

# *In Vitro* Colonic Fermentation and Glycemic Response of Different Kinds of Unripe Banana Flour

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**Abstract** This work aimed to study the *in vitro* colonic fermentation profile of unavailable carbohydrates of two different kinds of unripe banana flour and to evaluate their postprandial glycemic responses. The unripe banana mass (UBM), obtained from the cooked pulp of unripe bananas (*Musa acuminata*, Nanicão variety), and the unripe banana starch (UBS), obtained from isolated starch of unripe banana, plantain type (*Musa paradisiaca*) *in natura*, were studied. The fermentability of the flours was evaluated by different parameters, using rat inoculum, as well as the glycemic response produced after the ingestion by healthy volunteers. The flours presented high concentration of unavailable carbohydrates, which varied in the content of resistant starch, dietary fiber and indigestible fraction (IF). The *in vitro* colonic fermentation of the flours was high, 98% for the UBS and 75% for the UBM when expressed by the total amount of SCFA such as acetate, butyrate and propionate in relation to lactulose. The increase in the area under the glycemic curve after ingestion of the flours was

90% lower for the UBS and 40% lower for the UBM than the increase produced after bread intake. These characteristics highlight the potential of UBM and UBS as functional ingredients. However, *in vivo* studies are necessary in order to evaluate the possible benefic effects of the fermentation on intestinal health.

**Keywords** Dietary fiber · Glycemic response · *In vitro* fermentation · Resistant starch · Unavailable carbohydrates · Unripe banana

## Abbreviations

AS	available starch
AUC	area under the curve
DF	dietary fiber
IF	indigestible fraction
NTCD	non-transmissible chronic diseases
RS	resistant starch
SCFA	short-chain fatty acids
TS	total starch
UBM	unripe banana mass
UBS	unripe banana starch

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## Introduction

Changes in eating habits and physical activities have resulted in alterations in the regulatory mechanisms of human metabolism, whereas a crescent unbalance between energy intake and expenditure is evidenced. In addition, a significant number of non-transmissible chronic diseases (NTCD) is directly related with these behavior changes [1].

Unavailable carbohydrates can be represented by the indigestible fraction (IF), which contains the main sub-

strates for colonic fermentation, such as dietary fiber (DF), resistant starch, fructans, resistant proteins and other associated compounds [2]. Since these carbohydrates cause reduced digestion and/or absorption of the available carbohydrates, due to the viscous properties of DF, or substrate encapsulation by cellular wall or partial starch gelatinization, the intake of unavailable carbohydrates may result in a low increase in post-prandial glycemic response, interfering in the glucose metabolism [3]. In the large intestine, the microbiota ferments the carbohydrates that were not digested nor absorbed in the small intestine and produce short-chain fatty acids (SCFA), lactic acid and gases [4, 5]. SCFA have several benefic properties for the human organism and intestinal health [6, 7], which can be related with systemic effects in the metabolism of glucose and lipids, can increase glucose tolerance [8], contribute to the host's daily energetic demands, stimulate colonic blood flux and the use of fluids and electrolytes [5].

Resistant starch (RS) escapes digestion in the human upper gastrointestinal tract and is classified into four general subtypes called RS1–RS4 [5]. RS1 consists of physically inaccessible starch such as whole or partly milled grains; RS2 describes starch granules such as found in unripe banana or raw potato; retrograded starch obtained by food processing is classified as RS3 and RS4 consists of chemically modified starches, such as cross linked starches. RS has been identified as the main substrate for the human colonic microbiota and the rate of RS fermentation varies according to the type of RS. Some types disappear in a total of 24 h *in vitro* fermentation and produce high concentrations of SCFA, such as raw potato starch (RS2), while others (such as RS3) are more resistant to bacterial fermentation and take more than 24 h to be completely fermented [9]. RS *in vitro* fermentation has shown to increase the proportion of butyrate in relation to acetate and propionate [10]. Products of the *in vitro* fermentation of RS2 and RS3 are capable of inhibiting the initiation and promotion stage in colon carcinogenesis *in vitro* [11].

Bananas are mainly produced in tropical and subtropical developing countries. Approximately one-fifth of all bananas harvested in Brazil are wasted, which occurs mainly due to the lack of funds and technology of small producers to guarantee high quality fruit. When bananas are still unripe, they are easily transported, can be stored longer and have high concentration of starch, so the processing of unripe banana could provide a means to minimize losses and increase market share [12, 13]. According to Juarez-Garcia et al. [14], unripe banana flour produced under specific conditions is composed as follows: 73.4% total starch, 17.5% resistant starch and 14.5% dietary fiber. Besides its high starch content (60–80% dw), when it is raw it 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 [15]. An innovative approach has been taken to explore unripe banana flour as a functional ingredient, and to prove that its regular consumption can confer health benefits in humans [12, 14].

The objective of this work was to determine the extent of *in vitro* colonic fermentation of unavailable carbohydrates of two kinds of unripe banana flour and evaluate their glycemic response in healthy volunteers.

## Materials and Methods

### Banana Flour Elaboration

Two kinds of unripe banana flour at the first stage of maturation, which was determined according to Tribess et al. [16], were evaluated: the unripe banana mass (UBM) and the unripe banana starch (UBS). The UBM was obtained from the cooked pulp of unripe bananas (*Musa acuminata*, Nanicão variety) with peel. For the preparation of the UBM, unripe bananas were cooked with peel in water at 98 °C for 8 min, then peeled, homogenized, dried in a tray dryer (Proctor and Schwartz, model K11556, Philadelphia, USA) at 60 °C until constant weight and grounded in a mill MA 680 (Marconi, Piracicaba, Brazil) to particles <0.250 mm. The UBM was prepared in the Chemical Engineering Department of Escola Politécnica, USP, and the unripe bananas were provided by the Associação de Bananicultores from Vale do Ribeira, São Paulo, Brazil. The UBS is composed of isolated starch of unripe banana, plantain type (*Musa paradisiaca*) *in natura*. It was isolated from the pulp of raw bananas (citric acid solution (1 gL<sup>-1</sup>)) using a pilot scale procedure, by the Instituto Politecnico Nacional, CEPROBI, México, according to Flores-Gorosquera et al. [17]. Samples of the flours produced were packaged aseptically in aluminized plastic bags and stored at 25 °C until further analysis and assays.

### Chemical Analysis

All samples used were previously dried and milled to fine powder (particle size less than 0.250 mm) and all analyses were done in triplicate.

### Carbohydrates and Moisture Analysis

The resistant starch (RS) and available starch (AS) were quantified according to AOAC method 2002.02 [18, 19]. In the analysis of RS, a sample of dried boiled beans was used as in-house reference material. The glucose was quantified in the final supernatants with a GOD/POD/ABTS (glucose-oxidase/peroxidase/2,2'-Azino-di-[3-ethylbenzothiazoline] sulfonate (Sigma Chemical Co., St. Louis, MO,

USA)) mixture as described by Cordenunsi and Lajolo [20]. For AS quantification, the flasks containing supernatants stored at 4 °C, obtained in the RS analysis, had their volume completed to 50 mL or 100 mL (for AS higher than 20%) with Milli-Q water. In the supernatant, glucose was determined by the GOD/POD/ABTS mixture. Starch from Sigma Chemical Co. (St. Louis, MO, USA) was used as a standard reference material. The starch was calculated as glucose  $\times$  0.9. The total starch (TS) was calculated as the sum of the content of AS and RS.

Dietary fiber (DF) was quantified by the enzymatic-gravimetric method according to AOAC method 991.43 [21] with modifications. The modifications were proposed by McCleary and Rossiter [22] in order to exclude RS from the DF fraction. Total DF (without RS) was determined as the sum of soluble DF and insoluble DF fraction.

The moisture content of the samples was determined by a gravimetric method after dehydration at 60 °C and low pressure until a constant weight was achieved.

#### Indigestible Fraction Quantification and Isolation

Quantification and isolation of indigestible fraction (IF) were done according to the methods proposed by Saura-Calixto et al. [23] and Serrano et al. [24], with modifications. The procedure of quantification was constituted of sample homogenization in acid medium, followed by enzymatic treatment with 0.2 mL pepsin (65,000 U/mL KCl/HCl buffer 0.1 M, pH=1.5) (P-7012, Sigma, St. Louis, Mo, USA) and 1 mL  $\alpha$ -amylase (3,480 U/mL Trismaleate/NaOH buffer 0.1 M, pH=6.9) (A-3176, Sigma, St. Louis, MO, USA) to eliminate available compounds. After incubation (37 °C, 16 h), samples were centrifuged (25 °C, 1,500 g, 15 min). The residues were dried (105 °C, 16 h) and quantified gravimetrically as the insoluble IF. The supernatants were dialyzed (12,000–14,000 MWCO molecular porous membrane tubing, Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) against water (25 °C, 48 h). Dialysis retentates were vacuum-dried in speedvac and soluble IF was quantified by gravimetric method. The IF was calculated by the sum of insoluble IF and soluble IF of each replicate. In order to isolate the IF, the quantity of the initial sample to be isolated was equal to the amount necessary for obtaining 0.8 g of IF and the solutions and enzymes added were proportionally increased. The IF was obtained combining the final residues of insoluble IF and soluble IF and stored at -20 °C for *in vitro* colonic fermentation.

#### *In Vitro* Colonic Fermentation

The *in vitro* fermentation assay was elaborated according to the method described by Goñi and Martín-Carrón [4] and

Serrano et al. [24], with modifications [25]. The inoculum was prepared with fresh rat cecum contents of male Wistars (body weight of 300 $\pm$ 10 g). The collection of cecal material was approved by the Committee of Ethic in Animal Research and Experimentation of the Faculty of Pharmaceutical Sciences of the University of São Paulo (Protocol 54). Before using the inoculum, the contents of diluted cecum (100 g/L of sterilized anaerobic medium of fermentation) were mixed for 15 min in a Stomacher blender (Logen Scientific) and filtered (particles <1 mm). The anaerobic medium of fermentation was composed of tryptone, micromineral and macromineral solutions and resazurin as anaerobic redox indicator [26].

In glass flasks, previously dried (105 °C, 16 h) and weighed, 6 replicas of each sample (100 mg of the IF of flours, 3 for  $t=0$  h and 3 for  $t=24$  h), lactulose controls (100 mg, Sigma L-7877, St. Louis, MO, USA) and blanks (no sample) were hydrated with anaerobic fermentation medium (8 mL) at 4 °C for 16 h in totally sealed flasks with sleeve stoppers (Wheaton, Millville, NJ, USA). After hydration, 2.5 mL of inoculum were added and the headspace was rinsed with CO<sub>2</sub> (1 min). The samples ( $t=24$  h), controls and blanks were placed in a shaking water bath (37 °C, 24 h). Samples  $t=0$  h, after hydration, had pH quantified and were stored for SCFA quantification. The internal pressure of the flasks was measured with a 0–15 psi manometer (Ashcroft, Stratford, CT, USA). The pH was measured in incubated (24 h fermentation) and non-incubated (0 h fermentation) samples. Fermentation was stopped with 3 mL of 1 M NaOH. Flasks were centrifuged (4 °C, 1,500 g, 15 min). Residues were dried (105 °C) for quantification of the non fermented residue (NFR) and supernatants were totally collected for SCFA analysis.

#### SCFA Analysis

A mixture of 0.5 mL supernatants produced in the fermentation, 0.4 mL internal standard (2-metil-valeric acid, Aldrich 10987-8, Milwaukee, WI, concentration 0.05–5.0 mM) and 0.1 mL HClO<sub>4</sub> (in a concentration enough to keep pH of all samples the same) was centrifuged (4 °C, 19,000 g, 15 min) and supernatants were transferred to gas chromatography (GC) vials. 3  $\mu$ L (split 1:30) of supernatant were automatically injected (HP 7683, Wilmington, DE, USA) into a Plus HP 6890 CG (Wilmington, DE, USA), equipped with a flame ionization detector and capillary fused silica column (WCOT, CP7747, Varian, Lake Forest, CA, USA). The injector and detector temperatures were 270 °C and 300 °C, respectively. The analysis was made in a temperature ramp from 115 °C to 250 °C (13 min) under constant pressure. SCFA were identified and quantified by comparison with a volatile acid standard mix (Supelco, Bellefonte, PA, USA). SCFA

produced were expressed as molar proportions (%) of acetate:propionate:butyrate. Results obtained from blanks (both 0 h and 24 h fermentation) were subtracted from the samples to correct the SCFA production from the inoculum.

#### Evaluation of Glycemic Response

The glycemic responses produced by the intake of foods were evaluated according to the FAO protocol [27] and the experimental protocol was approved by the Ethical Research Committee of the School of Pharmaceutical Science, University of São Paulo (USP), according to the rules of the National Committee for Ethical Research of the Brazilian Health Ministry. All subjects signed informed consent to participate.

The *in vivo* study was done in two different steps. All healthy volunteers were aged between 22 and 40 years old, both genders, and had a body mass index (BMI) of 18.5–24.9 kg/m<sup>2</sup>, classified as eutrophic according to the World Health Organization [28], without previously diagnosed diabetes or diabetics in the family. The volunteers came to the laboratory after a 10–12-h overnight fast. In the first step, UBM was tested: nine volunteers received a portion of white bread (standard food) or UBM. In the second step, UBS was tested: nine other volunteers received a portion of white bread (standard food) or UBS. The flours were dissolved in 250 mL of water (25 °C) and the volunteers had 10 min to ingest each portion. Blood glucose was determined in each subject fasted (time zero) and after ingesting the flours (15, 30, 45, 60, 90 and 120 min) in order to make the glycemic response curve and calculate the area under curve (AUC) [27, 29]. Glucose was measured in capillary whole blood by Accu-Check Go, Roche Diagnostics® (Indianapolis, IN, USA).

The quantity of flour to be ingested was calculated based on a portion containing 50 g of TS; therefore, the volunteers received portions of 59 g of UBS and 81 g of UBM, which had 40 and 6.5 g of RS; 10 and 43 g of AS; and 1.0 and 8.3 g of total DF, respectively. In relation to the white bread (standard food), the portion ingested was 120 g, which contained 50 g of TS. The content of soluble sugars was not considered in the calculation of the quantity of flour to be ingested in the *in vivo* assays, since the concentration of soluble sugars in the maturation stage of the unripe bananas used is lower than 2% [30].

#### Statistical Analysis

The AUC of glucose concentration was expressed as means ± SEM and the other values as means ± SD. Data were subjected to Student's *t* test of Statistica 8.0 software (Stat Soft. Inc., Tulsa, OK, USA). The level of significance was set at  $p < 0.05$ .

## Results and Discussion

### Profiles of Unavailable Carbohydrate and *In Vitro* Colonic Fermentation of Banana Flours

The content of starch, DF and IF of the unripe banana mass (UBM) and the unripe banana starch (UBS) are shown in Table 1. The moisture of the UBM and UBS was 4.00±0.3% and 8.6±0.9%, respectively. Both UBM and UBS can be considered sources of RS. The UBS is a concentrated source of RS (67% dw) that was obtained from the pulp of raw unripe bananas, plantain type, which contain essentially RS2 and a very low amount of RS1 [15]. The UBM was made of cooked pulp of unripe bananas with peel and contains 8% (dw) of RS. Muir et al. [31] showed that when the RS of unripe bananas was measured in the raw and cooked pulp flours, the amount of RS in cooked unripe banana flour was reduced from 52% to 3%, and it was attributed to starch gelatinization. The resistant starch in granules (RS2), present in raw unripe bananas, is easily gelatinized when submitted to high temperatures in the presence of water [32]. When elaborating the UBM, unripe bananas were cooked with peel so that the peel could act as a physical barrier to the water and avoid total gelatinization of the starch. Using this step in the process resulted in a final concentration of RS equal to 8%. Although the UBM presents lower content of RS than the UBS, its content of total DF (10.3% dw) is significantly higher than the content of DF in the UBS (1.6% dw) (Table 1). Also, the UBM presents a similar proportion between soluble and insoluble DF, with high content of soluble DF (4.5% dw).

In relation to the content of unavailable carbohydrates (dw), considering the sum of total DF (without RS) and RS, the UBS contains 68.3% and the UBM 18.5%, which are significant quantities. These different kinds of flour are of great importance in Latin American countries, where the

**Table 1** Content of starch, dietary fiber and indigestible fraction in the unripe banana mass (UBM) and the unripe banana starch (UBS)

Compound (% dw) <sup>a</sup>	UBM	UBS
Total starch	61.6±1.3 <sup>a</sup>	83.8±2.5 <sup>b</sup>
Resistant starch (RS)	8.2±0.2 <sup>a</sup>	67.0±2.3 <sup>b</sup>
Available starch	53.2±1.2 <sup>a</sup>	16.8±0.9 <sup>b</sup>
Total dietary fiber (DF) (without RS)	10.3±0.2 <sup>a</sup>	1.3±0.2 <sup>a</sup>
Insoluble DF	5.8±0.2 <sup>a</sup>	0.0±0.0 <sup>b</sup>
Soluble DF	4.5±0.2 <sup>a</sup>	1.3±0.1 <sup>b</sup>
Indigestible fraction (IF)	21.0±0.5 <sup>a</sup>	69.4±1.5 <sup>b</sup>
Insoluble IF	14.2±0.7 <sup>a</sup>	57.7±2.5 <sup>b</sup>
Soluble IF	6.8±0.8 <sup>a</sup>	11.6±0.6 <sup>b</sup>

<sup>a</sup> Results expressed on dry weight (% dw) as mean ± SD of triplicates. Means in row sharing different letters are significantly different ( $p < 0.05$ )



ingestion of dietary fiber is below the nutritional requirement [33]. However, it is important to consider that the UBM and UBS must not be heated, in order to preserve their RS content.

According to the IF definition [2], the insoluble IF is basically constituted of RS, insoluble DF, resistant protein, tannins and others, while the soluble IF is mainly consisted of soluble DF, soluble polysaccharides and associated bioactive compounds. Due to the starch gelatinization during the elaboration of the UBM, its content of IF was 3.3 lower than the UBS content (Table 1). Comparing the IF contents with the contents of unavailable carbohydrates (calculated by summing DF and RS) of the flours, the UBS presented similar values (69.4% and 68.3%, respectively), once it is isolated starch, mainly composed of RS. On the other hand, the UBM presented a small difference (21.0% and 18.3%, respectively), possibly due to the presence of compounds associated to DF [2, 34].

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 [5]. Both flours presented high fermentability, expressed by several parameters, as shown in Table 2. For example, the fermentability expressed by total SCFA was 98% for UBS and 75% for UBM. The UBS was more fermentable than the UBM ( $p<0.05$ ), with results next to the ones of lactulose, for several parameters. The UBM fermentability varied from 50 to 75% in relation to lactulose. These results are due to different quantities of unavailable carbohydrates in the flours, as described in Table 1. The UBS is composed of RS, the main component of the unavailable carbohydrate, which reaches the colon and is almost totally fermented [35, 36]. The UBM is composed of both RS and DF, whereas the soluble DF is highly fermented, while the insoluble fibers are partially fermented in the large intestine [3].

Comparing the production of SCFA, expressed in molar proportion, significant differences were evidenced between

**Table 3** Molar proportions of short chain fatty acids (SCFA) produced in the *in vitro* colonic fermentation of indigestible fraction of the unripe banana mass (UBM) and the unripe banana starch (UBS)

	Molar proportion <sup>a,b</sup>		
	Acetate	Propionate	Butyrate
UBM	57.4±3.1 <sup>a</sup>	23.0±1.4 <sup>a</sup>	19.6±1.8 <sup>a</sup>
UBS	68.3±1.9 <sup>b</sup>	14.4±0.7 <sup>b</sup>	17.3±1.1 <sup>a</sup>

<sup>a</sup> Percentage of each SCFA in relation to the sum of acetate, propionate and butyrate. <sup>b</sup> Results expressed as means ± SD. Means in columns sharing different letters are significantly different ( $p<0.05$ )

the two different flours regarding acetate and propionate (Table 3). The molar proportion of acetate:propionate:butyrate of UBM (57:23:20) was close to the one presented in the fermentation of grape seed and peel (59:27:14) [36]. In the UBS, the molar proportion was 68:14:17, with a high amount of acetate. The results of the UBS are similar to the ones found by Campos-Vega et al. [37] in the fermentation of extract of polysaccharides from cooked common bean seeds (for example, for the cultivar Pinto Durango, the molar proportion was 68:13:19). The UBM presented a decrease in the acetate:propionate ratio (2.4) in relation to the UBS (4.8), which indicates a possible effect in the reduction of serum lipids [7]. In relation to butyrate, both UBS and UBM produced higher molar proportion than pectin (79:13:7) or cellulose (73:21:4) [38] and a molar proportion close to the one presented by RS2 (70:09:21) [11]. This butyrate molar proportion produced by UBM and UBS, which is similar to the one produced by RS2, suggests that these flours may promote intestinal health, although the effects of their *in vivo* fermentation need to be investigated. Butyrate has been reported to influence the promotion of differentiation, induction of apoptosis and inhibition of proliferation in colon tumor cell lines [11].

**Table 2** *In vitro* colonic fermentability of indigestible fraction of the unripe banana mass (UBM) and the unripe banana starch (UBS)

	Fermentability <sup>a</sup> (%)			
	ΔpH <sup>b,f</sup>	Pressure <sup>c,f</sup>	NFR <sup>d,f</sup>	Total SCFA <sup>e,f</sup>
UBM	50.0	52.9±8.3 <sup>a</sup>	47.8±0.1 <sup>a</sup>	74.6±8.2 <sup>a</sup>
UBS	83.7	107.8±4.2 <sup>b</sup>	71.0±7.0 <sup>b</sup>	98.1±7.5 <sup>b</sup>

<sup>a</sup> Having values of lactulose as reference (100%). <sup>b</sup> (average 24 h pH)—(average 0 h pH). <sup>c</sup> Pressure in 24 h. <sup>d</sup> Percentage of non-fermented residue =  $100 \times (\text{final dry weight of the residue after fermentation})/(\text{initial dry weight of the sample})$ . <sup>e</sup> Sum of the SCFA production (acetate, propionate and butyrate). <sup>f</sup> Results expressed as means ± SD. Means in columns sharing different letters are significantly different ( $p<0.05$ )

**Table 4** Area under the curve (AUC) of blood glucose until 2 h after intake of unripe banana mass (UBM) or unripe banana starch (UBS) in relation to the intake of white bread

Groups	AUC <sup>a,b</sup> (mmol.min/L)
Bread	158.9±22.0 <sup>a</sup>
UBM	94.5±14.8 <sup>b</sup>
Bread	153.4±17.7 <sup>a</sup>
UBS	16.1±3.5 <sup>b</sup>

<sup>a</sup> Calculated from the glycemic response in times: 0, 15, 30, 45, 60, 90 and 120 min. <sup>b</sup> Results expressed as mean ± SEM (nine volunteers per group). Means in columns of the same group sharing different letters are significantly different ( $p<0.05$ )

## Glycemic Response of Banana Flours

Among the several necessary requirements to consider a benefic ingredient, one of them is its capacity of producing a low increase in the postprandial glycemic response. Therefore, the glycemic responses produced by the ingestion of unripe banana flours, in relation to white bread (standard food), were evaluated in healthy subjects. The glycemic curves were made using the means of glycemic responses of the volunteers in each time of blood collection. The areas under the glycemic curves produced after the ingestion of either the UBM or UBS, in equivalent quantities of total starch, were significantly different in relation to the areas produced by the intake of white bread (Table 4).

These results show that both flours have the characteristic of not increasing the postprandial glycemic response in relation to bread (standard food), whereas the UBS had a strongest effect. There is a different explanation for this effect in each kind of flour. In the case of the UBS, the high content of RS (40 g) in the ingested portion of 59 g is the main reason. In the case of the UBM, although the portion (81 g) contained 43 g of available starch, it still has 6.5 g of RS (due to the presence of banana peel during the cooking process) and 8.3 g of dietary fiber (3.6 g are soluble dietary fiber), which may difficult the *in vivo* availability of starch [3]. Therefore, both flours do not produce high increase of the postprandial glycemic response in healthy volunteers, presenting favorable characteristics for their addition to food products or diets.

## Conclusions

Both unripe banana mass (UBM) and unripe banana starch (UBS) present high *in vitro* fermentability due to the high content of unavailable carbohydrates (resistant starch and/or dietary fiber), which, in its turn, do not produce high increase in the postprandial glycemic response in healthy volunteers. These characteristics highlight the potential of UBM and UBS as functional ingredients. However, intervention studies testing the regular intake of the flours, in humans, are needed in order to investigate the effect of their fermentation on intestinal health.

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