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Structural Characterization of Peruvian Carrot (*Arracacia xanthorrhiza*) Starch and the Effect of Annealing on Its Semicrystalline Structure

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ABSTRACT: Structural characteristics of native and annealed Peruvian carrot (*Arracacia xanthorrhiza*) starches were determined and compared to those of cassava and potato starches. Peruvian carrot starch presented round and irregular shaped granules, low amylose content and B-type X-ray pattern. Amylopectin of this starch contained a large proportion of long (DP > 37) and short (DP 6−12) branched chains. These last ones may contribute to its low gelatinization temperature. After annealing, the gelatinization temperatures of all starches increased, but the ΔH and the crystallinity increased only in Peruvian carrot and potato starches. The annealing process promoted a higher exposure of Peruvian carrot amylose molecules, which were more quickly attacked by enzymes, whereas amylopectin molecules became more resistant to hydrolysis. Peruvian carrot starch had structural characteristics that differed from those of cassava and potato starches. Annealing affected the semicrystalline structure of this starch, enhancing its crystallinity, mainly due to a better interaction between amylopectin chains.

KEYWORDS: starch, *Arracacia xanthorrhiza*, annealing, structure, amylose, amylopectin

INTRODUCTION

The demand for native starches from unconventional sources is increasing due to the interest in using native starches for food production instead of using chemically modified starches. Peruvian carrot (*Arracacia xanthorrhiza*) roots are a potential source of starch. Recent studies have been completed that characterize the functional properties of the Peruvian carrot starch for its use in the food industry.[1] This starch formed clear pastes with high viscosity and low retrogradation and syneresis, along with low gelatinization temperature.[2,3] However, there is still little knowledge about its structural characteristics.

The structural characteristics of granular starches have been under investigation for the last several years. It is widely known that starch granules are composed mainly of amylose and amylopectin, which form a semicrystalline structure with alternating higher and lower refractive indices, crystallinity, and resistance to acids and enzymes.[4] However, the location of amylose and amylopectin within the starch granules and how they are arranged are still not completely understood. Due to their semicrystalline structure, starch granules can undergo molecular reorganization when subjected to annealing. Annealing is a hydrothermal treatment during which suspensions of starch in water (>60% w/w) are held for a set period of time at a temperature higher than the glass transition temperature but lower than the onset gelatinization temperature.[5] This simple technique causes significant changes in the physicochemical properties of starches without destroying their granular structure, and it can provide useful information toward the understanding of granular structure in general.

The molecular reorganization caused by annealing occurs due to an increase in amylose and amylopectin mobility in the presence of water. More pronounced effects are obtained at subgelatinization temperatures.[6] Mobility of amorphous regions, increased by granule hydration, induces chain movements in both amorphous and crystalline domains facilitating ordering of double helices and, probably, of the amorphous areas too.[7,8] This reorganization results in higher gelatinization temperatures and a narrowing of the gelatinization temperature range due to the higher granular stability obtained.[5,9] These changes have been extensively described, but there is still no consensus regarding the mechanism of the process of annealing. Some authors discuss it based on increased crystallinity and perfection of starch crystallites due to an optimization of double helix packing and/or elongation of double helices from amylopectin chains.[9,10] Others have proposed new interactions between amylose/amylose, amylose/amylopectin and/or amylopectin/amylopectin.[11,12]

The aim of this study was to determine the structural characteristics of Peruvian carrot starch when compared to those of cassava and potato starches. The annealing of starches was performed in order to aid in the understanding of the starch's granular structure and also to investigate the mechanism of action of this hydrothermal treatment.

MATERIALS AND METHODS

Peruvian carrot (*Arracacia xanthorrhiza*) roots of the "Amarela de Senador Amaral" variety, cassava (*Manihot esculenta*) roots of the "Fécula Branca" variety, and potato (*Solanum tuberosum*) tubers of the "Monalisa" variety were used within 24 h after harvesting. Amyloglucosidase from *Aspergillus niger* (A7420, Sigma Chemical Co., USA), α-amylase from *Bacillus* sp. (A6380, Sigma Chemical Co., USA) and

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isoamylase from *Pseudomonas* sp. (Megazyme International, Ireland) were used. All other chemicals used were of analytical grade.

Isolation of Starches. The roots and tubers were peeled, sliced and ground for 2 min at high speed in a blender with two volumes of distilled water at 8 °C. The homogenate was passed through a sieve (0.250 mm screen), and the solids retained were washed three times on the sieve. The filtrate was passed through a sieve (0.180 mm screen) and left to stand overnight at 5 °C for decantation. The starch sediment was washed with distilled water and ethanol, recovered by centrifugation and dried at 38 °C in an air circulation oven.

Annealing. Starches were subjected to one-step annealing, following the procedure of Jacobs et al.,[6] with modifications. Native starch samples, in triplicate, were suspended in water (5% w/v) and incubated in a thermostatically controlled water bath for 24 h at a temperature of approximately 3 °C below the onset gelatinization temperature of each starch (54 °C for Peruvian carrot, 56 °C for cassava, and 59 °C for potato starches). After that, the samples were vacuum filtered and air-dried overnight at 38 °C.

Amylopectin Branched-Chain Length Distribution. Amylopectin was fractioned from amylose using *n*-butanol and was then debranched using isoamylase, as described by Jane and Chen.[13] The debranched chains were labeled with 0.2 M aqueous 8-amino-1,3,6-pyrenetrisulfonic acid (APTS) and 1 M aqueous sodium cyanoborohydride. The branched-chain length distribution was analyzed using fluorophore-assisted capillary electrophoresis (P/ACE MDQ, Beckman Coulter, Fullerton, CA, USA), according to Morrel et al.[14] The capillary used was a 50 μm diameter eCAP that was neutral coated with the supplied carbohydrate separation Buffer-N Gel. The sample was introduced by pressure injection at 0.5 psi for 5 s. Maltohexaose and maltoheptaose were used as reference standards. The analysis was performed in duplicate.

Wide-Angle X-ray Diffraction (WAXD). Native and annealed starches were stored for 10 days at 25 °C in a desiccator where a saturated solution of $BaCl_2$ containing 1% of sodium azide maintained 90% of relative humidity. The WAXD patterns of the starches were then determined using a wide angle goniometer unit (RINT2000, Rigaku, Tokyo, Japan), with copper Kα radiation ($\lambda = 0.1542$ nm). The scanning speed was 1°/min at 50 kV and 100 mA. The relative crystallinity was quantitatively estimated based on the relationship between the peak and total areas following the method of Nara and Komiya,[15] using the Origin software (version 7.5, Microcal Inc., Northampton, MA, USA). The analysis was performed in triplicate.

Average Amylopectin Molar Mass and Gyration Radius. Weight-average molar mass and the *z*-average gyration radius of amylopectin were determined using a HPSEC-MALLS-RI. Native and annealed starch samples were prepared as described by Yoo and Jane.[16] The HPSEC system consisted of an isocratic pump (HP 1050 series, Hewlett-Packard, Valley Forge, USA), a multiangle laser light scattering detector (Dawn DSP-F, Wyatt Tech. Co., Santa Barbara, CA, USA) and a refractive index detector (HP 1047A, Hewlett-Packard, Valley Forge, PA, USA). To separate amylopectin from amylose, a Shodex OH pack KB-guard column and the KB-806 and KB-804 analytical columns (Showa Denko K.K., JM Science, Grand Island, NY, USA) were used. Operating conditions and data analysis were the same as described by Yoo and Jane,[16] except that the flow rate used was 0.5 mL min^{-1} and the sample concentration was 0.15 mg mL^{-1}. The analysis was performed in duplicate.

Amylose Content and Amylose Leached. Amylose and amylopectin were separated according to Jane and Chen.[13] Iodine affinities of defatted starches and of isolated amylopectins were determined using an automatic potentiometric titrator (702 SM Titrino, Metrohm, Herisau, Switzerland). Absolute amylose contents were calculated following the method of Kasemsuwan et al.[17] The analysis was performed in triplicate. Amylose that leached to the supernatant during annealing was evaluated following the procedure of Palav and Seetharaman,[18] with some modifications. An aliquot of the supernatant (0.05 mL) was treated with 0.25 mL of iodine solution (0.2% KI and 0.02% I_2). The blue color was quantified after 20 min at 630 nm using an absorbance microplate reader (ELx808, Bio-Tek, Winooski, VT, USA). Pure amylose (A0512, Sigma Chemical Co., USA) was used to prepare a standard curve. The analysis was performed in triplicate.

Scanning Electron Microscopy. Native and annealed starches, rinsed with ethanol, were mounted on a metal plate covered with a carbon double-sided adhesive tape, submitted to a 20 nm gold layer application and observed in a scanning electron microscope (DSM 960, Zeiss, Oberkochen, Germany) operating at an acceleration voltage of 20 kV. Images were obtained with magnification of 1,000 and 2,000 times.

Confocal Laser Scanning Microscopy. Native and annealed starch suspensions (1% w/v) were stained with Rhodamine B (0.01% w/v), and a glass slide was mounted with a cover glass for viewing under a microscope, as described by Jane et al.[19] A confocal laser-scanning microscope (CLSM) (TCS SP2, Leica Microsystems, Wetzlar, Germany) was used with an Ar/Kr laser-light source. The excitation wavelength was 568 to 625 nm and the objective lens used was 100×/UV/oil immersion.

Thermal Properties. Gelatinization properties of starch samples were determined using a differential scanning calorimeter (DSC-Pyris 1, Perkin-Elmer, Norwalk, CT, USA). Starch samples (2 mg, dry basis) were weighed in aluminum pans, mixed with distilled water (6 μL) and sealed. The sealed pans were kept at room temperature for 2−3 h to equilibrate and scanned at a rate of 5 °C/min over a temperature range of 25 to 100 °C. An empty pan was used as a reference. The analysis was performed in triplicate.

Enzymatic Hydrolysis. Native and annealed starches were hydrolyzed with α-amylase (40 SKB/g of starch) and amyloglucosidase (8 U/g of starch) according to Franco and Ciacco,[20] with modifications. Starch suspensions (10% w/v) were incubated at 37 °C for 120 h. The amount of reducing sugar in the supernatant was determined according to Somogyi,[21] every 6 h. The enzymatic hydrolysis was interrupted following the procedure of Vieira and Sarmento,[22] and the hydrolysis residues, which were collected after 12, 36, and 96 h, were air-dried overnight at 38 °C. The molar mass of amylopectin and the amylose content after hydrolysis were determined as described above.

Statistical Analysis. The software Statistica for Windows (v. 5.0, Statsoft, Tulsa, OK, USA) was used to analyze mean values using analysis of variance (ANOVA). Differences were evaluated using the *t*-test with Tukey's adjustment. The significance level was set at a p value <0.05.

RESULTS AND DISCUSSION

Amylopectin Branched-Chain Length Distribution. Normalized chain length distributions of amylopectins isolated from Peruvian carrot, cassava and potato starches are shown in Figure 1, and the results are summarized in Table 1. Peruvian carrot starch (Figure 1a) had a gradual increase in chains of polymerization degree (DP) 6−12, and formed a peak at DP 12. This starch displayed a shoulder at DP 18−24. Cassava starch (Figure 1b) had a distribution similar to that of Peruvian carrot starch, but the relative intensity of the shoulder was lower, and there was a lower proportion of very long chains. These results are in agreement with those reported by Santacruz et al.[3] for Peruvian carrot starch, and by Jane et al.[23] for cassava starch. Shoulders on the branched-chain length distribution of amylopectins indicate that chains with DP <10, unable to form double helices, might be located in crystalline regions, which would result in defects in starch structure.[23,24] The potato starch (Figure 1c) had the lowest population of chains with DP 8,

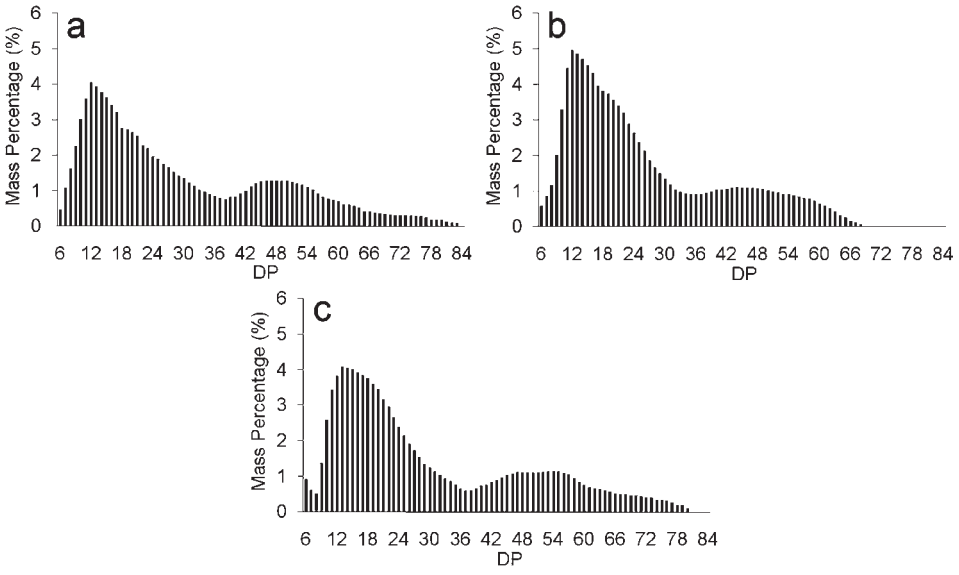

Figure 1. Branched-chain length distribution of amylopectins of native starches: (a) Peruvian carrot, (b) cassava, and (c) potato.

Table 1. Branched-Chain Length Distribution of Amylopectin of Starches in Mass-Basis, Measured by Fluorophore-Assisted Capillary Electrophoresis, before and after Annealing[a]

	branch chain length distribution (%)					
starches	DP 6−12	DP 13−24	DP 25−36	DP > 37	av DP	highest detectable DP
			Before Annealing			
Peruvian carrot	16.2 b	37.2 c	13.9 b	33.7 a	29.8 b	83
cassava	17.6 a	45.6a	15.6 a	21.2 c	24.7 c	68
potato	10.9 c	41.8 b	15.5 a	31.8 b	30.6 a	80
			After Annealing			
Peruvian carrot	15.6 b	36.2 c	13.4 b	34.8 a	29.6 b	80
cassava	18.3 a	44.9 a	14.6 ab	22.2 c	25.0 c	66
potato	10.5 c	41.1 b	15.8 a	32.6 ab	30.3 ab	80

[a] Values with the same letter in the same column are not significantly different at $p < 0.05$.

and this starch did not show a shoulder on its distribution. A similar distribution was found by Jane et al.[23] in potato starch.

Peruvian carrot starch had the largest proportion of long chains (DP > 37), followed by potato starch (Table 1). However, the average chain length of Peruvian carrot amylopectin was smaller than that of potato amylopectin due to the large proportion of short chains (DP 6−12) present in the first one. The large proportion of short chains and the small proportion of chains of DP 13−36 of Peruvian carrot starch may form a weak crystalline structure, which is unable to hold the clusters together or maintain the integrity of starch granules, as observed by Jane et al.[19] in the case of sugary-2 maize starch.

Alterations on the branched-chain length distribution of amylopectins after annealing were not expected, since this treatment does not change the chemical structure of the starch granules. The results shown in Table 1 confirm that the branched-chain length distribution of amylopectins remained unchanged in all starches. Similar results were found by Lan et al.[25] in normal, waxy, and high-amylose wheat starches after annealing at 10 °C below the onset gelatinization temperature for 72 h.

X-ray Pattern and Crystallinity. Peruvian carrot starch showed a typical B-type X-ray pattern (Figure 2 a), a result that agrees with those of Rocha et al.,[2] Santacruz et al.,[3] and Vieira and Sarmento.[22] According to Hizukuri et al.,[26] the average DP of the branched chains of amylopectin is generally associated with the polymorphism A, B or C type. A-type starches usually consist of a large proportion of short chains (DP 6−12), B-type starches normally have a large proportion of long chains (DP > 37), and C-type starches can have substantial amounts of both short and long chains. Potato starch showed a B-type X-ray diffraction pattern (Figure 2 c), whereas cassava starch showed an X-ray diffraction pattern similar to A-type. However, this last one exhibited a small peak at 5.5° at 2θ, which is typical of B-type patterns (Figure 2 b). Therefore, this starch was classified as a C_A-type due to the greater similarity of the peaks to A-type. Hoover[27] also reported a C_A-type pattern in cassava starch. It was expected that Peruvian carrot starch displayed a C-type X-ray diffraction pattern due to the large amounts of both short and long chains observed in this starch (Table 1). However, this starch displayed a typical B-type pattern, and proved to be an exception.

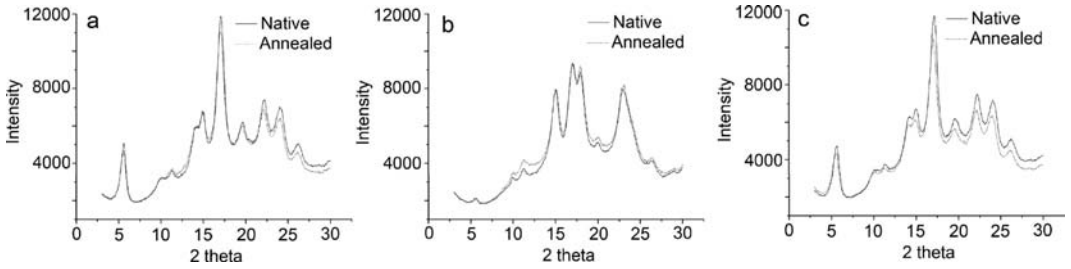

Figure 2. X-ray patterns of starches before and after annealing: (a) Peruvian carrot, (b) cassava, and (c) potato.

Table 2. Percentage of Crystallinity and Amylose Content of Starches before and after Annealing[a]

	percentage of crystallinity (%)		amylose content (%)			
starches	before annealing	after annealing	apparent[b]	absolute[c]	apparent[b] after annealing	leached[d]
Peruvian carrot	37.1 cd	43.1 a	20.0 c	9.1 f	20.3 c	0.18 B
cassava	36.2 d	37.0 cd	23.2 b	17.0 e	23.4 b	0.50 A
potato	38.3 c	41.1 b	30.4 a	18.4 d	30.3 a	0.19 B

[a] Values with the same lowercase letter are not significantly different at $p < 0.05$, for each analysis; values with the same uppercase letter are not significantly different at $p < 0.05$. [b] Calculated as $C = 100 \times IA_S/0.20$ where C is the percentage of apparent amylose content and IA_S is the iodine affinity of the whole defatted starch. [c] Calculated as $C = (IA_S - IA_{AP+IC})/[0.20 - (IA_{AP+IC}/100)]$ where C is the percentage of absolute amylose content, IA_S is the iodine affinity of whole defatted starch, and IA_{AP+IC} is the iodine affinity of the amylopectin and the intermediate component mixture. [d] Amylose leached to supernatant during annealing.

None of the X-ray diffraction patterns of the starches were influenced by the annealing, and then, they remained unchanged. These results agree with those of Hoover and Vasanthan,[12] who also subjected different starches to different annealing conditions.

The relative crystallinity of the three starches was similar and close to 37% (Table 2). After annealing, the crystallinity in Peruvian carrot and potato starches increased, whereas it did not change in cassava starch. Lan et al.[25] found increased crystallinity in annealed normal, waxy, and high amylose bread wheat starches, whereas Jacobs et al.[6] found no significant changes in crystal type and degree of crystallinity of annealed wheat, potato and pea starches. Annealing is a treatment that causes a rearrangement of starch molecules leading to either an increase in crystal perfection, an increase in crystallite size[9,10] or the formation of new crystallites.[11,12] The higher crystallinity found in annealed Peruvian carrot and potato starches may be attributed to crystal growth (increase in crystallite size), in function of the higher amount of chains of DP > 37 displayed in these starches, which would have a structure that is more susceptible to reorganization than that of cassava starch.

Amylose Content. Peruvian carrot starch showed 20.0% of apparent amylose content (Table 2), which is a value close to those obtained by Rocha et al.[2] and Vieira and Sarmento,[22] whose values were based on iodine affinity of whole starch. However, the iodine affinity of amylopectin isolated from this starch was high (2.40). This indicated that the large proportion of long branched chains of amylopectin (DP > 37) found in this starch (Table 1) contributed to its increased amylose content by forming a complex with iodine. The absolute amylose content of this starch was only 9.1%, which is a value close to that found by Santacruz et al.,[3] who used size exclusion chromatography. As observed in Peruvian carrot starch, potato starch also showed a great difference between apparent and absolute amylose contents due to the large proportion of long branched chains found in this starch as also observed by Jane et al.[23]

To verify if the gelatinization of the starches occurred during annealing, the apparent amylose content of the annealed starches and the leached amylose were determined (Table 2). After annealing, the apparent amylose content did not change in any starch. The amount of amylose leached to supernatant during annealing varied from 0.2% to 0.5%. Tester et al.[10] reported similar values of amylose leached during annealing of wheat starch at 35 and 45 °C. The loss of amorphous material during the hydrothermal treatment can be considered insignificant, since there was no change in apparent amylose content of the starches. These results indicate that the gelatinization of the starch granules did not occur under the annealing conditions used.

Morphology and Internal Structure. Granular size and shape are related to the botanical source of the starches. Peruvian carrot starch showed round and irregular shaped granules. Fragile granules, which tended to separate into pieces, were also found (Figure 3-1a). Cassava starch showed round shaped granules with some convex−concave shapes (Figure 3-1b), whereas potato starch predominantly showed oval shaped granules (Figure 3-1c). The surface of Peruvian carrot starch granules displayed some depressions, whereas smooth surfaces were found in cassava and potato starches.

After annealing, the granular shape of the starches remained unchanged. The granular surface of Peruvian carrot and potato starches was not altered after treatment. However, in cassava starch, the presence of some fissures was observed (Figure 3-1e).

Peruvian carrot starch granules could not be well visualized using confocal laser scanning microscopy (CLSM) (Figure 3-2a). Only a few large granules were stained with rhodamine B, and these did not show voids or cavities. According to Han and Hamaker,[28] rhodamine B, which was used in this study, stains starch granules by labeling starch granule-associated proteins.

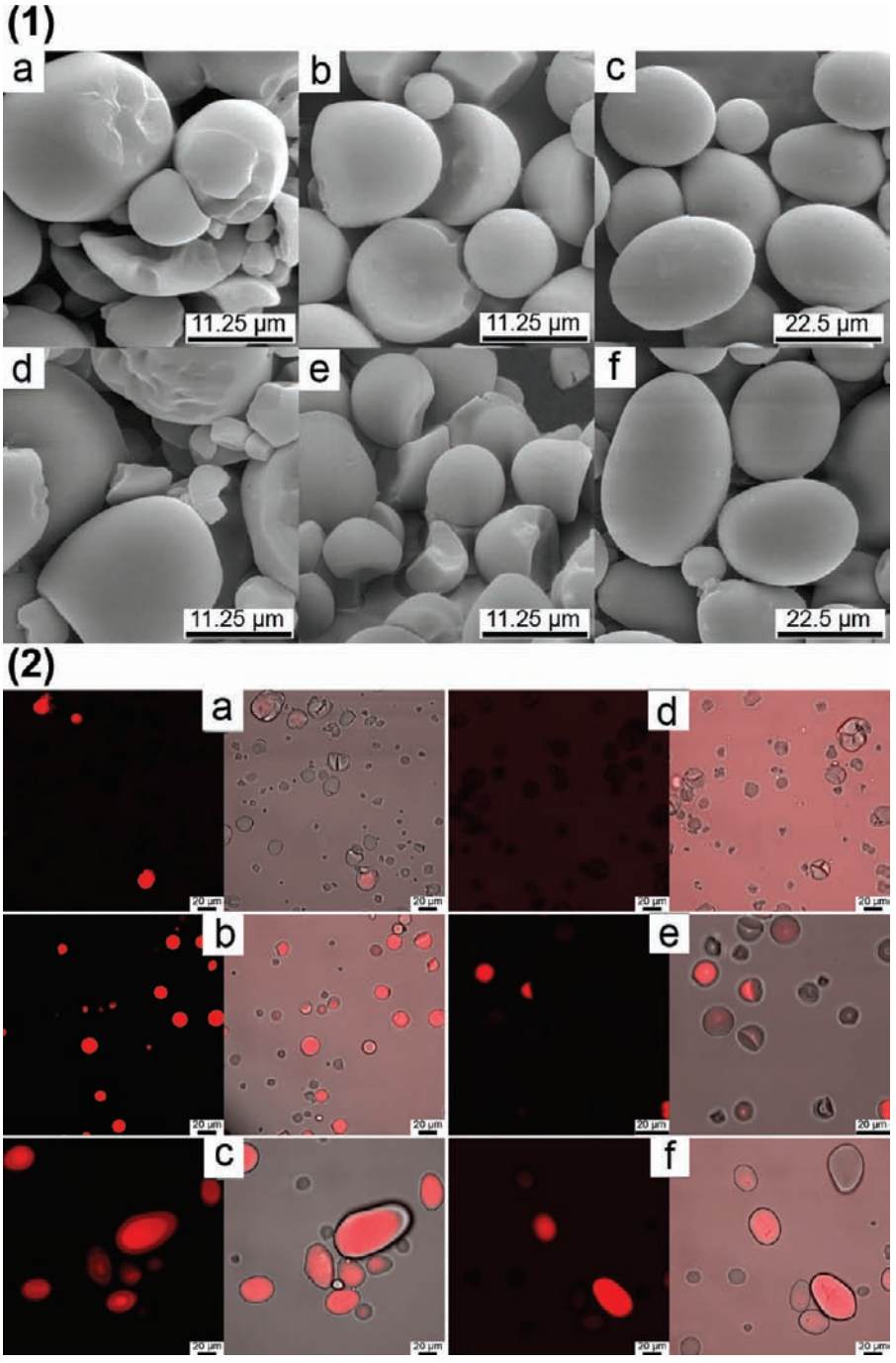

Figure 3. Micrographs of starches: (1) scanning electron micrographs, (2) confocal laser scattering micrographs (left) and overlaid optical and confocal laser scattering micrographs (right). Native starches: (a) Peruvian carrot, (b) cassava, and (c) potato. Annealed starches: (d) Peruvian carrot, (e) cassava and, (f) potato.

These proteins, present in trace amounts in starch granules, are mainly granule-bound starch synthase (GBSS), which is responsible for amylose production. Then, it is possible that Peruvian carrot starch has a very low concentration of GBSS, which could also explain the low absolute amylose content (9.1%) found in this starch (Table 2).

Fragile and brittle granules of Peruvian carrot starch were also found in the overlaid optical and confocal laser scattering micrograph (Figure 3-2a), resulting from the crystalline structure defects of this starch, which could lead to formation of less stable crystallites that can disrupt easily. Similar observations were made by Jane et al.[19] in the case of sugary-2 maize starch. The overlaid optical and confocal laser scattering micrographs also revealed that none of the small-diameter granules of all starches stained with rhodamine B. Cassava starch granules stained with rhodamine B showed an internal structure with no voids or cavities (Figure 3-2b). Potato starch showed granules with pronounced internal layers, which represent the growth rings (Figure 3-2c). Internal structures of both cassava and potato starches agree with the findings from Velde et al.[29]

Table 3. Thermal Properties of Starches before and after Annealing[a]

starches	T_0[b] (°C)	T_p[b] (°C)	ΔT[b] (°C)	ΔH[b] (J/g)
		Before Annealing		
Peruvian carrot	56.99 e	60.29 d	7.43	16.8 b
cassava	59.10 d	67.28 b	15.53	13.6 d
potato	62.04 c	64.75 c	6.55	15.5 c
		After Annealing		
Peruvian carrot	61.85 c	64.45 c	5.55	17.8 a
cassava	65.30 b	69.28 a	8.95	13.9 d
potato	67.71 a	69.93 a	5.20	17.9 a

[a] Values with the same letter in the same column are not significantly different at $p < 0.05$. [b] T_0 = onset temperature, T_p = peak temperature, ΔT = range of gelatinization temperature, ΔH = enthalpy change.

After annealing, the capacity to retain the dye decreased for all starches. The crystalline structure reorganization caused by the hydrothermal treatment made the diffusion of the dye into the granule difficult; thus, the visualization of the internal structure was compromised. None of the Peruvian carrot starch granules were observed (Figure 3-2d). Only a few granules of cassava (Figure 3-2e) and potato (Figure 3-2f) starches were visualized, and these displayed compact structures, with no voids, cavities or internal layers.

Thermal Properties. Peruvian carrot starch showed the lowest onset gelatinization temperature (T_0), followed by cassava starch (Table 3). Starches with a large proportion of short chains have lower gelatinization temperatures.[13,23,30] These starches also presented a shoulder on the amylopectin branched-chain length distribution at DP 18−24, which is considered a crystalline structural defect and contributes to the starch's low gelatinization temperatures.[9,23] On the other hand, the larger amount of long branched chains (DP > 37) of amylopectin displayed in Peruvian carrot and potato starches contributed to their higher enthalpy changes (ΔH). Narrow temperature ranges (ΔT) as those shown by Peruvian carrot and potato starches indicate a less heterogeneous distribution of crystals.[24] Despite the defects in crystalline structure of Peruvian carrot starch, the distribution of its imperfect crystallites is less heterogeneous than that in cassava starch.

Gelatinization temperatures increased in all starches, and decreased ranges of gelatinization temperatures were found as a result of annealing. However, the ΔH increased only in Peruvian carrot and potato starches, and remained unchanged in cassava starch (Table 3). A higher gelatinization temperature accompanied with a narrowing of the gelatinization temperature range occurs in all native starches after annealing, whatever their polymorphic structure or amylose content[10] in function mainly of an improvement of the most disordered crystallites in the starch structure.[24]

The enthalpy change (ΔH) is mainly related to the loss of order in the double helices.[27] Thus, the increase in the ΔH of annealed Peruvian carrot and potato starches may be attributed to a greater resistance of double helices from crystalline regions to melt, which also caused the increase in the crystallinity of these starches (Table 2).

Enzymatic Hydrolysis. In native and annealed starches, the hydrolysis percentage increased progressively, reaching a plateau at the end of incubation period (120 h) (Figure 4).

After 120 h of reaction, Peruvian carrot starch had 53% of hydrolysis, a value that was similar to the one observed in cassava starch (56%). Potato starch was the most resistant and displayed only 10% of hydrolysis. It is widely known that B-type starches are more resistant to enzymatic hydrolysis due to the large proportion of long branched chains of amylopectin (DP > 37), which extend through two or more clusters and stabilize the internal structure of granules.[31] However, Peruvian carrot starch showed a profile of hydrolysis similar to that of the cassava starch. This may be because Peruvian carrot starch also consists of a large proportion of short branched chains of amylopectin, making it more susceptible to enzyme action since the large proportion of chains of DP 6−12 promotes the formation of short double helices, which result in a less stable crystalline structure that is more susceptible to enzymatic attack.[31] Additionally, the granule surface area for enzyme adsorption must be considered during enzymatic hydrolysis.[32] Peruvian carrot starch granules have an average diameter of 15 μm,[2] whereas potato starch granules have an average diameter of 40 μm.[4] Small granules have a higher surface area per unit of weight than large granules, which, when combined with the other factors already cited, may have contributed to the greater susceptibility of Peruvian carrot starch to enzymes.

After annealing, Peruvian carrot starch became slightly more susceptible to enzymatic hydrolysis in the first 48 h of incubation, at which point it maintained a profile that was similar to the native starch. However, cassava starch became much more vulnerable to enzyme action, whereas no difference was visualized in potato starch. Jacobs et al.[33] observed less enzyme (pancreatin) resistance for annealed wheat and pea starches only in the second phase of hydrolysis, whereas annealed potato starch was more resistant to degradation than native starch.

Because annealing enhances the interaction of starch molecules, the expected result was that modified starches would show a greater resistance to enzymatic hydrolysis. However, the greater susceptibility of annealed Peruvian carrot starch in the first 48 h of hydrolysis suggests that, for this starch, the amorphous regions became more accessible to enzymes after treatment. For annealed cassava starch, the appearance of fissures on the granule surface (Figure 3-1e), which allowed for degradation caused by endocorrosion, seems to have also contributed for the greater increase in the rate of hydrolysis. Higher enzymatic susceptibility was also found for annealed sago starch[34] in relation to the native starch, due to fissures on the granules surface. Finally, potato starch, which was the most resistant before annealing, still showed the same resistance after treatment.

Amylose Content of the Enzymatic Hydrolysis Residues. The apparent amylose content of native and annealed starches, obtained at 12 h, 36 h, and 96 h of enzymatic hydrolysis, were evaluated (Table 4). Before annealing, the amylose molecules of Peruvian carrot and cassava starches showed progressive degradation over time of hydrolysis. The apparent amylose content of Peruvian carrot starch decreased from 20% at 0 h to 14.1% at 96 h of hydrolysis. The native cassava starch initially had 23.2% of apparent amylose, and at 96 h of hydrolysis, it had 21.6%. These results show that the amylose molecules of the Peruvian carrot starch were more available to enzymatic attack than those of cassava.

After the hydrothermal treatment, the amylose degradation of Peruvian carrot starch was most intense at first 12 h of hydrolysis, decreasing from 20.3 to 13.5% at this point and remaining

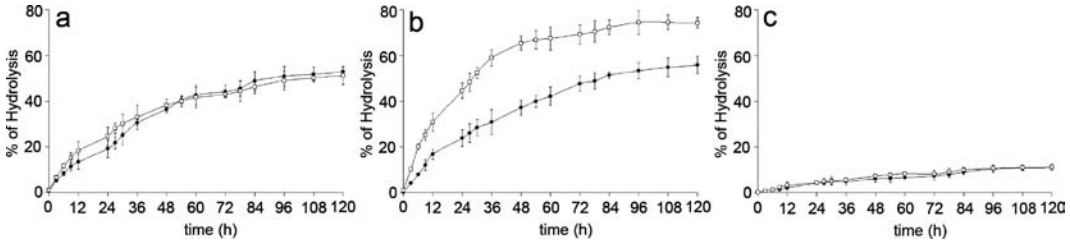

Figure 4. Enzymatic hydrolysis of starches before (●) and after (□) annealing: (a) Peruvian carrot, (b) cassava, and (c) potato.

Table 4. Amylose Content and Average Amylopectin Molar Mass of the Enzymatic Hydrolysis Residues, before and after Annealing[a]

	Amylose Content (%)							
	before annealing				after annealing			
starches	0 h	12 h	36 h	96 h	0 h	12 h	36 h	96 h
Peruvian carrot	20.0 a	17.6 b	15.9 c	14.1 d	20.3 a	13.5 d	14.1 d	14.1 d
cassava	23.2 a	23.2 a	22.0 b	21.6 bc	23.4 a	20.3 c	19.4 c	16.5 d
potato	30.4 a	30.8 a	29.3 b	30.1 a	30.3 a	30.1 a	29.9 ab	30.3 a
	Average Amylopectin Molar Mass ($M_w \times 10^8$ (g/mol))							
	before annealing				after annealing			
starches	0 h	12 h	36 h	96 h	0 h	12 h	36 h	96 h
Peruvian carrot	2.2 ab	2.4 a	1.9 b	1.5 c	2.4 a	1.9 b	1.9 b	1.8 b
cassava	5.1 a	5.1 a	4.6 b	5.1 a	5.0 a	5.0 a	4.5 b	5.1 a
potato	1.5 a	1.5 a	1.2 ab	0.9 b	1.3 ab	1.2 ab	1.0 b	1.2 ab

[a] Values with the same letter in the same line are not significantly different at $p < 0.05$.

constant after that. At 12 h of hydrolysis the annealed Peruvian carrot starch contained the same amylose content as the native starch at 96 h of hydrolysis, indicating that amylose molecules were more available to enzyme after treatment. In annealed cassava starch, the apparent amylose content gradually reduced over time of hydrolysis; however, the amylose molecules were attacked more after annealing when compared to the native starch. At 96 h of hydrolysis, the apparent amylose content of this starch was only 16.5%. No difference was observed in the apparent amylose content of native and annealed potato starches during the enzymatic hydrolysis, probably due to the low hydrolysis rate of this starch.

The location of amylose and amylopectin within the starch granules is still not completely understood. However, these results suggest that, in native Peruvian carrot and cassava starches, the amylose would be interspersed with amylopectin chains, and somehow protected from the enzymatic attack on the semicrystalline structure. The structural rearrangement provoked by the increase of mobility of the molecules in water during the treatment may have decreased the interaction between amylose and amylopectin and thus promoted a higher exposure of amylose molecules of these starches to the enzyme action.

Average Amylopectin Molar Mass of the Enzymatic Hydrolysis Residues. The weight-average molar mass (M_w) of amylopectin of the native and annealed starches subjected to enzymatic hydrolysis is shown in Table 4. The amylopectins of Peruvian carrot and potato starches showed an M_w that was smaller than that of cassava starch. The highest gyration radius (R_z) was observed in cassava starch, which also showed the largest dispersed-molecular density (ρ) (data no shown). These results are consistent with previous studies, which reported a higher M_w and ρ for amylopectins of A-type starches than that of B-type starches.[16]

The amylopectin M_w of Peruvian carrot and potato starches before annealing decreased over time of enzymatic hydrolysis. In cassava starch, the M_w decreased at 36 h of hydrolysis, but at 96 h a M_w similar to that at 0 h was observed. The ΔT of cassava starch (Table 3) indicated a heterogeneous population of its crystallites. These results suggested that amylopectin molecules that took part of the defective crystallites were preferentially attacked inducing the formation of lower molecules at 36 h of hydrolysis, which would continue being preferentially attacked in the next hours of hydrolysis. This way, the molecule population present in a sample with 96 h of hydrolysis would mainly consist of molecules that took part of the most perfect crystallites and then would be more resistant to enzyme.

Peruvian carrot and potato starches had a crystalline structure that was more prone to reorganization, which allowed for an increase of the percentage of crystallinity with annealing (Table 2). It is possible that, before annealing, the enzymes had greater access to amylopectin chains of these starches, allowing for the hydrolysis. However, in potato starch, the degradation of amylopectin molecules did not influence the apparent amylose content of this starch, which most likely remained unchanged due to its low rate of hydrolysis.

After annealing, the amylopectin M_w of Peruvian carrot starch decreased at 12 h of hydrolysis, and remained constant thereafter. However, even at 96 h of incubation, the amylopectin M_w of this annealed starch was higher than that of the native starch at the same point in the hydrolysis process. In cassava starch, the enzymatic attack on the amylopectin molecules was similar to that in the starch before annealing. The amylopectin M_w of annealed potato starch did not reduce during or after enzymatic hydrolysis. These results showed that amylopectin molecules of Peruvian carrot and potato starches became more resistant to the action of the enzymes as a result of the hydrothermal treatment. However, the higher reduction in the apparent amylose content found in the annealed Peruvian carrot starch after the first 12 h of the hydrolysis may be due to the degradation of amylose molecules and also to the degradation of very long branched chains of amylopectin.

Hoover and Vasanthan[12] and Jacobs et al.[6] state that mobility of the amylose chains increases during annealing, resulting in double helix formation due to the interaction of amylose/amylose and/or amylose/amylopectin chains. However, the findings herein showed an increase on the percentage of

crystallinity of Peruvian carrot starch after annealing (Table 2), in addition to a greater resistance of amylopectin molecules, as well as a greater susceptibility of amylose molecules to the enzymatic action. This suggests increased interaction between amylopectin/amylopectin chains. The annealing also caused more exposure of amylose molecules of cassava starch to enzymatic hydrolysis, but no significant effect on amylopectin susceptibility was observed. Despite the low rate of hydrolysis of the potato starch, this starch's amylopectin molecules still showed a greater resistance to the action of enzymes after annealing.

In summary, Peruvian carrot starch had structural characteristics that differed from cassava and potato starches. It is a B-type starch that consists of a large amount of both short (DP 6−12) and long (DP > 37) branched chains of amylopectin. This starch showed fragile granules that are likely formed due to a weak crystalline structure. It also had a lower amylose content, a lower gelatinization temperature, and a higher enthalpy change when these values were compared to those of cassava and potato starches. Amylose molecules from the Peruvian carrot may be interspersed among the amylopectin molecules. Annealing promoted the formation of new interactions between amylopectin chains, which enhanced the crystallinity of this starch and protected them from enzymatic attack. These new interactions may have exposed the amylose chains, which became more susceptible to hydrolysis.

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