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Composition, Digestibility and Application in Breadmaking of Banana Flour

E. JUAREZ-GARCIA,[1] E. AGAMA-ACEVEDO,[1] S.G. SÁYAGO-AYERDI,[2] S.L. RODRÍGUEZ-AMBRIZ[1] & L.A. BELLO-PÉREZ[1,*]

[1] *Centro de Desarrollo de Productos Bióticos del IPN. Km 8.5 carr. Yautepec-Jojutla, colonia San Isidro, apartado postal 24, 62731 Yautepec, Morelos, México;* [2] *Instituto Tecnológico de Acapulco, Calzada Instituto Tecnológico S/N Crucero Cayaco-Puerto Marqués, 39905 Acapulco, Guerrero, México (*author for correspondence; e-mail: labellop@ipn.mx)*

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Abstract. Banana flour (BF) was obtained from unripe banana (*Musa paradisiacal* L.) and characterized in its chemical composition. Experimental bread was formulated with BF flour and the product was studied regarding chemical composition, available starch (AS), resistant starch (RS) and rate of starch digestion *in vitro*. The chemical composition of BF showed that total starch (73.36%) and dietary fiber (14.52%) were the highest constituents. Of the total starch, available starch was 56.29% and resistant starch 17.50%. BF bread had higher protein and total starch content than control bread, but the first had higher lipid amount. Appreciable differences were found in available, resistant starch and indigestible fraction between the bread studied, since BF bread showed higher resistant starch and indigestible fraction content. HI-based predicted glycemic index for the BF bread was 65.08%, which was significantly lower than control bread (81.88%), suggesting a "slow carbohydrate" feature for the BF-based goods. Results revealed BF as a potential ingredient for bakery products containing slowly digestible carbohydrates.

Key words: Banana, Bakery product, Resistant starch, Starch digestibility, Indigestible fraction

Introduction

One of the current tendencies in nutrition and health is to consume low-carbohydrate food products. Consumers are demanding foods showing two main properties: the first one deals with the traditional nutritional aspects of the food, whereas, as a second feature, additional health benefits are expected from its regular ingestion. In a rapidly changing world, with altered food habits and stressful life styles, it is more and more recognized that a healthy digestive system is essential for overall quality of life [1]. Carbohydrates constitute the main fraction of unripe fruits, of these, starch and non starch polysaccharides (dietary fibre) are the major constituents. In the 80's, dietary fibre (DF) was identified as an important component of a healthy diet, and the food industry looked for palatable ways to increase the fibre content of their products. From a chemical point of view, dietary fibre (DF) consists of cellulose, hemicellulose, lignin, pectin, β-glucans and gums [2, 3]. In the case of fruits and vegetables, the parenchymatous tissues and cell walls are the DF supply. The interest in foods rich in DF increased in the recent decades, and the importance of this food constituent has led to the development of a large market for fibre-rich products and ingredients. A high DF intake has been related to several physiological and metabolic effects [4]. In the digestive tract, DF exerts a buffering effect that links excess of acid in the stomach, increases the fecal bulk and stimulates the intestinal evacuation; besides, it provides a favorable environment for the growth of the beneficial intestinal flora. Fibre can also bind diverse substances including cholesterol [5, 6]. It has been reported that these specific properties of DF play an important role in the prevention and treatment of obesity, atherosclerosis, coronary heart diseases, colorectal cancer and diabetes [7, 8]. Consumption of certain fibres, particularly highly viscous SDFs, is usually associated with moderate postprandial glycemic responses, a property of importance in the dietetic treatment of diabetes [9, 5].

Resistant starch (RS) was defined by EURESTA (the Flair Concerted Action on Resistant Starch) as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals [10]. RS has a reduced caloric content and is characterized by physiological effects that make it comparable to DF [11]. Resistant starches are not digested in the human small intestine and are fermented by bacterial microflora in the large bowel, affecting a number of physiological functions and thus having different effects on health, e.g., reduction of the glycemic and insulinemic responses to food, hypocholesterolemic action and protective effects against colorectal cancer [12]. In recent years, there has been a considerable interest in the possibility of improving control of diabetic patients by altering the glycemic impact of the carbohydrates ingested. A tool for ranking foods with respect to their potential blood glucose raising is the glycemic index (GI) concept [13] (Jenkins et al., 1981), which leads to the preferred selection of "slow carbohydrate" food items/diets [13, 14]. Although it cannot be regarded as a general rule, a nutritional variable that may be linked to low GI properties is the RS content of a food or complete meal [15].

In this sense, the nutritional/nutraceutical potential of unripe banana starch and fiber have been claimed by several authors [16–19].

Banana is a climacteric fruit that in México and many other countries is consumed in ripe state. For this reason, large quantities of fruit are lost during commercialization, as consequence of deficient postharvest handling. Unripe banana fruit represents an alternative source of indigestible carbohydrates, due to a number of reasons such as, starch content of the pulp, high cellulose, hemicellulose and lignin levels, as well as the low cost of the fruit that may allow the preparation of BF with attractive chemical and functional characteristics.

The aim of the present work was to evaluate the chemical and functional properties of BF from unripe banana fruit and its application in a bakery product.

Materials and Methods

Banana Flour Preparation

Commercial hard green **(unripe)** preclimacteric banana (*Musa paradisiaca* L.) fruits were purchased from the local market of Cuautla, Morelos, México. They were peeled and cut into 1cm slices, immediately rinsed in citric acid solution (0.3% w/v). The slices were dried at 50 °C, ground using a commercial grinder (Mapisa Internacional S.A. de C.V., México, D.F.) to pass a US 50 sieve and stored at 25 °C in sealed plastic containers for further analyses.

Chemical Composition

Moisture content was determined by gravimetric heating (130 ± 2 °C for 2 h) using a 2–3 g sample. Ash, protein and lipid were analyzed according to AACC methods 08-01, 46-13 and 30-25, respectively [20]. Dietary fiber (DF) was tested using the 985.29 AOAC method [21]. Total starch (TS) was determined by the method of Goñi, García-Alonso & Saura-Calixto [22]; in brief, 50 mg of sample were dispersed in 2M KOH (30 min) to hydrolyze all the starch, then samples were incubated with amyloglucosidase (Boehringer, No. 102857, 60 °C, 45 min, pH 4.75), and glucose was determined using the glucose oxidase assay GOD-POD. TS was calculated as glucose (mg) $\times$ 0.9; potato starch was used as a control. Available starch content in bakery products was measured with the multienzymatic method described by Holm et al. [23]. Resistant starch was measured by the method proposed by Goñi et al. [24]. In brief, removal of protein with pepsin P-7012 (Sigma Chemical CO., St Louis MO) at 40 °C, 1 h, pH 1.5, incubation with amylase A-3176 (Sigma Chemical CO., St Louis MO) at 37 °C for 16 h to hydrolyze digestible starch, treatment of the precipitate with 2 M KOH, incubation with amyloglucosidase A-7255 (Sigma Chemical CO., St Louis MO) at 60 °C, 45 min, pH 4.75, and determination of glucose using glucose oxidase peroxidase assay (SERA-PAK® Plus, Bayer de México, S.A. de C.V., Edo. De México).

Table 1. Formulation of banana flour and control breads

Ingredient	Banana flour bread	Control bread
Banana flour (g)	100	—
Wheat flour (g)	—	100
Gluten (g)	20.3	—
Water (ml)	60	80
Onion powder (g)	1.5	1.5
Garlic powder (g)	0.5	0.5
Salt (g)	0.7	0.7
Sugar (g)	2.5	2.5
Baking powder (g)	2.8	2.8
Concentrate milk (ml)	50	50
Butter (g)	25	25

Preparation of Bread

For the preparation of the bread, raw materials were acquired in local supermarkets and stored in glass/plastic containers at room temperature (25 °C), or under refrigeration depending on the stored requirements of the material. The formulation used for the bread is shown in Table 1. Butter was beaten until creaming, mixed with sugar, onion, garlic, salt, milk and baking powder added onto the wheat flour or the BF and mixed perfectly until having a 2 cm height surface. Bread were cut with a circular mould (6 cm diameter) and placed on greased aluminum bread mold. The bread was baked in a household oven (Hotpoint, 6B4411LO. Leisser S.A. de C.V., San Luis Potosi, México), at an approximate temperature of 200 °C for 20 min. Once baked, bread were allowed to cool down during 30 min and stored in a plastic container with hermetic cover. Control product, based on wheat flour, was chosen as a pragmatic approach.

Chemical Composition of the Bread

The chemical composition was carried out using the same methodologies described for the BF analysis. Soluble (SIF) and insoluble (IIF) indigestible fractions were assessed using the sequential pepsin/amylase hydrolysis method of Saura-Calixto et al. [25] in samples boiled in tap water during 90 min; this method has been proposed as an alternative to the enzymatic dietary fiber assays, aiming to include most of the physiologically indigestible part of foods, regardless their chemical nature.

Starch Hydrolysis Index of Products 'as eaten' (chewing/dialysis test)

Starch hydrolysis index (HI) of products 'as eaten' (chewing/dialysis test) was assessed with the protocol developed by Granfeldt et al. [26]. Samples of cookies containing 1g of available starch were tested. Six healthy subjects participated in the chewing phase of the experiments, which was followed by pepsin digestion (P-7012, Sigma Chemical CO. St Louis. MO) and incubation with porcine pancreatic amylase (A-6255, Sigma Chemical CO. St Louis. MO) in a dialysis bag. The reducing amylolysis products appearing in the dialysate were measured colorimetrically, and expressed as maltose equivalents. Data were plotted as 'hydrolysis degree' vs. 'time' curves and the Hydrolysis Index (HI) was calculated as the area under the curve (0-180 min) for the test product, expressed as a percentage of the corresponding area for a white bread reference sample, chewed by the same person.

Statistical Analysis

Results were expressed by means of values ± standard error of three separate determinations. Comparison of means was performed by one-way analysis of variance (ANOVA) followed by Tukey's test. The average HI was calculated from six digestion replicates runs for each sample and means were compared by the Wilcoxon matched-pair signed-rank test, each person being his own control. The predicted Glycemic Index (pGI) was calculated from HI values, using the empiric formula proposed by Granfeldt [27]: $\text{pGI} = 0.862\ \text{HI} + 8.198$, for which the correlation coefficient (r) is 0.026 ($p < 0.00001$). Statistical analyzes were run using the computer SPSS V. 6.0 software (SPSS Institute Inc., Cary NC).

Results and Discussion

Chemical Composition of Banana Flour

Banana flour (BF) had low moisture content (Table 2), similar to those determined in commercial dry products such as wheat germ, oat cookies and bran flakes [28]. An important parameter of an ingredient used in bakery formulations is its lipid content. The BF analysed in this work had a lipid content of 2.69%, higher than the values reported by others for BF prepared from diverse unripe banana, being estimated to be between 0.33 and 0.82% [17]. The high lipid content reported here (in this work) could be due to that in our study a complete banana was used (peel and pulp). Lipid components present in the peel, as the carotenoids, were recovered during extraction of the lipids. This could be anticipated by the colour of the extract obtained after extraction with hexane. However, the lipid content of our BF was lower than those reported in grape skins flour (6.87–7.78%) [29], commercial grape fiber (6.9%) [30] and the flour of peel of *Citrus sinensis* (22.2%) [31]. Extrudates were prepared using unripe BF obtained from the pulp and for this preparation the lipids content ranged between 0.30 and 1.0% [32]. Protein content in BF was also low (3.27%) as compared to other preparations such as fiber grape skins (11.6–14.4%) [29] and a commercial fiber (14.4%) obtained from red grape peels [30]. The protein content in BF from eight different varieties ranged between 2.5 and 3.3% [17] and it was similar (3.8%) to a previous report for a preparation of BF [33]. These values of protein content are close to that determined in BF obtained in this study. It was reported a lower protein value (2.2%) for a fiber concentrate from *Citrus sinensis* peel [31]. Fruits are characterized by their content of certain mineral components. BF had 4.70% of ash content, which is higher than the ash content determined in unripe BFs (2.6–3.5%). Fiber concentrate prepared from *Citrus sinensis* peel showed a lower ash amount (3.3%) [31], but other fiber concentrate of grape skins (between 5.7 and 9.2%) had a higher ash level [29, 30].

Table 2. Chemical composition, Available starch (AS) and Resistant starch (RS) contents in banana flour (BF) prepared from unripe fruit (%)[a]

Component	Amount
Moisture	7.1 ± 0.05
Ash[b]	4.7 ± 0.13
Protein[b,c]	3.3 ± 0.4
Lipid[b]	2.7 ± 0.38
Dietary fiber[b]	14.5 ± 0.46
Total starch[b]	73.4 ± 0.92
AS[b]	56.3 ± 0.74
RS[b]	17.5 ± 0.14

[a]Dry matter basis. Values are means of three replicates ± standard error. Means in row not sharing the same letter are significantly different ($p < 0.05$).
[b]Dry basis.
[c]N × 5.85.

Total dietary fiber (TDF) content in BF was 14.52% (Table 2), which is close to the highest level measured in eight different unripe BFs (15.54%). Nevertheless, some reports indicate that other varieties have a lower TDF composition (6.28%) [17]. Lower amount of TDF (9.2%) was reported for a preparation of BF [33]. Due to the TDF content determined in our preparation of BF, this finding was compared with the value reported for some antioxidant dietary fiber preparations, being lower than the values reported for guava (48–49%) [34], grape skins (54.1–64.6%) [29, 30], citrus peel (57%) [31], fiber from two Mexican lime peels (66.7 and 70.4%) [35] and in mango peel fiber (65–71%) [36]. The relatively low TDF content in BF is related with the high starch content. However, for certain kind of food products, such a starch level might be of importance, given the additional functional properties imparted by starch. As was mentioned above, the BF preparation studied in this work was obtained from the whole fruit (pulp and peel). Pulp of unripe banana features a high content of starch [35, 37, 38]. Total starch (TS) content in the BF was 73.36% (Table 2), which is similar (77.0%) to that reported in BF as α-glucans (starch polysaccharides) [33]. In other work in which unripe BFs were characterized, TS content between 61.3 and 76.5% was reported, having three of the varieties of banana TS content similar to that determined in this study. Such high starch level might contribute to the formation of resistant starch (RS) during processing of BF-based products, as it has been shown in banana starch extrudates [39]. Although the total starch amount in BF is high, a good proportion is not available for hydrolysis by digestive enzymes, since available starch recorded was of 56.29% and resistant starch content of 17.50%. Higher RS content was determined in BF (57.2 and 47.3%) analyzed by two methods [33], that were different from the one used in this study.

Chemical Composition of Breads

Moisture content was higher in BF bread than in control bread (Table 3). This high moisture content in BF bread might be related to the high protein and starch composition and low lipid level, since the former components are hydrophilic and have the capacity to join more water molecules. Bread with high moisture content has a softer texture than bread with low moisture level; however, the microorganisms growth could be favoured, affecting the stability and shelf-life of the product [40].

The high ash content of the BF had implications in the ash level founded in the bread formulated with this flour, since ash content was higher in BF bread than in control bread. Protein and starch contents were higher in BF bread than in control, which is of importance due to the valuable metabolic role that both nutrient play in our body. However, lipid content was lower in BF bread than in the control, an interesting finding taking into account the possibility of formation of amylose-lipid complex. This type of complex has been related with decreased lipid content and an increase of the indigestible fraction of the starch, as it was mentioned thereafter. Total starch was higher in BF bread than control bread, but available starch was lower in the former sample. This fact agree with the possibility of amylose-lipid complex formation during starchy products processing as it was mentioned above [41]. Bread product added with BF presented lower available starch (AS) fraction than the control bread. Absolute AS value in the BF appears to be lower than in control sample. However, when AS is calculated as a percentage of total starch in the bread, such a difference is ruled out, indicating that the variation is mainly due to starch dilution created by the use of gluten, a non-starch material.

Table 3. Chemical composition, Available starch (AS) and Resistant starch (RS) contents in a bread elaborated with banana flour (BF) prepared from unripe fruit (%)[a]

Sample	BF bread	Control bread
Moisture	26.6 ± 0.87[a]	13.7 ± 0.07[b]
Ash[b]	3.3 ± 0.01[a]	2.0 ± 0.0[b]
Protein[b,c]	9.8 ± 0.02[a]	4.1 ± 0.07[b]
Lipid[b]	14.2 ± 0.96[a]	18.4 ± 0.06[b]
Total starch[b]	63.1 ± 0.41[a]	59.4 ± 0.16[b]
AS[b]	54.9 ± 0.15[a]	59.1 ± 0.63[b]
RS[b]	6.7 ± 0.07[a]	1.0 ± 0.06[b]
Dietary fiber[b]	5.1 ± 0.11[a]	2.3 ± 0.09[b]
Insoluble indigestible fraction[b]	22.3 ± 0.83[a]	12.4 ± 0.55[b]
Soluble indigestible fraction[b]	3.8 ± 0.19[a]	5.9 ± 0.25[b]
Total indigestible fraction[b]	26.1 ± 0.92[a]	18.4 ± 0.73[b]

[a] Dry matter basis. Values are means of three replicates ± standard error. Means in row not sharing the same letter are significantly different ($p < 0.05$).
[b] Dry basis.
[c] N × 5.85.

The bread added with BF had higher level of resistant starch (6.74%) that the control bread (1.0%). Regular wheat bread contains high amounts of digestible starch fractions [27]; also it was reported in bread produced with ordinary baking a RS content of 3.0%, and when a long-time baking and different ingredients were tested the RS level ranged between 5.1 and 7.7% [42]. The data reported here and by other authors suggest the BF is an alternative for product formulation with reduced digestible starch contents.

The product formulated with BF exhibited dietary fiber content higher than control bread sample (Table 3). In BF bread the dietary fiber content was more than 100% higher. This pattern agrees with the higher RS level present in BF bread, since there is a RS fraction associated with the insoluble residue of dietary fiber that can be quantified as dietary fiber by the most analytical procedures [43, 44]. This pattern agrees with the higher DF content in cooked beans than in raw beans [44]. Commercial integral bread samples have dietary fiber contents from 4.99% to 6.11% and in fibre-rich cookies it has been reported 3.73 and 5.95% [28].

The indigestible fraction (IF) focuses on the food main constituents unavailable for digestion in the small intestine, which therefore reach the colon becoming substrate for the fermentative microflora. This fraction comprises not only dietary fiber and RS but also protein, certain polyphenols, and other associated compounds [25]. The total IF (TIF) of BF bread (26.11%) was higher than for the control bread sample (18.36%) and similar to the reported value for white bread (13.8%) [25]. An important difference was in regard to the insoluble IF composition (IIF), since the BF bread had a higher content (approximately 50%) than the control bread. Conversely, the former sample showed a lower soluble IF (SIF) content. Similar pattern for IIF and SIF was reported in white bread, although the SIF value was lower (2.61%) than that determined in this study for BF bread (3.82%). Bakery product prepared here with BF feature a significative IF content and may be an alternative for people with special caloric requirements.

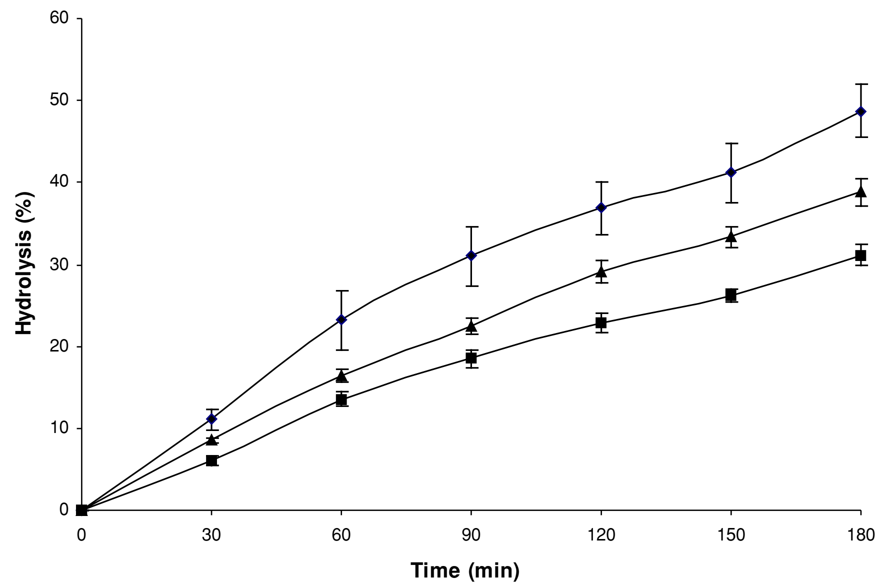

Figure 1. Average hydrolysis curves of starch in Banana Whole Flour bread (BWF) (*Musa paradisica* L.) and bread of Wheat Flour (WF). Values are means of three chewing and digestion experiments. Areas under curves were used for calculation of HI figures reported in Table 3. White Bread (♦), bread of Wheat Flour (▲), and Banana Whole Flour bread (BWF) (*Musa paradisica* L.) (■).

Starch Hydrolysis

Curves for combined digestion/dialysis process for the different products are depicted in Figure 1. Commercial white bread, used as reference, showed a digestion value of about 50% after 180 min, which agrees with the data reported in the original protocol [26]. Control bread baked in the laboratory exhibited a 39% hydrolysis at 180 min, which was lower than in BF bread (32%). Control bread was digested more rapidly that BF added products. In similar experiments testing another cereal product (corn tortilla) a 38.8% hydrolysis was recorded after 180 min [45], which is higher than those determined in the cereal products added with BF studied here.

Hydrolysis Indices (HI) calculated from the hydrolysis curves and the corresponding predicted Glycemic Indices (pGI) are presented in Table 4. BF bread showed a lower HI than control bread; a result that agrees with the chemical characteristics of both products (e.g. RS, DF, IF, etc.). BF exerts a significant effect on the rates of digestion and absorption (simulated by the dialysis phase) of the starch component of the meals. It is well known that degree starch gelatinization, the indigestible polymers (dietary fiber) and some associated non-fibrous compounds may reduce the rate of starch digestion *in vitro and in vivo*, resulting in low metabolic responses [26]. A recently reported 75% pGI value for fresh baked tortilla [45] is similar to that determined here for control sample, which suggests a moderate glycemic response for these products, because BF bread might be considered a product with low glycemic response.

Table 4. Hydrolysis index (HI) and predicted glycemic index (pGI) of bread elaborated with banana flour (BF) prepared from unripe fruit[a]

Sample	HI (%)[a]	pGI (%)[b]
BF bread	65.1 ± 2.4[a]	64.3
Control bread	81.9 ± 3.2[b]	78.8
White bread reference	100c	94

[a]Hydrolysis index (IH) was related to bread = 100% (Granfeldt et al., 1992). Values are mean of six chewing and dialysis replicates ± standard error ($p < 0.05$).
[b]Predicted glucemic index (pIG) = 0.862 HI + 8.198 (Granfeldt, 1994) Means in columns not sharing the same letter are significantly different ($p < 0.05$).

The decreased indices exhibited by BF product may permit further reduction of metabolic responses following ingestion of these experimental food items.

Conclusions

The chemical composition of BF showed low lipid and high starch levels, valuable features for elaborating certain food product types. BF bread presented significantly higher resistant starch, dietary fiber and indigestible fraction content than control bread. Bakery product with BF had low glycemic index, thus it might be used as dietary aid by people with special low caloric requirements. Unripe banana fruit appears suitable source for obtaining banana flour with high resistant starch and indigestible fraction.

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