RESEARCH ARTICLE

Morphometric and cristallinity changes on jicama starch (*Pachyrizus erosus)* during gelatinization and their relation with in vitro glycemic index

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The effect of gelatinization on the in vitro glycemic index (GI) of starch obtained from jicama tubers was investigated, and the relationships between the starch crystallinity, granule morphology, and the GI were measured. Samples were prepared by heating an aqueous dispersion of starch (1:3 w/v) at different baking process temperatures (60, 65, 70, 75, or 80°C). Native starch granules showed spherical and polyhedral shapes, with a morphometric aspect ratio (AR) of 0.89–1.0 and sizes ranging from 3 to 21 μm. During the thermal process a change in the AR (0.21–0.88) and size (3.0–46 μm) was observed, as well as the formation of agglomerates. Native jicama starch exhibited a C_A-type X-ray diffraction pattern, while those thermally treated showed a transition from the C_A-type to C_B-type, decreasing their crystallinity (20.8–11.5%) at higher temperatures. The gelatinization degree of the starch samples and the glycemic index showed the largest values (97.4% and 98.6% respectively) at the highest temperature (80°C), while the crystallinity percentage followed an inverse correlation to temperature (11.5%, 80°C), indicating that the thermal history affected the behavior of the starch enzymatic digestion. The in vitro glycemic index correlated positively with the gelatinization degree based on aspect ratio ($R^2 = 0.977$), DSC gelatinization enthalpy ($R^2 = 0.969$), and crystallinity ($R^2 = -0.988$). Based on these results a mathematical model is proposed to determine the glycemic index as a function of the aspect ratio. However, more studies are required to validate this information.

Keywords:
Crystallinity degree / Gelatinization degree / In vitro glycemic index / Jicama starch / Starch aspect ratio

1 Introduction

Jicama (*Pachyrhizus erosus*) is a leguminous crop that grows well in tropical and subtropical regions. Furthermore, it has a low production cost, a high potential for nitrogen fixation and a high agronomic yield (70–120 t/ha) [1, 2]. However, the current interest in this crop lies in its tuberous root, also known as jicama, as a result of its starch content (20–50 g/100 g dry basis) [3, 4], making this root a possible new source of this polysaccharide.

Jicama starch has been analyzed by different authors in its native form [1, 3, 4] reporting that it has similar physical properties regarding its size (3–35 μm), shape (round and polygonal), diffraction pattern (A-type crystallinity pattern),

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and some thermal and rheological properties when compared to starches from other sources such as corn, rice, and wheat. Its functional properties have also been studied with respect to their botanical source, from the ratio of amylose/amylopectin (82–18%) to the degree of chain branching, and to the length of amylopectin outer chain [3]. However, the changes on the structure of the starch granule during a thermal process (gelatinization) and their relation to functional, morphometric, and nutritional characteristics (in vitro glycemic index) have not been reported [5, 6].

Starch gelatinization has been defined as the disruption of the molecular order within the starch granule, as a result of irreversible morphometric changes and different physicochemical, thermal, and rheological properties as compared to those of native starch [7]. These changes are controlled in part by the molecular structure of amylopectin [8], starch composition, and granule architecture (crystalline to amorphous ratio) and, according to Di Paola [9], several methods can be used to follow the gelatinization process, where birefringence (quantified by polarized light microscopy), double-helical structure (quantified by NMR), and crystallinity (quantified by X-ray diffraction, DSC) are the most readily quantifiable parameters to follow the behavior of starch granules during heating [10–11].

During the heating process, the chemical, textural, and structural changes that the starch granule undergoes, not only have an effect on its physical quality, but they are also reflected in the starch glycemic response [12]. Hence, in this study, native starch from jicama was isolated and thermally treated and its crystallinity, morphology, thermal behavior, size and aspect ratio, and glycemic index (GI) were investigated. Correlations that might exist between these parameters and GI were also explored.

2 Materials and methods

2.1 Native starch

Native starch was extracted from Jicama tubers (*P. erosus L. Urban*, variety "San Juan," Nayarit, Mexico) as reported by Novelo-Cen and Betancur-Ancona [13]. Lipid (0.76 g/100 g sample (db)), and ash contents (5.3 g/100 g sample(db)) [14], as well as amylose (26.4%) [15], and damaged starch (3%) [16] were evaluated.

2.2 Sample preparation

Thermally processed samples were acquired by subjecting jicama native starch slurries (12.2 g starch db/36.6 g water) to a heating process in a convective heating chamber (Henry Simon Limited, Cheshire, UK) at 185°C. Independent gelatinized starch samples were extracted from the oven immediately after reaching 60°C (13 min), 65°C (16 min), 70°C (18 min), 75°C (20 min), and 80°C (22 min) at their center (K-type thermocouple, 0.81 mm diameter, T/C wire, Digi-Sense, Vernon Hills, IL, USA). The extracted samples were cut into a cylindrical shape (3.5 cm diameter from the center) and immediately frozen. They were then grounded (Braun, ksm2) and freeze-dried (−50°C, Labconco, Kansas, MO, USA). Dried samples (moisture 6%) were stored at −24°C for less than a week.

The target temperatures were selected based on a preliminary DSC study, where the gelatinization temperature range of native jicama starch varied from 60 to 77°C, assuring by this way different degrees of gelatinization.

2.3 Scanning electron microscopy (SEM)

Jicama tissue (perimedullary parenchyma) and jicama starch granules were structurally characterized by applying scanning electron microscopy (JSM 5800 Jeol LV, Jeol LTD, Tokyo, Japan).

Jicama tissue (0.5 mm × 0.5 mm × 0.5 mm) was fixed in buffered 3% glutaraldehyde solution (w/v, G5882 Sigma–Aldrich) for 24 h, extensively rinsed with the same buffer (0.1 M phosphate buffer, pH 7.2), post-fixed with 1% OsO_4 solution (w/v, 1 h, 20°C, 75632 Sigma–Aldrich), dehydrated using a series of acetone–water solutions (25%, 50%, 75%, 95% for 10 min and at 100% for 2 h) and dried (LEICA EM CPD030, Vienna, Austria), while the starch granules were directly mounted on aluminum pans without any previous fixing or drying treatment. Both samples were mounted on the pans using a carbon double sided tape and were covered with gold (1 min, 50–100 mTorr, Denton Vacuum Desk II). Micrographs were obtained at 15 kV and 2000× [17] for parenchymal tissue and at 2000× and 25 kV for starch granules [18].

2.4 Laser confocal scanning microscopy

Samples (0.5 cm × 0.5 cm × 0.5 cm) were stained with 2 mL of 0.15% Rhodamine B (83689-1G Sigma) solution in water [19, 20]. The excess of dye was removed (deionized water) and the sample was dried (Binder, 53 FD, Germany) at 30°C for 24 h. Micrographs were obtained using a confocal laser scanning microscope (LSM 710, Carl Zeiss, Wurttemberg, Germany) with 20× lenses at a wavelength of 518 nm for excitation of Rhodamine B, and maximum emission at 568 nm. Images were acquired in RGB color.

2.5 X-ray diffraction

An X-ray diffractometer (Rigaku Miniflex 600) was used. Native and thermally treated starch samples (5%, moisture,) were placed in the sample holder and the diffraction patterns were obtained (45 kV, 40 mA, CuK, $\lambda = 0.154$ nm, 3° to 35° (2θ°)). The degree of crystallinity (Eq. 1) was evaluated [21].

$$\text{Degree of crystallinity } (\%) = \frac{A}{A+B} \times 100 \quad (1)$$

where A is the integrated intensity of the crystalline phase and $(A + B)$ is the total area of the diffractogram.

2.6 Particle size analysis

The particle sizes were measured using a laser diffraction particle size analyzer (CILAS 1090 LD, France; laser diode 830 nm, liquid mode with distilled water). To prevent undesired settling, the mixture suspension was continuously sonicated (60 s).

Two different Feret diameters were measured (minimum and maximum), which are defined as the minimal and maximal distance between two tangents to the contour of the particle after consideration of all possible orientations. Using the ratio of these diameters (minimum/maximum) the aspect ratio (AR) was calculated. Expert Shape program (Version 4.14 CILAS, 2011) was used to analyze data.

2.7 Differential scanning calorimetry (DSC)

A Mettler Toledo DSC823e was used. Sample (3 mg, moisture content 6%) was weighed into an aluminum capsule (100 μL) adding water (9 μL). The hermetically sealed containers were allowed to stand for 24 h at room temperature. Analysis was carried out by heating the sample from 30 to 120°C at a rate of 10°C/min [19]. The STARe software v9.00 was used to analyze data. Onset (T_o), peak (T_p) and end (T_e) temperatures and gelatinization enthalpy (ΔH_g) were recorded. The gelatinization percent or gelatinization degree (Eq. 2) was calculated in relation to that of native jicama starch [6].

$$\mathrm{GD}(\%) = \left(1 - \frac{\Delta H_{g\ \mathrm{sample}}}{\Delta H_{g\ \mathrm{native}}}\right) \times 100$$

$\mathrm{GD}(\%) =$ Gelatinization percent or gelatinization degree

$\Delta H_{g\ \mathrm{sample}} =$ Gelatinization enthalpy of the sample

$\Delta H_{g\ \mathrm{native}} =$ Gelatinization enthalpy of the native jicama starch (2)

2.8 Glycemic index (GI)

Starch samples (10 mg db) were mixed with 2 mL of HCl-KCl buffer (pH = 1.5) and added to 0.04 mL of a 1% pepsin solution (pH = 1.5 HCl-KCl buffer), keeping the samples in a water bath (40°C, 1 h), and completing the volume to 5 mL (tris maleate buffer solution, pH = 6.9) obtaining the sample solution [22].

Subsequently, 5 mL of tris maleate solution (pH = 6.9) containing alpha-amylase (2.6 U, A3176 Sigma) was added to the sample solution, incubating (37°C) with agitation in a water bath (Aqua bath analog 18020AQ, Barnstead, In.), taking 0.5 mL aliquots every 30 min over a period of 0–3 h. Each aliquot was placed in another water bath at 100°C and stirred vigorously for 5 min to inactivate the enzyme. Then 0.6 mL sodium acetate buffer (pH = 4.75) and 12 μL of amyloglucosidase from Aspergillus niger (A7420, Sigma) were poured to each aliquot to hydrolyze the starch. The samples were heated (60°C, 45 min) under stirring, adjusting the volume of the aliquot to 2 mL with distilled water. Finally, the glucose content was determined (GAGO-20, SIGMA), and results multiplied by a factor of 0.9 to convert glucose to starch [22]. These results were transformed to percentage of hydrolyzed starch.

To obtain the glycemic index (eGI, Eq. 3 [23]):

$$\mathrm{eGI} = 8.189 + (0.0862 \times \mathrm{HI}) \quad (3)$$

it was necessary to evaluate the hydrolysis index (HI) (Eq. 4):

$$\mathrm{HI} = \frac{\mathrm{AUC_{sample}}}{\mathrm{AUC_{reference}}} \quad (4)$$

which depends on both, the area under the starch hydrolysis curve of the sample ($\mathrm{AUC_{sample}}$, Eq. 5), and the curve of a reference product ($\mathrm{AUC_{reference}}$) based in wheat flour white bread.

$$\mathrm{AUC_{sample}} = C_\infty(T_f - T_0) - \left[\left(\frac{C_\infty}{k}\right)\left[1 - e^{-k(T_f - T_0)}\right]\right] \quad (5)$$

To solve Eq. (5), it was necessary to know the concentration at equilibrium (C_∞), the kinetic constant (k), and the final ($T_f = 180$ min), and initial ($T_0 = 0$ min) hydrolysis times, which were obtained from plotting and fitting to an exponential curve of two parameters the starch hydrolysis percent data versus hydrolysis times (SigmaPlot 12.0 software) (Eq. 6).

$$C = C_\infty(1 - e^{-kt}) \quad (6)$$

where C is the concentration at time t, C_∞ is the concentration at equilibrium, k is the kinetic constant and t is the hydrolysis time.

2.9 Statistical analysis

All experiments were carried out at least in triplicates, reporting the average and standard deviation values. In some results, the minimum and maximum were also reported. Data were statistically analyzed (SigmaPlot V.12.0. Systat Software Inc., San José, CA, USA). The $p < 0.05$ values were considered significantly different. Mathematical models were calculated using the software Table Curve 3D v4.0.01 for windows (Systat Software Inc.).

3 Results and discussion

3.1 Microscopic analysis

Figure 1A, B show that jicama starch granules tended to form aggregates, having polyhedral form. Once these granules

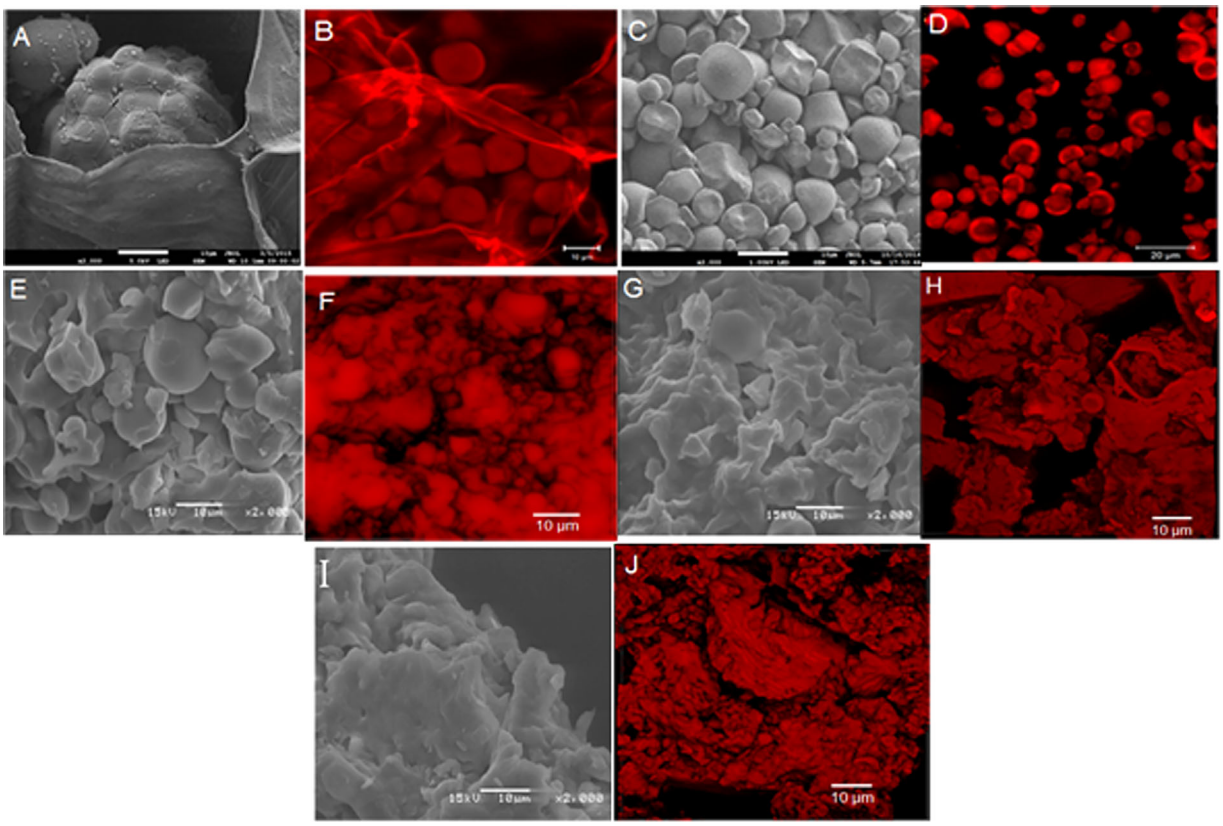

Figure 1. SEM and confocal micrographs of jicama native and thermally processed starch. (A and B) Starch grains in raw jicama cells, (C and D) isolated native starch granules, (E and F) starch heated up to 60°C, (G and H) starch heated up to 70°C, and (I and J) starch heated up to 80°C.

were released (Fig. 1C, D), different sizes and shapes (polyhedral and spherical) were observed. Polygonal shapes can be related to the compactness degree of the starch granules forming the conglomerates, as well as to their size and maturity degree. In this regard, it has been published that compound starch granules are the result of the fusing of different granules, developing simultaneously within a single amyloplast [24], while the different sizes occur as a result of the different initiation stages during starch biosynthesis [3]. In this process, the larger granules are the firsts to be synthesized, while the smaller ones are generally synthesized later during development [25].

The effect of heating the sample up to 60°C (Fig. 1E, F) in the presence of water caused the starch granules to swell, losing their distinctive features, but in an uneven way, as some of them did not appear to be gelatinized or swollen, maintaining their size and shape. On reaching 70°C, the granules continued to swell, resulting in the loss of some physical characteristics. (Fig. 1G, H). At 80°C, the shape of the granules had completely changed. This effect was also studied using polarized light and confocal laser microscopes, where loss of birefringence (Fig. 2A, B) and granular structure (Fig. 3A, B) were also observed. It is important to mention that some granules, even when the samples were heated up to 80°C, did not change their shape and size, and continue presenting birefringence, which could mean that the granules still remained native (NS) (Figs. 2B and 3B). In this regard, Muñoz et al. [26] reported that the loss of birefringence and the change in the granule size (swelling) began almost simultaneously, with only a small difference in temperature, and when the granule had reached 50% swelling, birefringence was zero.

3.2 X-ray diffraction analysis

Native starches (Fig. 4A and A*) showed strong diffraction peaks at 15° and 23° (2θ), an unresolved doublet at 17° and 18° (2θ), few small peaks at around 5.6°, 9.6°, 11.6°, 20°, and 26.4° (2θ), and the lack of a split peak at 22° and 24° (2θ), which is a clear indication of a C_A-type X-ray diffraction [6]. These results are similar to those reported by Stevenson et al. [3] for jicama starch regarding type of starch and percentage of crystallinity ($34.3 \pm 0.3\%$) and within the range of other starches reported and cited by different authors [27–30].

Thermally treated starches, independently of the testing temperature, showed a modification of their X-ray diffraction pattern as well as a decrease in their intensity counts (Fig. 4B–F). The main changes corresponded to the split of 23° (2θ) peak, resulting in the formation of two smaller ones at 22.2° and 24.1° (2θ), and the disappearance of the shoulder

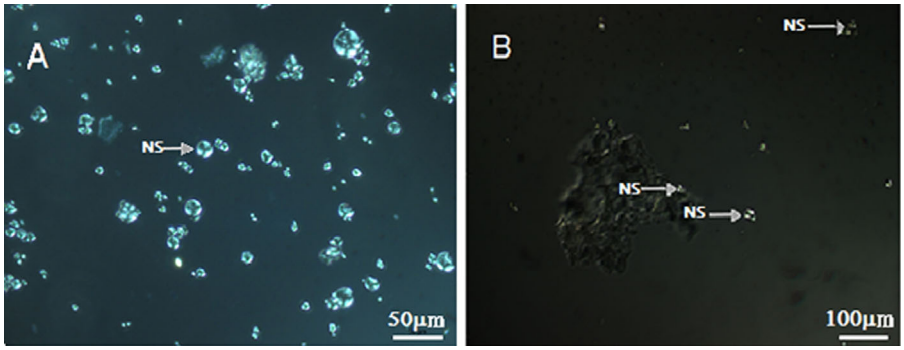

Figure 2. Polarized light microscopy images. (A) Native jicama starch, (B) starch thermally treated up to 80°C. NS, native starch.

(18°, (2θ)), leaving only the single peak at 17.3° (2θ). This change in the crystalline path indicates the prevalence of C_B-type crystallites. This modification could be related to the rearrangement of the starch molecules, as has been reported by Zhang et al. [31] who observed this transition from A to B polymorphic type in C-type kudzu starch as a result of the annealing process. It has also been published [6] that in the interior of C-type starch granules the B-type allomorph exists, and it is surrounded by the A-type allomorph at its periphery, promoting a faster A-type degradation that will result in the obtained C_B-type starch.

With respect to crystallinity percentage, as expected, all thermal treated samples decreased their crystallinity as their temperature increased, varying from 20.8% (60°C) to 11.5% (80°C), but without completely losing their crystalline character. It was also observed from diffraction patterns that heated samples presented a much more defined C_B-type crystalline structure than native jicama starch. According to Tester and Karkalas [27], the application of heat causes an irreversible breakdown of the structure of the starch, decreasing its crystallinity.

3.3 Particle size distribution

From the morphometric evaluation, it was found that jicama native starch granules follow a unimodal distribution. Granule sizes varied from a minimum diameter of 3 μm to a maximum of 21 μm with an average value of 10.04 ± 3.54 μm. The largest proportion of granules in the sample however, had diameters ranging between 9 and 12 μm representing about 47% of the sample.

Figure 5B–F showed the mean diameter frequency distributions of thermal treated samples. This figure also shows a micrograph, where the change in the shape of the starch granules due to the thermal process can be observed.

From Fig. 5B, a significant increment in the percentage of particles smaller than 9 μm, and a decrement in the percentage of the larger ones as compared to control can be observed. In these thermally treated particles, the size varied from 3.04 to 36 μm, where the highest proportion was observed between 4 and 9 μm. At higher temperature (Fig. 5C–F), a change in the shape and number of granules was noticeable. At 65°C, the number of granules larger than 9 μm also decreased, giving rise to larger particles but in a smaller proportion. As heating continued (80°C), most of the starch was disaggregated into smaller particles, and a few big agglomerates were formed. However, some heat-resistant starch remained in the samples. The increment in the number of small particles could be related to the gelatinization of granules and their disaggregation, enhanced by the mechanical work (friction and shear forces develop during liquid displacement) and sound energy

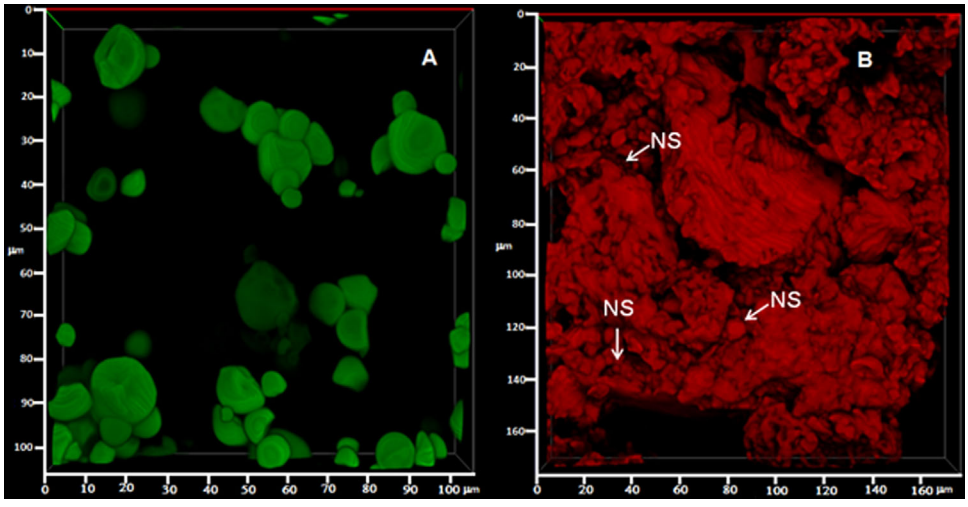

Figure 3. Confocal laser scanning microscopy of native and thermally treated jicama starch. (A) Isolated native starch granules, (B) starch thermally processed up to 80°C. NS, native starch.

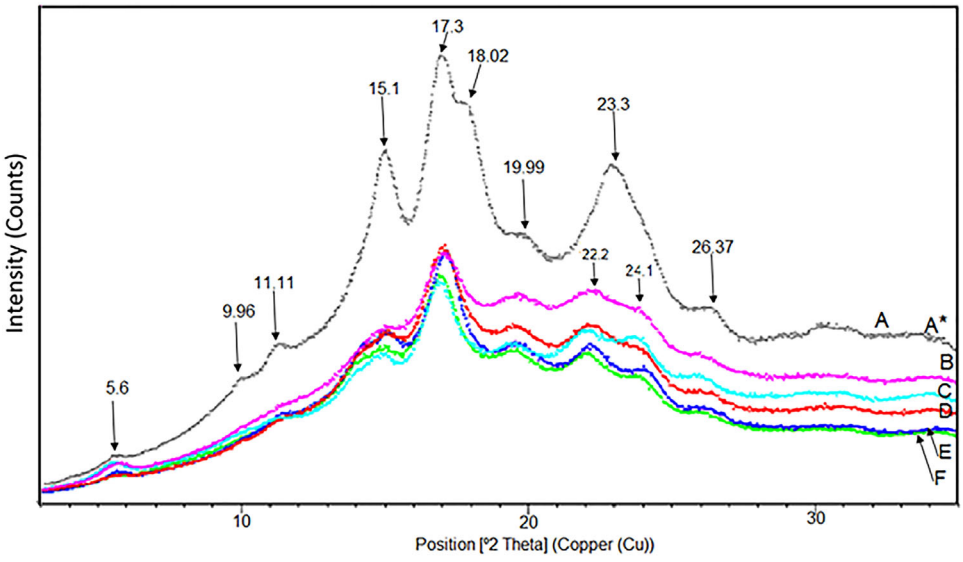

Figure 4. X Ray diffraction patterns of jicama tuber root, native and thermal treated starches. (A) Jicama tuber root (black line), (A*) jicama native starch (gray line), (B) 60°C (pink line), (C) 65°C (light blue line), (D) 70°C (red line), (E) 75°C (dark blue line), and (F) 80°C (green line).

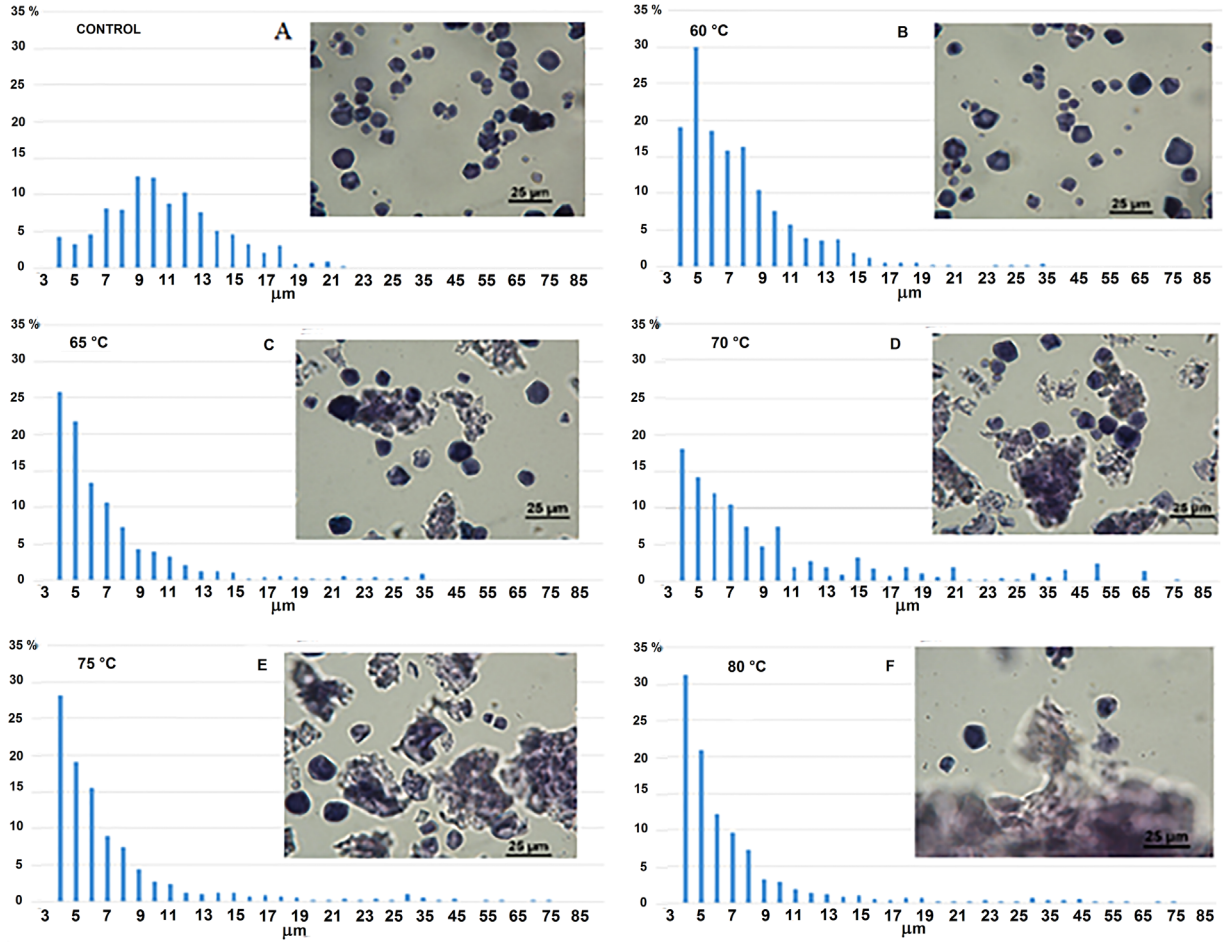

Figure 5. Diameter frequency distributions (laser diffraction particle size analysis) and light microscopy images of native and thermal treated jicama starch stained with lugol as examples of the changes in the shape of starch during thermal processing. (A) Native starch, (B) 60°C, (C) 65°C, (D) 70°C, (E) 75°C, and (F) 80°C.

(sonication) applied to disperse particles of the samples during particle size analysis.

In this regard, Parada and Aguilera [5] mentioned that larger granules tend to gelatinize before the smaller ones, and that the survivors, with higher gelatinization temperatures, remain native.

The frequency size distribution histograms give information on how the sizes of starch particles (native, partially, or fully gelatinized) change in number during the heating process, but it is not possible to specify what degree of gelatinization the starch granules have suffered with that information.

The aspect ratio (AR) is the ratio of the minimum Feret diameter to a maximum Feret diameter and quantifies the "squareness" or "roundness" of an object in an image [32].

Based on the results of the analysis of particle size, where jicama native starch granules presented spherical or polyhedral shapes, and sizes ranging from 3 to 21 μm, and according to Olson [32], who reported that such forms have an aspect ratio (AR) ranging from 0.89 to 1, we assumed that native starches from jicama will have sizes and AR values between these ranges. We also supposed that as temperature increases, starch granules will deform, changing their AR to lower values. Hence, if these assumptions are true, all those particles with AR values smaller than 0.89 and larger diameters than those observed in native starches, can be considered to have suffered some degree of gelatinization.

Based on the given assumptions, we proposed that the gelatinization degree could be measured by the aspect ratio data (Eq. 7) by relating the number of total starch particles and the number of non deformed heated particles (native starch, intact particles):

$$GD_{AR} = \frac{TS_p - IP}{TS_p} \times 100 \quad (7)$$

where: GD_{AR}, gelatinization degree obtained by aspect ratio data; TS_p, total starch particles. Data obtained from laser diffraction particle size analysis; IP, intact particles corresponds to non deformed-heated particles that remain in the sample after the heating treatment.

Figure 6 shows a dot plot of the aspect ratio of starch particles versus their diameters. For native starches (NS, Fig. 6A), the aspect ratio (AR) for almost all granules reached values from 0.89 to 1, while for those granules heated up to 60°C (Fig. 6B), a significant decrement in the proportion of intact particles as compared to control was observed. In these thermally treated particles, the size varied from 3.04 to 46 μm, with the highest concentration being between 4 and 9 μm. At higher temperatures (Fig. 6C–F), a change in the shape and number of granules was noticed. At 65°C, the number of intact particles continued to decrease and as heating continued (70–80°C), most of the samples were disaggregated into small particles, and a few big agglomerates were formed, increasing the percentage of particles with an aspect ratio from 0.21 to 0.88. It is important to mention that in all thermally treated samples, granules presenting aspect ratio values from 0.89 to 1 and diameters between 3 and 21 μm were found. This type of granules was also observed by microscopy analysis. Based on the size and AR values, these starch jicama granules could be considered as heat-resistant starch.

3.4 Differential scanning calorimetry (DSC)

The gelatinization onset (T_o), peak (T_p), and end (T_e) temperatures and the gelatinization enthalpies (ΔH_g) of native, and thermal treated starches (60, 65, 70, 75, and 80°C) as measured by DSC are presented in Table 1.

Native starch presented onset and peak temperatures of 60.7°C, and 66.6°C, respectively, as well as a gelatinization enthalpy of 13.0 ± 0.2 J/g. These values are higher than those reported by Stevenson et al. [3] ($T_o = 52 \pm 0.9$°C, $T_p = 58.6 \pm 1.7$°C, $\Delta H_g = 15.1 \pm 0.1$ J/g) and Martinez-Bustos et al. [33] ($T_o = 54.6$°C, $T_p = 61.6$°C, $\Delta H_g = 10.2$ J/g), but similar to those reported by Martinez-Bustos et al. [34] ($T_o = 60.4$°C, $T_p = 66.2$°C, $\Delta H_g = 11.0$ J/g). These differences can be related to the origin of the raw materials (Queretaro, Guanajuato, and Nayarit), where different jicama varieties are produced, as well as the way of preparing the samples (water content, time to equilibrate the sample before analysis).

Based on this enthalpy information, it was noticed that most of the starch granules were gelatinized during heat treatments, leaving just a low percentage unaffected, as they required a smaller amount of energy (1.42–0.34 J/g) during DSC test to gelatinize as compared to the energy required when analyzing native starch (13.04 J/g).

Since the gelatinization degree during heat treatments did not reach the 100%, it is assumed that some granules were more heat-resistant than others, which was also confirmed by microscopy and laser diffraction particle size analysis. It was also observed that the largest degree of gelatinization was obtained at the highest heating temperatures (75 and 80°C).

In this regard, Parada and Aguilera [5] mentioned that the temperature of gelatinization may reflect the fact that a population of starch granules has a distribution of melting temperatures, where larger granules tend to gelatinize before small ones, which indicates that, after heating, the survivor granules have, at any processing temperature, gelatinization temperatures higher than those that had already gelatinized, resulting in intact starches after heat treatment.

Regarding the effect of heat treatment, jicama starch, as expected, presented high degree of gelatinization, reaching values from 89.1 ± 0.09% at 60°C to 97.4 ± 0.13% at 80°C (Table 1) and these results were very similar between both methodologies, which means that the AR methodology could be an alternative way to determine gelatinization degree. It is important to mention that the high degree of gelatinization obtained at low temperature (60°C) could be

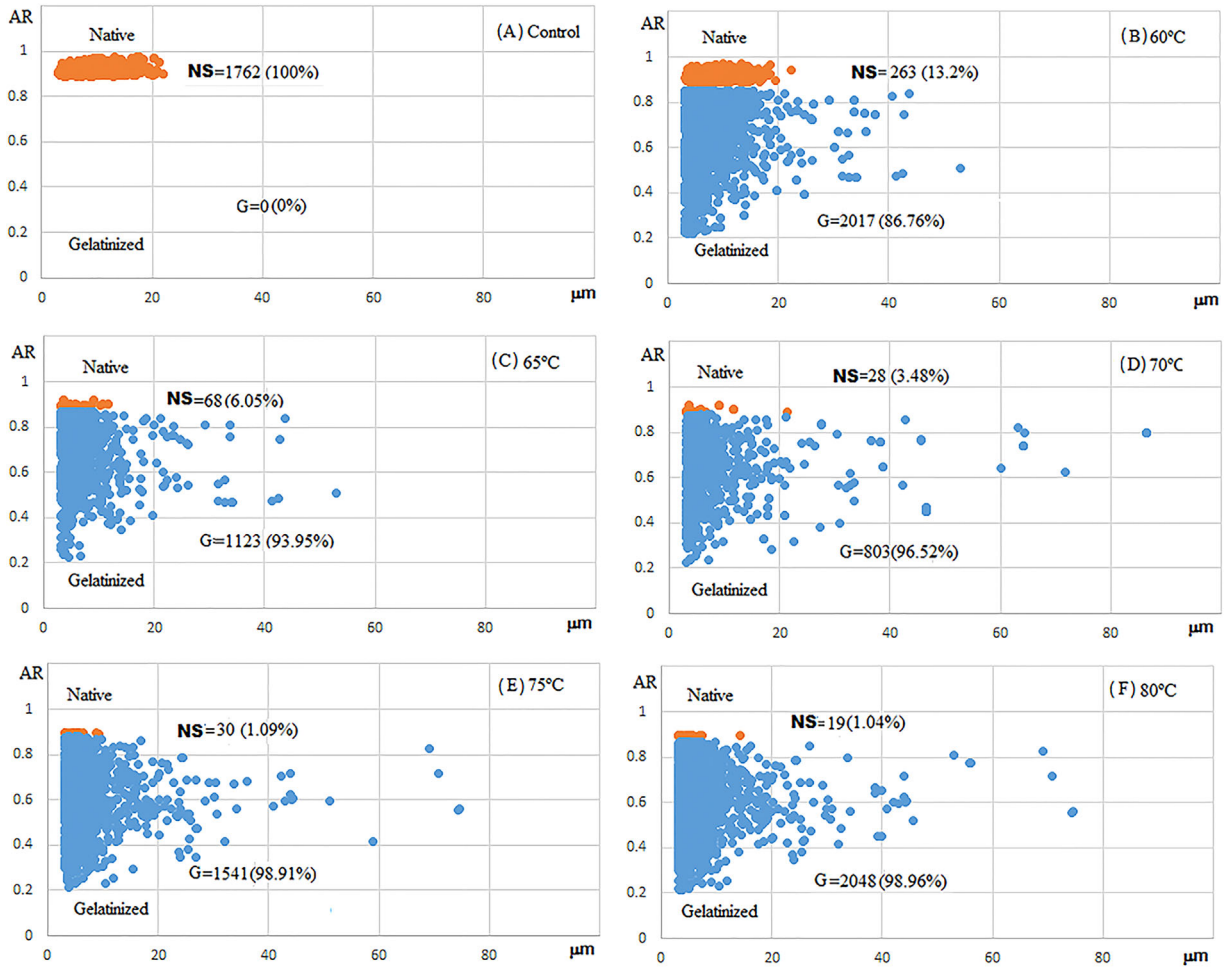

Figure 6. Aspect ratio scatter plot of native and heat treated starches versus starch diameter. (A) Native starch (control); (B–F) heat treated samples at 60°C, (B) 65°C, (C) 70°C, (D) 75°C, and (E) 80°C (F). NS, native starch particles number; G, gelatinized starch particles number.

the result of a thermal inertia of the system after reaching the target-temperature in the core of the samples when extracted from the oven.

3.5 Glycemic index

The results show that native starch has a high glycemic index (GI >70) [22], but similar to that of rice, pea [22], sweet potato [35], and the thermally processed samples presented even higher GI values, which correlated with a higher gelatinization degree; these results are consistent with those obtained by microscopy, DSC, and X-ray diffraction analysis. In this regard, Jane et al. [36] expressed that depending on the type (A or B) and the degree of starch crystallinity (%), the enzymatic response to starch hydrolysis will change. They reported that starches with A-type crystallinity are more

Table 1. Differential scanning calorimetry parameters of native and heated jicama starch, gelatinization degree, and glycemic index

Sample	ΔH_g (J/g)	T_o (°C)	T_p (°C)	T_e (°C)	GD_{DSC}%	GD_{AR} %	eGI (%)
Native starch	13.0 ± 0.02^a	60.7 ± 0.2^a	66.6 ± 0.4^a	77.8 ± 0.9^a	–	–	74.1 ± 0.01^a
60°C	1.4 ± 0.01^b	68.7 ± 0.4^b	73.4 ± 0.02^b	77.9 ± 1.4^b	89.1 ± 0.09^a	86.8 ± 0.16^a	90.2 ± 0.15^c
65°C	1.2 ± 0.02^c	69.5 ± 0.7^b	74.0 ± 0.10^b	77.7 ± 0.6^a	91.1 ± 0.12^b	93.9 ± 0.02^b	95.5 ± 0.02^d
70°C	0.6 ± 0.01^d	70.8 ± 0.2^c	74.0 ± 0.94^b	77.5 ± 0.7^a	95.2 ± 0.10^c	96.6 ± 0.02^c	96.4 ± 0.29^e
75°C	0.3 ± 0.02^e	71.7 ± 0.3^d	74.1 ± 0.11^b	77.9 ± 2.0^a	97.4 ± 0.13^d	98.2 ± 0.01^d	97.1 ± 0.01^f
80°C	0.3 ± 0.02^e	71.1 ± 0.1^{cd}	74.5 ± 1.86^b	77.0 ± 2.0^a	97.4 ± 0.13^d	98.5 ± 0.01^e	98.6 ± 0.30^g

ΔH_g, gelatinization enthalpy; T_o, onset temperature; T_p, peak temperature; T_e, end temperature; GD_{DSC}, gelatinization degree from DSC; GD_{AR}, gelatinization degree from aspect ratio; eGI, glycemic index.
Data is the average value ± standard deviation. Values followed by a different letter in the same column are significantly different ($p < 0.05$).

Table 2. Pearson correlation factors

Variables	Correlation
GI vs. %C	$R = -0.988$; $p = 0.001$
GI vs. GD_{DSC}	$R = 0.969$; $p = 0.025$
%C vs. GD_{AR}	$R = -0.950$; $p = 0.048$
%C vs. GD_{DSC}	$R = -0.940$; $p = 0.050$
GI vs. GD_{AR}	$R = 0.977$; $p = 0.017$
GD_{DSC} vs. GD_{AR}	$R = 0.999$; $p = 0.005$

GI, glycemic index; %C, crystallinity percentage; GD_{AR}, gelatinization degree evaluated by aspect ratio (Cilas); GD_{DSC}, gelatinization degree evaluated by enthalpy of gelatinization (DSC data); *R*, correlation values; $p < 0.05$.

susceptible to hydrolysis than B-type, which is consistent with the change of C_A-type to C_B-type observed in our samples and the glycemic index values obtained.

3.6 Correlation analysis

The correlations among crystallinity and gelatinization degree with glycemic index were evaluated (Table 2). These data indicated a significant negative correlation between glycemic index and crystallinity (−0.988), as well as a significant positive correlation between glycemic index and degree of gelatinization (0.969, 0.977), indistinctly of the method employed to determine the gelatinization degree (GD_{DSC}, GD_{AR}). Therefore, there is a possibility to relate the degree of gelatinization of starches to their aspect ratio.

Looking for an expression that allows an easy way to determine glycemic index, those parameters that presented high correlation coefficient values with glycemic index were evaluated. Best results were found with crystallinity percentage and both degrees of gelatinization (GD_{DSC}, GD_{AR}) as independent variables and glycemic index as dependent variable (Fig. 7A, B). Mathematical modeling shows that as crystallinity decreased, and the gelatinization degree increased, no matter how this parameter was evaluated (DSC or AR) the glycemic index increased. The mathematical behavior of this effect is presented in Eqs. (8 and 9), where the determination coefficients were 0.9859 when using DSC methodology and 0.9895 when evaluating GD by AR.

$$GI = 110.03 - 10.19\text{Ln}\ C + 0.1338\ GD \quad (8)$$

$$GI = 106.85 - 9.30\text{Ln}\ C + 0.1415\ GD_{AR} \quad (9)$$

where: GI, glycemic index; GD, gelatinization degree by DSC (%); GD_{AR}, gelatinization degree by aspect ratio (%); *C*, crystallinity percent. Both equations are much alike, which means that both methodologies render the same results.

4 Conclusions

Native jicama starch granules presented aspect ratio values, as measured by dynamic light scatering, from 0.89 to 1 which are in accordance with their shape. The degree of starch gelatinization based on the aspect ratio data showed a minimum value of 87.7% for samples treated at 60°C and maximum of 98.9% for samples heated up to 80°C. Native jicama starch exhibited a C_A-type X-ray diffraction pattern, while those thermally treated showed a transition from the C_A-type to C_B-type, and a decreasing crystallinity. Jicama native starch presented a high glycemic index, while thermal treatment increased this value as expected. Glycemic index showed high correlation coefficients (>0.98) with the degree of gelatinization, calculated from AR or DSC, and

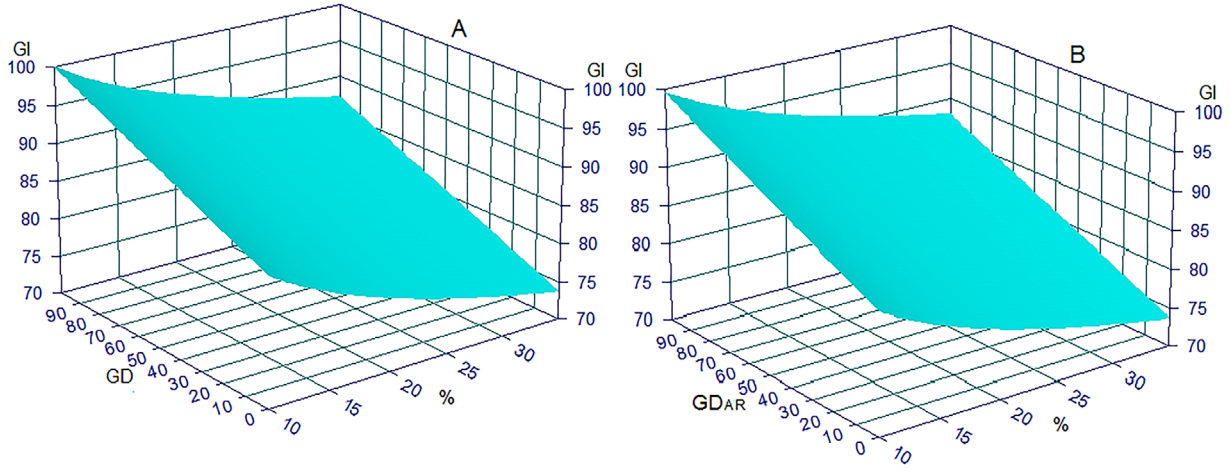

Figure 7. Mathematical model correlating crystallinity, gelatinization degree and glycemic index. %, crystallinity; GD, gelatinization deegre by DSC; GD_{AR}, gelatinization degree by aspect ratio; GI, glycemic index.

crystallinity. The result of this work indicates that the aspect ratio of starch granules measured by dynamic light scattering can be used as a simple methodology to quantitatively determine glycemic index. More studies in different matrices are required to validate the mathematical models to establish the relation of glycemic index and the aspect ratio of starch granules.

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5 References

[1] Martinez-Bustos, F., Lopez-Soto, M., Zazueta-Morales, J. J., Morales-Sanchez, E., Preparation and properties of pregelatinized cassava *(Manihot esculenta Crantz*) and jicama (jícama) using ohmic heating. *Agrociencia* 2005, *39*, 275–283.

[2] Heredia, Z. A., Jicama guide to grow in the Bajio. Celaya, Guanajuato, Mexico. *INIFAP Bajío Experimental Field* 1996, 24.

[3] Stevenson, D. G., Jay-lin, J., Inglett, G. E., Characterisation of jicama (mexican potato) (jícama *L. Urban*) starch from taproots grown in USA and Mexico. *Starch/Stärke* 2007, *59*, 132–140.

[4] Ramos De La Peña, A. M., Renard, C. M. G. C., Wiker, L., Contreras-Esquivel, J. C., Advances and perspectives of *Pachyrhizus spp.* In food science and biotechnology. *Trends Food Sci. Technol*. 2013, *29*, 44–54.

[5] Parada, J., Aguilera, J. M., Effect of native crystalline structure of isolated potato starch on gelatinization behavior and consequently on glycemic response. *Food Res. Int*. 2012, *45* (1), 238–243.

[6] Cai, C., Wei, C., In situ observation of crystallinity disruption patterns during starch gelatinization. *Carbohydr. Polym*. 2013, *92*, 469–478.

[7] Knorr, D., Heinz, V., Buckow, B., High pressure application for food biopolymers. *Biochim. Biophys*. 2006, *1764*, 619–631.

[8] Tester, R. F., Morrison, W. R., Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chem*. 1990, *67*, 551–557.

[9] di Paola, R. D., Asis, R., Aldao, M. A. J., Evaluation of the degree of starch gelatinization by a new enzymatic method. *Starch/Stärke* 2003, *55*, 403–409.

[10] Chiang, Y., Johnson, J. A., Measurement of total and gelatinized starch by glucoamylase and o-toluidine reagent. *Cereal Chem*. 1977, *54*, 429–435.

[11] Mendes Da Silva, C. E., Ciacco, C. F., Barberis, G. E., Solano, W. M. R., Rettori, C., Starch gelatinization measured by pulsed nuclear magnetic resonance. *Cereal Chem*. 1996, *73*, 297–301.

[12] Parada, J. A., Rozowski, J., Relationship between glycemic response of starch and its microstructural state. *Chil. J. Nutr. (Revista Chilena de Nutrición)* 2008, *35*, 84–92.

[13] Novelo-Cen, L., Betancur-Ancona, D., Chemical and functional properties of *Phaseolus lunatus* and *Manihot esculenta* starch. *Starch/Stärke* 2005, *57*, 431–441.

[14] American Association of Cereal Chemists, (Ed) *Approved Methods of the American Association of Cereal Chemists, Method: 76-30A*, 10th edn., AACC, St. Paul, MN, USA 2000.

[15] Hoover, R., Ratnayake, W., Starch characteristics of black bean, chick pea, lentil, navy bean, and pinto bean cultivars grown in Canada. *Food Chem*. 2002, *78*, 489–498.

[16] AOAC International *Official Methods of Analysis of AOAC International, Methods: 923.03, 44.19*, 17th edn., AOAC International, Gaithersburg, MD, USA 2002.

[17] Bordoloi, A., Kaur, L., Singh, J., Parenchyma cell microstructure and textural characteristics of raw and cooked potatoes. *Food Chem*. 2012, *133*, 1092–1110.

[18] Amaya-Llano, S. L., Martínez-Bustos, F., Martínez-Alegría, A. L, Zazueta-Morales, J. J., Comparative studies on some physicochemical, thermal, morphological, and pasting properties of acid thinned jicama and maize starches. *Food Biop. Technol*. 2011, *4*, 48–60.

[19] Díaz-Ramírez, M., Calderón-Domínguez, G., Chanona-Perez, J. J., Janovitz-K, A., et al., Modelling sorption kinetic of sponge cake crumb added with milk syrup. *Int. J. Food Sci. Technol*. 2013, *48*, 1649E–1660E.

[20] Kolmakov, K., Belov, V. N., Bierwagen, J., Ringemann, C., et al., Red-emitting rhodamine dyes for fluorescence microscopy and nanoscopy. *Chem. Eur. J*. 2010, *16*, 158–166.

[21] Ribotta, P. D., Cuffini, S., León, A. E., Añón, M. C., The staling of bread: An X-ray diffraction study. *Food Hydrocolloids* 2004, *18*, 305–313.

[22] Goni, I., Garcia-Alonso, A., Saura-Calixto, F., A content starch hydrolysis procedure to estimate glycemic index. *Nutr. Res*. 1997, *17*, 427–437.

[23] Granfeldt, Y., Bjorck, I., Drews, A., Tovar, J., An in vitro procedure based on chewing to predict metabolic response to starch in cereal and legume products. *Eur. J. Clin. Nutr*. 1992, *46*, 649–660.

[24] Buleon, A., Colonna, P., Planchot, V., Ball, S., Starch granules: Structure and biosynthesis. *Int. J. Biol. Macromol*. 1998, *23*, 85–112.

[25] Parker, M. L., The relationship between A-type and B-type starch granules in the developing endosperm of wheat. *J. Cereal Sci*. 1985, *3*, 271–278.

[26] Muñoz, L. A., Pedreshi, F., Leiva, A., Aguilera, J. M., Loss of birefringence and swelling behavior in native starch granules: Microstructural and thermal properties. *J. Food Eng*. 2015, *152*, 65–71.

[27] Tester, R. F., Karkalas, J., Starch-composition, fine structure, and architecture. *J. Cereal Sci*. 2004, *39*, 151–165.

[28] Cheetham, N. W. H, Tao, L., Variation in crystalline type with amylose content in maize starch granules: An X-ray powder diffraction study. *Carbohydr. Polym*. 1998, *36*, 277–284.

[29] Morrison, W. R., Tester, R. F., Gidley, M. J., Karkalas, J., Resistance to acid hydrolysis of lipid complexed amylose and

lipid free amylose in linterized waxy and non-waxy barley starch. *Carbohydr. Res*. 1993, *245*, 289–302.

[30] Pantindol, J., Wang, Y. J., Fine structures and physico-chemical properties of starches from chalky and translucent rice kernels. *J. Agric. Food Chem*. 2003, *51*, 2777–2784.

[31] Zhang, B., Wu, C., Li, H., Hu, X., et al., Long term annealing of C-type kudzy starch: Effect on crystalline type and other physicochemical properties. *Starch/Stärke* 2015, *67*, 577–584.

[32] Olson, E., Particle shape factors and their use in image analysis – part 1: Theory as published in GXP. *Summer* 2011, *15*, 3.

[33] Martínez-Bustos, F., López-Soto, M., San Martín-Martínez, E., Zazueta-Morales, J. J., Velez-Medina, J. J., Effects of high energy milling on some functional properties of jicama starch (*Pachyrhizus erosus L. Urban*) and cassava starch (*Manihot esculenta Crantz*). *J. Food Eng*. 2007, *78*, 1212–1220.

[34] Martínez-Bustos, F., Amaya-Llano, S. L., Carbajal-Arteaga, J. A., Chan, Y. K., Zazueta-Morales, J. D. J., Physicochemical properties of cassava, potato, and jicama starches oxidised with organic acids. *J. Sci. Food Agric*. 2007, *87*, 1207–1214.

[35] Foster-Powell, K., Holt, S. H. A., Brand-Miller, J. C., International table of glycemic index and glycemic load values 2002. *Am. J. Clin. Nutr*. 2002, *76*, 5–56.

[36] Jane, J. L., Kasemsuwan, T., Leas, S., Zobel, H., et al., Anthology of starch granule morphology by scanning electron-microscopy. *Starch/Stärke* 1994, *46*, 121.

