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Mechanically fractionated flour isolated from green bananas (*M. cavendishii* var. nanica) as a tool to increase the dietary fiber and phytochemical bioactivity of layer and sponge cakes

Running tittle: Nutritional enhancement of cakes through fractionated banana flours

Cristina Segundo[1,2], Laura Román[2], Manuel Gómez[2], Mario M. Martínez[2,3]*

[1]Facultad de Ingeniería, CIT Jujuy. Universidad Nacional de Jujuy. CONICET. Italo Palanca, 4600 Jujuy, Argentina

[2]Food Technology Area. College of Agricultural Engineering. University of Valladolid, 34004 Palencia, Spain.

[3]Whistler Center for Carbohydrate Research, Department of Food Science, Purdue University, West Lafayette, IN 47907, U.S.A.

*Corresponding author e-mail: mart1269@purdue.edu: mariomartinez175@gmail.com

Abstract

This article describes the effect of mechanically fractionated flours from green bananas on the nutritional, physical and sensory attributes of two types of cakes (sponge and layer). A plausible 30% replacement of banana flour in the formulation of layer cakes is demonstrated, finding only a small decline in the sensory perception. On the contrary, sponge cakes were noticeable worsened with the use of banana flours (lower specific volume, worse sensory attributes and higher hardness), which was minimized when using fine flour. Both layer and sponge cakes exhibited an enhancement of the resistant starch and dietary fiber content with the replacement of green banana flour (up to a five-fold improvement in RS performance). Moreover, sponge cakes yielded more polyphenols and antioxidant capacity with banana flours, especially with the coarse fraction. Therefore, results showed that a mechanical fractionation allowed a feasible nutritional enhancement of cakes with the use of banana flours.

Keywords: unripe banana, dietary fiber, cake, particle size, antioxidant capacity, resistant starch

1. Introduction

Dietary fiber (DF) represents a broad range of carbohydrate compounds varying in structure that scape digestion and absorption within the upper gastrointestinal part. High fiber diets may also be helpful for fecal bulking, decreased transit time, reduction of postprandial glycemic response, maintenance of normal blood cholesterol levels, and a lower risk of developing coronary heart disease (Verspreet, et al., 2016). Despite its physiological benefits, diets contain a low amount of DF, on average ranging from 12-14g of dietary fiber per day in United States and 18-20 grams in Europe (Schmitz, Marquart, and van de Kamp, 2007). Among dietary fibers, resistant starch (RS) is the starch fraction that scape luminal digestion. It encompasses five different starch sub-types based on the mechanism by which they resist digestion. In particular, resistant starch type 2 (RSII) is defined as starch granules that are inherently resistant due to the composition and conformation of the granule. For most sources of RSII, including potato and banana, normal cooking temperatures, such as those found in baking, will tend to disrupt the structure of the granule, potentially leading to gelatinization and increasing its digestibility (Birkett and Brown, 2007). This loss of dietary RS in western diets contributes to what has been termed the "carbohydrate gap", which is the observed deficit in the amount of fermentable nutrients compared with what is required by the intestinal microflora to maintain digestive health. In this scenario, diets that reverse the current trends and increase dietary RS intake are important strategies to rectify this gap for improving overall health (Birkett and Brown, 2007).

Banana is a general term embracing a number of species or hybrids in the genus *Musa* of the family *Musaceae*. In particular, cavendish subgroup banana cultivars (*M. cavendishii*) are the mainstays of the export trades (Zhang, Whistler, BeMiller, and Hamaker, 2005).. With a global production of 106 million tons in 2013 (FAO, 2015), about one-fifth of all bananas harvested become culls. When banana bunches arrive at central collection stations, bananas too small for shipping are removed, along with those that have damaged or spoiled areas that could cause microbial contamination of the bunch. Rejected bananas are normally disposed of improperly. Therefore, a successful industrial use of the culled bananas, such as the production of a low-cost banana flour ingredient, would alleviate the problem.

The trigger for the industrial use of banana flour could be based on its nutritional properties. In particular, banana flour contains an important fraction of dietary fiber [up to 15% in some varieties, (da Mota, Lajolo, Ciacco, and Cordenunsi, 2000)], consisting mainly of pectin (soluble fraction) and cellulose, lignin and hemicellulose (insoluble fraction) (Thebaudin, Lefebvre, Harrington, and Bourgeois, 1997). Among the dietary fiber, native raw banana starch is also known to be resistant to the attack of digestive enzymes, with *in vivo* results showing that 75-84% of the starch granules ingested reached the terminal ileum (Englyst and Cummings, 1986; Faisant, et al., 1995). Although it was found that the resistance was largely overcome by cooking to gelatinize the starch, other studies showed that the 'easily hydrolysable starch' could be lower after cooking compared to other starch sources (Zhang, and Hamaker, 2012). Another important nutritional benefit of bananas is their antioxidant capacity attributed to the role in the scavenging of free radicals of phenolic compounds, which convert existing free radicals into less harmful molecules and repairing oxidative damage (Du, Li, Ma, and Liang, 2009). Even though the antioxidant properties have been especially attributed to banana peels (Kondo, Kittikorn, and Kanlayanarat, 2005), accumulating evidence has revealed that banana pulp also contains various antioxidants, such as phenolic compounds, comprising catechin, epicatechin, lignin and tannins and anthocyanins (Sulaiman, et al., 2011).

All these nutritional benefits have made banana flour to be explored as a functional ingredient in various products such as slowly digestible cookies (Aparicio-Saguilan, et al. 2007), high-fiber bread (Ho, Aziz, and Azahari, 2013; Juarez-Garcia, Agama-Acevedo, Sayago-Ayerdi, Rodriguez-Ambriz, and Bello-Perez, 2006), pasta with higher antioxidant capacity (Ovando-Martinez, Sayago-Ayerdi, Agama-Acevedo, Goñi, and Bello-Perez, 2009, Almanza-Benitez, Osorio-Díaz, Mendez-Montealvo, Islas-Hernandez, and Bello-Perez, 2015), noodles (Choo, and Aziz, 2010), tortillas (Aparicio-Sanguilan, et al., 2013) and snacks (Wang, Zhang, and Mujumdar, 2012). However, to the best of our knowledge, the use of banana flour as an ingredient in baked emulsions, such as cakes, has been not considered so far.

Banana flour counts with 61-76.5% starch (da Mota, et al., 2000), which will determine some of the flour functionality. However, the intrinsic properties of flour particles, such as particle size, will strongly influence their emulsifying and hydration properties and therefore affecting the quality of the resultant cakes. In particular, rice flours with coarse particle size did not allow the correct emulsion formation by halting air incorporation in form of fine and homogenous bubbles, worsening cake volume and texture (de la Hera, Martínez, Oliete, and Gomez, 2013b). However, the optimum flour particle size distribution for cake-making depends, in turn, on the type of cake. In particular, Dhen, et al., (2016) reported a negative influence of course soy flour on the quality of sponge cake, whereas no significant differences were observed for layer cakes. Therefore, our hypothesis was that the use of flour isolated from green bananas with an optimum particle size could: 1) improve the nutritional properties of cakes while maintaining its physical and sensory attributes, and, 2) minimize the waste of banana culls.

The objective was to study the effect of a wheat flour replacement (15 and 30%) by green banana flour with three different particle sizes [80µm (fine), 80-156µm (medium) and 156-200 µm (coarse)] on the nutritional (dietary fiber, resistant starch, phenolic compounds, antioxidant capacity), physical (specific volume, texture and color) and sensory properties of layer and sponge cakes. To do this, banana flour was obtained from green (unripe) bananas through a low-cost procedure and subsequently sieved. Nutritional and physicochemical properties of flours as well the rheological properties of batters were also analyzed in order to enhance the discussion about the different phenomena involved.

2. Materials and methods

2.1 Materials

Wheat flour (10.3 g/100 g moisture; 8.88 g/100 g protein; 84.23 µm mean particle size) was supplied by Harinera la Castellana S.A (Medina de Campo, Valladolid, Spain). Sugar, sunflower oil, whole liquid milk, powdered milk and liquid pasteurized egg were purchased from the local market. Baking powder 2x1 and the emulsifier SuperMixo T500 were provided by Puratos (Gerona, Spain). Bananas (*M. cavendishii* var. nanica) were purchased at ripening state 2 (yellow with black spots) according to standard color chart, http://ripening-fruit.com/banana).

2.2 Methods

2.2.1 Isolation of banana flour from green bananas and flour characterization

Bananas were peeled and ground in a Liliana AM523 juicer (Cheffy, Argentina) at 1 speed for 3 min. The paste obtained was poured in a tray and dried in an oven with forced convective flow at 60°C for 24 hours. Dried paste was ground in a Numak FW 100 laboratory mill (Ojalvo, Argentina) and subsequently sieved in a Bühler MLI 300B screening machine (Uzwil, Switzerland) for 15 min, obtaining a fine (<80µm), medium (80-156µm) and coarse fractions (156-200µm). The different fractions were sealed in plastic bags and stored.

2.2.2 Proximate analysis and characterization of flours

Banana flour was characterized following AACC (2016) methods for moisture content (44.15.02), protein (46.11.02), lipids (30.25.01), dietary fiber (32.05.01), ash (08.12.01) and resistant starch (32-40.01). Carbohydrate content of flours was obtained by weight difference with the other flour components. The amylose content was determined by the Megazyme amylose assay kit (Megazyme, Bray, Ireland) using the Concanavalin A precipitation procedure. It is noteworthy that the resistant starch contained in green banana is comprised mainly by starch granules that are inherently resistant due to the composition and conformation of the granule (RSII). However, this type of RS is disrupted by high temperatures, such as those in the initial step of total fiber analysis, potentially leading to gelatinization (Birkett and Brown, 2007). Therefore, RSII was not included in the fiber analysis (method 32.05.01, AACC, 2016). All analyses were run in duplicate. The characteristics of the different banana flour fractions are shown in Table 1.

Flour photomicrographs were taken with a Quanta 200FEI (Hillsboro, Oregon, USA) ESEM (Fig. 1). Photomicrographs were taken in beam deceleration mode (BDM) at 1.5 keV in high vacuum mode with a backscattered electron detector (BSED).

2.2.3 Cake making

Layer and sponge cake were made as follows. Layer cakes consisted of 350 g flour, 315 g sucrose, 210 g whole milk, 175 g liquid pasteurized egg, 105 g sunflower oil and 10.5 g baking powder. All ingredients were mixed in the Kitchen Aid Professional mixer KPM5 (St. Joseph, Michigan, USA) for 1 min at speed 4 and 9 min at speed 6. Cake batter (185 g) was placed into disposable oil-coated aluminum pans (109 x 159 x38 mm) and the cakes were baked in an electric oven for 25 min at 185 °C.

Sponge cake formula consisted on 245 g flour, 240.5 g sugar, 344 g liquid pasteurized egg, 55 g water, 14 g emulsifier, and 25 g powdered milk. A creaming-mixing procedure was used. All ingredients, except for the flour and milk were mixed for 2 min at speed 6 using a Kitchen-Aid Professional mixer (Kitchen Aid, St. Joseph, Michigan). After the addition of the milk and flour, the mixing process was continued for 3 min at speed 8. Cake batter (125 g) was placed into oil-coated aluminum pans (109x 159 x 38 mm) and baked as described above.

After baking, cakes were removed from the pans and left to cool for 60 min before being placed in coded plastic bags that were sealed to prevent drying. For each type of cake (layer or sponge), a 15 and a 30% of wheat flour replacement by fine, medium and coarse banana flour was studied, which together with the control (100% wheat flour) gave 7 formulations. All cakes were made in duplicate.

2.2.4 Density and rheological properties of batters

Batter density was determined with a standard container (100 cc) of known weight filled with batter.

Before conducting any rheological measurement, samples were allowed to rest in the measurement position for 5 min as equilibration time, i.e. the necessary time to allow the stresses induced during sample loading to relax. The exposed edges of the samples were always covered with vaseline oil (Panreac Química S.A., Castellar del Valles, Spain) to avoid sample drying during measurements. Rheological properties were recorded using a rheometer (Haake RheoStress 1, Thermo Fischer Scientific, Scheverte, Germany). All rheological tests were run in duplicate.

2.2.4.1. Flow properties.

Shear stress versus shear rate data were recorded using a concentric cylinder geometry programmed to increase the shear rate from 1 to 100 s-1 for 400 s (up curve), then to maintain this shear rate for 300 s and immediately followed by a reduction from 100 to 1 s-1 for 100 s (down curve). Data from the up curve of the shear cycle were fitted to the Ostwald-de Waele model ($\sigma = K.\gamma^n$), where σ is the shear stress (Pa), γ is the shear rate (s^{-1}), K is the consistency coefficient ($Pa.s^n$), and n is the flow behavior index (dimensionless). All measurements were made in duplicate.

2.2.4.2 Viscoelastic properties

Linear viscoelastic properties were studied at 20°C by small amplitude oscillatory test (SAOS) using a 60 mm steel serrated plate-plate sensor geometry with a 1 mm gap. Dynamic linear viscoelastic range was estimated by performing a stress sweep from 0.05 to 10 Pa at a frequency of 1 Hz. Frequency dependence experiments were conducted from 10 to 0.01 Hz at 20°C. The applied stress was always selected to guarantee the existence of linear viscoelastic response. The storage modulus (G'), loss modulus (G'') and loss factor (tan δ= G''/G') at 1Hz were obtained. All the analyses performed on the batter were carried out in duplicate.

2.2.5 Physical properties of cakes

Cake characteristics were made 24 hours after baking. Specific volume was determined with the Volscan Profiler volume analyzer (Stable Mycrosystems, Surrey, UK) and calculated as the ratio between the volume of the cake and its weight. Weight loss was calculated as the ratio between weight of batter in the mold and the weight of the cake after baking, expressed as a percentage. Measurements were run in two cakes for each formulation.

Crumb texture was determined using a TA-XT2 texture analyzer (Stable Microsystems, Surrey, UK) with the "Texture Expert" software. A 25-mm-diameter cylindrical aluminum probe was used in a 'texture profile analysis' (TPA) double compression test to penetrate to 50 % depth, with a test speed of 2 mm/s and a 30-s delay between the first and second compressions. Hardness (N), cohesiveness, and springiness were calculated from the TPA graphic. Measurements were made on two central slices (20 mm thickness) from two cakes for each formulation.

2.2.6 Cake sensory evaluation

Hedonic sensory evaluation of cakes was conducted with 72 volunteers, regular cake eaters, of 18-55 years of age and various socioeconomic backgrounds. Cakes were evaluated on the basis of acceptance of their appearance, odor, taste, aftertaste, texture, and overall liking on a nine-point hedonic scale. The scale of values ranged from "like extremely" to "dislike extremely" corresponding the highest and lowest scores to "9" and "1" respectively. Samples were analyzed one day after baking. Samples were presented as half-pieces for appearance and 2-cm slices for texture, odor, taste, taste persistency and overall acceptability, on white plastic dishes coded with four-digit random numbers and served in a randomized order. Water was available for rinsing.

2.2.7 Resistant starch and dietary fiber in cakes

Resistant starch and total dietary fiber were analyzed in cakes following the methods 32-40.01 and 32.05.01, respectively (AACC, 2016). Analyses were run in duplicate.

2.2.8 Phenolic compounds

Phenolic compounds were extracted with methanol-water acidified with HCl (50:50 v/v, pH 2, 50 mL g^{-1} sample, 16 h) and acetone-water (70:30 v/v, 50 mL g^{-1} sample, 60 min) at room temperature (25°C) under constant stirring. After centrifugation (15 min, 25°C, 3000 × g) supernatants were pooled and used to determine extractable polyphenols content and antioxidant capacity. Extractable polyphenols were determined by the Folin-Ciocalteu procedure. The sample (0.1 mL) was mixed with 1 mL of Folin-Ciocalteu reagent and swirled for 10 min. Then, 1 mL of sodium carbonate solution (7% w/w) was added and mixed, and then it was completed to 3 mL with distilled water. Determination was performed at a wavelength of 765 nm in a spectrophotometer (UV/VIS Spectronic 4001/4, Spectronic Instrument, USA), and total polyphenol content was expressed as mg of gallic acid equivalents (GA)/100g dry matter. All analyses were carried out in duplicate

2.2.9 Free radical-scavenging assay (DPPH)

The antioxidant activity of the extracts from the cakes was determined by DPPH free radical elimination. Three aliquots (100, 300 and 500µl) of each sample extract were taken and added to 3 mL of methanolic DPPH solution. The decrease in absorbance at 517nm was determined spectrophotometrically during 300seg. The inhibition activity of the DPPH radical by the samples was calculated according to the equation 1.

Inhibition activity (%)=100×(1-(A_{ss}/A_0)) (1)where A_{ss} is the absorbance of the solution in a steady state and A_0 is the absorbance of DPPH without the sample.

IC_{50} value (mg extract/ml) is the effective concentration at which the antioxidant activity was 50%, i.e., the amount of sample required for 50% of free radical scavenging activity IC_{50} value was obtained by interpolation from linear regression analysis. Each antioxidant attribute of ethanolic extracts from cakes was averaged from two replications.

2.2.10 Statistical analysis

Analysis of variance (ANOVA) was used to study the differences between batters and cakes characteristics according the percentage and kind of unripe banana flour. Fisher's least significant difference (LSD) test was used to describe means with 95% confidence. The analysis was performed using Statgraphics Plus V5.1 software (Statpoint Technologies, Warrenton, USA).

3. Results and Discussion

3.1. Chemical composition of green banana flours

The effect of mechanical fractionation on the chemical composition of the three fractions of banana flours is shown in Table 1. Among the three fractions, fine flours showed lower protein and ash content as well as higher moisture and carbohydrate content. As for the coarse fraction, higher lipid content was observed. No significant differences were observed in the amylose percentage, whereas resistant starch, total dietary fiber and phenolic compounds displayed the most outstanding differences.

The resistant starch content of banana flours ranged from 24.88 to 37.9%, which is lower than that reported by Zhang et al. (2012) in pure banana starch. However, the low-cost method used in the present work for the production of banana flour, including a dry-milling step, was expected to be more aggressive in terms of damaging starch granules than the conventional extraction with chemical reagents for the production of pure starch. The damage of starch during milling could also explain the lower amount of resistant starch in the medium and coarse fractions. The presence of particles with different size suggests differences in the hardness between different parts of the dried pulp (similar to milling of cereal kernels). The coarse fractions could come from the hardest parts of the dried pulp, resulting in prolonged times inside the laboratory mill before escaping through the mesh and therefore causing some damage at granular level. This effect was already observed by de la Hera, Gomez, and Rosell (2013a) in coarse fractions of rice flour.The three fractions of green banana flour showed a considerable amount of total dietary fiber (6.43-10.41 g/100 g) and the presence of soluble fiber (1.92-2.04

g/100 g). These results are similar to the values for fiber content of seven types of banana flours reported by da Mota et al. (2000) (6.0-15.5 g/100 g for total fiber and 2.10-3.05 g/100 g for soluble fiber).This would suggest that these flours and their resultant products could have the physiological benefits of soluble and insoluble fibers. No significant differences were found in the content of soluble fiber among fractions with different particle size. However, a significant increase in the total dietary fiber content with the particle size was observed. This was expected since the fine fraction was formed basically by starch granules loosed from the coarser particles that comprise the coarse fractions (Fig. 1). On the other hand, large particles possessed other components associated with the banana pulp, such as dietary fiber and protein attached to the granule surface and filling the intergranular space within the particle.

Coarse fractions of banana flours showed a higher content of phenolic compounds. During banana flour-making, dehydration of pulps through drying process may contribute to the successive extraction of the phenolic compounds. At cellular level, the phenolic compounds are located in the vacuoles and are separated from oxidative enzymes in an intact fruit (Sulaiman, et al., 2011). This structure collapses during dehydration or drying process leading to a release of more phenolic compounds that may be then degraded by the oxidative and hydrolytic enzymes. The coarser size of coarse flour particles with better integrity of their plant cells could contribute to a better protection of phenolic compounds to excessive degradation. Nevertheless, it is noteworthy that the drying step at 60 °C will denature these degrading enzymes, making all the banana fractions possess a plausible amount of phytochemicals in the final dried flour (Table 1). Moreover, coarser fractions of banana flour can also be associated with a higher content of dietary fiber, which is related to higher concentration of bioactive compounds such as polyphenols (Almanza-Benitez, et al. 2015).

3.2 Batter properties

Batter density is shown in Table 2. The results showed that banana flour inclusion gave rise to a reduction in the batter density for both layer and sponge cakes, especially in the later one. This could be due to either a better air incorporation into the batter or to the breakage of bubbles as a result of the interactions with the rest of the ingredients in the different cakes. In layer cakes, a lower density was found with higher flour replacement, whereas no significant trend was observed in the case of sponge. Dhen et al. (2016) already observed a similar effect using soy flour in cake making. In the same way, Wilderjans, Pareyt, Goesaert, Brijs, and Delcour (2008) reported a decline in the density value of pound cakes when the amount of starch content was reduced. As for the particle size, no significant effects were found. de la Hera et al. (2013b) reported a decrease in the density of rice batters with coarse fractions, for both layer and sponge cakes. However, in that study, cakes were made with 100% of sieved rice flour, whereas in the present study a maximum of 30% replacement by sieved banana flour fractions is used.

Flow behavior of batters with 15 and 30% banana flour replacement are shown in Table 2. The effect of the different fractions of banana flour was different in each type of cake. In general, the addition of banana flour produced a decrease in the consistency coefficient (K) of sponge cakes batters, with lower values for higher replacement. However, this low K was minimized with the fine fraction and even enhanced with a 15% replacement of the fine fraction of banana flour. As for layer cakes, an opposite trend was observed, increasing the batter consistency (K) with the use of medium and coarse particles. Differences in K are crucial since a high consistency will be related to a high capacity of batters to retain air, resulting in a high volume of the cakes. However, the increase of K should be achieved taking into account that a very high consistency could also diminish cake quality since it would impede the correct batter dosing and expanding

(Lakshminarayan, Rathinam, and KrishnaRau, 2006). The different trend reported for each type of batter (sponge and layer) suggests that the effects of banana flour depend on the type of emulsion. In sponge batters, the emulsion is formed by a greater number of air bubbles formed during mixing, which are more closely packed than in layer cakes, as a result of the emulsifier action (Gómez, Ruiz, and Oliete, 2011). On the other hand, the baking powder in layer cakes, responsible for the air bubbles growth, does not act until a certain temperature is reached in the oven. In this way, in sponge cake emulsion, coarse particles would difficult the emulsifier action by halting bubble stabilization (Dhen, et al., 2016) and promoting their rupture during the shear stress produced during the rotational rheological test. This hypothesis is endorsed in turn by the significant loss of pseudoplastic behavior (higher flow behavior index, n) observed in sponge cakes substituted by the medium and coarse fractions of banana flour, which was less evident in layer cakes. A lower pseudoplastic behavior entails a less complex structure of these batters, leading to an emulsion behavior more similar to water, which is, in fact, in agreement with the theory of the air bubbles escaping from the batter during the rotational flow behavior test.

In order to better characterize the structure of batters, the small amplitude oscillatory test was expected to provide more accurate information. The influence of the addition of sieved banana flours on the viscoelasticity is shown in Table 2. In the available frequency window, the so-called plateau relaxation zone was observed in all the batters (data not shown). This region is characterized by the fact that the storage modulus (G') is higher than the loss modulus (G''), both moduli depending on frequency but following a different pattern (Martinez, Sanz, and Gomez, 2015). This region is characteristic of the occurrence of physical entanglements in polymeric materials (Ferry, 1980). In sponge cakes, a significant increase of G' was observed with a 15 and 30% replacement of medium banana fractions, whereas in layer cakes, the coarsest fraction led to the most noticeable increase of both viscoelastic moduli. This would suggest that batters containing medium and coarse banana flours have the same rheological units than the rest but with an enhanced contribution to increase the batter consistency (Martinez, et al., 2015). However, a 30% substitution by the medium fraction (sponge cake) and the coarse fraction (layer cake) of banana flour had a significantly lower loss tangent (tanδ), indicating different viscoelasticity and therefore the presence of new rheological units. This can be attributed to the fact that viscoelastic properties are also dependent on the concentration of air trapped during mixing (Kalinga, and Mishra, 2009). According to the flour composition (Table 1), it seems that fiber content, having a much higher water absorption capacity than intact starch, could diminish the free water content enhancing the structure and increasing both dynamic moduli. The result would be a masking of the effect of the intrinsic higher surface to volume ratio of fine flours. The fact that these results follow a different trend than those for K in sponge cakes would confirm that during the rotational test the shear stress was enough to promote the air bubbles breakage, while it did not happen during the oscillation sweeps. On the other hand, the parallel increase between the viscoelastic moduli and K would suggest that in layer emulsion, no bubble scape was produced.

3.3 Cake characteristics

In order to assess the effect of the incorporation of the different fractions of banana flour on the physical properties of cakes, specific volume and crumb texture were analyzed (Table 3). The effects of the different banana flour fraction were conditioned to the type of cake. The specific volume of layer cakes was barely modified with the inclusion of banana flour (less than 8% of increase/decrease compared to control), appreciating a slightly improve with fine and medium fractions. Conversely, the incorporation of green banana displayed a clear negative effect on the specific volume of the sponge cakes, which was reduced greatly when increasing the particle size of banana flour and the level of replacement. In actual fact, the only cake which was not significantly different from the control was the cake made with 15% of fine banana flour, whereas with 30% coarse banana flour replacement the reduction in the specific volume reached 36%. This results are in agreement with the consistency and flow behavior index of sponge batters, indicating that higher consistency (higher K) and pseudoplasticity (lower n) bring about high volume cakes. However, this correlation was not clear for layer cakes, where bubbles are created during baking. In this way, changes in volume may not be solely related to the air incorporated during the mixing, but to differences in the air retention and expansion during baking process. It is known that bubble size is crucial for batter stability in such a way that, big average bubble size is related with low stability, especially during the first stages of baking, when the batter viscosity is reduced (Stauffer, 1990). The internal structure of sponge cakes consists of a greater number of bubbles with smaller average size than layer cakes (Gómez, et al., 2011). This structure could be more sensitive to the presence of coarse flour particles, which could impact more negatively to the volume of sponge cakes than the volume of layer cakes (de la Hera, et al., 2013b; Dhen, et al., 2016).

Crumb hardness of sponge cakes was highly correlated to the specific volume of cakes, observing a negative correlation between specific volume and crumb hardness (r=-0.71), which agrees with that observed in other studies (Gómez, Ruiz-París, Oliete, and Pando, 2010; de la Hera et al., 2013b). These studies have already proven that this correlation was more significant in sponge than in layer cakes. In fact, in our study, the decrease in crumb hardness of layer cakes was not correlated to the specific volume and therefore the phenomena cannot be ascribed to changes in volume. We hypothesize that it should be related to structural changes in the crumb, possibly influenced by the differences between the starches from banana and wheat in terms of gelatinization and retrogradation behaviors. In this way, despite significant differences were hardly seen in the springiness of layer cakes, a decline in the cohesiveness was found for the cakes made with 30% of banana flour, which may be related to the differences between wheat and banana starch. A similar trend was obtained for sponge cakes, but in this case, both a reduction in springiness and cohesiveness was appreciated, being this effect clearer as the level of replacement was increased, especially for the coarsest particle sizes.

3.4 Sensory evaluation

The sensory analysis (Table 4) was performed in layer cakes with 30% banana flour replacement and in sponge cakes with 15% replacement, since sponge cakes with 30% replacement showed a low specific volume. The inclusion of banana flour in layer cakes led to a slight worsening in the acceptability of the cakes, but no effect was found for the particle size, which in general, is in accordance with the results observed for the physical characteristics of cakes. The most important differences among the parameters studied were noticed in appearance, possibly due to colour differences (supplementary material), and odor. Changes in the color of the crust should be explained by the presence of higher sugars in banana flours, such as glucose, fructose and sucrose (Emaga, Andrianaivo, Wathelet, Tchango, and Paquot, 2007). These sugars are precursors for Maillard reactions and caramelization because of the high temperatures reached on the external part of the cake during baking (Purlis, 2010). As for the inner part of the cake, i.e., crumb, which do not exceed 100°C during baking, Maillard and caramelization reactions do not take place, and color differences can be attributed to differences in the color of the flour. In this case banana flour is less whitish than wheat flour, with slight differences in the particle size (see supplementary material). In general, differences for overall cake acceptability of cakes made with banana flour were not greater than 0.7 compared to the control.

In the case of the sponge cakes, despite the fact that the level of replacement chosen was lower, a lower general acceptance of cakes was observed with banana flour replacement (except for cakes made with the fine fraction), being differences more noticeable when particle size was increased. These results are in agreement with the physical characteristics of cakes reported in the previous section.

According to the results, layer and sponge cakes were obtained with a low decline in their acceptability with a 30% and 15% banana flour replacement, respectively. Furthermore, the effect of flour particle size depended, in turn, on the type of cake. In particular, layer cakes were not influenced by the particle size whereas sponge cakes with no significant differences with the control were only obtained with banana flour with fine particle size.

3.5 Resistant starch, dietary fiber, phenolic compounds and antioxidant capacity

Layer and sponge cakes made with the different fractions of green banana flour were analyzed for resistant starch, total dietary fiber, phenolic compounds and antioxidant capacity (Fig. 2). Both layer and sponge cakes exhibited an enhancement of the dietary resistant content with the replacement of green banana flour, especially with the higher level of substitution (30%). In particular, the use of green banana flours yielded up to a five-fold improvement in RS performance (Fig. 2a). Although it has been reported that the resistance of banana starch in their native state to the luminal digestion is overcome by hydrothermal processing, our results show that there is an important fraction that persists in the final cake. This would indicate that either the integrity of some granules could resist the high temperatures at relatively high moisture levels during baking or that after cooling some leached amylose chains would retrograde creating resistant starch type 3 (RSIII). However, we believe that the former is a more plausible mechanism, since cake systems are also composed by other ingredients such as sugars, that could delay starch gelatinization and therefore keeping the granular integrity. The inherent resistance of the integral granules is in turn reinforced by its B-type crystallinity (entailing lack of superficial pores) and therefore without channels for the access for pancreatic α-amylase during intraluminal digestion. Our results are in agreement with other works after processing banana hydrothermally (Zhang, et al., 2012). Results also showed that 30% replacement with fine flours gave rise to the highest RS, which seems logical regarding the higher RS content of these flours in their native state (Table 1). Explanations for this occurrence are given in section 3.1.

The enhancement in the RS was also complemented with the increase of other sources of dietary fiber through the substitution of green banana flour (Fig. 2a). In particular, these fibers would encompass pectin, cellulose, lignin and hemicellulose, typical from banana flours (Thebaudin, et al., 1997). In this case, a higher amount of dietary fibre was found for the coarsest fractions of green banana flour, which was also expected according to the fiber content of the initial flours (Table 1). Explanations for this occurrence are also given in section 3.1.

In sponge cakes, a significant increase of the polyphenol content was observed for the medium and coarse fractions of banana flours (Fig. 2b), which is in accordance with polyphenol content of the initial flours. As for layer cakes, even though a higher phenolic content can be observed, the effect was not as noticeable as in sponge cakes. The reasons for this different behavior must be approached from the fact that high temperatures (above 50°C) can destroy phenolic compounds (Azizah, Ruslawati, and Swee-Tee, 1999). As we mentioned, the internal structure of the different type of cake varies (Gómez, et al., 2011). The greater number of bubbles with smaller average size in sponge cakes brings about batters with higher consistency and solid-like behavior than layer batters (see Table 3), which can lead to moderating the mass and heat transfers and therefore diminishing the loss of phenolic compounds during baking. In other words, the short baking time and the gluten-starch-non starch polysaccharides matrix in the cake substituted with banana flours could protect the polyphenols against excessive destruction (Almanza-Benitez, et al., 2015). This occurrence would happen with less strength in layer cakes, in which the loss of phenolic compounds from the raw banana flours would be higher as a consequence of the absence of entities that protect phenolic compounds from destruction during baking.

The incorporation of green banana flours brought about sponge and layer cakes with lower values of IC_{50}, i.e., lower effective concentration at which the antioxidant activity was 50% and therefore indicating higher phytochemical activity. In particular, cakes made with the coarsest fractions of banana flours had the highest antioxidant capacity. This followed the same trend as the polyphenols content. Fruits are usually rich in polyphenols such as anthocyanins and proanthocyanindins which can increase the scavenging activity of the stable DPPH radical model, as a consequence of the hydrogen donating ability (Von Gadow, Joubert, and Hannsman, 1997). In addition, those phenolic compounds could be associated with the higher presence of dietary fiber, since it was reported that dietary fiber retains antioxidant capacity. During fermentation of dietary fiber in the colon, the phenolic compounds with antioxidant capacity can exert its physiological activity (Saura-Calixto, 1998). The significant antioxidant capacity of cakes, and in particular of sponge cakes substituted with green banana flours, is an additional nutraceutical characteristic to that of the high RS and dietary fiber content. In this sense, consumption of cakes substituted with green banana flours could give more beneficial effects to people's health.

4. Conclusion

In this study, we showed that flour isolated from green bananas can be used to improve the nutritional properties of layer cakes without negatively affecting their physical properties, resulting in only a small decline in the sensory perception. On the other hand, the physical and sensory attributes of sponge cakes were noticeably worsened with the use of banana flours, but this effect was minimized when using fine flour. Increases of RS and dietary fiber (other than RS) content were attained in both type of cakes with green banana flour replacement. Furthermore, the incorporation of green banana flours also enhanced the content of phenolic compounds and the antioxidant capacity of cakes, especially of sponge cakes, where a 40% increase of antioxidant capacity was achieved. As for the particle size, coarse flours yielded cakes with higher content of dietary fiber, phenolic compounds and antioxidant capacity than those made with the fine counterparts. However, they also led to a significant decline in the volume and textural properties of sponge cakes, even though no significant differences in the sensory perception were found between the different particle sizes. On the other hand, fine flours yielded cakes with significantly higher resistant starch content (up to a five-fold improvement), higher volume and lower hardness than the course counterparts. Thus, banana fine flours would be more suitable in general. However, the manufacturer should also consider that the optimum particle size of banana flour for cake-making is dependent on the nutritional target as well as the type of cake.

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Figure captions

Figure 1: Micrographs of the different fractions of flour extracted from green bananas. Fine (left), medium (center) and coarse (right) fractions.

Figure 2: Effect of the addition of green banana flour on the content of resistant starch and total dietary fiber (a); and the polyphenols content and half-inhibition concentration, IC_{50} (b) of cakes. Sponge and layer cakes are shown on the left and on the right, respectively.

Table 1. Chemical composition of banana flours

	Moisture	Protein	Lipids	Ash	Carbohydrates	Resistant starch (%)	Total fiber (%)	Soluble fiber (%)	Amylose (%)
Fine	11.31^{b}	3.54^{a}	0.19^{a}	2.36^{a}	82.61^{b}	37,9^{b}	6.43^{a}	2.04^{a}	11,82^{a}
Medium	10.31^{a}	5.07^{b}	0.18^{a}	3.10^{b}	81.19^{a}	28,41^{a}	7.69^{b}	1.92^{a}	13,9^{a}
Coarse	10.33^{a}	5.24^{b}	0.25^{b}	3.15^{b}	81.22^{a}	24,88^{a}	10.31^{c}	2.02^{a}	15,03^{a}

Different letters in the same column mean significantly different ($p<0.05$)

Table 2. Density and rheological properties of batter with 15 and 30% banana flour replacement

	Sponge batter						Layer batter				
	ρ	G'(Pa)	G''(Pa)	tanδ	$K(Pa\cdot s^n)$	n	ρ	G'(Pa)	G''(Pa)	tanδ	$K(Pa\cdot s^n)$
Control	0.370^{c}	1080^{a}	327^{ab}	0.31^{c}	33.13^{b}	0.57^{b}	1.10^{e}	134^{a}	111^{a}	0.82^{bc}	20.31^{ab}
Fine 15	0.355^{a}b	1195^{ab}	337^{abc}	0.29^{bc}	36.93^{c}	0.55^{a}	1.08^{d}	97^{a}	81^{a}	0.83^{c}	22.60^{bc}
Medium 15	0.350^{a}	1430^{b}	409^{c}	0.29^{bc}	30.85^{b}	0.60^{c}	1.05^{c}	161^{a}	138^{a}	0.85^{c}	18.67^{a}
Coarse 15	0.360^{b}	1110^{a}	318^{ab}	0.29^{bc}	26.49^{a}	0.63^{d}	1.08^{d}	148^{a}	125^{a}	0.84^{c}	17.21^{a}
Fine 30	0.360^{b}	1085^{a}	310^{a}	0.29^{bc}	30.78^{b}	0.59^{bc}	1.06^{c}	75^{a}	64^{a}	0.85^{c}	18.77^{a}
Medium 30	0.360^{b}	1455^{b}	394^{bc}	0.27^{b}	27.38^{a}	0.64^{de}	1.01^{b}	453^{b}	346^{b}	0.77^{b}	24.53^{cd}
Coarse 30	0.346^{a}	1305^{ab}	318^{ab}	0.24^{a}	25.61^{a}	0.65^{e}	0.99^{a}	3220^{c}	593^{c}	0.18^{a}	27.34^{d}

Different letters in the same column mean significantly different ($p<0.05$)

ρ, batter density; G', storage modulus; G'', loss modulus; tanδ, loss tangent; K, consistency coefficient; n, flow behavior index

Table 3: Physical characteristics of sponge and layer cakes substituted with banana flour of different particle sizes

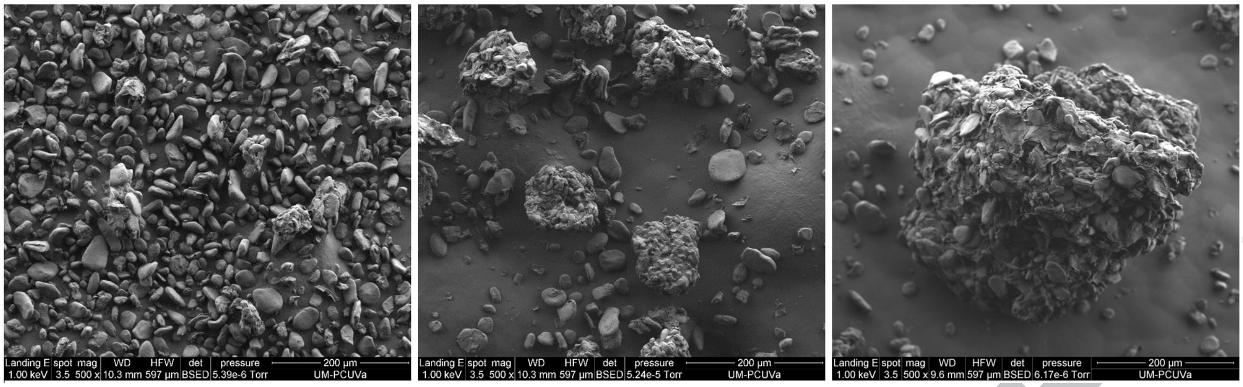
	Sponge cake				Layer cake			
	Specific volume (cm^3/g)	Hardness (N)	Springiness	Cohesiveness	Specific volume (cm3/g)	Hardness (N)	Springiness	Coh
Control	4.08^{d}	3.08^{a}	0.92^{d}	0.67^{e}	2.41^{b}	6.0^{d}	0.89^{b}	
Fine 15	3.94^{d}	3.38^{a}	0.88^{bc}	0.57^{d}	2.34^{a}	6.18^{d}	0.87^{ab}	
Medium 15	3.16^{c}	3.18^{a}	0.90^{cd}	0.57^{d}	2.6^{d}	4.36^{a}	0.88^{b}	
Coarse 15	3.02^{bc}	4.32^{b}	0.89^{bc}	0.51^{cd}	2.41^{ab}	4.90^{b}	0.86^{ab}	
Fine 30	3.34^{c}	4.53^{b}	0.89^{bc}	0.49^{bc}	2.60^{d}	5.15^{bc}	0.88^{b}	[illegible]
Medium 30	2.73^{ab}	6.93^{c}	0.87^{ab}	0.42^{a}	2.42^{b}	5.40^{c}	0.83^{a}	
Coarse 30	2.61^{a}	4.815^{b}	0.85^{a}	0.44^{ab}	2.5^{c}	4.19^{a}	0.85^{ab}	

Different letters in the same column mean significantly different ($p<0.05$)

Table 4. Sensory profile of sponge and layer cakes substituted with banana flour of different particle sizes

	Sponge cakes (15%)						Layer cakes (30%)					
	Appear	Od	Text	Tas	Persist	Overall acceptab...	Appear	Od	Text	Tas	Persist	Overall acceptab...
Contr	7.08^{c}	6.2	6.43^{a}	6.6	6.30^{a}	6.78^{b}	7.54^{b}	6.5	6.32^{b}	6.6	6.37^{a}	6.93^{b}
Fine	6.67^{bc}	5.8	6.29^{a}	6.4	6.19^{ab}	6.52^{ab}	5.97^{a}	5.6	5.59^{a}	6.2	6.25^{a}	6.41^{a}
Medi	5.94^{a}	5.7	6.11^{a}	6.3	6.00^{ab}	6.24^{a}	6.03^{a}	5.5	5.80^{a}	6.0	6.01^{a}	6.23^{a}
Coar	6.14^{ab}	5.7	5.97^{a}	6.1	5.75^{a}	6.11^{a}	5.94^{a}	5.3	5.80^{a}	6.3	6.08^{a}	6.38^{a}

Different letters in the same column mean significantly different ($p<0.05$)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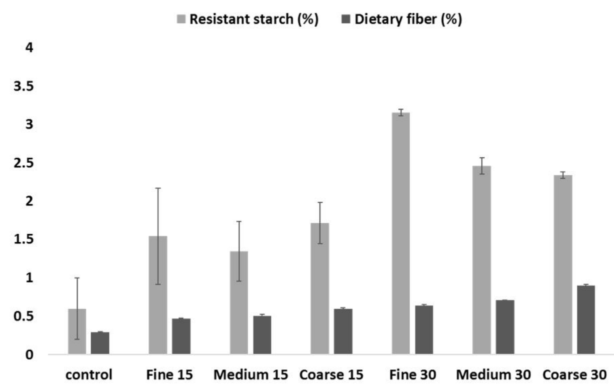
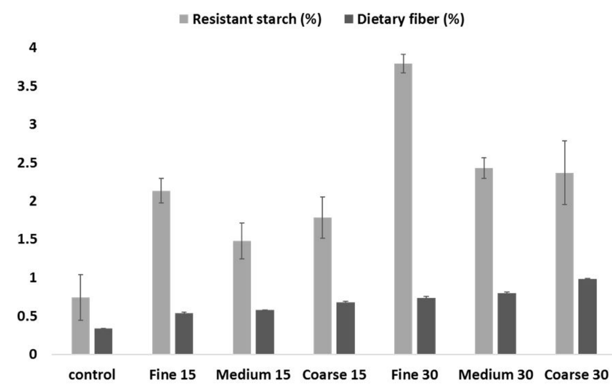

Resistant starch (%)
Dietary fiber (%)
4
3.5
3
2.5
2
1.5
1
0.5
0
control
Fine 15
Medium 15
Coarse 15
Fine 30
Medium 30
Coarse 30


Resistant starch (%)
Dietary fiber (%)
4
3.5
3
2.5
2
1.5
1
0.5
0
control
Fine 15
Medium 15
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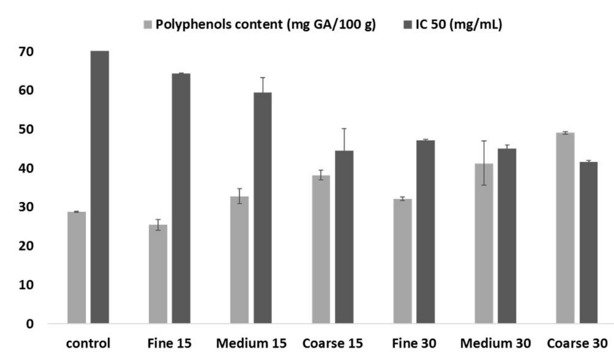
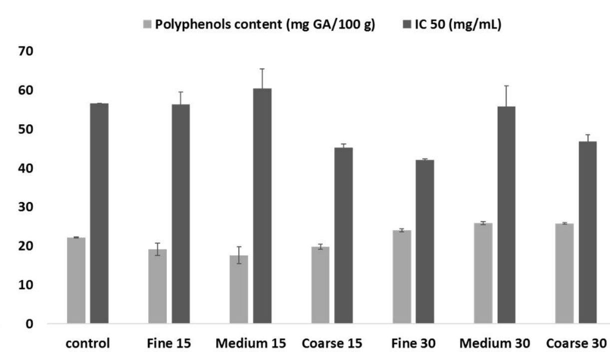
Fine 30
Medium 30
Coarse 30


Polyphenols content (mg GA/100 g)
IC 50 (mg/mL)
70
60
50
40
30
20
10
0
control
Fine 15
Medium 15
Coarse 15
Fine 30
Medium 30
Coarse 30


Polyphenols content (mg GA/100 g)
IC 50 (mg/mL)
70
60
50
40
30
20
10
0
control
Fine 15
Medium 15
Coarse 15
Fine 30
Medium 30
Coarse 30

Highlights

The coarser fractions of banana flour yielded more fiber and phenolic compounds

30% replacement of banana flour in layer cakes is proved without a quality worsening

The particle size of banana flours strongly influenced the quality of sponge cakes

Banana flours increased the content of resistant starch and dietary fiber of cakes

Sponge made with banana flours yielded more polyphenols and antioxidant capacity

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