

The Effect of in Vitro Digestion on the Structural, Morphologic Characteristics and Fermentability of Raw Banana Starch

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Abstract This study aimed to observe the effect of in vitro digestion on the structural and morphologic characteristics, and find the relationship between in vitro digestion and colonic fermentation of raw banana starch. After digested by artificial gastric juice and artificial intestinal juice, the starch granules were damaged, but not drastic. X-ray diffractograms showed that the crystal polymorph (C-type) was not changed and the degree of crystallinity was increased after digestion. There were no significant differences in chemical structure according to the infrared spectrum analysis. The swelling power and solubility of the digested starches were greater than that of raw banana starch at all temperatures. Banana starches digested by gastric and intestinal juice presented high fermentability, expressed by total short chain fatty acids. The results may be due to the slight changes on structural and morphologic characteristics. To conclude, passage through the stomach and small intestine increased susceptibility of raw banana starch to further fermentation, to increase the production of metabolically active short chain fatty acids.

Keywords: raw banana starch, in vitro digestion, in vitro fermentation, short-chain fatty acids

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1. Introduction

Bananas are mainly produced in tropical and subtropical developing countries, the fourth most important crop after rice, wheat and corn. It contains appreciable amounts of vitamins B and C, minerals like potassium and calcium, as well as various antioxidants, especially catechin, epicatechin, and gallic acid [1]. Other than that, banana is rich in fatty acids, phytosterols and steryl glucosides [2]. However, bananas are a very delicate commodity for economic, social, environmental and political reasons. About one-fifth of all bananas harvested become culls [3].

When bananas are still unripe, they are easily transported, can be stored longer. Raw banana is high in total dietary fiber, high amounts of essential minerals, such as potassium, and vitamins A, B1, B2, and C [4]. Apart from them, starch is the main component of raw banana, corresponding to 60-80 g/100 g (dw) of the fruit, a percentage range similar to that of corn or potatoes [3]. Besides, raw banana is also a concentrated source of resistant starch type 2 (RS2), which presents reduced susceptibility to amylase in vitro or in vivo, in rats or in humans [5,6], due to its high degree of crystalline intrinsic

structure [3]. Because of the high banana waste and the raw banana nutritional characteristics, the processing of raw banana could provide a means to minimize losses and increase market share [3,7].

From a nutritional point of view, starch has been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) [8]. RS is not digested in the upper gastrointestinal tract, but is fermented by microorganisms in the colon that produce short chain fatty acids (SCFA). SCFA provide additional energy to the body along with a high proportion of butyrate that is beneficial to colonic health [9]. Moreover, RS-unlike nonresistant starch is not digested and therefore not absorbed as glucose in the small intestine of healthy humans [9,10,11]. Banana starch is one of the few sources of RS2 that is available in an ordinary meal. Previous studies showed that native raw banana starch appeared to be highly resistant to hydrolysis by enzymes [3,10], since 84% of the starch ingested reached the terminal ileum [6]. Raw banana starch has been explored as a functional ingredient, and many studies have suggested that consumption of raw banana starch provides a beneficial effect on human health [12-16], associated with indigestible components such as resistant starch [17]. New economical strategy to increase utilization of banana includes the production of banana starch when the fruit is

unripe, and to incorporate the flour into various innovative products such as slowly digestible cookies [18-22].

It is well known that raw banana starch proved very resistant to amylase hydrolysis. The microscopic observations showed that raw banana starch contained irregularly shaped dense starch granules with smooth surfaces. After their passage through the small intestine, starch granules appeared exocorroded, with porous surfaces, and some exhibited several irregular pits, crevices or holes by which the enzymes had penetrated and hydrolyzed the inner part [6]. The fermentability of the starch was also reported, evaluated by different parameters, using rat inoculum. The in vitro colonic fermentation of the starch was high when expressed by the total amount of SCFA such as acetate, butyrate and propionate.

Less is known, however, regarding the relationship between the digestion and fermentation. We speculated that raw banana starch after digestion in the small intestine and stomach could be utilized more effectively by intestinal microorganisms, promoting the production of SCFA. Therefore, this study aimed to observe the changes of structural and morphologic characteristics of raw banana starch after in vitro digestion, and the relationship with in vitro colonic fermentation. Because colonic fermentation of dietary fiber increases the SCFA content and decreases the pH of colon contents, we also measured these characteristics and used them as indicators of banana starch fermentation.

2. Materials and Methods

2.1. Test Materials

The raw banana starch (RBS) was provided by Foshan Jiaoye Biological Technology Co., Ltd., and was obtained from unripe (green) bananas (*Musa spp. ABB Group, Dongguan dajiao*). Briefly, after washing, the bananas were peeled, cut into 3-4 cm³ pieces and immediately macerated in the color protecting solution composed of sodium sulfite anhydrous (0.05%), citric acid (0.4%) and vitamin C (0.1%). The homogenate was then centrifuged at 4,000 rpm. Sediment was further purified by washing and centrifugation. The white starch sediment was dried in a spiral vibrofluidized bed model dryer at 40-45°C, passed through a 100 mesh screen after pulverization, and in the end stored at room temperature using the vacuum package. The yield of banana starch was 25%.

2.2. In Vitro Digestion

According to the in vitro digestion model proposed by Jenkins [23], the digestion was performed in a dialysis bag placed inside a chamber containing a flowing stream of dialyzing solution. Banana starch digested by artificial gastric juice (GBS) was prepared as followed: 1 g of the sample and 30 mL of artificial gastric juice were placed in a dialysis bag, and were in rotary vibrator 200 r/min at a constant temperature of 37°C for 6 h. The residue in the dialysis bag was dried in an oven at 50°C for determination. Similarly, banana starch digested by artificial intestinal juice (IBS) and banana starch digested by artificial gastric juice and artificial intestinal juice (GIBS) were prepared.

Artificial gastric juice and artificial intestinal juice were prepared according to Pharmacopoeia of the People's Republic of China (2010 Edition).

2.3. In Vitro Colonic Fermentation

The in vitro fermentation assay was elaborated according to the method described by Goñi [24] and Serrano [25]. The inoculum was prepared with fresh rat cecum contents of male Wistars (body weight of 180 ± 10 g). The contents of diluted cecum (100 g/L of sterilized anaerobic medium of fermentation) were mixed for 15 min in a blender and filtered (particles < 1 mm). The anaerobic medium of fermentation was composed of tryptone, micromineral and macromineral solutions [26]. 5 mL diluted cecum and 0.3 g banana starch were blended and were in rotary vibrator 200 r/min at a constant temperature of 37°C for 16 h. Then SCFA in the fermentation broth were detected with gas chromatography.

2.4. Chemical Analysis

Moisture (925.45), total protein (960.52), fat (920.39) and ash (923.03) contents were determined according to AOAC methods [27]. The dietary fiber method (DF) was tested using 992.16 AOAC (2005). The determination of resistance in the banana starch was carried out based on the enzymatic method of AOAC 2002.02 [28].

Total starch was determined as follows: the starch from samples was solubilized in 0.5 mol/L NaOH and neutralized with 0.5 mol/L acetic acid. An aliquot was precipitated with 80 mL/100 mL ethanol. The precipitated starch was hydrolyzed with amyloglucosidase (Sigma A-7255), and the resultant glucose was determined with 3,5-dinitrosalicylic acid (DNS). Total starch was calculated as glucose × 0.9.

SCFA in the supernatant were detected with gas chromatography. A mixture of 5 mL supernatant produced in the fermentation, 0.4 mL internal standard (2-methyl-valeric acid) and 14 mL HClO₄ (in a concentration enough to keep pH of all samples the same) was centrifuged (4 °C, 12000 r/min, 15 min) and supernatants were transferred to gas chromatography (GC) vials. 1 µL of supernatant were automatically injected into GC (7890A-5975C, Agilent), equipped with a flame ionization detector and capillary fused silica column (Zebron™ ZB-WAX, GC Cap. Column 30 m × 0.32 mm × 0.25 µm, Ea). The detector temperatures were 260°C. The analysis was made in a temperature ramp from 50°C to 180°C under constant pressure. SCFA were identified and quantified by comparison with a volatile acid standard mix.

2.5. Scanning Electron Microscopy

The scanning electron microscopy (SEM) was performed with a scanning electron microscope (XL-30-ESEM, FEI, Holland), using an voltage of 15 kV. Samples were fixed with double-sided tape onto aluminium cylinders and coated with a layer of gold.

2.6. X-ray Diffraction

X-ray diffraction patterns were obtained with an X-ray diffractometer (Siemens, Germany) equipped with a

copper anode X-ray tube. The diffractometer was operated at 80 mA and 5 kV, and the spectra were scanned over a diffraction angle (2θ) range of 10-80° at 6°/min. Percent crystallinity was calculated as the percentage of peak area to the total diffraction area.

2.7. Infrared Spectrum Analysis

Infrared spectra was analyzed with fourier transform infrared spectrometer (Bruker, TENSOR27, France) in KBr pellets. Measurements were taken over a wavelength range from 400 to 4000 cm.

2.8. Swelling Power and Solubility

Approximately 5 g of the sample (W_s) and 100 mL of distilled water were placed in pre-weighed centrifuge tubes. The tubes were left at a constant temperature of 50, 60, 70 and 80°C for 15 min. The tubes were then centrifuged at 3000 r/min for 15 min. The supernatant was separated, and was placed in a previously weighed Petri dish. The sample on the plate was dried in an oven at 65°C for 12 h and the residue was calculated by weight difference (R). The centrifuge tubes were weighed, and calculated weight difference of the sample in the tube after centrifugation (W_c). The swelling power (SP) and solubility index (SI) were calculated according to Eqs. (1) and (2), respectively.

$$SP = \frac{W_c - W_s}{W_s} \times 100\% \quad (1)$$

$$SI = \frac{R}{W_s} \times 100\% \quad (2)$$

3. Results and Discussions

3.1. Chemical Compositions

The nutritional components in RBS were 8.62% of moisture content, 3.14% of protein, 0.51% of fat, 2.06% of ash, 62.32% of resistant starch and 12.3% of dietary fiber. The levels of proteins and lipids in the sample were low. There was a high carbohydrate content in the sample, with over 70% of starch and over 60% of resistant starch, with values similar to those found by Elizabete et al (67%) [4], more than those found by Ovando-Martinez et al [20], Carolina et al [29] and Juarez-Garcia [5]. These disagreements could be due to differences in cultivars used, the stage of maturity of the fruits and processing techniques. RS, the main component of the unavailable carbohydrate, which reaches the colon and is almost totally fermented [30,31]. The starch obtained in this study is an excellent source of resistant starch, and can be used as a new kind of adjunct for functional food. When the RS was measured in the raw and cooked pulp flours, the amount of RS in cooked raw banana flour was reduced from 52% to 3%, and it was attributed to starch gelatinization [32]. Therefore, it is important to consider that the RBP must not be heated, in order to preserve RS content. In this study, the digested starches (57.38% of GBS, 58.25% of IBS, 57.09% of GIBS) had no sharp decline in RS content, compared to RBS (62.32%). Therefore, RBS could fulfill the role as a source of DF,

once it presents physiological effects that are similar to those produced by DF. The high content of total DF (DF and RS) and low content of available carbohydrates of RBS highlight the potential of this ingredient to promote intestinal health, due to its physiological effects and profile of fermentation.

3.2. Scanning Electron Microscopy

The size and shape of starch granules are among the important factors in determining the potential use of starch. The microscope is an important tool in studies on the characteristics of starch granules, providing information on the origin of the granules, size characteristics, and information on the surface morphology. It can be used to analyze, morphologically, whether granules are influenced by a process. The SEM images of the starch granules obtained were shown in Figure 1. Most of the RBS granules presented similar shapes and sizes, triangular, rounded and smooth. These findings are different from those reported by Elizabete W M et al [4].

As showed in Figure 1B, the starch granules of GBS were still intact, without fractures, indicating that the damage was not drastic. Figure 1C showed that the damage of starch granules, with fractions, have been initiated by artificial intestinal juice. Figure 1D presented granules with depressions, with more fractions indicating that the intact structure were damaged and degradation of the granule starch had already been initiated.

3.3. X-ray Diffraction

Banana, potato, and some legume starches in the raw state are considered largely resistant, due to their particular crystallinity that makes them less susceptible to enzymatic hydrolysis. In this study, X-ray diffractometry was employed to study changes of the crystal polymorph and the degree of crystallinity after digestion. X-ray diffractograms of the starches were shown in Figure 2. The X-ray diffraction study showed starch granules of RBS with a pattern of a mixture between the A- and B-type polymorphs, also referred to as C-type, as well as the digested starches. The result did not agree with the diffraction pattern of starches from the banana varieties “macho” and “criollo”, A-type [33]. As is not expected, the starch digested by different ways resulted in an increase of crystallinity with different degrees (18.14 of RBS, 24.69 of GBS, 26.2 of IBS, 24.15 of GIBS), maybe due to the degradation of the amorphous region in RBS.

Raw banana starch appeared to be highly resistant to hydrolysis by enzymes. The banana starches, compared with those of cassava, corn and chayote, swell more slowly, which may indicate that a strong micellar arrangement needs to be broken.

3.4. Infrared Spectrum Analysis

FTIR spectra for starch samples was shown in Figure 3. Typical saccharide bands region located across of 1180 cm^{-1} to 953 cm^{-1} was noted, which is often considered as the vibration modes of C-C and C-O stretching and the bending mode of C-H bonds. These bands turned out to be the most intense in the IR-spectrum, as mentioned previously [34]. Bands at 3300 cm^{-1} , 1630 cm^{-1} and broader peaks centered around 2200 cm^{-1} were typical

bands that associated with individual biopolymer components in addition to the contribution of the water absorption [1]. In this study, all of the starches presented similar features in the FTIR spectral regions, without excepted peaks. It could be concluded that the digestion of

RBS did no damage to the bonds in starch, since there was no shift of bands, no disappearance of bonds and no presence of new bonds. However, most of absorption peaks of RBS were stronger compared with the digested starches.

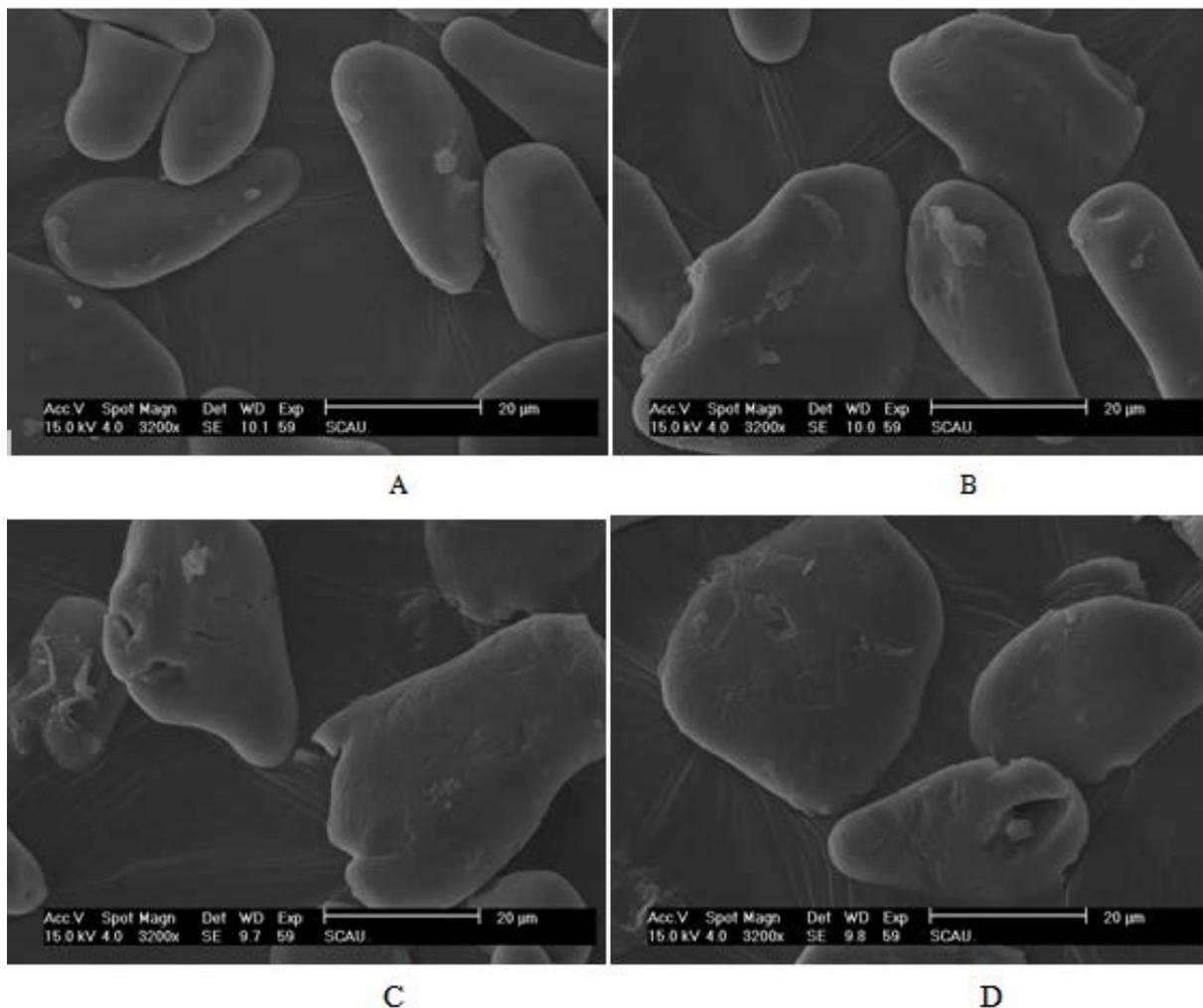


Figure 1. Scanning electron microscopy of banana starch granules (magnification 800×). A - raw banana starch (RBS), B - banana starch digested by artificial gastric juice(GBS), C - banana starch digested by artificial intestinal juice (IBS), D - banana starch digested by artificial gastric juice and artificial intestinal juice (GIBS)

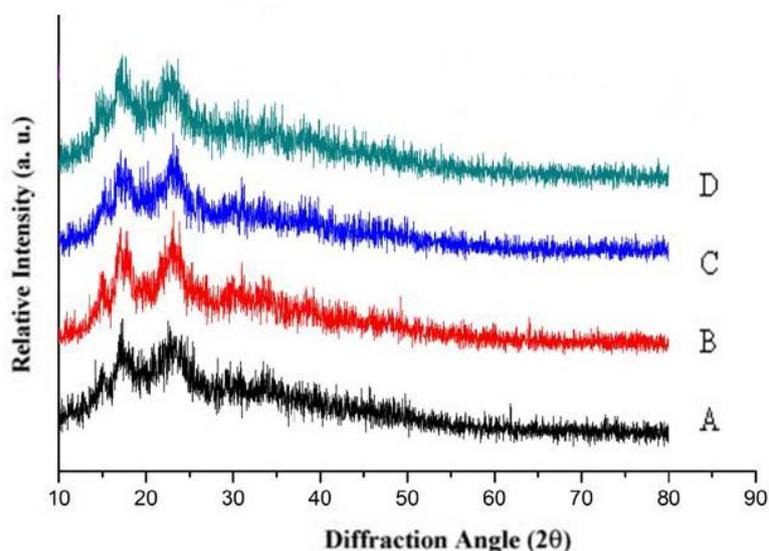


Figure 2. X-ray diffractograms of banana starches. A - raw banana starch (RBS), B - banana starch digested by artificial gastric juice(GBS), C - banana starch digested by artificial intestinal juice (IBS), D - banana starch digested by artificial gastric juice and artificial intestinal juice (GIBS)

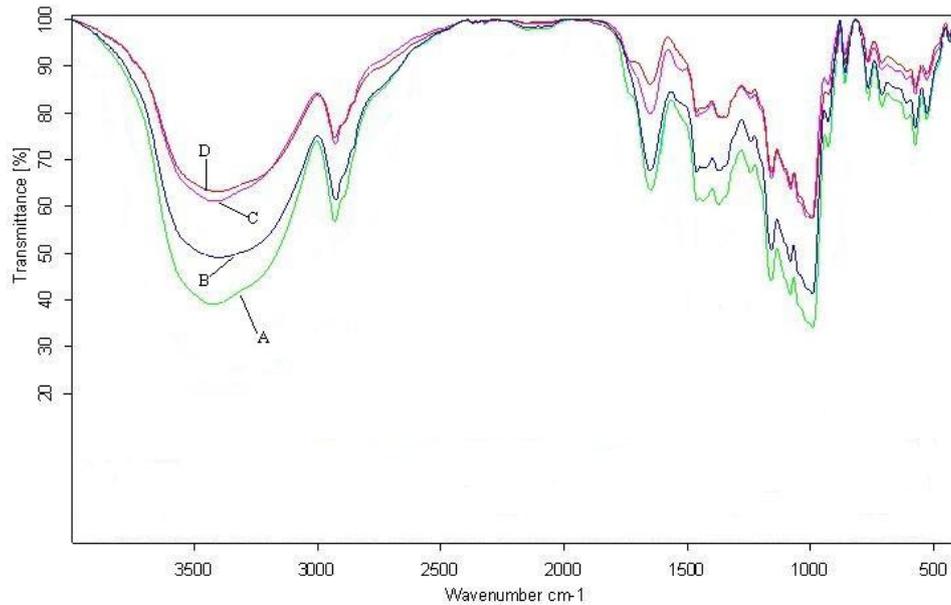


Figure 3. FTIR spectra for banana starches. A - raw banana starch (RBS), B - banana starch digested by artificial gastric juice (GBS), C - banana starch digested by artificial intestinal juice (IBS), D - banana starch digested by artificial gastric juice and artificial intestinal juice (GIBS)

3.5. Swelling Power and Solubility

The swelling power and solubility index are good parameters through which to evaluate the integrity of the starch granule. The swelling power is related to the cold paste viscosity, because only the damaged and modified starch granules absorb water and swell at room temperature [35]. The solubility is related to the amount of soluble solids in the dry sample. The values obtained for the swelling power and solubility index of the starches are shown in Figure 4 and Figure 5. The swelling power and solubility index were directly related to increasing temperature. RBS obtained in this study showed low

values of swelling power and solubility index at temperatures below 80°C. However, the application of temperatures above 80°C significantly increased these properties, since the hydrogen bonds were broken, the water molecules bind to hydroxyl groups released, the granule expanded and amylose is exuded [36]. The RBS exhibited lower solubility compared to the digested starches. Similar behaviour was observed by Gutierrez et al [37]. The results revealed that the starch granule digested by gastric and intestinal digestion had been damaged. The volatility of the starch granule maybe contribute to the fermentation performance.

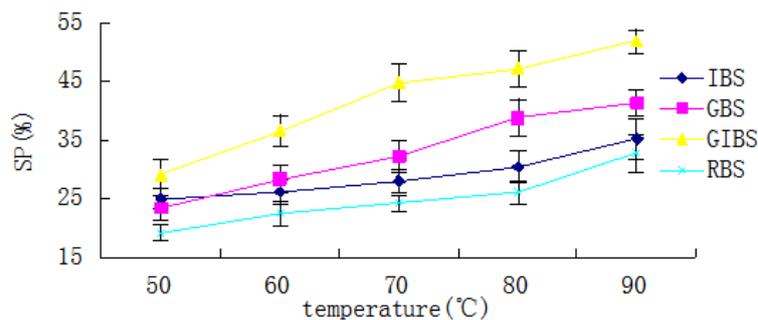


Figure 4. The swelling power (SP) of banana starches. RBS - raw banana starch, GBS - banana starch digested by artificial gastric juice, IBS - banana starch digested by artificial intestinal juice, GIBS - banana starch digested by artificial gastric juice and artificial intestinal juice

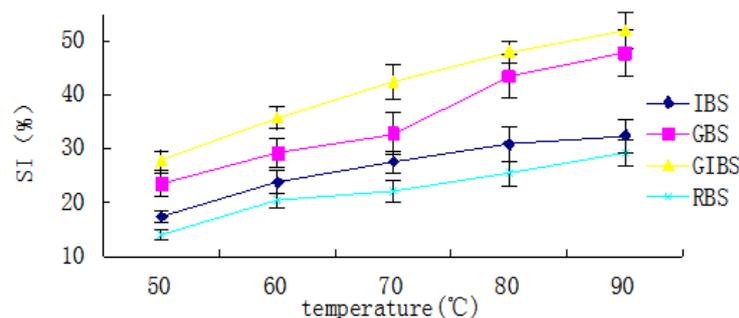


Figure 5. The solubility index (SI) of banana starches. RBS - raw banana starch, GBS - banana starch digested by artificial gastric juice, IBS - banana starch digested by artificial intestinal juice, GIBS - banana starch digested by artificial gastric juice and artificial intestinal juice

Moreover, this characteristic of RBS limits the use of starch products with instant features but opens the spectrum for use when the goal is to increase the viscosity, as bases for sauces, puddings and flans.

3.6. In Vitro Fermentation

The fermentability reflects the extension of the substrate degradation by the colonic microbiota, and a high fermentability of a substrate generally means a high in vitro production of SCFA [9]. In this study, the banana starches digested by artificial gastric and intestinal juice presented high fermentability, expressed by total SCFA (Figure 6). The GIBS was most fermentable, compared to GBS and IBS. These results may be due to different quantities of unavailable carbohydrates. Furthermore, it was speculated that slight changes on structural and morphologic characteristics contributed to the fermentability, including the damaged starch granules, an increase of degree of crystallinity, degradation to a small extent and the advanced swelling power and solubility.

Regarding acetate, propionate and butyrate, significant differences were evidenced among the digested starches compared to raw banana starch. GBS presented a high amount of acetate. IBS and GIBS presented a high amount of propionate. The results did not agree with that found by Campos-Vega et al [38], since in vitro fermentation of RS was shown to increase the proportion of butyrate. Butyrate has been reported to influence the promotion of differentiation, induction of apoptosis and inhibition of proliferation in colon tumor cell lines [39].

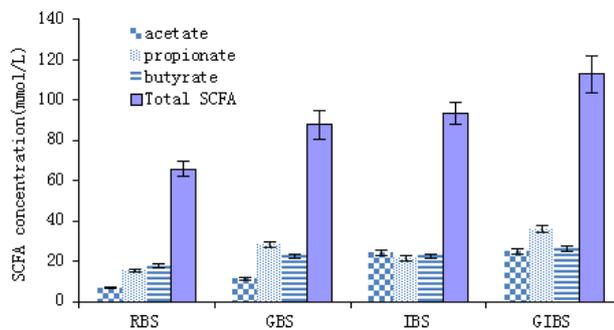


Figure 6. The acetate, propionate, butyrate and total SCFA concentration in the fermentation broth of starches. RBS - raw banana starch, GBS - banana starch digested by artificial gastric juice, IBS - banana starch digested by artificial intestinal juice, GIBS - banana starch digested by artificial gastric juice and artificial intestinal juice

4. Conclusions

Microscopic observations revealed that raw banana flour contained irregularly shaped starch granules with smooth surfaces. A smooth and dense surface of native banana starch granules could partially account for their resistance. In addition, larger blocklets, protected starches from enzymic attack. It is likely that both intrinsic resistance and encapsulation of starch granules are responsible for the low digestibility and the low rate of hydrolysis of RBP in gastric and intestinal juice. In this study, after their passage through the small intestine, starch granules appeared exocorroded, with porous surfaces, and some exhibited several irregular pits and crevices. However, the damage from gastric and intestinal

juice was not severe to impact the RS content and crystallinity of banana starch, resulting to higher susceptibility to colonic fermentation.

Our work revealed that raw banana starch granules are unique enough, related to the structural and morphologic characteristics in vitro digestion procedure. Slight changes on structural and morphologic characteristics of the starch that underwent digestion by artificial gastric and intestinal juice did not influence the micellar arrangement and was beneficial for utilization by the intestinal flora. This study provided further evidence to the potential of RBS as functional ingredients. However, the results of in vivo digestion and fermentation need to be investigated.

Statement of Competing Interests

The authors have no competing interests.

References

- [1] Someya, S., Yoshiki, Y., Okubo, K. "Antioxidant compounds from banana (*Musa cavendish*)." *Food Chem.* 79, 351-354. 2002.
- [2] Oliveira, L., Freire, C.S.R., Silvestre, A.J.D., Cordeiro, N. "Lipophilic extracts from banana fruit residues: A source of valuable phytosterols." *J. Agr. Food Chem.* 56, 9520-9524. 2008.
- [3] Zhang, P., Whistler, R., Bemiller, J., Hamaker, B. "Banana starch: Production, physicochemical properties and digestibility-a review." *Carbohydr. Polym.* 59, 443-458. 2005.
- [4] Elizabete, W.M., Carmen, C.T., Tatiana, B.T., Angela, Z., Julieta, B., Nelly, P., Gloria, V., Milana, C.T.D., Andréa, C., Bertolini, B.R.C., Franco, M.L. "Chemical Composition and Nutritional Value of Unripe Banana Flour (*Musa acuminata*, var. Nanicão)." *Plant Food. Hum. Nutr.* 66, 231-237. 2011.
- [5] Juarez-Garcia, E., Agama-Acevedo, E., Sayago-Ayerdi, S.G. "Composition, digestibility and application in breadmaking of banana flour." *Plant Food. Hum. Nutr.* 61, 131-137. 2006.
- [6] Faisant, N., Gallant, D.J., Bouchet, B. "Banana starch breakdown in the human small intestine studied by electron microscopy." *Eur. J. Clin. Nutr.* 49, 98-104. 1995.
- [7] Aurore, G., Parfaid, B., Fahrasmane, L. "Bananas, raw materials for making processed food products." *Trends Food Sci. Tech.* 20, 78-91. 2009.
- [8] Englyst, H.N., Kingman, S.M., Cummings, J.H. "Classification and measurement of nutritionally important starch fractions." *Eur. J. Clin. Nutr.* 46, S33-50. 1992.
- [9] Topping, D.L., Clifton, P.M. "Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides." *Physiol. Rev.* 81, 1031-1064. 2001.
- [10] Englyst, H.N., Cummings, J.H. "Digestion of the carbohydrates of banana (*Musa paradisiaca sapientum*) in the human small intestine." *Am. J. Clin. Nutr.* 44, 42-50. 1986.
- [11] Englyst, H.N., Cummings, J.H. "Digestion of polysaccharides of potato in the small intestine of man." *Am. J. Clin. Nutr.* 45, 423-431. 1987.
- [12] Mendez, J.D., Juarez-Rojop, I.E., Aguilar-Mariscal, H. "Potential Beneficial Effects of Native Banana Starch on Glycemia and Insulin Resistance in Obese Non-diabetic Women." *Interciencia.* 6, 470-476. 2012.
- [13] Rodriguez-Soza, E.J., Gonzalez, M.A., Calonie, I.B.D. "The preparation of green banana flour." *J. Agr. U. Puerto Rico.* 61, 470-478. 1977.
- [14] Suntharalingam, S., Ravindran, G. "Physical and biochemical properties of green banana flour." *Plant Food. Hum. Nutr.* 43, 19-27. 1993.
- [15] Kayisu, K., Hood, L.F., Vansoest, P.J. "Characterize ion of starch and fiber of banana fruit." *J. Food Sci.* 46, 1885-1890. 1981.
- [16] Langkilde, A.M., Champ, M., Andersson, H. "Effects of high-resistant-starch banana flour (RS2) on in vitro fermentation and the small-bowel excretion of energy, nutrients, and sterols: an ileostomy study." *Am. J. Clin. Nutr.* 75, 104-111. 2002.

- [17] Rodríguez-Ambríz, S.L., Islas-Hernández, J.J., Agama-Acevedo, E. "Characterization of a fiber-rich powder prepared by liquefaction of unripe banana flour." *Food Chem.* 107, 1515-1521. 2008.
- [18] Edith, A.A., José, J., Islas-Hernández, Luis, A.B. "Starch digestibility and glycemic index of cookies partially substituted with unripe banana flour." *LWT-Food Sci. Technol.* 46, 177-182. 2012.
- [19] Aparicio-Saguilan, A., Sayago-Ayerdi, S.G., Vargas-Torres, A. "Slowly digestible cookies prepared from resistant starch-rich linterized banana starch." *J. Food Compos. Anal.* 20, 175-181. 2007.
- [20] Ovando-Martínez, M., Sáyo-Ayerdi, S., Agama-Acevedo, E., Goñi, I., Bello-Pérez, L.A. "Unripe banana flour as an ingredient to increase the indigestible carbohydrates of pasta." *Food Chem.* 113, 121-126. 2009.
- [21] Hernandez-Nava, R. G., Berrios, J., Pan, J. "Development and characterization of spaghetti with high resistant starch content supplemented with banana starch." *Food Science and Technology Inter-national.* 15, 73-78. 2009.
- [22] Saifullah, R., Abbas, F.M.A., Yeoh, S.Y. "Utilization of green banana flour as a functional ingredient in yellow noodle." *Int. Food Res. J.* 16,373-379. 2009.
- [23] Jenkins, D.J.A., Thorne, M.J., Wolever, T.M.S. "The effect of starch-protein interaction in wheat on the glycemic response and rate of in vitro digestion." *Am. J. Clin. Nutr.* 45, 946-951. 1987.
- [24] Goni, I., Martín-Carrón, N. Fermentación colónica de fibra dietética y almidón resistente. In: Lajolo, F.M., Saura-Calixto, F., Penna, E.W., Menezes, E.W. (eds) *Fibra dietética em Iberoamérica: tecnología y salud-obtención, caracterización, efecto fisiológico y aplicación en alimentos*, Varela, São Paulo, 311-338. 2001.
- [25] Serrano, J., Goñi, I., Saura-Calixto, F. "Determination of β -carotene and lutein available from green leafy vegetables by an in vitro digestion and colonic fermentation method." *J. Agric. Food Chem.* 53, 2936-2940. 2005.
- [26] Goering, H.K., Van Soest, P.J. Forage fiber analyses (apparatus, reagents, procedures and some applications). In: United States Department of Agriculture (ed) *Agricultural handbook*, US Government Printing Office, Washington, vol. 379. 1970.
- [27] Horwitz W., Latimer G.W. *Official Methods of Analysis* edn. Maryland: AOAC International, 2006.
- [28] McCleary, B.V., Monaghan, D.A. "Measurement of resistant starch." *J AOAC Int.* 85, 665-675. 2002.
- [29] Carolina, V.B., Edna, R.A., Daiana, C., Antonio, M.C., Rodriguesa, Luiza, H.M. "Green banana (*Musa cavendishii*) flour obtained in spouted bed-Effect of drying on physicochemical, functional and morphological characteristics of the starch." *Ind. Crop. Prod.* 41, 241-249. 2013.
- [30] Asp, N.G., van Amelsvoort, J.M.M., Hautvast, J.G.A.J. "Nutritional implications of resistant starch." *Nutr. Res. Rev.* 9, 1-31. 1996.
- [31] Goni, I., Martín, N., Saura-Calixto, F. "In vitro digestibility and intestinal fermentation of grape seed and peel." *Food Chem.* 90, 281-286. 2005.
- [32] Muir, J.G., Birkett, A., Brown, I., Jones, G.O., Dea, K. "Food processing and maize variety affects amounts of starch escaping digestion in the small intestine." *Am. J. Clin. Nutr.* 61, 82-89. 1995.
- [33] Luis, A.B., Edith, A., Sonia, G., Sáyo-Ayerdi, Esther, M. "Some Structural, Physicochemical and Functional Studies of Banana Starches Isolated from two Varieties Growing in Guerrero." *México.* 52, 68-73. 2000.
- [34] Mousia, Z., Farhat, I.A., Pearson, M., Chesters, M.A., Mitchell, J.R., "FTIR. microspectroscopy study of composition fluctuations in extruded amylopectin-gelatin blends." *Biopolymers.* 62, 208-218. 2001.
- [35] De Sousa, D.W.L., Constancio, A.P., Melo, F.M. "Cardiac metastasis from osteosarcoma: a case report." *Pediatr Blood Cancer.* 53, 799. 2009.
- [36] Zavareze E.D., Silva C.M., Salas-Mellado M., "Functionality of bluewing searobin (*prionotus punctatus*) protein hydrolysates obtained from different microbial proteases." *Quimi. Nova.* 32, 1739-1743. 2009.
- [37] Gutierrez-Dorado, R., Ayala-Rodríguez, A.E., Milan-Carrillo, J. "Technological and nutritional properties of flours and tortillas from nixtamalized and extruded quality protein maize (*zea mays L.*)." *Cereal Chem.* 85, 808-816. 2008.
- [38] Campos-Vega, R., Reynoso-Camacho, R., Pedraza-Aboytes, G. "Chemical composition and in vitro polysaccharide fermentation of different beans (*Phaseolus vulgaris L.*." *Food Sci.* 74, 59-65. 2009.
- [39] Fassler, C., Gill, C.I.R., Arrigoni, E. "Fermentation of resistant starches: influence of in vitro models on colon carcinogenesis." *Nutr. Cancer.* 58, 85-92. 2007.