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Identification of carbohydrate parameters in commercial unripe banana flour

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SUMMARY

Unripe banana flour (UBF), which is rich in resistant starch (RS), has shown several positive physiological effects in clinical trials. Although such observations encourage the emergence of UBF in the food market, specific identity or quality standards for the product are still lacking. This work aimed to assess and propose characterization parameters for commercially available UBF. The results showed that three of the brands examined presented a RS content higher than 40%, whereas nine showed a lower content, with two having less than 10% RS and over 80% total starch, which was fully identified as cereal starch by light microscopy (LM). The presence of banana peel in the flour was correlated with the lipid ($r = 0.870$), ash ($r = 0.812$), protein ($r = 0.704$) and total starch ($r = -0.761$) contents. According to principal components analysis (PCA) and LM identification, the main parameters for the characterization of commercial UBFs are the contents of RS, dietary fiber, lipid and ash. The large variability in RS content (4 to 62%) found in commercial UBFs is one reason why consumers would benefit from additional labeling information, such as the inclusion of the RS and soluble sugar (SS) contents, the unripe banana cultivar used, and indications about use of the peel. Moreover, adulterations could be verified by food inspection agencies via LM, which can be used as a tool to identify the type and state of the starch present.

1. Introduction

Functional foods and ingredients with a high content of unavailable carbohydrates, such as resistant starch (RS), have shown promising potential for reducing the incidence of non-communicable diseases (NCDs) due to the reduced speed of digestion and absorption of these carbohydrates (Fuentes-Zaragoza, Riquelm-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010; Birt et al., 2013). Over the years, interest and knowledge about the biological utilization of RS have grown in regard to its effects on intestinal physiology (Englyst & Macfarlane, 1986; Topping & Clifton, 2001; Davis & Milner, 2009; Conlon et al., 2012) as well as its ability to promote a reduced glycemic response (Anderson et al., 2010; Klosterbuer, Thomas, & Slavin, 2012) and increased insulin sensitivity (Robertson, Bickerton, Dennis, Vidal, & Frayn, 2005). A cause-and-effect relationship exists between the intake of RS as a replacement for a portion of the available starch in baked goods and a reduction in post-prandial glycemic response (EFSA, 2011). Increased insulin sensitivity has also been observed in studies of prolonged RS ingestion (4 to 12 weeks) (Johnston, Thomas, Bell, Frost, & Robertson, 2010; Maki et al., 2012; Robertson et al., 2012).

Global banana production was approximately 102 million tons in 2012, and Brazil is the world's fifth largest producer, with an output of 6.9 million tons per year (FAO, 2014). However, of the total amount produced in the country, approximately 30% is lost during the post-harvest phase (Almeida, 2012). Thus, the development and utilization of any technology or process that enhances its use can be advantageous.

Different factors are involved in the production of unripe banana flour (UBF), as banana ripening stage and drying process. The carbohydrate composition of bananas is largely altered during ripening as the starch reserve is hydrolyzed to soluble sugars (Cordenunsi & Lajolo, 1995). As a large proportion of the starch in unripe bananas is in the form of resistant starch, it is important to assess the proper stage of fruit ripening to produce flour with a high RS content (Englyst & Cummings, 1986; Zhang, Whistler, BeMiller, & Hamaker, 2005). The process generally employed for unripe banana flour (UBF) drying utilizes a tray dryer or hot-air dryer (Haslinda, Cheng, Chong, & Noor Aziah, 2009). The amount of RS in UBF has been reported in early studies; Englyst, Kingman, and Cummings (1992) evaluated UBF samples and found 57.0% RS, whereas Faisant et al. (1995) reported a value of 54.0%. In Brazil, Tribess et al. (2009) established a process to produce

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UBF with a high RS content (51.0 ± 7.3%), whereby the process temperature remains below gelatinization.

Some authors have suggested the use of unripe banana peel due to its higher content of antioxidants (Haslinda et al., 2009). However, it is important to consider that pesticide residues might be found in commercial banana peels (Aurore, Parfait, & Fahrasmane, 2009), which may result in food safety issues.

The application of UBF in foods has been evaluated in several studies. A lower glycemic index and load as well as higher fermentability and short-chain fatty acid (SCFA) production in vitro have been reported for cereal bars with UBF compared to a control cereal bar (Santos, 2010). Clinical trials with UBF have demonstrated hunger/satiety positive responses when compared with controls, as well as improvement in bowel function in healthy volunteers (Giuntini et al., 2015; Menezes et al., 2010).

UBF has been applied (pilot scale) in several food products, such as cereal bars (Santos, 2010), crackers (Fasolin, Almeida, Castanho, & Netto-Oliveira, 2007), pasta (Agama-Acevedo et al., 2009), and noodles (Choo & Aziz, 2010). In addition, commercial UBF can be purchased from natural product stores and on the Internet in both Brazil (http://verdenutri.com.br, http://www.natue.com.br) and abroad (http://www.bananaflour.com/, www.bananaflour.com.au). In a globalized food environment, the current strategies available for monitoring and detecting economically motivated adulteration have relative strengths and weaknesses, and new approaches have been recommended (Manning & Soon, 2014). To date, there is no specific legislation for UBF identity and quality standards available in Brazil, Canada, Australia or the United States, among other countries. Although commercial UBF has been used in a clinical trial with metabolic syndrome volunteers (Tavares da Silva et al., 2014), its RS content has not been assessed.

Therefore, to fill this gap, the purpose of this work was to assess and propose characterization parameters for commercially available UBF.

2. Material and methods

2.1. Unripe banana flour (UBF) samples

Standard UBF was prepared from the pulp of unripe bananas, *Musa acuminata* (group AAA), sub-group *Cavendish* (called 'Nanicão' in Brazil), not subjected to a ripening chamber. The material was obtained from Vale do Ribeira and marketed in CEAGESP/SP, maturity stage I, and UBF production at the semi-industrial scale was performed according to a proposed process (Tribess et al., 2009). Three different batches were produced. Standard Peel UBF was produced in the same manner as Standard UBF with the peel retained. Cooked UBF was produced in the same manner as Standard UBF but was heated to 120 °C for 1 min before drying process as a standard of gelatinized starch. The UBF starch gelatinization temperature ranges from 67.9 to 71.3 °C (Rayo et al., 2015; Tribess et al., 2009). The commercial unripe banana flours (n = 12) examined were produced in the states of São Paulo (6), Rio Grande do Sul (2), Santa Catarina (2), Bahia (1) and Minas Gerais (1) and were purchased in stores that specialize in natural products. The commercial UBFs were labeled A to L.

2.2. Physical–chemical analyses

2.2.1. Unripe banana maturation stage

The unripe banana maturation stage, before UBF production, was assessed using 3 parameters: total soluble solids content (TSS), peel color and pulp firmness. Bananas have 7 ripening stages assessed by peel color. The color assessment was performed via visual assessment using randomly chosen bananas according to Von Loesecke's comparative standard (PBMH & PIF, 2006). The analysis of firmness was performed using a TA.XT2i *Texture Analyzer* (EqEP08TP) (*Stable Micro Systems* Ltd., Godalming, Surrey, UK) with a 6-mm diameter probe. For the analysis, unpeeled bananas were penetrated at three sites (right and left extremities and central). The penetration speed was 1 mm/s with a 12 mm depth (Ditchfield, 2004) and a pre-test speed of 5 mm/s. The analysis of TSS, expressed in °Brix, according to AOAC 932.12 methodology (Horwitz & Latimer, 2006), was determined using a digital refractometer (DR201-95, Krüss-Optronic GmbH, Hamburg, Germany) and a portable refractometer (model 950.032 B-ATC, Alla, France).

2.2.2. Chemical composition

All of the chemical analyses were performed in triplicate, and the results are expressed in %, equivalent to g/100 g, of dry (d.w.) and fresh weight (f.w.). All samples were ground to particles <0.250 mm prior to the chemical analyses. The determination of the moisture content of the samples was performed in a vacuum oven at 70 °C (AOAC 920.151). The protein content was determined by the total nitrogen present using the micro-Kjeldahl technique (AOAC 960.52) and considering a conversion factor 6.25; the lipid content was determined using the Soxhlet method (AOAC 920.39) and the ash content by calcination in a muffle furnace at 550 °C to a constant weight (AOAC 923.03) (Horwitz & Latimer, 2006). RS was quantified based on the AOAC 2002.02 method (McCleary, McNally, & Rossiter, 2002), and the method of Cordenunsi and Lajolo (1995) was used to quantify the total starch (TS). The amount of free glucose was determined using an enzymatic method (glucose oxidase/peroxidase/ABTS) (Bergmeyer & Bernet, 1974). The following reference materials were used: RS of corn and potato (Megazyme International Ireland Ltd., Wicklow, Ireland, Megazyme K-RSTCL) and cooked *carioca* beans (*in house*) were used to determine RS, and starch from potato (Sigma S-2004) was used for the determination of TS. The concentration of available starch (AS) was obtained from the difference between the total and resistant starch (AS = TS − RS). Soluble sugars (SS) were extracted and qualified according to a previous report (Cordenunsi, Shiga, & Lajolo, 2008). Glucose, fructose and sucrose (Sigma, Chemical CO, Saint Louis, MO, USA) were used as reference materials. The total available carbohydrate content was calculated by the sum of SS and AS. Dietary fiber (DF) was quantified by the enzymatic-gravimetric method according to AOAC method 991.43 (Horwitz & Latimer, 2006), with the modifications proposed by McCleary and Rossiter (2004) to exclude RS from DF. Total DF (TDF) was determined as the sum of DF (without RS) and RS analyzed.

2.3. Light microscopy (LM)

Five reference materials were used for LM: corn starch (Sigma S5296); potato starch (Sigma S-2004) and *in-house* UBF, Standard UBF, Standard Peel UBF and Standard cooked UBF described above. The samples were prepared as indicated in American and Brazilian Pharmacopeia (American Pharmacopeia, 2014; Brasil, 2010) and analyzed using a light microscope (Olympus model CHS, Olympus Optical Co Ltd., Japan). Qualitative analysis of structures was performed with the preparation of both Lugol and glycerin slides for each sample. Quantitative analysis of yellow structures and gelatinized starch was performed using 10 μl of the prepared solution for each sample (50 mg of sample + 1.5 ml Lugol's solution). Counts were performed in the 4 peripheral squares of a Neubauer chamber (Mirrored; 0.100 mm depth; 0.0025 mm^2; New Optics), and the average was calculated (Umerie & Ezeuzo, 2000).

For the histochemical analysis, different treatments were performed to identify the composition of the yellow structures: Calcofluor white for cellulose; Sudan IV for lipids; Ferric chloride for phenolic compounds; and Vanillin-hydrochloric acid for tannins (Demarco, 2014; Demarco, de Moraes Castro, & Ascensão, 2013; Ruzin, 1999). Observations and photomicrographs were achieved using a Leica DMLB light microscope equipped with a 100-W mercury vapor lamp, a UV filter cube (excitation filter BP340–380, dichromatic mirror RKP400, suppression filter LP425) and a blue filter cube (excitation filter BP420–490, dichromatic mirror RKP510, suppression filter LP515). The histochemical analysis flowchart is illustrated in Fig. S1 (Supplementary material).