Physicochemical and Nutritional Characteristics of Banana Flour During Ripening

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#### **Abstract**

Banana flour has been recognized as functional ingredient, owing to its healthy nutritional pattern. Nevertheless, unripe and ripe banana flours show different characteristics and scarce information is available about changes undergone during banana ripening. This study evaluates the changes on physiochemical (chemical composition, hydration properties, rheological properties and structural characteristic) and nutritional (resistant starch content, phenolic compound and antioxidant activity) characteristics of banana flour at the initial four ripening stages. The significant increase in protein content and decrease in carbohydrate and apparent amylose content, besides the reduction in pasting properties, between 2<sup>nd</sup> and 3<sup>rd</sup> stages suggested a climacteric peak during ripening. Between those stages, a significant decrease in total and resistant starch was produced together with an increase in total phenolic content and antioxidant activity. Therefore, the knowledge of the physicochemical and nutritional characteristics of banana flour at each ripening stage allows better selection depending on the industrial application.

### **Keywords**

Banana flour, ripening stage, physicochemical characteristics, nutritional characteristics.

### 1 1. Introduction

- 2 Banana reaches to the ripening state, when the fruit has a size for separation from the
- 3 plant, which is known as climacteric fruit. In this stage, the fruit has not sensory
- 4 characteristics due to that is considered as unripe. During climacteric, many
- 5 biochemical reactions take place in banana, like synthesis of ethylene, starch
- 6 degradation, sugar accumulation and change in bioactive compound (do Nascimento et

7 al., 2006; Fatemeh, Saifullah, Abbas, & Azhar, 2012; Wang, Tang, Chen, & Huang,

8 2014). The most visible change during that period is the peel color modification, which

allowed to define seven ripening stages that are used to industrialize banana fruits (Von

10 Loesecke, 1950).

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In the last decade, the use of green or unripe banana flour as a functional ingredient has

attracted much attention from research teams, because of its high content of resistant

starch, dietary fiber and potassium (Juarez-Garcia, Agama-Acevedo, Sáyago-Ayerdi,

Rodríguez-Ambriz, & Bello-Pérez, 2006; Pragati, Genitha, & Ravish, 2014). As a

result, it has been proposed as ingredient for breads (Juarez-Garcia et al., 2006; Noor

Aziah, Ho, Noor Shazliana, & Bhat, 2012), cookies (Agama-Acevedo, Islas-Hernández,

Pacheco-Vargas, Osorio-Díaz, & Bello-Pérez, 2012) and pasta (Ovando-Martinez,

Sáyago-Ayerdi, Agama-Acevedo, Goñi, & Bello-Pérez, 2009). Main consensus

indicates that the use of unripe banana flour increases antioxidant activity and reduces

glycemic index by increasing non-digestible starch. Indeed, Sardá et al. (2016b)

reported that because of its high resistant starch content, unripe banana flour produced a

reduction in the energy consumption, a feeling of satiety and a positive impact on

glucose homeostasis in healthy volunteers. On the other hand, studies about rheological

properties of unripe banana starch demonstrated its gelling and thickening properties

(Utrilla-Coello et al., 2014; Zhang & Hamaker, 2012).

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Despite the ongoing interest, some of previous studies lack to specify the ripening stage

of banana, provoking variation among research's results, for instance the amount of

resistant starch reported in unripe banana flour fluctuates between 30 to 57% dry base

31 (Liao & Hung, 2015; Sardá et al., 2016a). Indeed, the lack to consider the ripening stage

also affects the quality of commercial flours. For instance, Sardá et al. (2016a) found high variability in resistant starch content among commercial unripe banana flour (4-62%), likely due to their different ripening stage. In addition, when ripening is considered, studies only compared the behavior of flour from unripe and ripe stage regarding physicochemical characteristics (Pragati, et al., 2014, Alkarkhi, bin Ramli, Yong, & Easa, 2011), hygroscopic behavior (Cardoso & da Silva Pena, 2014) and bioactive compounds content (Fatemeh et al., 2012), showing significant differences between those stages. Despite these studies, there is no information about physicochemical and nutritional characteristics of banana flour obtained at different ripening stages. Recently, Yap, Fernando, Brennan, Jayasena, & Coorey (2017) reported the effect of banana ripening in puree production, studying five ripening stages of banana. Authors concluded that 5<sup>th</sup> stage of ripening was the most appropriated for puree industrialization, based on the organoleptic and physicochemical characteristics.

Considering the scarce information existing on ripening process and the divergences reported about banana flour characteristics, the aim of this research was to generate information on each ripening stage with a dual purpose, to reach proper industrialization and consumer's health benefit. Specifically, physicochemical and nutritional changes of banana flour at different ripening stages were evaluated.

# 2. Materials and methods

Banana Cavendish of *Musa acuminata* (AAA) was harvested in Los Ríos, Ecuador (1°27'39.0"S 79°29'19.0"W) between February and March 2016. The ripening stages of this fruit were established according to Von Loesecke (1950) scale, which defined seven ripening stages. That scale was based on the color of the peel and the amount of soluble

solids. Nevertheless, in the present study only bananas at stages 1 to 4 were used because bananas at stages from 5 to 7 have excessive high sugar content that prevents the drying process due to caramelization. The maturation was carried out in a room at 23 ±2 °C, without exposure to light. The ripening time after cutting the fruits from the plant were one day for 1<sup>st</sup> stage, three weeks for reaching 2<sup>nd</sup> stage, six weeks for 3<sup>rd</sup> stage and seven weeks were needed for reaching the 4<sup>th</sup> stage. All reagents were analytical grade

# 2.1. Banana flour preparation

Previously to flour preparation, the amount of soluble solids was determined to confirm the right ripening stage of the samples. For that 30 g of pulp was suspended in 90 mL of water, blended for two minutes and measured using a refractometer (Kruss, Hamburg, Germany) with a scale from 0 to 30 °Brix.

Banana, at different ripening stages, were washed, peeled and sliced (2 mm thick).

Slices were immersed into boiling water for 5 minutes, drained to eliminate the excess
of water and dried individually first at 70 °C for 30 minutes and then at 50 °C for 12
hours. Dried banana was ground and screened in a mesh #70 (212 µm). Flour was stored
at room temperature for further analysis.

# 2.2. Chemical composition

Moisture, fat, protein and ash content of each flour were determined following AOAC methods (925.10, 922.06, 920.87, 923.03, respectively) (AOAC, 2012). Carbohydrate content was obtained by difference of 100 g minus the sum of grams of moisture, fat, protein and ash.

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Apparent amylose content (AAC) was analyzed according to the method of Juliano et al. (1981). Absorbance was measured in a Multi-Mode Microplate Reader Synergy HTX (BioTek Instruments, Winooski, USA). Standard curve was made using blends of amylose from potato (Sigma-Aldrich, St. Louis, MO) and amylopectin from maize (Sigma-Aldrich, St. Louis, MO). Total sugar content was determined according to the method of Somogyi (1952).

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# 2.3. Hydration properties of flours and gels

Water holding capacity (WHC) and water binding capacity (WBC) were determined at different ripening stage to assess hydration properties of flours. On the other hand, water absorption index (WAI), water solubility index (WSI) and oil absorption capacity (OAC) were evaluated to characterize gel hydration properties. These properties were

determined according to the procedure described by Cornejo & Rosell (2015).

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# 2.4. Color analyses

- The flour color was analyzed by the computer vision system (Yam & Papadakis, 2004),
- 99 that counted with a light source, a camera (Nikon COOLPIX S2800, 20.1 megapixels)
- and a software (Adobe Photoshop CS5) to process an image and to obtain the  $L^*$ ,  $a^*$
- 101 and  $b^*$  values.

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### 2.5. X-ray diffraction (XRD)

104 The X-ray diffractometer model X'Pert PRO (PANalytical, Boulder, United States) was
105 used at 30 mA and 40 kV, a diffraction angle (2θ) range of 5-40 ° with a 0.03 ° step size
106 and measuring time of 15 s. The relative crystallinity (RC) was determined through the

equation of Rabek (1980): RC (%) = (Ac/(Ac+Aa))\*100; where Ac is the crystalline area and Aa is the amorphous area on the X-ray diffractograms. The areas were calculated in the software Origin®2017 (OriginLab Corporation, Northampton, USA).

### 2.6. Rheological properties

Rheological measurements were performed on a controlled stress Kinexus PRO rheometer (Malvern Instruments, Worcestershire, United Kingdom) operated under strain control mode at 25 °C. The measuring system consisted of cone-plate geometry (40 mm in diameter and 4° of angle). Gels were prepared with 0.5 g of flour and 4.5 ml of water, they were mixed and heated in a water bath at 90 °C for 10 minutes under continuous agitation, then cooled down to room temperature. The apparent viscosity was accomplished by increasing the shear rate (0.0001 to 450 s<sup>-1</sup>) in 15 min. Linear region was prior defined through amplitude sweep with constant frequency of 1 Hz and shear strain from 0.1 to 100%. The storage modulus (*G'*) and loss modulus (*G''*) were determined in the linear region varying the frequency from 0.01 to 10 Hz.

# 2.7. Pasting properties

Pasting properties were tested according ICC standard method No 162 (ICC, 1996) with a Rapid Visco Analyser model 4500 (RVA) (Perten Instruments, Hägersten, Sweden). Sample (3.5 g based on 14 g of moisture per 100g of flour) and deionized water (25 ml) were put into a canister. RVA settings were hold at 50 °C for 1 min, then heated up to 95 °C at 12.2 °C min<sup>-1</sup>, hold at 95 °C for 2.5 min and cooled down to 50 °C at 11.8 °C min<sup>-1</sup>. The rotational speed of the paddle was 960 rpm during 10 s and kept at 160 rpm during the rest of the assay. In order to inhibit alfa amylase activity, assays were also run in the presence of 0.001 M silver nitrate instead of water.

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133	2.8. Nutritional characteristics
134	The total phenolic content was determined following the Folin-Ciocalteau method
135	(Singleton & Rossi, 1965). The absorbance was measured with spectrophotometer
136	model DU530 (BECKMAN, Fullerton, USA). Results were expressed as mg of gallic
137	acid equivalent/100g.
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139	The antioxidant activity was evaluated by Oxygen Radical Absorbance Capacity
140	(ORAC) method proposed by Huang, Ou, Hampsch-Woodill, Flanagan, & Prior (2002).
141	In the method, fluorescein (40x10 <sup>-3</sup> mM) (Sigma-Aldrich, St. Louis, MO) was used as a
142	target of free radical damage and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic
143	acid (Trolox) (Sigma-Aldrich, St. Louis, MO) to prepare a standard curve from 200 $\mu\text{M}$
144	to 5 $\mu M$ . A Multi-Mode Microplate Reader Synergy HTX (BioTek Instruments,
145	Winooski, USA) was used to measure the fluorescence at 485 nm of excitation
146	wavelength and 528 nm of emission wavelength. Results were expressed as $\mu \text{mols}$
147	Trolox equivalent/100g.
148	Potassium content was analyzed according to AOAC method 975.03 (AOAC, 2016).
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150	Total starch (AOAC method 996.11) and resistant starch (AOAC method 2002.02) were
151	determined using Megazyme kits (Megazyme International Ireland Ltd., Wicklow,
152	Ireland) and absorbance was measured in a UV-Visible spectrophotometer model

# 2.9 Statistical analysis

Evolution 201 (Thermo Scientific, Madison, USA).

All analyses were carried out in triplicate. Statistical analyses were made using Statgraphics Centurion 16 (Statistical Graphics Corporation, UK). Normal distribution of the data was verified with standardized skewness and standardized kurtosis analyses. Multiple sample comparison was used to determine significant differences among samples by analysis of variance (ANOVA) and multiple range tests. Pearson correlation coefficient (in absolute value) was applied considering meaningful those coefficients greater than 0.67.

# 3. Results and discussion

Ripening stages used in this study were defined according to the scale of Von Loesecke (1950), based on the amount of soluble solids and giving different color scale. In line with it, 1<sup>st</sup> stage (totally green) showed between 1.2 - 2.1 °Brix, 2<sup>nd</sup> stage (green with yellow traces) between 2.2 - 6.9 °Brix, 3<sup>rd</sup> stage (more green than yellow) between 7.0 - 13.2 °Brix, and 4<sup>th</sup> stage (more yellow than green) ranged from 13.3 to 18.0 °Brix.

# 3.1. Physicochemical characteristics

The proximate composition is shown in Table 1. Results indicate that ripening stage of banana significantly influenced the flour chemical composition (P=0.000), confirming previous findings with unripe and ripe banana flours (Cardoso & da Silva Pena, 2014; Pragati et al., 2014; Sardá et al., 2016a). It is interesting to note the significant changes in protein, carbohydrate and AAC observed between 2<sup>nd</sup> and 3<sup>rd</sup> stages. Protein content increased and carbohydrate and AAC decreased their levels significantly (P=0.000). Those changes indicated the progress of biochemical reactions and the existence of a climacteric peak between those stages, which could be linked to ethylene production. In fact, Toledo et al. (2012) identified changes in defense, storage and biosynthetic

proteins involved in ripening, mainly during ethylene production. In the case of proteins, they were higher in the 3<sup>rd</sup> stage and then decreased. Proteins are involved in several metabolic pathways during ripening and senescence of the fruits (Shi et al, 2014). According to Toledo et al (2012) during pre-climacteric and climacteric stage some proteins enzymes are synthetized, and are involved in banana flavor, texture, defense and synthesis of ethylene. However, during climacteric peak and postclimateric these enzymes are down regulated, and protein could be used as a source of energy. The balance between the synthesis and degradation of protein determinates the total crude protein. Particularly significant were the changes in sugar content and AAC, which ranged from 6.3 to 52.7 g/100 g and 24.97 to 1.67 g/100 g, respectively. Certainly, a significant increment (P = 0.000) of sugar content was observed between  $2^{nd}$  and  $3^{rd}$  stages, due to carbohydrate enzymatic hydrolysis, affecting mostly to amylose, as indicated the rapid reduction of AAC after 2<sup>nd</sup> stage, corroborating Gao, Huang, Dong, Yang, & Yi (2016) findings. Liu et al. (2015) suggested that amylose content reduction was generated by hydrolysis in the amorphous part of the starch granule. In fact, a strong correlation was found between sugar and carbohydrate content (r=-0.94, P<0.001), sugar and AAC (r=-0.89, P < 0.005), as well as carbohydrate and AAC (r=0.89, P < 0.001). In addition, possible amylose lixiviation during the flour preparation might have affected the AAC

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It is interesting to note that between  $2^{nd}$  and  $3^{rd}$  stages, sugar, amylose as well as fat shift abruptly. Bearing in mind the correlation between fat and sugar (r=0.96, P<0.001) as well as fat and carbohydrate (r=-0.94, P<0.001), it might be possible that between these stages a conversion of carbohydrate to fat was taking place.

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Hydration properties of banana flour at different ripening stages (Table 2) showed a significant ( $P \le 0.005$ ) decrease in WHC, WBC and WAI at the end of ripening. As previously noted, between 2<sup>nd</sup> and 3<sup>rd</sup> stages an abrupt change was observed in WAI and WSI, being consistent with the existence of a climacteric peak. In fact, a strong inverse correlation was distinguished between WAI and WSI (r=-0.97, P=0.000). Also, a high correlation was observed between carbohydrate content and WAI (r=0.95, P=0.000) and WSI (r=-0.90, P < 0.001), as well as between sugar content and WAI (r=-0.93, P < 0.001) and with WSI (r=0.92, P<0.005). Consequently, the reduction of hydration properties may be attributed to starch degradation and sugar release; possibly sugar could interact with starch chains (Baek, Yoo, & Lim, 2004) or water, limiting the availability of water to hydrate the starch (Peroni-Okita et al., 2013). Present findings suggested that some reported results ascribed to unripe banana flour could correspond to some degree of ripening. For instance, Sarawong, Schoenlechner, Sekiguchi, Berghofer, & Ng (2014) assessed the WAI and WSI of unripe banana flour, obtaining values that could be located between 2<sup>nd</sup> and 3<sup>rd</sup> stages of ripening, supporting the necessity to define the ripening stage. On the other hand, OAC increased during ripening, which was correlated to the increase of fat (r=0.81, P<0.05), suggesting an emulsifier role of the fat.

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The color characteristics of banana flour  $(L^*, a^*)$  and  $b^*$  also significantly changed during ripening (Table 2). As ripening stages progressed, flour lightness  $(L^*)$  decreased, confirming previous report (Pragati et al., 2014). In addition, flour reduced its greenness tonality  $(a^*)$  and increased its yellowness  $(b^*)$ . This result could be related to enzymatic reactions.

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The X ray diffraction patterns of banana flour at different ripening stages are presented in Figure 1. The patterns show three prominent peaks between 15 to 25 diffraction angles, consistent with B type crystallinity. The type of diffraction pattern confirms previous results in banana starch (Zhang & Hamaker, 2012). The crystallinity index for banana flour from 1st stage of ripening agrees with previous results in green banana starch from different cultivars (Utrilla-Coello et al., 2014). Between 1st and 2nd stages of ripening, the crystallinity percentage decreased significantly (P<0.05), but no further significant changes were observed. Considering that the term crystallinity is more related to amylopectin (Buléon, Colonna, Planchot, & Ball, 1998), presumably at the beginning of ripening amylopectin was more degraded than amylose. Indeed, this finding agrees with the increase of AAC between 1st and 2nd stages previously mentioned. It must be also considered that the immersion in boiling water during flour preparation, might produce partial starch gelatinization of the outer layers and subsequent amylose lixiviation that could be more pronounced in the late ripening stages due to pulp softening. That loss in amylose could contribute to keep the crystallinity pattern of the remaining amylopectin.

A significant decrease in the viscoelastic properties of the banana flour was observed during ripening (P<0.05) (Table 3). The Ostwald equation  $\eta_{app} = K\dot{\gamma}^{n-1}$  was used to describe the apparent viscosity of banana flour, at a share rate range between ~10 a 450 s<sup>-1</sup>. The power law indexes (0<n<1) at different ripening stage indicated a pseudoplastic behavior, which agrees with results obtained with banana starch (Utrilla-Coello et al., 2014). In contrast to early findings, no significant correlation was found between AAC and n value (Utrilla-Coello et al., 2014; Xie et al., 2009), likely due to previous studies were carried out with starch. Banana flour is a more complex matrix and the rheological

behavior results from the interaction of all components. Indeed, a strong correlation was found between n value and protein (r=0.96, P=0.00). In addition, a correlation between apparent viscosity (K) and AAC (r=0.79, P<0.01) was observed, as well as K with carbohydrates (r=0.91, P<0.001) and K with sugar content (r=-0.91, P<0.005), which agree with findings obtained with starch (Utrilla-Coello et al., 2014). These results were expected, since as the sugar increase, it became diluted in water, affecting the rheological behavior of the solution.

The elastic (G') and the viscous (G'') moduli decrease during ripening (Table 3), likely due to starch breakdown might decrease the interactions between molecules that form a three-dimensional network. Additionally, the higher G' values vs G'' values, showed that banana flour led to soft gels, that are more elastic than viscous, like the ones obtained with banana starch (Utrilla-Coello et al., 2014).

In relation to pasting properties recording the apparent viscosity during heating and cooling, flours showed a significant high viscosity peak in 1<sup>st</sup> and 2<sup>nd</sup> ripening stages that decreased when ripening progressed. It must be commented that during flour preparation the immersion of banana slices into boiling water for five minutes may produce partial gelatinization of the external layer. Even though the results in 1<sup>st</sup> and 2<sup>nd</sup> ripening stages differed from previous study in banana starch (Agama-Acevedo, Nuñez-Santiago, Alvarez-Ramirez, & Bello-Pérez, 2015), they are quite consistent with those of Bertolini, Bello-Pérez, Méndez-Montealvo, Almeida, & Lajolo (2010) and Bakare et al. (2017) carried out with unripe banana flour. Divergences might be explained either by varietal reasons or the undefined ripening stage of the flours used. The most plausible explanation for the changes observed between initial two stages and

the later ones (3<sup>rd</sup> and 4<sup>th</sup>) might be the enzymatic activity. However, when silver nitrate was used to inhibit the α-amylase during the RVA analysis (Figure 2), no differences in the apparent viscosity were observed in the flours from 1<sup>st</sup>, 3<sup>rd</sup> and 4<sup>th</sup> ripening stages, revealing the absence of  $\alpha$ -amylase activity. Recently, Gao et al. (2016) proposed that  $\beta$ amylase plays a significant contribution to starch degradation during banana ripening and α-amylase has an assistant role. Also, do Nascimento et al. (2006) noticed that βamylase is highly induced during ripening and suggested that its activity was highly correlated to starch degradation. Present results corroborate their findings. However, an unexpected behavior was observed in 2<sup>nd</sup> stage in the presence of silver nitrate, because the curve was significantly lower than in the absence of the inhibitor. With the increase in the amount of reducing sugars (from 0.09 a 0.25 g/100g) obtained at 2<sup>nd</sup> stage, it would be expected an increase of the amylase activity and consequently an increase of the apparent viscosity of the flour in the presence of the inhibitor. Nevertheless, the lower viscosity obtained with the flour at 2<sup>nd</sup> stage suggested the action of any other amylase or carbohydrate hydrolase that was activated in the presence of silver nitrate. Likewise, the formation of protein-starch and lipid-starch complexes might also be having some effect on the apparent viscosity, principally during cooling. Despite the numerous approaches to explain that behavior, the question still unanswered is what enzyme or reaction could be activated with silver nitrate.

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In addition, when recording the apparent viscosities, the values of final viscosity, setback and breakdown in 1<sup>st</sup> and 2<sup>nd</sup> ripening stages were higher than those obtained in 3<sup>rd</sup> and 4<sup>th</sup> ripening stages. Results indicate that flour from 1<sup>st</sup> and 2<sup>nd</sup> ripening stages led to firmer gels with higher tendency to retrograde. Banana gels obtained from stages 3<sup>rd</sup>

and 4<sup>th</sup> were more stable during heating, mixing and cooling, due to the decrease in the amount of starch able to gelatinize (Bakare et al., 2017).

As mentioned earlier, between  $2^{nd}$  and  $3^{rd}$  ripening stages, an abrupt change in all rheological properties was noticed (Table 3), likely linked to chemical composition of the flours. In fact, a strong positive correlation was found between pasting properties and carbohydrates content (r>0.91, P<0.001) as well as a strong negative correlation between pasting properties and sugar content (r>-0.91, P<0.005). Thus, results are strongly related to starch degradation during ripening, supporting again that between  $2^{nd}$  and  $3^{rd}$  ripening stages, a climacteric peak is developed. So far, the broad difference in rheological performance between  $2^{nd}$  and  $3^{rd}$  stages, confirms the significance of defining ripening stage for research and industrial purposes.

# 3.2. Nutritional characteristics

Table 4 presents the nutritional characteristics of banana flour at different ripening stages. It has been previously described that green banana is rich in starch, majorly in the form of resistant starch type 2 (RS2), which corresponds to native uncooked starch that is barely susceptible to hydrolysis (Gonzalez-Soto et al., 2007). As noticed above, between 2<sup>nd</sup> and 3<sup>rd</sup> ripening stages a strong change was observed in total starch, resistant starch and total phenolic content. In contrast to these findings, Gao et al., (2016) mentioned a gradual reduction of total starch and resistant starch during ripening process in Cavendish cultivar. Probably, samples were collected during the first five days of storage time and it might be possible that the 3<sup>rd</sup> stage of ripening was not reached. Nevertheless, Wang et al. (2014) indicated a rapid reduction of Cavendish's resistant starch content during the first four stages of ripening. Present results

corroborate that there was an intense starch degradation during the  $2^{nd}$  stage of ripening. Likewise, it must be stressed that the ratio resistant starch/total starch is nearly constant through the ripening process. On the other hand, as remarked Gao et al. (2016), a significant correlation between amylose and total starch (r>0.91, P=0.00) and amylose and resistant starch (r>0.89, P=0.005) was found. Taking into account the importance of resistant starch as a functional ingredient, contributing to prevent cancer in intestinal cells (Sardá et al., 2016b), banana flour from  $2^{nd}$  and  $3^{rd}$  ripening stages would be advisable for nutritional purpose.

Total phenolics content increase during fruit maturity (Table 4), contradicting Fatemeh et al. (2012) results. This divergence might be ascribed to genetic factors, geographic region of production, farming practice, post-harvest handling and processing and storage conditions (Rodriguez-Amaya, 2010). The antioxidant activity obtained for unripe banana flour (1<sup>st</sup> stage) was lower than the one reported (261.00±8.80 μmol of Trolox equivalent/100g dw) by Menezes et al. (2011), indicating an early stage of ripening in the present study. The antioxidant activity increased with ripening stage, like total phenolic content, except in 4<sup>th</sup> stage, in which a decline was observed, this might be due to a biochemical reaction taking place between stages 3<sup>rd</sup> and 4<sup>th</sup>.

During ripening, potassium content remained stable; this behavior agrees with results of Yap et al. (2017), whom analyzed the content of this mineral in the pulp. Also, values are similar to those published by USDA (2016) for green banana flour (1100 mg/100 g). A strong positive correlation was found between total phenolic content with potassium content (r=1, P<0.00), which might indicate the participation of potassium in the phenolic production pathway. In fact, a study in *Labisia pumila* Benth (Ibrahim, Jaafar,

Karimi, & Ghasemzadeh, 2012) treated with high potassium fertilization, an enhancement of secondary metabolites such as total phenolics in different parts of plant biosynthesis was observed. Authors obtained a significant positive correlation between total non-structural carbohydrate (TNC) (total soluble sugar and starch content) with total phenolics, possibly potassium contributes to increase the translocation of carbohydrate that indirectly enhanced the production of total phenolics. Nevertheless, potassium content remained constant and total phenolics content increased during banana ripening. We suggest that this mineral enhanced the availability of TNC in 3<sup>rd</sup> and 4<sup>th</sup> stages pertaining to total soluble sugar content, in special for glucose, that is converted into aromatic amino acids through the shikimic acid pathway (Seigler, 1998).

# 4. Conclusions

Banana flour from  $1^{st}$  and  $2^{nd}$  ripening stages, due to their rheological and nutritional properties, such as high peak viscosity, high final viscosity and high resistant starch content, could be used as a functional ingredient for bakery products. In addition, the pseudoplastic behavior (n < 1) of banana flours at  $1^{st}$  and  $2^{nd}$  stages, make them appropriate components to elaborate emulsions, due to both their high starch and low sugar contents. There is scarce information about the use of banana flour from  $3^{rd}$  and  $4^{th}$  ripening stages, but they might be used to prepare beverage or baby food due to the content of bioactive compounds as well as, its sugar and fat content that improve taste and palatability. Finally, continuing research on the effect of ripening in physicochemical characteristic of banana starch appears fully justified, because it will explain deeper starch degradation process and the composition results of modified starch by ripening.

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#### **Conflict of interest statement**

We have no conflicts of interest to disclose.

### References

- Agama-Acevedo, E., Islas-Hernández, J. J., Pacheco-Vargas, G., Osorio-Díaz, P., & Bello-Pérez, L. A. (2012). Starch digestibility and glycemic index of cookies partially substituted with unripe banana flour. *LWT-Food Science and Technology*, 46(1), 177–182.
- Agama-Acevedo, E., Nuñez-Santiago, M. C., Alvarez-Ramirez, J., & Bello-Pérez, L. A. (2015). Physicochemical, digestibility and structural characteristics of starch isolated from banana cultivars. *Carbohydrate Polymers*, 124, 17–24.
- Alkarkhi, A. F., bin Ramli, S., Yong, Y. S., & Easa, A. M. (2011). Comparing physicochemical properties of banana pulp and peel flours prepared from green and ripe fruits. *Food Chemistry*, *129*(2), 312–318.
- Baek, M. H., Yoo, B., & Lim, S. T. (2004). Effects of sugars and sugar alcohols on thermal transition and cold stability of corn starch gel. *Food Hydrocolloids*, *18*(1), 133–142.

- Bakare, A. H., Ogunbowale, O. D., Adegunwa, M. O., & Olusanya, J. O. (2017). Effects of pretreatments of banana (Musa AAA, Omini) on the composition, rheological properties, and baking quality of its flour and composite blends with wheat flour. *Food Science & Nutrition*, 5(2), 182–196.
- Bertolini, A. C., Bello-Pérez, L. A., Méndez-Montealvo, G., Almeida, C. A., & Lajolo, F. (2010). Rheological and functional properties of flours from banana pulp and peel. *Starch-Stärke*, *62*(6), 277–284.
- Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules*, 23(2), 85–112.
- Cardoso, J. M., & da Silva Pena, R. (2014). Hygroscopic behavior of banana (Musa ssp. AAA) flour in different ripening stages. *Food and Bioproducts Processing*, 92(1), 73–79.
- Cornejo, F., & Rosell, C. M. (2015). Physicochemical properties of long rice grain varieties in relation to gluten free bread quality. *LWT-Food Science and Technology*, 62(2), 1203–1210.
- do Nascimento, J. R. O., Júnior, A. V., Bassinello, P. Z., Cordenunsi, B. R., Mainardi, J. A., Purgatto, E., & Lajolo, F. M. (2006). Beta-amylase expression and starch degradation during banana ripening. *Postharvest Biology and Technology*, 40(1), 41–47.
- Fatemeh, S. R., Saifullah, R., Abbas, F. M. A., & Azhar, M. E. (2012). Total phenolics, flavonoids and antioxidant activity of banana pulp and peel flours: influence of variety and stage of ripeness. *International Food Research Journal*, 19(3), 1041–1046.
- Gao, H., Huang, S., Dong, T., Yang, Q., & Yi, G. (2016). Analysis of resistant starch degradation in postharvest ripening of two banana cultivars: Focus on starch

- structure and amylases. Postharvest Biology and Technology, 119, 1–8.
- Gonzalez-Soto, R. A., Mora-Escobedo, R., Hernandez-Sanchez, H., SanchezRivera, M., & Bello Perez, L. A. (2007). The influence of time and storage temperature on resistant starch formation from autoclaved debranched banana starch. *Food Research International*, 40(2), 304–310.
- Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry*, 50(16), 4437–4444.
- Ibrahim, M. H., Jaafar, H. Z., Karimi, E., & Ghasemzadeh, A. (2012). Primary, secondary metabolites, photosynthetic capacity and antioxidant activity of the Malaysian Herb Kacip Fatimah (Labisia Pumila Benth) exposed to potassium fertilization under greenhouse conditions. *International Journal of Molecular Sciences*, *13*(11), 15321–15342.
- Juarez-Garcia, E., Agama-Acevedo, E., Sáyago-Ayerdi, S. G., Rodríguez-Ambriz, S. L.,
   & Bello-Pérez, L. A. (2006). Composition, Digestibility and Application in
   Breadmaking of Banana Flour. *Plant Foods for Human Nutrition*, 61(3), 131–137.
- Juliano, B. O., Perez, C. M., Blakeney, A. B., Castillo, T., Kongseree, N., Laignelet, B.,
  ... Webb, B. D. (1981). International cooperative testing on the amylose content of milled rice. *Starch-Stärke*, 33(5), 157–162.
- Liao, H. J., & Hung, C. C. (2015). Chemical composition and in vitro starch digestibility of green banana (cv. Giant Cavendish) flour and its derived autoclaved/debranched powder. *LWT-Food Science and Technology*, 64(2), 639–644.

- Liu, Y., Xu, Y., Yan, Y., Hu, D., Yang, L., & Shen, R. (2015). Application of Raman spectroscopy in structure analysis and crystallinity calculation of corn starch. Starch-Stärke, 67((7-8)), 612–619.
- Menezes, E. W., Tadini, C. C., Tribess, T. B., Zuleta, A., Binaghi, J., Pak, N., ... Lajolo, F. M. (2011). Chemical composition and nutritional value of unripe banana flour (Musa acuminata, var. Nanicão). *Plant Foods for Human Nutrition*, 66(3), 231–237.
- Noor Aziah, A. A., Ho, L. H., Noor Shazliana, A. A., & Bhat, R. (2012). Quality evaluation of steamed wheat bread substituted with green banana flour. *International Food Research Journal*, 19(3), 869–876.
- Ovando-Martinez, M., Sáyago-Ayerdi, S., Agama-Acevedo, E., Goñi, I., & Bello-Pérez, L. A. (2009). Unripe banana flour as an ingredient to increase the undigestible carbohydrates of pasta. *Food Chemistry*, 113(1), 121–126.
- Peroni-Okita, F. H., Cardoso, M. B., Agopian, R. G., Louro, R. P., Nascimento, J. R., Purgatto, E., ... Cordenunsi, B. R. (2013). The cold storage of green bananas affects the starch degradation during ripening at higher temperature. *Carbohydrate Polymers*, 96(1), 137–147.
- Pragati, S., Genitha, I., & Ravish, K. (2014). Comparative Study of Ripe and Unripe Banana Flour during Storage. *Journal of Food Processing & Technology*, 5(11).
- Rabek, J. F. (1980). Applications of wide-angle X-ray diffraction (WAXD) to the study of the structure of polymers. In *Experimental methods in polymer chemistry* (1st ed., pp. 505–508). Chichester: Wiley-Interscience.
- Rodriguez-Amaya, D. B. (2010). Quantitative analysis, in vitro assessment of bioavailability and antioxidant activity of food carotenoids—A review. *Journal of Food Composition and Analysis*, 23(7), 726–740.

- Sarawong, C., Schoenlechner, R., Sekiguchi, K., Berghofer, E., & Ng, P. K. (2014). Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. *Food Chemistry*, 143, 33–39.
- Sardá, F. A. H., de Lima, F. N., Lopes, N. T., Santos, A. D. O., Tobaruela, E. D. C., Kato, E. T., & Menezes, E. W. (2016a). Identification of carbohydrate parameters in commercial unripe banana flour. *Food Research International*, 81, 203–209.
- Sardá, F. A. H., Giuntini, E. B., Gomez, M. L. P., Lui, M. C. Y., Negrini, J. A., Tadini,
  C. C., ..., & Menezes, E. W. (2016b). Impact of resistant starch from unripe banana
  flour on hunger, satiety, and glucose homeostasis in healthy volunteers. *Journal of Functional Foods*, 24, 63–74.
- Seigler, D. S. (1998). Shikimic Acid Pathway. In *Plant Secondary Metabolism* (pp. 94–105). Boston, MA: Springer.
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158.
- Somogyi, M. (1952). Notes on sugar determination. *Journal of Biological Chemistry*, 195, 19–23.
- Toledo, T. T., Nogueira, S. B., Cordenunsi, B. R., Gozzo, F. C., Pilau, E. J., Lajolo, F. M., & do Nascimento, J. R. O. (2012). Proteomic analysis of banana fruit reveals proteins that are differentially accumulated during ripening. *Postharvest Biology and Technology*, 70, 51–58.
- USDA, U. S. D. of A. (2016). Full Report (All Nutrients): 45020579, WEDO, BANANA FLOUR, UPC: 867297000003. Retrieved July 11, 2017, from https://ndb.nal.usda.gov/ndb/foods/show/25042

- Utrilla-Coello, R. G., Rodríguez-Huezo, M. E., Carrillo-Navas, H., Hernández-Jaimes, C., Vernon-Carter, E. J., & Alvarez-Ramirez, J. (2014). In vitro digestibility, physicochemical, thermal and rheological properties of banana starches. *Carbohydrate Polymers*, 101, 154–162.
- Von Loesecke, H. W. (1950). *Bananas: chemistry, physiology, technology*. (No. LOE 634.77 (BR 571)): Interscience Publishers, Inc.
- Wang, J., Tang, X. J., Chen, P. S., & Huang, H. H. (2014). Changes in resistant starch from two banana cultivars during postharvest storage. *Food Chemistry*, *156*, 319–325.
- Xie, F., Yu, L., Su, B., Liu, P., Wang, J., Liu, H., & Chen, L. (2009). Rheological properties of starches with different amylose/amylopectin ratios. *Journal of Cereal Science*, 49(3), 371–377.
- Yam, K. L., & Papadakis, S. E. (2004). A simple digital imaging method for measuring and analyzing color of food surfaces. *Journal of Food Engineering*, 61(1), 137–142.
- Yap, M., Fernando, W. M., Brennan, C. S., Jayasena, V., & Coorey, R. (2017). The effects of banana ripeness on quality indices for puree production. *LWT-Food Science and Technology*, 80, 10–18.
- Zhang, P., & Hamaker, B. R. (2012). Banana starch structure and digestibility. Carbohydrate Polymers, 87(2), 1552–1558.

### **Figure Captions**

Figure 1. X-ray diffraction patterns of banana flour at different ripening stages

**Figure 2.** Pasting curves of banana flour at different ripening stages using rapid viscoanalyser in water and silver nitrate solution