample were prepared and a hundred granules were randomly chosen and measured per slide.

### 2.5 Crystallinity of the starches

The pattern of crystallinity of starches was determined using X-rays in a RINT2000 Wide Angle Goniometer unit with copper radiation, line K,  $L=1,542 \text{ \AA}$ . The scanning speed was  $1^\circ \text{ min}^{-1}$  at 50 kV and 100 mA.

### 2.6 Enzymatic hydrolysis

The starches were hydrolyzed with bacterial  $\alpha$ -amylase, in duplicate, according to Franco and Ciacco (1992) with modifications. The starches (15%, w/v) were dispersed in phosphate buffer (0.2 M pH 6.0) with 3 mL of bacterial  $\alpha$ -amylase solution (0.2% w/v). An aliquot of 1 mL of sodium azide solution (10% w/v) was added to avoid microbial growth. The starch dispersions were incubated for 48 hours at 37 °C in an orbital shaker. After solids decant, aliquots of the supernatant were removed at 6, 9, 24, 30, and 48 hours, and the amount of reducing sugars was determined (SOMOGYI, 1945).

After 48 hours of incubation, the enzyme in the dispersion was inactivated by adding HCl (0.1 N) to reach pH 3.0 followed by stirring for 15 minutes, neutralization with NaOH (0.1 N), and centrifugation at 2100 g for 20 minutes. The hydrolysis residues were washed with distilled water and ethanol, recovered by filtration, and dried in an air circulation oven at 40 °C. The

hydrolysis percentage was calculated in relation to the dry basis (db) starch weight by Equation 1:

$$\text{Percentage of hydrolysis} = \frac{100 \times \left[ \frac{\text{weight of starch (g, db)} - \text{weight of hydrolyzed starch (g, db)}}{\text{weight of starch (g, db)}} \right]}{\text{weight of starch (g, db)}} \quad (1)$$

### 2.7 Scanning Electron Microscopy (SEM)

An aliquot of sample, previously dehydrated with ethanol was placed on a metal plate, previously covered with a carbon double sided adhesive tape, submitted to a 20 nm gold layer application, and observed with a scanning electron microscope, model DSM 960 ZEISS – “Digital Scanning Microscope”.

### 2.8 Amylose content

The starch samples were defatted by dispersing starch in 90% DMSO solution which was boiled and stirred for one hour (FRANCO et al., 2002). The iodine affinities of defatted whole starch were determined according to Kasemsuwan et al. (1995) using a potentiometric autotitrator (716SM Titrino, Brinkmann Instrument, Westbury, NY). The amylose contents were calculated dividing the iodine affinity of starch by 20.0% (TAKEDA; HIZUKURI, 1987).

### 2.9 Intrinsic viscosity

The starch solutions were prepared as proposed by Lansky, Kooi and Schoch (1949), and the intrinsic viscosity was analyzed according to Leach (1963). A capillary viscosimeter (Cannon-Fenske, number 50, Fisher Scientific, USA) was used and maintained at 35 °C by using a thermostated bath.

### 2.10 Thermal properties

The gelatinization properties of starch samples were determined by using a differential scanning calorimeter (DSC-Pyris 1, Perkin Elmer, Norwalk, CT) (FRANCO et al, 2002 ). The heating rate used was 5 °C min<sup>-1</sup>.

### 2.11 Pasting properties

The pasting properties of starches were obtained using a Rapid Visco Analyzer (RVA-4, Newport Scientific, Australia) according to Franco et al., (2002).

### 2.12 Statistical analysis

All samples were analyzed in duplicate or in triplicate. The statistical analysis was performed using the data analysis tools of Statistics for Windows (v. 5.0, Statsoft, Tulsa, OK). The analysis of variance was conducted using Tukey’s studentized range test at the 5% of significance.

## 3 Results and discussion

### 3.1 Chemical composition

As expected for root and tuber starches, all samples displayed low contents of ash, protein, and lipid (< 0.5%, Table 1) indicating that the starches were pure enough to be used.

### 3.2 Enzymatic hydrolysis

The cassava was the most susceptible to hydrolysis followed by sweet potato and Peruvian carrot starches (Figure 1), which presented similar behavior during hydrolysis, mainly after 15 hours of treatment. The potato starch was the most resistant to enzyme action and it practically did not suffer degradation under the conditions of this experiment. The percentage of hydrolysis, calculated according to Equation 1 described in the material and methods section of this work, was of 20.9% for the cassava starch, whereas the potato presented 5.9% of hydrolysis (Table 2). Sweet potato and Peruvian carrot starches showed hydrolysis percentages of 15.5, and 13.2%, respectively. According to Rickard, Asaoka and Blanshard (1991), for starches from non-cereal sources, the cassava starch is the most susceptible to  $\alpha$ -amylase, and the potato starch, the most resistant. It is well known that cassava and sweet potato starches display an A-type X-ray diffraction pattern, whereas potato starch shows B-type pattern (COTTRELL et al., 1995; JANE et al., 1999; MCPHERSON; JANE, 1999; HOOVER, 2001; SRICHUWONG et al., 2005). We also found

**Table 1.** Chemical composition of granular starches\*.

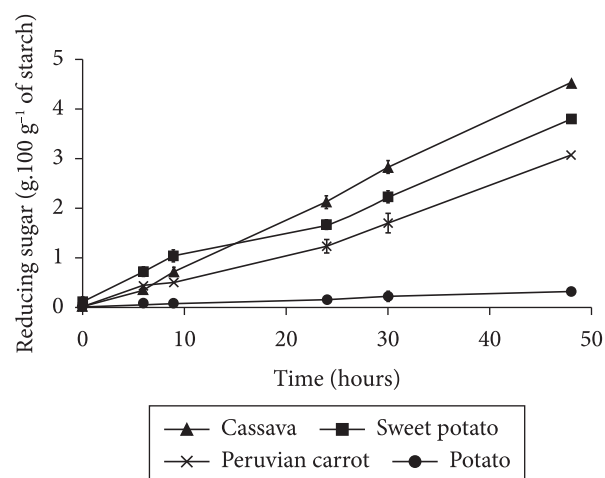
Starch	Ash	Protein	Lipid
Cassava	0.19 ± 0.01	0.18 ± 0.03	0.12 ± 0.03
Sweet potato	0.21 ± 0.01	0.14 ± 0.03	0.14 ± 0.01
Peruvian carrot	0.18 ± 0.03	0.12 ± 0.01	0.13 ± 0.03
Potato	0.15 ± 0.02	0.12 ± 0.01	0.10 ± 0.02

\* Results expressed in dry basis; mean values of three replicates per sample.

**Table 2.** Percentage of hydrolysis of starches submitted to  $\alpha$ -amylase action for 48 hours.

Starch	% Hydrolysis *
Cassava	20.9 <sup>a</sup>
Sweet potato	15.5 <sup>b</sup>
Peruvian carrot	13.2 <sup>c</sup>
Potato	5.9 <sup>d</sup>

\*Percentage of hydrolysis = 100 × [weight of starch (g) – weight of hydrolyzed starch (g)/weight of starch (g)]; and values followed by the same letter are not significantly different ( $P < 0.05$ ).

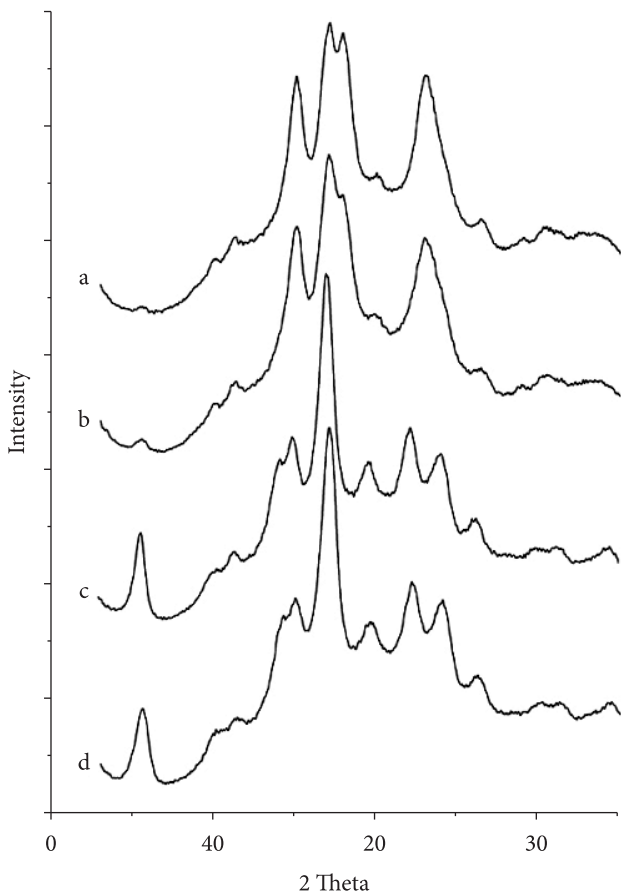


**Figure 1.** Amount of reducing sugar produced during the hydrolysis of different starches with bacterial  $\alpha$ -amylase, as a function of time.

an A-type X-ray diffraction pattern for cassava and sweet potato starches (Figure 2 a, b) while Peruvian carrot and potato starches showed a B-type pattern (Figure 2 c, d). In a previous study, Rocha, Demiate and Franco (2008) reported a B-type pattern for Peruvian carrot starch which was also reported by Santacruz et al. (2003). Starches with A-type polymorphism are more susceptible to amylases action than starches with B-type polymorphism (JANE, 2006; HOOVER; ZHOU, 2003; GÉRARD et al., 2001). In the A-type starches, A and B<sub>1</sub> short chains in the crystalline structure are less stable and more susceptible to rearrangements, whereas B-type starches present higher proportions of long B chains, which extend for two or more clusters and stabilize the internal structure of granules becoming more resistant to enzymatic action (JANE, 2006).

### 3.3 Shape and size distribution of granules

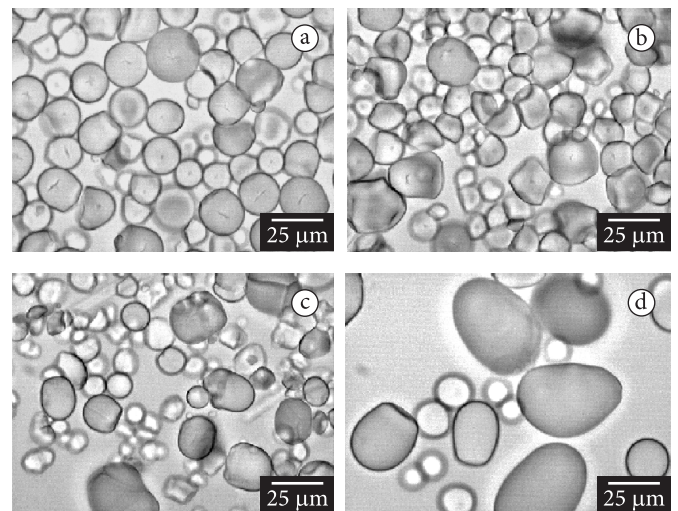
Starch granules from different sources showed distinct shape and size. Cassava and Peruvian carrot starch granules displayed round shapes, whereas the sweet potato starch showed granules with round and polygonal shapes, and potato starch granules were elliptical (Figure 3). Cassava and sweet potato starches displayed granules with similar average size (15.6



**Figure 2.** X-ray diffraction pattern of native starches: a) cassava; b) sweet potato; c) Peruvian carrot; and d) potato.

and 13.4  $\mu\text{m}$ , respectively) presenting more than 70% of the granules with diameters between 10.1 and 20.0  $\mu\text{m}$ , agreeing with Peroni, Rocha and Franco (2006)'s findings. A lower granule average diameter for the Peruvian carrot (10.8  $\mu\text{m}$ ) was observed, whereas the potato starch displayed an average diameter of 23.4  $\mu\text{m}$  and proximately 50% of the granules with diameter higher than 20.0  $\mu\text{m}$  (Table 3). It is possible that, among the B-type diffraction pattern presented by the potato starch (Figure 2d), its highest granule size has also contributed for its highest resistance to hydrolysis. The enzymatic digestion pattern of starch granules of a smaller size differs from those of a larger size (LINDEBOOM; CHANG; TYLER, 2004), and the small ones are hydrolyzed faster than the large ones (OATES, 1997; FRANCO; CIACCO, 1992; YONEMOTO; CALORIDOMINGUES; FRANCO, 2007). According to Tester, Qi and Karkalas (2006), the enzymatic hydrolysis of granular starch involves enzymes in solution acting on a solid substrate and then, the surface area accessible to enzymes becomes an important kinetic parameter.

A smooth granule surface of all native starches was observed using scanning electron microscopy, (Figure 4). After hydrolysis, cassava, sweet potato and Peruvian carrot starches showed some granules degraded on their external part (Figures 4b, d, and f), indicating that hydrolysis occurred by exocorrosion, and it was not uniform for all granules of each starch, in which some regions were much more susceptible to enzymes than others, probably due to the structural characteristics of each starch. According to Oates (1997), enzymes cause alteration on the granule surface and degrade the external part by exocorrosion. When endocorrosion occurs, the internal part of the granule is corroded forming small pores through which enzymes penetrate into the granule. In this study, it was not possible to observe any endocorrosion of the starches after hydrolysis, except for the Peruvian carrot starch that showed some granules with internal degradation (Figure 4f  $\downarrow$ ). The hydrolyzed potato starch granules showed only a slight signal of exocorrosion



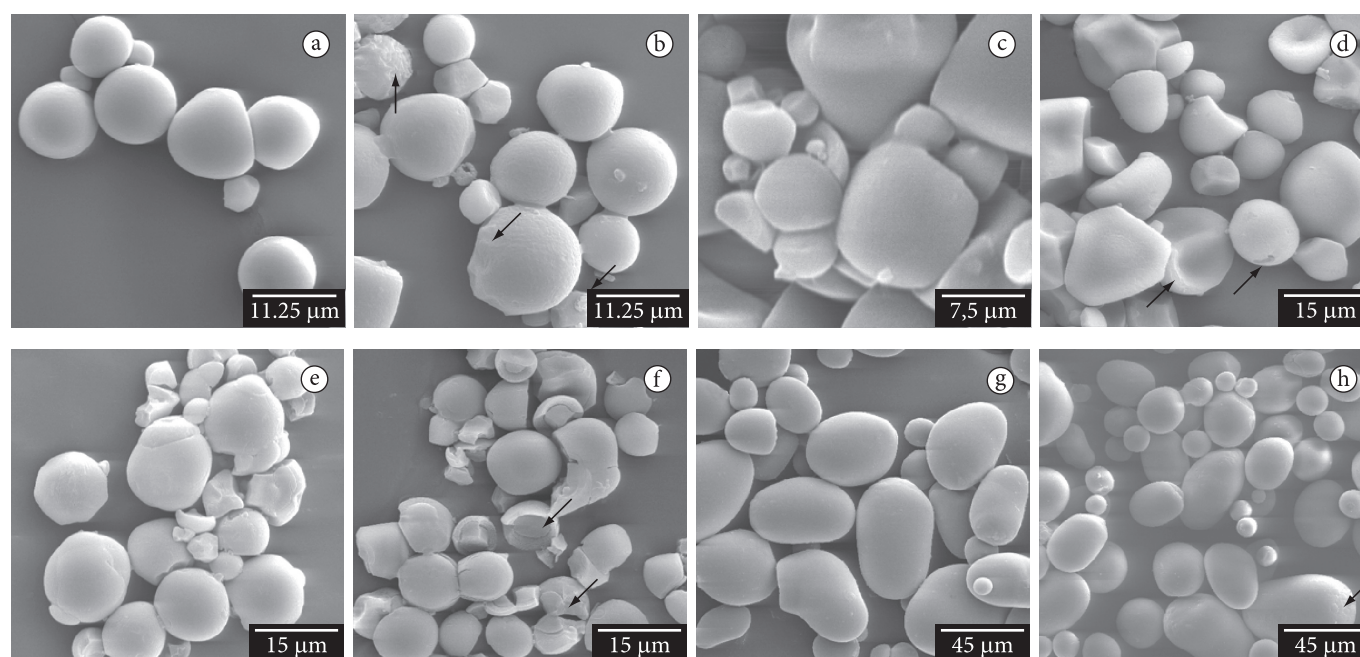
**Figure 3.** Photomicrographs of native starches observed in an optical microscope: a) cassava; b) sweet potato; c) Peruvian carrot; and d) potato.



**Table 3.** Overall and average sizes and granular size distribution of starches.

Starch	Overall size ( $\mu\text{m}$ )	Average size ( $\mu\text{m}$ )	Size distribution		Shape
			Size ( $\mu\text{m}$ )	%	
Cassava	7.3 – 23.7	15.6 <sup>b</sup>	5.0–10.0	8.4	Round
			10.1–20.0	72.0	
			> 20.0	19.6	
Sweet potato	7.2 – 28.3	13.4 <sup>c</sup>	5.0–10.0	21.4	Round and polygonal
			10.1–20.0	70.8	
			> 20.0	7.8	
Peruvian carrot	1.4 – 30.2	10.8 <sup>d</sup>	1.0–5.0	12.7	Round
			5.1–15.0	67.6	
			> 15.0	19.7	
Potato	6.7 – 66.0	23.4 <sup>a</sup>	5.0–20.0	51.4	Elliptical
			20.1–30.0	25.3	
			> 30.0	23.3	

\* Values followed by the same letter are not significantly different ( $P < 0.05$ ).



**Figure 4.** Photomicrographs of starches observed in SEM before (a, c, e, and g) and after hydrolysis (b, d, f, and h) with bacterial  $\alpha$ -amylase: (a and b) cassava; (c and d) sweet potato; (e and f): Peruvian carrot; (g and h): potato.

(Figure 4h↓). Gallant, Mercier and Guilbot (1972), who also reported higher resistance of the potato starch to enzymatic action, observed by SEM that the external part of the granules were kept intact after hydrolysis, and when the attack reached the internal regions of the granules, the hydrolysis occurred faster. In our experiments, no degradation signal in internal areas of the potato starch granules was observed.

### 3.4 Amylose content

The amylose content affects gelatinization and retrogradation properties, swelling power, and enzymatic susceptibility of starches (GÉRARD et al., 2001; YOU; IZYDORCZYK, 2002).

There was no significant difference between the amylose contents of cassava and sweet potato starches, which showed values around of 20%. The Peruvian carrot starch displayed amylose content of 18.7%, whereas the potato presented the highest amylose content (28.9%). Most starches contain 20-30% amylose depending on the botanical source. Hoover (2001) found values varying from 18.6 to 25.6% for the cassava starch depending on the plant variety. Also, according to Moorthy (2001), the sweet potato starch was found to have an amylose content of 20-25% depending on the variety, and Rocha, Demiate and Franco (2008) observed values of 21.7 and 17.8% for starches from two varieties of Peruvian carrot. Cottrell et al.

(1995) reported amylose contents varying from 24.4 to 27.3% for four potato cultivars, whereas Ganga and Corke (1999) showed values varying from 20.1 to 35.1% for twenty-four potato genotypes.

The enzymatic hydrolysis caused a decrease in the amylose contents of all starches, except for the potato starch, which kept the amylose content unchanged (Table 4).

The higher the enzymatic susceptibility of the starches to  $\alpha$ -amylase, the higher the reduction of amylose content of these starches. Therefore, it was possible to observe that 18.6% of cassava amylose was degraded by enzyme, whereas 7.2 and 7.5% of sweet potato and Peruvian carrot amylose molecules, respectively, suffered degradation during hydrolysis. Zhang and Oates (1999) observed that the molecular weight of sweet potato amylose decreased after the treatment of the starch with bacterial  $\alpha$ -amylase.

It is not clear whether amylose or amylopectin fraction is more affected by enzymatic hydrolysis. Yamada et al. (1995) reported that amylose could exist in the starch granules independently of the amylopectin suggesting that these linear molecules prevail in the amorphous areas of granules. Nevertheless, Jane (2006) reported that amylose, which is part of amorphous areas, is concentrated mainly in the granule periphery improving the interaction between amylose and amylopectin in those regions. Amylose and amylopectin molecules strongly associated in the periphery of the granules would be less susceptible to enzymatic attack justifying why starches with high proportions of amylose are more resistant to enzymes.

### 3.5 Intrinsic viscosity

Enzymatic hydrolysis caused a reduction of intrinsic viscosities of 9.8, 8.7, 4.9, and 1.3% for cassava, sweet potato, Peruvian carrot, and potato starches, respectively (Table 4). This reduction in the intrinsic viscosities of hydrolyzed starches in relation to the original starches can be explained by the enzyme action on the amylose and amylopectin molecules. These results confirmed the highest enzymatic susceptibility for the cassava starch and the lowest one for the potato starch. According to

**Table 4.** Amylose content\* and intrinsic viscosity of starches before and after hydrolysis with bacterial  $\alpha$ -amylase\*\*.

Starch	Amylose content (%)		Intrinsic viscosity	
	Before hydrolysis	After hydrolysis	Before hydrolysis	After hydrolysis
Cassava	19.9 <sup>bc</sup>	16.2 <sup>c</sup>	2.44 <sup>a</sup>	2.20 <sup>b</sup>
Sweet potato	20.7 <sup>b</sup>	19.2 <sup>cd</sup>	1.83 <sup>c</sup>	1.67 <sup>d</sup>
Peruvian carrot	18.7 <sup>d</sup>	17.3 <sup>c</sup>	2.43 <sup>a</sup>	2.31 <sup>b</sup>
Potato	28.9 <sup>a</sup>	28.8 <sup>a</sup>	2.28 <sup>b</sup>	2.25 <sup>b</sup>

\*Iodine affinity (IA) for pure amylose was assigned as 20% (TAKEDA; HIZUKURI, 1987); Amylose content = IA/0.20; \*\* Each value represents average of two replicates for amylose content and three replicates for intrinsic viscosity. Values followed by the same letter, for each analysis, are not significantly different ( $P < 0.05$ ).

Leach and Schoch (1961), intrinsic viscosity of  $\alpha$ -amylase in vitro hydrolysis residues was slightly lower than that of the original starch, which can also be observed in this study, in which the maximum decrease observed for the cassava starch did not reach 10%.

### 3.6 Pasting properties

Native starches displayed a typical viscosity pattern, an increase of viscosity up to a peak followed by a drop of viscosity under agitation and heating (Figure 5). The peak viscosity of hydrolyzed starches dropped; such reduction was larger for the starches that were more degraded by enzyme. Like what was observed for intrinsic viscosity, the reduction of peak viscosity of hydrolyzed starches was expected since the  $\alpha$ -amylase acts on the starch molecules breaking  $\alpha$ -(1-4) linkages and providing dextrin which presents a lower swelling during gelatinization. Since the peak viscosity is related mainly to the swelling of amylopectin molecules (TESTER; QI; KARKALAS, 2006), these results suggest that besides some amylose molecules, some amylopectin molecules were also degraded during the hydrolysis of cassava, sweet potato, and Peruvian carrot starches. These starches did not present any viscosity during the constant heating and cooling cycles of pastes.

### 3.7 Thermal properties

The gelatinization temperatures and enthalpy changes of native starches are shown in Table 5, which displays values close to those found in the literature (HOOVER, 2001; ROCHA; DEMIATE; FRANCO, 2008; GANGA; CORKE, 1999). After statistical analysis, it was observed that the gelatinization temperatures ( $T_o$ ,  $T_p$ ,  $T_c$ ) and  $\Delta H$  for all starches remained unchanged after hydrolysis, except for the peak temperature of the hydrolyzed cassava starch that was slightly lower when compared with the native cassava starch. These results suggested that either the enzyme attacked simultaneously the crystalline and amorphous areas of the granules of cassava, sweet potato and Peruvian carrot starches, or the hydrolysis was not enough to alter the amorphous and organized areas of these granules because both starches that suffered hydrolysis and the potato starch did not have their thermal properties changed. From the amylose content, intrinsic viscosity, and pasting property analysis, it was observed that some amylose and amylopectin molecules of cassava, sweet potato, and Peruvian carrot starches were degraded by enzyme, and thus it is probable that for these starches there was degradation of amorphous and crystalline areas.

Zhou, Hoover and Liu (2004) observed slight increase of the gelatinization temperatures and decrease in  $\Delta H$  when legume starches were treated with pancreatic  $\alpha$ -amylase. The decrease in the  $\Delta H$  of starches after hydrolysis suggests that  $\alpha$ -amylase is able to hydrolyze crystallites from branch chains of amylopectin and retrograded amylose (formed by hydrolyzed amylose chains) (ZHOU, HOOVER, LIU; 2004). In our experiments, the extent of hydrolysis was not enough to cause these effects.

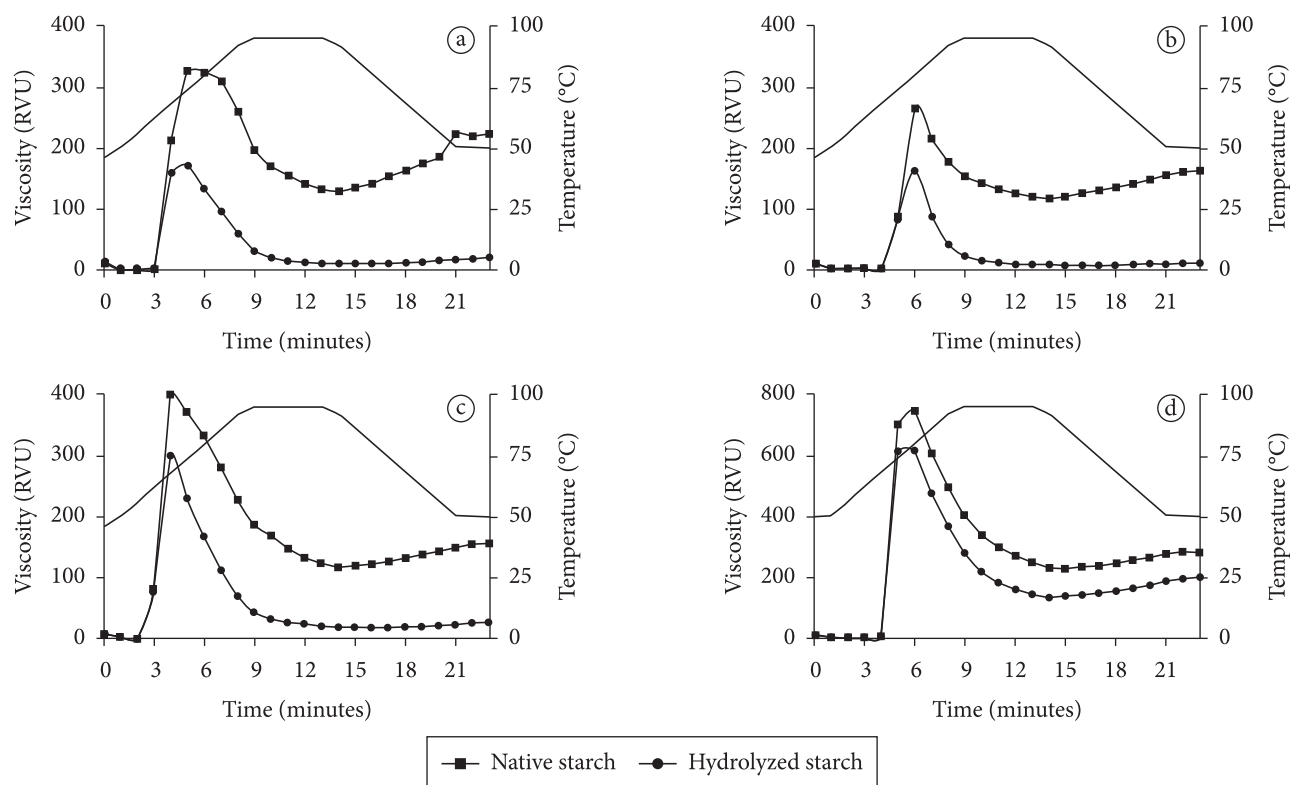


Figure 5. Viscosity pattern of native and hydrolyzed starches a) cassava; b) sweet potato; c) Peruvian carrot; and d) potato.

Table 5. Thermal properties \* of native and hydrolyzed starches.

Starch	T <sub>o</sub> (°C)	T <sub>p</sub> (°C)	T <sub>c</sub> (°C)	ΔH (J.g <sup>-1</sup> )
Native cassava	58.54 <sup>c</sup>	63.01 <sup>c</sup>	68.14 <sup>c</sup>	11.5 <sup>b</sup>
Hydrolyzed cassava	58.24 <sup>cd</sup>	62.40 <sup>d</sup>	67.50 <sup>c</sup>	11.9 <sup>b</sup>
Native sweet potato	67.49 <sup>a</sup>	71.70 <sup>a</sup>	75.29 <sup>a</sup>	12.3 <sup>b</sup>
Hydrolyzed sweet potato	67.73 <sup>a</sup>	71.75 <sup>a</sup>	75.41 <sup>a</sup>	11.7 <sup>b</sup>
Native Peruvian carrot	57.02 <sup>e</sup>	60.30 <sup>e</sup>	63.33 <sup>d</sup>	16.5 <sup>a</sup>
Hydrolyzed peruvian carrot	57.37 <sup>de</sup>	60.56 <sup>e</sup>	63.52 <sup>d</sup>	16.7 <sup>a</sup>
Native potato	64.18 <sup>b</sup>	67.44 <sup>b</sup>	73.11 <sup>b</sup>	15.7 <sup>a</sup>
Hydrolyzed potato	64.06 <sup>b</sup>	67.18 <sup>b</sup>	73.60 <sup>b</sup>	15.8 <sup>a</sup>

\*T<sub>o</sub>, T<sub>p</sub>, T<sub>c</sub>: onset, peak, and conclusion temperatures, respectively; ΔH: enthalpy change; and "The data represent average of three replicates. Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

## 4 Conclusion

Starches from different botanic sources behave differently towards enzymatic action because of their distinct structural characteristics. The cassava starch was the most susceptible to α-amylase hydrolysis followed by sweet potato and Peruvian carrot starches. The Potato starch was the most resistant to the enzyme. For the three first starches, hydrolysis occurred in the amorphous and crystalline areas of the starch granules. There was exocorrosion of the granule surface for all starches and the Peruvian carrot starch also showed granules with some degradation of its internal part. The extension of hydrolysis on the starches, except for the potato starch, was enough to modify some physicochemical characteristics of the starches such as

pasting viscosity and intrinsic viscosity, but it did not modify their thermal properties.

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