Ingeniería de alimentos

Revista Mexicana de Ingeniería Química

Vol. 13, No. 1 (2014) 151-163

EFFECT OF COOKING, ANNEALING AND STORAGE ON STARCH DIGESTIBILITY AND PHYSICOCHEMICAL CHARACTERISTICS OF UNRIPE BANANA FLOUR

EFECTO DEL COCIMIENTO, ANILLADO Y ALMACENAMIENTO EN LA DIGESTIBILIDAD Y LAS CARACTERÍSTICAS FISICOQUÍMICAS DE HARINA DE PLÁTANO VERDE

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Received October 7, 2013; Accepted November 5, 2013

Abstract

The effect of cooking (C) unripe banana fruits at different times (5, 15 and 25 min), followed by annealing (ANN) and annealing + storage (ANN+S) were evaluated. Cooking of unripe banana fruit for longer times (15 and 25 min) decreases the RS content, but increases the SDS fraction. Granular aggregates were produced, which could be formed by partially gelatinized granules. ANN and ANN+S increased the RS content, while the SDS value did not change. The pasting behaviour of ANN and ANN+S showed low viscosity without breakdown or setback due to the restricted amylose leaching, which was higher in flours with ANN+S. The ANN and ANN+S increased the gelatinization temperatures while the annealed and stored samples showed an increase in the crystallinity level due to reorganization of the granular structure compared with native and cooking treated samples. It is possible to combine different treatments to modify the starch digestibility of unripe banana flour, due to that changes in the granular conformation were produced, which decrease its ability to swell and to gelatinize.

Keywords: annealing; starch digestibility; banana flour; resistant starch.

Resumen

Se evaluó, en frutos de plátano verde, el efecto de la cocción (C) a diferentes tiempos (5, 15 y 25 min) seguido de *annealing* (ANN), y *annealing* + almacenamiento (ANN+A). La cocción de los frutos durante tiempos prolongados (15 y 25 min) disminuyó el contenido de almidón resistente (AR), pero incrementaron la fracción de almidón de digestión lenta (ADL). Se produjeron agregados granulares, los cuales pueden estar formados por gránulos parcialmente gelatinizados. El ANN y ANN+A incrementaron el contenido de AR, mientras que el ADL no cambió. La viscosidad de pastas con el ANN y el ANN+A fueron bajas, sin rompimiento granular o re-asociación, debido a restricción en el hinchamiento de los gránulos y la baja lixiviación de amilosa, la cual fue mayor en las muestras tratadas con ANN+A. El ANN y el ANN+S incrementaron las temperaturas de gelatinización, así como su cristalinidad, debido a la reorganización de la estructura granular comparada con las muestras nativas y con cocción. Es posible combinar diferentes tratamientos para modificar la digestibilidad del almidón del plátano verde, debido a los cambios que se producen en la conformación granular, los cuales disminuyen su hinchamiento y gelatinización.

Palabras clave: anillado; digestibilidad del almidón; harina de plátano; almidón resistente.

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1 Introduction

Starch is the main carbohydrate in the human diet and has been widely used as an ingredient in the food industry. Nowadays, consumers are in search of products that can improve their health and well-being. It is well known that unripe banana starch has a very low digestion rate when raw (Faisant *et al*., 1995). And that this characteristic has a beneficial effect on colonic health. According to its rate of digestion and glucose release, starch has been classified into three fractions: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). After ingestion, the RDS fraction causes a sudden increase in blood glucose level, the SDS is hydrolyzed completely in the small intestine at a lower rate compared to the RDS; and the RS is the portion that cannot be digested in the small intestine, but rather is fermented in the large intestine (Englyst *et al*., 1992). However, it is important to keep in mind that when the unripe fruit is cooked, as occurs in many countries of South America and Africa, its native RS is rendered digestible. Different methods and processing conditions have been used to increase or modify starch fractions; annealing (ANN) and storage (S) are hydrothermal treatments that have proven to increase SDS and RS. During ANN starch granules in excess water (>60% w/w) are incubated at a temperature above the glass transition temperature (T_g) but below the onset (T_o) temperature of gelatinization in a given period of time (Hoover and Vasanthan 1994; Tester and Debon 2000). Depending on the source of the starch, the susceptibility to enzyme hydrolysis increases, decreases, or remains unchanged after ANN (Hoover and Manuel 1996; Jacobs and Delcour 1998; Waduge *et al*., 2006). Otherwise, different S conditions have been used to promote retrogradation of starch compounds mainly to increase the RS fraction. Incubation of starch gels at low temperatures (refrigeration) for a set period of time promotes reorganization of leached amylose chains from starch granules during gelatinization. However, the RS and SDS in the granular starch produced by ANN and/or S are not heat stable. The lack of thermal stability of SDS and RS represents a drawback of functionality as a food ingredient. The use of banana flour instead of isolated starch has several advantages because some components present in the flour can help to promote thermal stability and decrease the rate of enzymatic hydrolysis, and also be a source of antioxidant compounds such as polyphenols (Ovando-Martínez *et al*., 2008), and non-starch polysaccharides, such as dietary fiber (14.5%) (Juárez-García *et al*., 2006). The nutritional fractions of starch as well as the pasting and thermal properties of banana flour are crucial for food application. The objectives of this study were to determine the impact of ANN and S on the SDS and RS fractions of unripe banana flour, and to evaluate their physicochemical characteristics.

2 Materials and methods

Unripe bananas (*Musa paradisiaca* L.) were purchased at the local market of Cuautla, Morelos, Mexico. Green fruits without yellow spots and from 25 to 30 cm in length were obtained.

2.1 Cooking of green banana fruit

Banana fruits were separated in four groups of similar weight; three of those groups were separated for cooking treatment by submersion in a boiling water bath (100 °C), the first group for 5 min, the second for 15 min and the third for 25 min. After the time, fruits were removed from the water bath and submerged in an ice bath (3-4 °C) until the temperature in the center of the fruit reached 20 °C; temperature profile during the cooling step was determined using a digital thermometer (Radioshack, USA), the fourth uncooked group was used as control.

2.2 Flour preparation

The groups of cooked and uncooked green banana were peeled and sliced (1 cm thick), then were washed with a citric acid solution (0.5 g/L), afterwards were dried in a convection oven at 40 °C for 24 hr. The dried slices were grounded with a manual grinder (Mapisa Internacional S.A de C. V., México, D.F.) until pass mesh n°50.

2.3 Chemical analysis

Moisture content was determined by gravimetric heating (130 °C for 2 h) using 3 g of flour. Ash and protein were analyzed according to AACC methods 08?01 and 46?13, respectively (AACC 2000). Total starch (TS) was determined by the Goñi *et al*. (1997) method; in brief, 50 mg of sample were dispersed in 2 M KOH during 30 min to hydrolyze starch, then the samples were incubated with amyloglucosidase (Roche, No. 102857, 60 °C,

45 min, pH 4.75), and glucose was determined using the glucose oxidase peroxidase assay GOD-POD (Sera-Pak ® Plus glucose, Buenos Aires, Argentina). Glucose concentration (μg/mL) was determined by linear regression analysis. TS were calculated as glucose (mg) × 0.9; potato starch was used as a control.

2.4 Annealing and storage

Banana flours were weighed into hermetic glass containers. Moisture content of flour was carried to 70% by adding the appropriate amount of distilled water. The containers were tight sealed, kept for 24 h at room temperature, and then were annealed (A), incubating the containers at 65 °C for 24 h in a convection oven. Afterwards the containers were opened, and the flour samples were dried at 40 °C for 24 h. After ground and passed through a 50 mesh screen, annealed flours were kept into hermetic plastic bags until analysis. For storage treatment (S), after the incubation period at 65 °C, tight sealed glass containers were kept in refrigeration at 4 °C for 7 days, afterwards the flours were vacuum dried at room temperature (25 °C) and were kept in plastic bags until analysis.

2.5 Polarized light microscopy

Birefringence of native, ANN, and ANN+S starch granules was observed under polarized light with a light microscope (Leitz-Wetzlar, Germany) The images were recorded at the same magnification (100X) for all flour samples (1.0% flour suspension).

2.6 Scanning electron microscopy (SEM)

For SEM studies, the samples of native, C, ANN, and ANN+S flours were fixed to a conductive double glued carbon tape; a film piece was mounted on an aluminum stub. Samples were covered in a metal ionizer (JEOL) with a 50 nm thick gold layer; afterwards sample was deposited under vacuum in a JEOL JSMP 100 (Tokyo, Japan) electron microscope. All samples were examined using an accelerated voltage of 15 kV (Viveros-Contreras *et al*., 2013).

2.7 Amylose leaching (AML)

To determine amylose leaching due to heating, 25 mg of flour (d.s.b.) were weighted, and mixed with 10 mL of deionized water, and then mixture was heated at 50, 65, 80 and 95 °C in sealed cap tubes for 30 min with constant magnetic stirring, tubes were cooled at room temperature and centrifuged at 2000 × g for 10 min. The supernatant (1.0 mL) was withdrawn, and amylose content was determined as described by Hoover and Ratnayake (2004). AML was expressed as percentage of amylose leached per 100 g of dry flour.

2.8 X-ray diffraction

X-ray diffraction patterns were obtained using an Advance D8 diffractometer (Bruker, Coventry, UK) at 35 kV with CuK α radiation (1.542 Å). The samples were scanned in the angular range 3-37 ° (2θ). The crystallinity percentage (% C) was determined from the diffractogram calculating the area corresponding to the crystalline peaks Ap; from the difference between the area under the curve and the area of the amorphous halo, the total area under the curve (At), and the instrumental noise (N) according to the following equation (Rodríguez-García, 1995):

$$\%C = \frac{Ap}{At - N} \tag{1}$$

The amorphous halo was determined with the amorphous component of starch obtained with an extraction procedure reported elsewhere (Bogracheva *et al*., 2001).

2.9 Starch digestibility

2.9.1 Ungelatinized flour

In vitro starch digestibility was determined following the method described by Englyst *et al*. (1992) with slight modifications. Flours (800 mg) were hydrated with 8 mL of distilled water; pepsin (52.1 mg) was dispersed in water (10 mL) and was added to each tube, which was incubated in a shaking water bath (37 °C, 200 strokes/min) for 30 min. At 20 min of incubation, porcine pancreatic ?-amylase (1 g) was dispersed in water (6.7 mL), and centrifuged at 1500 × g for 12 min. The supernatant (4.6 mL) was transferred to a beaker, and amyloglucosidase (0.26 ml) and invertase (0.33 mL) were added to the solution. This enzyme solution was freshly prepared for each digestion. After 30 minutes of protein digestion, 10 mL of 0.5M sodium acetate buffer (pH 5.2) were added to each test flask. The enzyme solution (1 mL) and 5 glass beads (1 cm diameter) were then added into each tube, which was incubated in a shaking water bath (37 °C, 200

strokes/ min). Aliquots (0.5 mL) were taken at 1 minute intervals between samples and mixed with 4 mL of 80% ethanol. The glucose oxidase-peroxidase reagent measured the hydrolyzed glucose content. Starch classifications based on the rate of hydrolysis were: rapidly digestible (digested within 20 min) starch (RDS), slowly digestible (digested between 20 and 120 min) starch (SDS) and resistant starch (RS) (undigested after 120 min).

2.9.2 Gelatinized sample

The native, ANN, and ANN+S flours (800 mg) and water (8 mL) were added to Erlenmeyer flasks. The flasks were sealed to avoid water loss, and the contents were mixed by manual stirring for 1 min. The flasks were heated in a boiling- water bath for 30 min with shake at low speed (100 strokes/min). After heating, the flask was placed in a water bath at 37 °C for 10 min to equilibrate the temperature. Same procedure of enzymatic digestion (above) was followed.

2.10 Rapid viscosity analysis

The paste viscosities of treated and untreated green banana flour were determined by using a Rapid Visco Analyzer (RVA) (Newport Scientific Inst., Warriewood, Australia). The flour slurry (7.0% w/w, d.s.b) was heated from 50 to 95 °C for 3.5 min, held at 95 °C for 5 min, cooled to 50 °C for 3.5 min and then held at 50 °C for 4 min.

2.11 Differential scanning calorimetry

The thermal properties of starch were studied using a differential scanning calorimeter (DSC, TA Instruments, model 2010, New Castle, NJ, USA) previously calibrated with indium. Samples (2 mg, d.s.b) were weighed in an aluminum pan and 7 mL of deionized water was added. The pan was sealed tightly and allowed to stand for 1 h before analysis. An empty aluminum pan was used as reference. The sample was subjected to a heating program ranging from 20 to 120 °C at a heating rate of 10 °C/min. Onset (To), peak (Tp), conclusion temperature (Tc), and enthalpy of gelatinization (ΔH) were obtained directly from the data analysis using the software TA Instruments Universal Analysis 2000 for Windows version 3.2. For starch retrogradation samples were stored at 4 °C for 7 days and then re-scan as above. Triplicate measurements were done for each sample.

2.12 Statistical analysis

One-way analysis of variance (ANOVA) at significance level of 5% ($\alpha = 0.05$) was applied to data and when statistical differences were found, Tukey's test of multiple comparisons was applied analysis was done using the statistical program Sigma-stat (SYSTAT Software 2005).

3 Results and discussion

3.1 Chemical analysis

The total starch content of native and thermal treated flours did not show significant differences, with values around 75%. A similar total starch content has been reported in unripe banana flour without treatment and is used for preparation of spaghetti (73.7% and for preparation of fiber-rich powder (76.8%) (Ovando-Martínez *et al*., 2008; Rodríguez-Ambríz *et al*., 2008).

3.2 Polarized light and scanning electron microscopy

The raw unripe banana flour (figs. 1A and 1B) showed intact starch granules with characteristic birefringence. This pattern shows that the preparation of the flour did not affect the main component of the pulp. Other components (protein and fiber) are present in the flour (Fig. 1A). The banana starch granules are elongated with lenticular shape, characteristics that were reported in isolated banana (Espinosa-Solís *et al*., 2009; Bello-Pérez *et al*., 2005). At longer cooking times (Fig. 1C-1F), aggregates were observed, and they were more evident at the longest cooking time (25 min). The aggregates reveal that they were formed of starch granules that were not completely gelatinized due to the presence of birefringence (Fig. 1D). A decrease in birefringence was observed when the cooking time increased (Fig. 1F). The presence of protein and components of the fiber protected the starch granules, preventing the gelatinization of the starch granules (Debet and Gidley 2007). The annealing treatment in the raw unripe banana flour (Raw+ANN) (Fig. 2A) produced changes in the shape of the starch granules; some starch granules maintained an elongated shape but they appeared as swollen granules. The birefringence characteristics continued, indicating the order of starch components in the granular structure. The sample was cooked for 15 min and annealed (15+ANN), which did not change the morphology of the aggregates, but the

birefringence appeared to decrease (figs. 2C and 2D). A similar pattern was found in the same sample with additional storage (15+ANN+S), where an increase in the birefringence was detected. This pattern is due to the fact that ANN produces a granular re-ordering and S produces a retrogradation of starch components (Krueger *et al*., 1987; Tester and Morrison 1990).

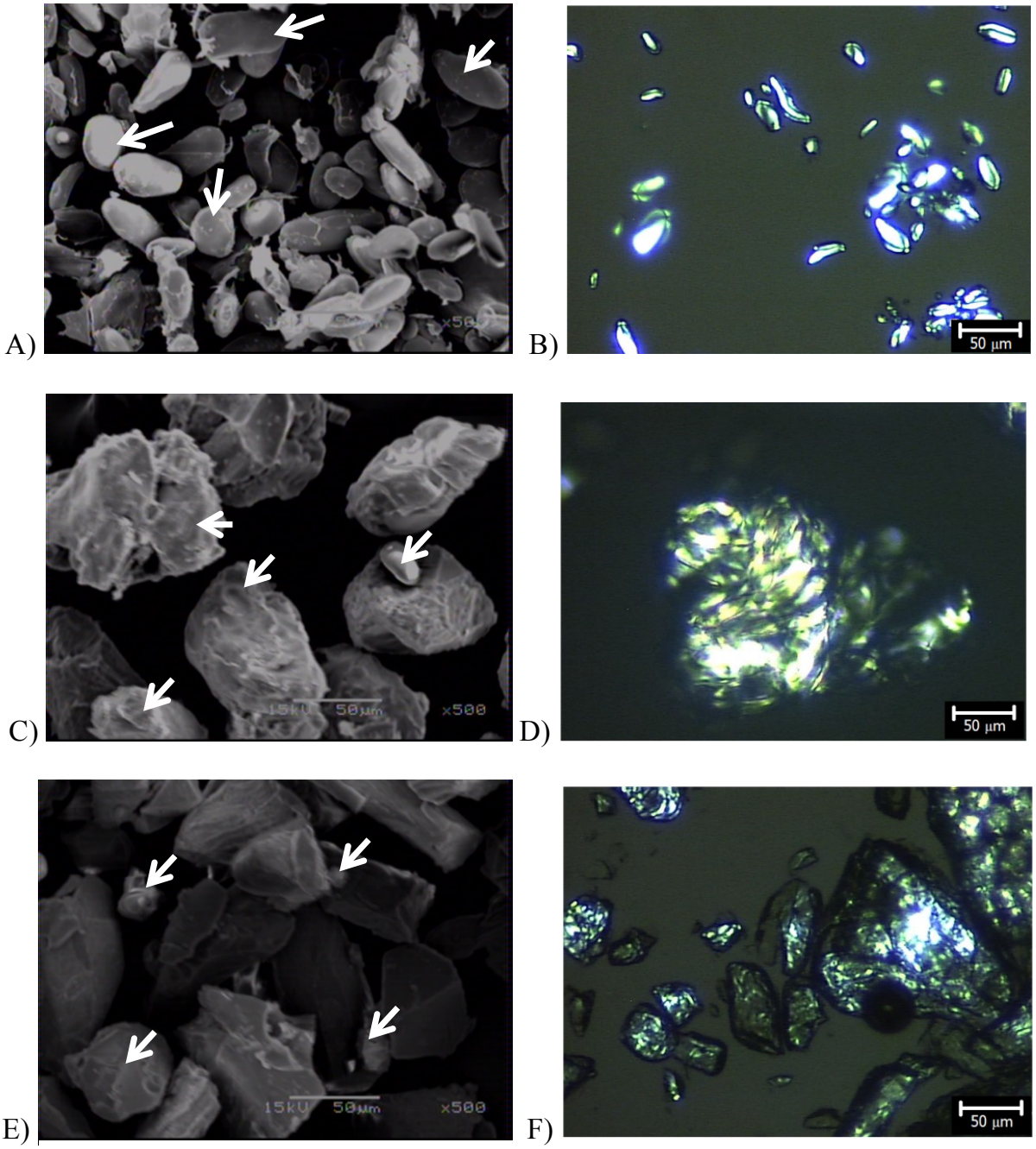


Fig. 1. Unripe banana flour samples; A, C and E, electron scanning microscopy images of Raw unripe banana flour, cooked for 15 and 25 min respectively; B, D and F polarized light microscopy images of Raw unripe banana flour, and cooked for 15 min and 25 min respectively.

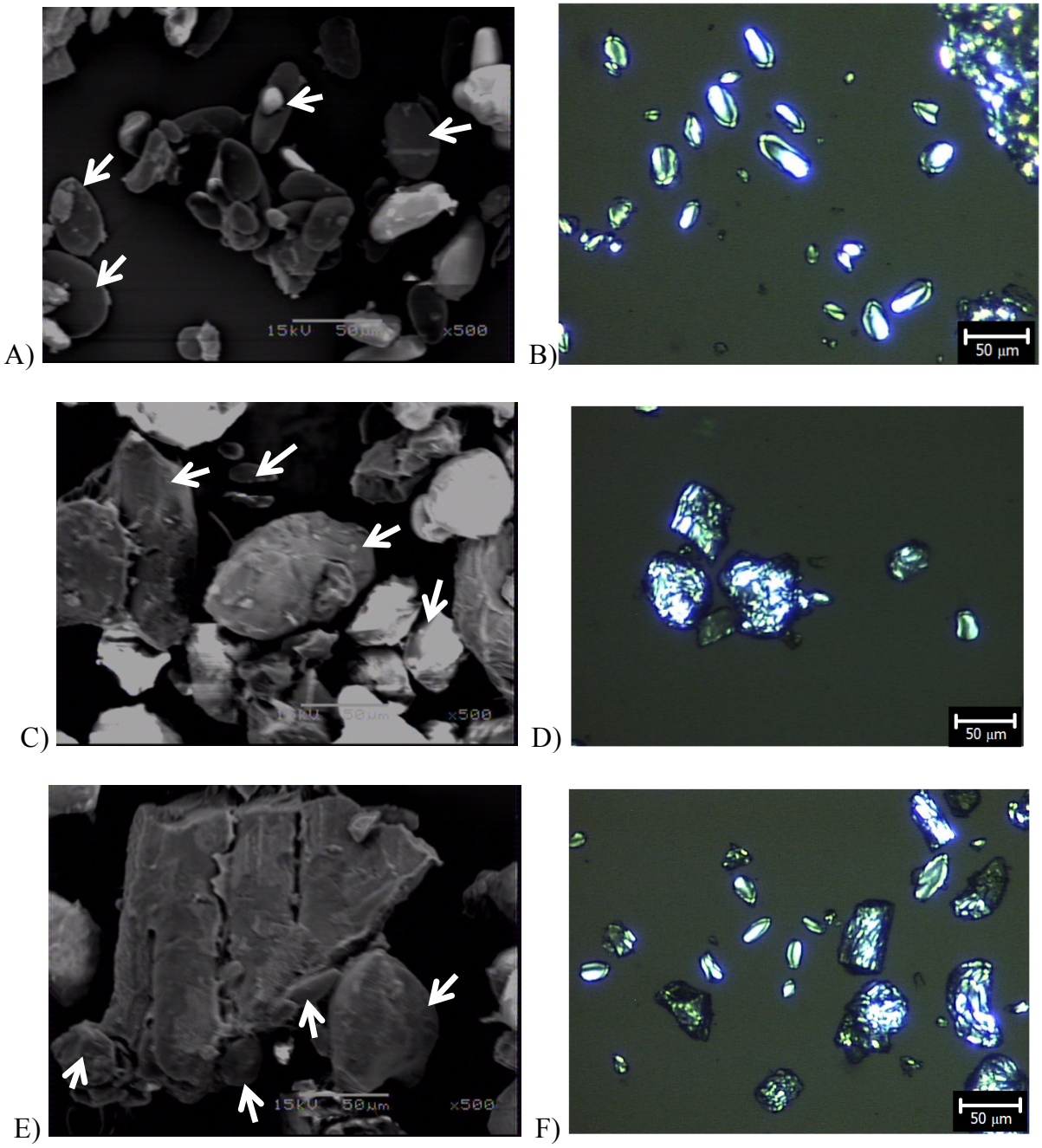
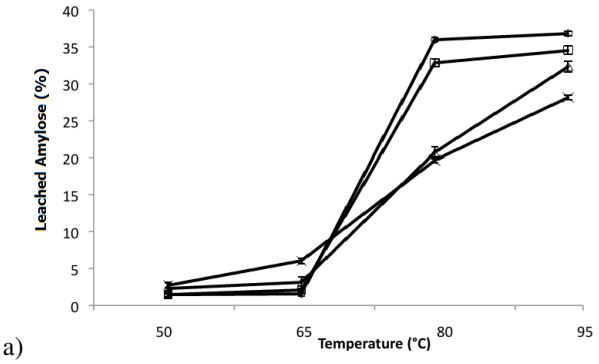


Fig. 2. Thermal treated banana flours; A, C and E, electron scanning microscopy images of Raw+ANN, 15+ANN, and 15+ANN+S respectively; B, D and F light microscopy images of Raw+ANN, 15+ANN, and 15+ANN+S respectively.

3.3 Amylose leaching

Heating treatment such as cooking and annealing produces amylose leaching from the starch granules. This parameter is important in the functionality and the starch digestibility of unripe banana flours. At low heating temperatures (50 and 65 °C) the amylose leaching value (Fig. 3) was similar for the raw unripe



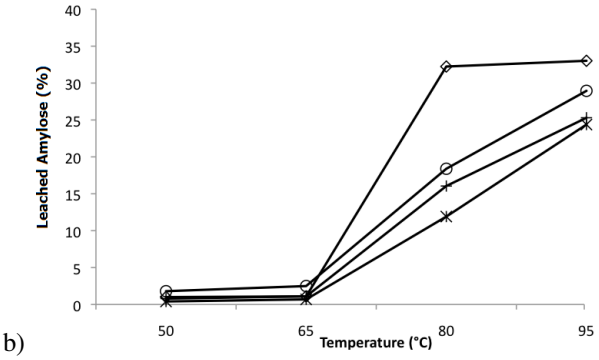
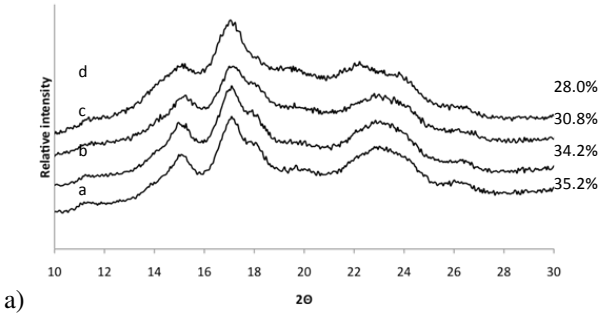


Fig. 3. Amylose leaching of thermal treated unripe banana flours; A) Raw unripe banana flour (⋄), cooked for 5 min (□), 15 min (Δ) and 25 min (X), respectively; B) Raw unripe banana flour (⋄), cooked for 15 min (○), 15+ANN (+) and 15+ANN+S (X), respectively.



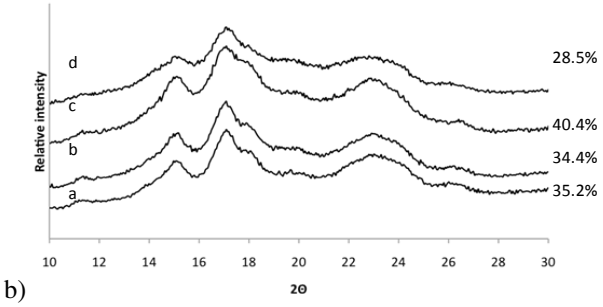


Fig. 4. X-ray diffraction patterns of unripe banana flour under different treatments: A) Effect of cooking time, a) Raw unripe banana flour, cooked for: b) 5 min, c) 15 min and d) 25 min; B) Effect of annealing, a) Raw unripe banana flour, b) Raw+ANN, c) 15+ANN, and d) 25+ANN, respectively.

banana flour to that cooked for 5 and 15 min, but a higher amylose leaching value was obtained at 65 °C in the sample cooked for 25 min. Cooking for a long period of time produced higher starch disorganization and when this unripe banana flour is heated, a higher amount of amylose is leached. The test at 80 °C produced two kinds of behavior in the samples; those with a minimum heating treatment (raw and cooked for 5 min) had the highest amount of leached amylose and those with a longer cooking time (15 and 25 min) had the lowest amount (around 18%). Heating at a higher temperature (95 °C) produced a plateau of leached amylose content (around 34%) for the raw and the samples cooked for 5 minutes and those cooked for a longer time (15 and 25 min) increased, reaching a value of approximately 27%. The microscopy study showed the presence of aggregates when the cooking time increased; the aggregates could retard the amylose leaching when the flour was heated in water. ANN and ANN+S treatments produced a decrease in the amylose leaching (Fig. 3B). At lower temperature of the test (50 and 65 °C) there was no difference in the amylose leaching content, but at higher temperature (80 °C), ANN and mostly ANN+S decrease the amount of leached amylose. However, at the highest temperature in the test (95 °C) no difference was detected between the samples with ANN and ANN+S. However, a plateau was not observed in cooked for 15 min, 15 + ANN, and 15 + ANN + S samples. This indicates that amylose leaching from the starch granule continued after 80 °C, and perhaps no more amylose will be apart out to the continuous phase because the "ghosts" starch granules keep amylopectin and perhaps some lineal chains. It is very difficult to broke the "ghosts" and liberates the starch components; high shear rate could produce the complete leaching of lineal chains (amylose and some external long chains of amylopectin) from "ghosts" (Zhang *et al*., 2013). Similar results have been found in native starches, as shown by Tester and Morrison (1990) and Radosta *et al*. (1992). Some authors have attributed this pattern to the interaction between amylose and amylopectin chains (Hoover and Vasanthan 1994; Tester and Debon 2000), and also to the presence of aggregates with a higher order in the samples with ANN and ANN+S, as evidenced by the microscopy study, which may explain this pattern.

3.4 X-ray diffraction

The raw unripe banana flour and that cooked for different times showed a B-type X-ray diffraction pattern (Fig. 4A). Raw unripe banana flour and the 5 min cooked sample showed a similar crystallinity level. Cooking for 15 and 25 min produced a decrease in the crystallinity, indicating disorganization of the starch components, as evidenced in the microscopy study, thermal analysis and pasting behavior. The change in the crystallinity level can be following in the main peak at $2\theta = 17°$. The wideness of this peak increased at longest cooking time due to higher disorganization of starch structure. The samples with ANN and ANN+S showed a similar X-ray diffraction pattern, but a different crystallinity level (Fig. 4B). Raw unripe banana flour and Raw+ANN had similar crystallinity levels, where no change was observed in the peak at $2\theta = 17°$. This treatment in the raw sample did not modify the crystalline structure of the starch, perhaps because of the other components of the unripe banana flour such as cellulose, hemicellulose, and pectins which produce a physical barrier that decreases the ANN effect on the starch granules. Jayakody and Hoover (2008) found that a slight reduction in potato starch crystallinity by ANN may be due to reflection of crystalline disruption or reorientation, as reported by Vermeylen *et al*. (2006). According to Gomes *et al*. (2004), annealed starches exhibit a reduced intensity of the crystallinity peak. These authors reported that the increase in helical order was produced by interactions between amylose (AM) and amylopectin (AMP) chains. Reduced inter-crystalline spacing may indicate that helical packing becomes more compact and organized. The sample 15+ANN showed an increase in the crystallinity level compared with the sample with lower cooking time (data not shown). This heat treatment creates disorganization in the physical barrier and produces a reorganization of the starch components; it is known that ANN could arise due to the interplay of the increase in crystalline order, the formation of new crystallites by the interactions among (AM-AM, AM-AMP, AMP-AMP) starch chains, and the increase in crystallite size and crystallite reorientation (Lan *et al*., 2008). At the longest cooking time (25 min), the effect is the inverse due to the decrease in the crystallinity level recorded as is observed in the peak at $2\theta = 17°$. Cooking for 25 min produced starch gelatinization, but during annealing, it is not possible to reorganize the starch components. The crystallinity level of the unripe banana flour with different treatments could be important in starch digestibility because it could affect the rate at which enzymes degrade the starch granules (Han and BeMiller, 2007).

3.5 Starch digestibility

The starch fractions after enzymatic digestion of the ungelatinized and gelatinized samples are shown in Table 1. The raw unripe banana flour showed high resistant starch (RS) content and the 5 min cooking time of the fruit did not affect this content. At longer cooking times, an important decrease in RS amount and increase in slowly digestible starch (SDS) were obtained. This pattern is due to the fact that cooking of the fruit for 15 and 25 min produced partial gelatinization of the starch granules, which could decreased the RS content (Aparicio-Saguilan *et al*., 2005; Hernandez-Nava *et al*., 2011), as well as a production of short chains that can be reorganized in some minutes, producing an increase in SDS content. The ANN and the ANN+S of the cooked unripe banana flour produced an increase in RS content compared with their counterparts without these treatments, with slight but significant differences observed in the SDS content. ANN produced reorganization of the granular structure and increased the granular stability that decreased the starch hydrolysis by the digestive enzymes (Tester *et al*., 1998). 25+ANN+S showed an increase in RS content compared with 25+ANN. The storage process of gelatinized banana starch at low temperatures produced retrogradation with the increase in the RS content (Bello-Pérez *et al*., 2005). Raw unripe banana starch without gelatinization before the Englyst?s test showed an SDS content of 10.8% and RS of 85% (Carlos-Amaya *et al*., 2011). Gelatinization of samples before the Englyst's test decreased the RS content but increased the SDS level in the raw unripe banana flour (Table 1). In general cooking of samples at different times showed a lower RS level but a higher SDS content. Samples with ANN and ANN+S presented a similar pattern, showing that these treatments maintained the SDS fraction even with the additional gelatinization treatment before the Englyst's test. The analysis of unripe banana flour with different treatments and an additional heating treatment could give more information on the digestibility properties and the possible applications in foods that need thermal treatment during their preparation. The gelatinization treatment applied on native banana starch before the Englyst's test produced a reduction in RS content but a slight increased in the

SDS level (Carlos-Amaya *et al*., 2011).

3.6 Pasting characteristics

The pasting profiles of the unripe banana flour with the different treatments are shown in Fig. 5. Samples cooked for longer times (15 and 25 min) showed lower peak viscosity than the raw sample and sample cooked for 5 min (Fig. 4A), but the former samples presented the peak at higher temperatures. Cooking and cooling produced arrangement of starch components that increased the thermal stability of those banana flours as was observed in the thermal analysis by DSC. This pattern agrees with the microscopy and amylose leaching studies where no appreciable effect on starch and other components of the pulp was produced with the shortest cooking time used. However, the breakdown was higher in the 5 min cooked sample than in the raw sample due to the conformation of starch granules which apparently presents a slight disorganization during heating. Raw unripe banana starch showed a similar pasting profile to raw unripe banana flour (Espinosa-Solís *et al*., 2009; Carlos-Amaya *et al*., 2011), but the viscosity values were higher in the former due to the flour presenting other components such as fiber. Samples with 15 and 25 min of cooking showed lower values of breakdown and setback, but the final viscosity upon cooling was similar for raw, cooked for 5, and 15 min samples, indicating similar gel structure. Disorganization of the starch components is responsible for this behavior. ANN and ANN+S produced an important change in the organization of starch components as evidenced in the pasting profile (Fig. 5B). A well-defined peak viscosity was not found in samples with both treatments, a pattern that agrees with the microscopy study where aggregates of disorganized and melt-like starch granules were observed. However, the maximum peak was observed during the holding step at 95 °C, indicating that the remnant structure is disorganized at higher temperature than the raw flour and that cooked for 15 min. This was also showed in the thermal analysis. The samples 15+ANN and 15+ANN+S did not show breakdown and setback, indicating a soft gel structure upon cooling. According to Lineback and Rasper (1988), several factors control the viscosity during the pasting of starch, including the swelling of the granules and the amount of solubilized starch; therefore, it can be stated that the formation of a tightly packed array of swollen, granular aggregates and the leaching of macromolecules can contribute to the development of viscosity in starch paste.

Table 1. Digestibility values of unripe banana flour samples cooked for different times and treated under different conditions.

	Ungelatinized samples			Gelatinized samples		
Sample	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
R	2.5±0.1^{j}	5.9±0.1 ^{g}	66.5±0.1^{b}	54.2±0.6^{c}	10.9±0.9^{c}	9.9±0.8^{b}
5*	5.9±0.3^{f}	7.5±0.4^{e}	61.5±0.3^{d}	52.1±0.4^{de}	15.4±0.6^{a}	7.5±0.5^{d}
15*	42.8±0.2^{b}	14.2±0.5^{bc}	17.9±0.3^{gh}	53.6±0.4^{d}	14.2±0.4^{ba}	7.2±0.4^{d}
25*	49.7±0.7^{a}	13.0±0.4^{bc}	12.1±0.5^{i}	55.8±0.3^{c}	10.4±0.7^{c}	8.8±0.5^{c}
Raw+ANN	2.4±0.1^{j}	3.7±0.4^{h}	68.8±0.3^{a}	52.8±0.7^{d}	15.4±1.0^{a}	6.8±0.9^{d}
5+ANN	3.9±0.1^{h}	6.8±0.1^{f}	64.1±0.1^{c}	51.3±0.7^{e}	15.2±0.8^{a}	8.5±0.7^{c}
15+ANN	35.9±0.3^{d}	15.2±0.6^{b}	23.9±0.4^{e}	58.0±0.5^{b}	9.5±0.6^{dc}	7.5±0.5^{d}
25+ANN	42.4±0.5^{b}	13.8±0.2^{cb}	18.7±0.3^{g}	59.4±0.6^{a}	8.2±0.3^{e}	7.4±0.4^{d}
Raw+ANN+S	2.8±0.2^{i}	3.8±0.5^{h}	68.3±0.3^{a}	50.8±0.2^{f}	14.8±0.6^{a}	9.4±0.6^{b}
5+ANN+S	4.6±0.3^{g}	6.0±0.3^{g}	64.3±0.3^{c}	52.1±0.5^{de}	11.6±0.5^{c}	11.3±0.5^{a}
15+ANN+S	33.5±0.4^{e}	18.2±0.1^{a}	23.2±0.2^{ef}	57.1±0.4^{b}	9.0±0.4^{d}	8.9±0.4 ^{cb}
25+ANN+S	41.1±0.3^{c}	9.9±0.2^{d}	23.9±0.2^{e}	58.9±0.7^{a}	10.6±1.1^{c}	5.5±0.7^{ed}

Values in same column with different letter are different ($p < 0.05$).

R= Raw unripe banana flour.

RDS: Rapid digestible starch, SDS: Slow digestible starch, RS: Resistant starch.

ANN= Annealing (65 °C, 24 h, 70 % water content);

ANN + S= Annealing plus Storage (7 days, 4 °C).

*Time of the heating treatment of green banana fruits (min)

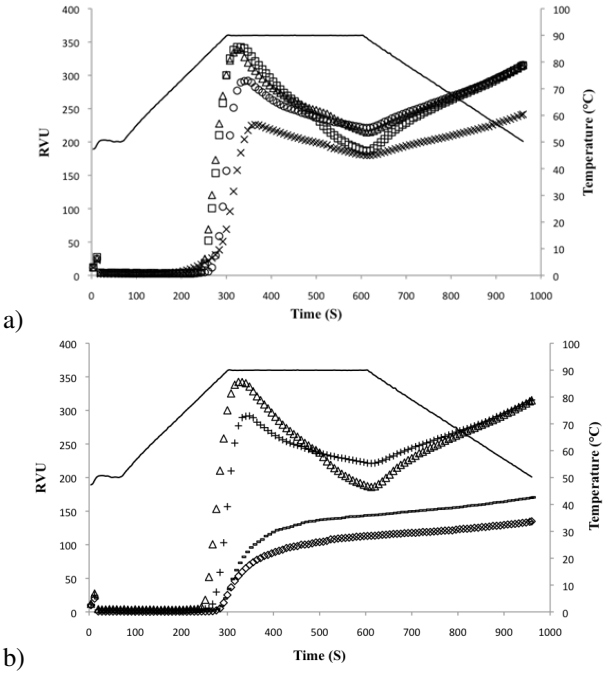


Fig. 5. Rapid viscosity profiles of unripe banana flour: A) Raw unripe banana flour (Δ), cooked for 5 min (□), 15 min (○) and 25 min (X), respectively; B) Raw unripe banana flour (Δ), cooked for 15 min (+), 15+ANN (-) and 15+ANN+S (⋄), respectively.

So we speculate that the granular aggregates and other fruit components indeed contribute to viscosity; also, ANN and ANN+S may make the swollen gelatinized granules more rigid and resistant to shear, resulting in a higher viscosity when the dispersion is cooling down (Jacobs *et al*., 1995).

3.7 Thermal properties

The gelatinization properties of unripe banana flour with different treatments are shown in Table 2. Raw unripe banana flour presented temperatures and enthalpy of gelatinization similar to those determined previously (Espinosa-Solís *et al*., 2009). The sample cooked for 5 min presented similar gelatinization parameters to its raw counterpart, but a longer cooking time (15 min) produced an increase in the gelatinization temperatures but a decrease in the enthalpy value due to starch gelatinization. A similar trend was reported previously (Tester y Debon 2000; Waduge *et al*., 2006; Jacobs *et al*., 1995; Adebowale *et al*., 2005). The sample with the longest cooking time (25 min) showed two endotherms, the first one a low temperature (60.5 °C) with a higher enthalpy value (5.0 J/g) and the second a higher temperature (86.3 °C) with a lower enthalpy value (0.5 J/g). Whether the two enthalpy values are considered, the calculated value of both parameters is higher than that of the 15 min cooked sample. This pattern could be due to the fact that intact and partially gelatinized granules could form granular aggregates and the former could be protected during the heating, producing an increase in the temperatures and the enthalpy values of gelatinization. The ANN and ANN+S treatments increased the gelatinization temperatures, but a slight change in the gelatinization enthalpies was found. This pattern is due to the increase in the granular stability that produces the formation of a more organized structure with lower free energy (Blanshard 1987). After the retrogradation of the raw and thermal treated banana flours (Table 3), an increase in the onset temperature of the retrogradation was detected in the thermal treated flours, but the peak and conclusion temperatures associated with this phase transition were similar. In general, the enthalpy of retrogradation increased in samples that were cooked at different times compared with in raw flour, but no effect of the cooking time was found on this parameter. Retrogradated Raw+ANN+S flour had similar retrogradation temperatures but higher enthalpy values than those determined in the raw unripe banana flour (Espinosa-Solís *et al*., 2009). In general; ANN and ANN+S did not affect significantly the retrogradation parameters of the cooked samples when they were compared with their non-treated counterpart. This pattern shows that gelatinization of starch disorganizes its granular structure, and that thermal treatment does not change the way in which starch is reorganized.

Conclusions

Cooking of unripe banana flour produced changes in the starch structure and in the flour matrix that decreased the resistant starch (RS) and increased the slowly digestible starch (SDS) contents. The hydrothermal treatment (ANN) and storage (S) increased the SDS and RS content. The changes in the starch digestibility due to cooking, hydrothermal treatment, and storage were related with the morphologic characteristics, crystallinity level, enthalpy, and pasting profile. Cooking, annealing, and storage can modify the starch digestibility characteristics of unripe banana flour, and it is possible to produce unripe banana flour with specific starch digestibility features combining the treatments.

Table 2. Thermal parameters of unripe banana flour cooked for different times and treated under different conditions.

Sample	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	To_R (°C)	Tp_R (°C)	Tc_R (°C)	ΔH_R (J/g)
Raw	70.3±0.2^{f}	76.6±0.2^{h}	86.6±0.4^{g}	14.8±0.1^{b}	40.9±0.5^{d}	56.6±0.2^{c}	71.8±0.8^{cd}	7.7±0.6^{b}
5*	70.7±0.2^{f}	76.7±0.3^{h}	85.7±0.3^{g}	14.3±0.5^{b}	45.8±0.7^{cb}	57.3±1.2^{b}	72.0±1.8^{cd}	8.6±0.7^{a}
15*	76.9±0.2^{c}	83.2±0.1^{d}	89.6±0.4^{f}	3.9±0.1^{g}	44.5±0.4^{c}	58.3±0.1^{b}	69.8±0.8^{d}	9.7±0.5^{a}
25*	52.8±1.6^{h}	60.5±1.2^{k}	74.2±0.3^{h}	5.0±0.9^{f}	45.3±0.3^{c}	57.6±0.3^{b}	70.6±0.3^{d}	9.4±0.4^{a}
25$^{\&}$	82.7±1.0^{b}	86.3±1.5^{cb}	93.0±0.6^{b}	0.5±0.2^{j}	——	——	——	——
Raw+ANN	76.6±1.3^{c}	80.4±0.2^{f}	88.0±0.1^{f}	13.5±0.0^{cb}	38.3±0.3^{g}	57.3±0.3^{b}	71.4±0.3^{d}	7.9±0.0^{b}
5+ANN	78.3±0.1^{d}	81.3±0.0^{gf}	89.8±0.6^{f}	14.2±0.1^{b}	39.4±0.3^{f}	56.8±0.1^{c}	70.7±0.1^{c}	7.7±0.0^{b}
15+ANN	78.6±0.3^{cd}	84.7±0.4^{c}	93.0±0.2^{cd}	3.9±0.1^{g}	41.6±0.5^{c}	56.7±0.2^{c}	71.7±0.3^{c}	8.3±0.3^{ab}
25+ANN	62.0±0.2^{j}	72.5±0.3^{j}	94.9±0.3^{i}	4.5±0.3^{f}	44.2±0.4^{c}	57.70.2^{cb}	73.7±0.3^{b}	6.7±0.2^{c}
25+ANN$^{\&}$	83.2±0.5^{b}	87.9±0.2^{bc}	93.8±0.3^{cd}	0.7±0.0^{j}	——	——	——	——
Raw+ANN+S	79.3±0.3^{c}	82.2±0.1^{dc}	88.2±0.1^{gf}	13.6±0.2^{cb}	40.3±0.4^{c}	57.8±0.7^{cb}	72.8±0.3^{c}	7.3±0.3^{b}
5+ANN+S	79.8±0.1^{c}	82.4±0.1^{dc}	92.1±0.1^{d}	16.6±0.0^{a}	49.6±0.2^{b}	59.0±0.2^{b}	72.3±0.1^{c}	9.2±0.1^{a}
15+ANN+S	79.5±0.2^{c}	84.4±0.3^{c}	91.5±0.1^{cd}	4.2±0.2^{g}	50.2±0.3^{a}	60.0±0.1^{a}	73.1±0.1^{b}	8.1±0.1^{b}
25+ANN+S	63.2±0.2^{j}	76.3±0.1^{i}	96.0±0.1^{i}	5.4±0.1^{i}	50.5±0.2^{a}	60.3±0.2^{a}	71.9±0.2^{c}	7.3±0.2^{b}
25+ANN+S$^{\&}$	85.1±0.0^{a}	89.4±0.1^{a}	92.8±0.1^{d}	0.3±0.0^{j}	——	——	——	——

Values in same column with different letter are different ($p < 0.05$).

Raw=Raw unripe banana flour.

To: Gelatinization onset temperature; Tp: Gelatinization temperature; Tc: Gelatinization conclusion temperature; ΔH: Gelatinization enthalpy; To_R: Retrogradation onset temperature; Tp_R: Retrogradation temperature; Tc_R: Retrogradation conclusion temperature; ΔH_R: Retrogradation entalphy;

ANN= Annealing (65 °C, 24 h, 70 % water content);

ANN+S= Annealing plus storage (7 days, 4 °C);

*Time of the heating treatment of green banana fruits (min)

&= Second transition

—— = Not determined

Acknowledgements

We appreciate the financial support from SIP-IPN, COFAA-IPN and EDI-IPN. One of the authors (JdeR-M) also acknowledges the scholarship from CONACYT-México.

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