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Effect of green banana pulp on physicochemical and sensory properties of probiotic yoghurt

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Abstract

In order to investigate the potential of the green banana as a prebiotic, and for its content of resistant starch, fermented yogurts were produced by cultures composed of *Lactobacillus delbrueckii*, *Streptococcus thermophilus, Bifidobacterium bifidum* and *Lactobacillus acidophilus* as well as being enriched with three concentrations of industrialized green banana pulp (GBP) (3%, 5% and 10% w/v). The green banana pulp added to the yogurt stimulated the multiplication of *L. acidophilus* after the first day of fermentation and *B. bifidum* after seven days in cold storage compared to the control that consisted of yogurt without the addition of green banana pulp. The dose-response effect was not observed; however, the results show that the green banana pulp has a prebiotic potential without interfering with either the physicochemical or sensorial characteristics.

Keywords: resistant starch; probiotic yogurt; green banana; *Bifidobacterium bifidum*; *Lactobacillus acidophilus*.

Practical Application: In this work we applied green banana in yogurt to improve probiotic counting, indicating a possible prebiotic effect.

1 Introduction

There are currently several food companies engaged in producing various functional products. In addition, these companies are investing in food production researches to fulfill the high consumer demand (Raud, 2008). One of the most important is the probiotic yogurt. Probiotic is a live microorganism that, when consumed by humans, is capable of modulating the metabolic activity of replacing the intestinal microbiota and promoting health benefits (Spanhaak et al., 1998).

For a microorganism to be classified as probiotic it must be a bacteria of human origin and be resistant to hydrochloric acid in the stomach, digestive enzymes, bile salts in the duodenum and processing conditions like dehydration, freezing and lyophilization (Ferreira & Teshima, 2000).

Nowadays the dairy technology science has made many important advances that led foods with high nutritional properties that confer health.

Probiotic food development involves the use of different microbial and prebiotic species; the latter consists of substances acting as a substrate to probiotic microorganisms. The industry has been searching for prebiotics capable of stimulating the multiplication as well as promoting the maintenance of such microorganisms in order to preserve food (Fuentes-Zaragoza et al., 2011) and its benefits to health. Prebiotics have gelling properties, promoting best viscosity for products such as yogurt.

A component food that presents a function similar to the fibers is the resistant starch, its consumption is associated with several changes in metabolis which may confer some health benefits in cases of colitis, ulcerative colitis, gastric ulcer, uremia, nephritis, gout, cardiovascular problems and celiac disease, besides delaying the gastric emptying and decreasing the blood cholesterol levels (Mastro et al., 2007).

The resistant starch is present in several foods especially in green bananas, containing between 40.9 g/100g and 58.5 g/100g on a dry basis (Tribess et al., 2009).

Acording to Padam et al. (2014) banana starches which are relatively low in amylase content, have high resistance to heating and amylase attack, low swelling properties, and low retrogradation, giving a aplication in the food industry as gelling agent, thickening agent and stabilizer. These characteristics are spetialy important to dairy industry, for its odorless and soluble capacity, green banana pulp (GBP) could be aplyed to formulate several kinds of desserts. The objective of this work is to evaluate the physicochemical features of the commercial GBP, which is the pulp of the cooked green banana, and to verify a possible probiotic effect on the development of a functional yogurt. This work is motivated by the extensive production of bananas in Brazil and the need for new researches into the uses of resistant starch.

2 Materials and methods

2.1 Materials

Commercial green banana pulp (Vale Mais Alimentos, Brazil) and homogenized and pasteurized cow milk (Fazenda Bela Vista, Brazil) were purchased from the local market of Santos, São Paulo, Brazil.

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Other materials including in this study were lyophilized yogurt starter culture used in the preparation of the yogurts, consisted of a blend of *Lactobacillus acidophilus* LA-5™ (1×10^6 UFC/g), *Bifidobacterium* Bb-12™ (1×10^6 UFC/g), *Streptococcus thermophilus* and *L. delbrueckii* spp. Bulgaricus (Bio Rich, Chr. Hansen, Denmark). All other reagents were analytical grade or electrophoresis grade.

2.2 Experimental design

Pasteurized whole milk was firstly boiled, when the temperature reached 65 °C it was homogenized with GBP at different concentrations (0%, 3%; 5% or 10%; w/v). A commercial lactic culture (Bio Rich™ Probiotic, CHR Hansen) was added at 45 °C, according to the manufacturer's instructions (400 mg per L), and then, mixed thoroughly, and incubated at 40 °C for 6 hours without pH monitoring. After fermentation they were kept under refrigeration at 6 °C, until analysis.

2.3 Chemical composition

To the GBP and yogurts centesimal composition, the official AOAC methods were followed (Association of Official Methods Analytical Chemists, 2012). Dry matter was obtained by desiccation at 105 °C until constant weight, whereas the ash content was given by incineration at 550 °C, crude protein was determined by the Kjeldahl procedure, using 6.25 as converting factor to GBP and 6.38 to yogurt (specific for dairy products).

Bligh & Dyer (1959) method was used for lipids quantification. The determination of the carbohydrates was carried out by difference.

The enzymatic-gravimetric method described by Chisté et al. (2009), based on the weighing of sample after the starch and protein enzymatic removal, was used for determining the total fibers of GBP. The analyte, insoluble in ethanol 95%, was determined gravimetrically.

The determination of the resistant starch content on GBP was carried out using the methodology adopted by Freitas & Tavares (2005), where the samples were successively submitted to incubations with enzymes, centrifugation cycles and dialysis.

The titratable acidity determination in the yogurts was carried out with 0.1N NaOH, using 5% phenolphthalein in alcoholic solution as indicator (Instituto Adolfo Lutz, 2005). The final pH was measured using a digital potentiometer (pHmeter Quimis l, model SC 09, Diadema, Brazil). Both determinations were performed at the 2nd and 10th day after producing the yogurts.

The analysis of the tannins in the yogurts was performed by the Fólin-Denis method, which is based on the reduction of phosphomolybdate-phosphotungstate under alkaline conditions (Agostini-Costa et al., 1999).

2.4 Sensory analysis

The Ordination Preference Method was applied for the yogurts sensory analysis (Minim, 2006). Three randomly selected encoded samples were used. One sample was commercial and two others had GBP with smaller and bigger concentrations, respectively (3% and 10%), to which the taster had, according to a crescent order of preference, assigned number 1 for a greater preference sample, number 2 for the second, and 3 for the third sample.

The tasting of the samples was performed at the Gastronomy and Nutrition Laboratory of the Catholic University of Santos in closed chambers with red lights so that the tasters were not influenced by the color of the product. The Free and Informed Consent Term was handled by the participants and approved by the local Research Ethics Committee (protocol: 4408.7.2007).

At the sensory evaluation moment, the natural yogurts containing green banana pulp were mixed with strawberry jam to present a similar flavor to the commercial sample. The experimental yogurts were prepared about 24h before the sensory tests.

2.5 Rheological analysis

All rheological analysis were performed with a texture analyzer (TA-XT Plus, UK) at a crosshead spead of 1.0 mm/s. The texture analyzer was fitted by compression using a 35 mm diameter cylindrical acrylic probe and extension bar using 5kg load cell, and it was programmed to compress 30 mm.

These analysis were performed in triplicate, using 50g of yogurt for each rum, and were maintained at 6 °C. Probe returned to initial position at 10 mm/s, according to protocol used to Antunes et al. (2004) with feel modifications.

To calculate the firmness results it was considered the peak force during the first compression cycle in stress-strain curve during the compression test. Furthermore, the area of the curve up to this point is taken as a measure of consistency.

2.6 Microbiological analyses

For the isolation and counting of probiotic bacteria each one of the yogurt samples were aseptically diluted by adding 25 g to 225 mL of a 0.1% (w/v) sterile peptone water. Aliquots of these suspensions were transferred to test tubes containing 9 mL with the same diluent, so as to obtain serial decimal dilutions (10^{-1} to 10^{-6}).

Later, an aliquot of each diluted sample was transfered to plate dishes containing specif Agar. Agar LP-MRS (Propionate of Lithium-MRS), to enumerate *B. bifidum* under anaerobic incubation using GasPak™ systems (Probac, Brazil) at 37 °C for 72 hours. *Lactobacillus acidophilus* levels were enumerated with Agar Bile-MRS using an aerobic incubation at 37 °C for 72 hours, as described by Vinderola & Reinheimer (1999). The counting was performed by a Manual Colony Counter apparatus.

2.7 Statistical analyses

The descriptive statistics were made using Microsoft Excel for Windows. The differences between the average values at a 5% significance level was obtained by ANOVA and Tukey test or Student *t*-test. The sensory analysis results were analyzed through the non-parametrical statistical test of Friedman (Minim, 2006), using the software Prism 5 for windows (GraphPad Softaware).

3 Results and discussion

The GBP is a food ingredient used as a thickening agent. Its role as a functional food has been demonstrated to help gastrointestinal disorders, partly attributed to the resistant starch present in the unripe fruit (Rabbani et al., 2009).

The starch resistance suffers interference from thermal treatment, being reduced after heating, and rising after the retrogradation and cooling (Keim et al., 2009).

The GBP used in this work consists of the cooked green banana pulp which presented a content of resistant starch about 4.3% (w/w), and 3.2% (w/w) of dietary fiber. The estimated concentrations of resistant starch in fermented milks obtained in this work with 3%, 5% and 10% of GBP, were 0.13%, 0.21% and 0.43%, respectively. Walter et al. (2005) found resistant starch in green banana (*Musa sinensis* L.) varying from 0.68% to 0.74%. Lii et al. (1982) analyzed the green banana pulp of the Taiwan species and observed 0.49% of crude fiber.

The results of the GBP composition and yogurts are presented in Table 1. The use of GBP did not significantly altered the mainly compositional components of yogurt, except to the moisture on yogurt 10% GBP, However, Hauly et al. (2005) produced soy yogurt added to the fructooligosaccharides that presented a smaller moisture value of 77.08%, when compared to those added with GBP (3%, 5% and 10%).

The lactic probiotic beverages developed by Cunha et al. (2008) with different concentrations of milk and cheese whey presented the smaller lipid contents (1.68%). Silva (2007) elaborated a symbiotic yogurt that presented a greater protein value (5.01%) when compared with the yogurts containing GBP. Torres et al. (2000) found 4.29% of proteins in commercial yogurts.

According to the Technical Regulation for Identity and Quality of Dairy Beverages of the Ministry of Agriculture and Supply in Brazil, they must have at least 1.6% of proteins in their composition. The yogurts elaborated in this work fulfill this recommendation (Brasil, 2000a).

Hauly et al. (2005) produced a soy yogurt with sweetened FOS that presented a bigger carbohydrates content (16.97%).

In the yogurt concentrations of 3%, 5% and 10% it was found 0.03%, 0.03% and 0.73% of tannins, respectively. Onibon et al. (2007) evaluated the presence of antinutritional factors (tannin, phytate and oxalate) in Nigerian fruts, including banana, these authors considered the antinutrients within tolerable levels, even when consumed in right portion.

The acidity indicates the degree of lactose conversion in lactic acid (Thamer & Penna, 2005). According to Table 2, it is possible to observe that the acidity increases in different concentrations of GBP, indicating a growth in lactic bacteria and the production of lactic acid in the three types of yogurt.

The average lactic acid was 1.5% in the yogurt produced by Fuchs et al. (2006) was higher than the amount of lactic acid found in the yogurts in this study. According to the Brazilian legislation, the minimum amount of lactic acid in a yogurt must be 0.6% and a maximum 1.5% (Brasil, 2000b; Faria et al., 2006b; Silva, 2007).

The yogurts present pH above 4.3, a value near to the value found in the probiotic yogurt protease inhibitors) and zinc in Caribbean foods, which were found in irrelevant quantities in the green banana.supplemented with inulin and oligofructose produced by Fuchs et al. (2006) that presented a pH in around 4.2. The pH values are important because a yogurt with a low acidity (pH > 4.6) promotes whey separation. On the other hand, a pH < 4.0, the clot contraction, due to a reduction of the protein hydration, also causes a conversion into whey (Thamer & Penna, 2005).

With regard to sensory analysis, the ordination test, which is an affective test is used to improve products and evaluate alterations in food production, focusing on comparing several products in relation to consumer preference. The results are presented in Table 3.

The sensory test was applied with the yogurts prepared using the maximal and minimum concentration of GBP and the results presented no statistically significant difference with the control at the 5% level. The sample containing 5% GBP was not tested, because the main goal is the acceptance of the most concentrated product, and this was observed without fatigating the tasters.

Table 1. Composition of the green banana pulp (GBP) and yogurt, with different concentrations of GBP (g/100g; w/v).

	GBP	3%	5%	10%
Moisture (%)	$76.7^b \pm 0.76$	$88.0^a \pm 0.05$	$87.0^a \pm 0.02$	$86.0^b \pm 0.20$
Ash (%)	$0.8^a \pm 0.07$	$0.8^a \pm 0.07$	$0.8^a \pm 0.03$	$0.7^a \pm 0.02$
Protein (%)	$0.8^b \pm 0.04$	$4.4^a \pm 0.11$	$2.7^a \pm 0.02$	$2.6^a \pm 0.42$
Lipid (%)	$0.2^b \pm 0.07$	$3.2^a \pm 0.02$	$2.6^a \pm 0.50$	$2.7^a \pm 0.40$
Carbohydrate (%)	21.9	3.5	6.8	8.0

Analyses permormed in triplicate. Different letters in each row differ at a 5% significance level of the Tukey's test.

Table 2. Values of lactic acid and pH observed in the 1st and the 10th days in the yogurts containing 3%, 5% and 10% (w/v) of GBP.

Days	GBP 3%		GBP 5%		GBP 10%	
	Lactic acid (%)	pH	Lactic acid (%)	pH	Lactic acid (%)	pH
1	0.63 ± 0.00	4.5 ± 0.0	0.57 ± 0.01	4.4 ± 0.0	0.74 ± 0.01	4.5 ± 0.0
10	0.64 ± 0.01	4.4 ± 0.0	0.69* ± 0.07	4.3 ± 0.0	0.79 ± 0.00	4.4 ± 0.0

*$p < 0.05$ *vs* first day value. There was no statistically significant difference beetwen the other results according to ANOVA test.

Faria et al. (2006a) elaborated fermented buffalo milk with green-apple, orange and peach flavorings, and evaluated the preference and acceptance of the product by means of sensorial analysis by the ordination test. The results were evaluated by the statistical test of Friedman and it was verified that the differences among the averages were not significant at 5% level among the yogurts.

According to Figure 1 the concentration of GBP added in the yogurts up to 5% caused a significant ($p < 0.05$) increase in both firmness and consistency. This parameters are important to new kind of yogurts that has been developed in the last years confirming the technological functionality of GBP, very useful to texture improving of dairy products.

In this analysis the addition of GBP does not caused a linear rose in the texture profile, other analysis also shown the same, probably by complexes interactions between protein and starch.

Table 3. Sum of the yogurt order added of GBP and commercial yogurt, according to the ordination test.

Yogurts	Sum of orders*
Yogurt 3%	92[a]
Yogurt 10%	94[a]
Commercial Yogurt	83[a]

*The same letters do not differ among themselves by Friedman test ($p < 0.05$)

A probiotic analysis was carried out in the 1[st] and 7[th] day after the yogurt production. There was a microorganism growing in both the Agars, LP-MRS to *B. bifidum,* and Bile-MRS to *L. acidophilus*. Table 4 presents the results of the counts.

When analyzing Table 4, it is observed that the GBP added to the yogurt has favored the grow of probiotic microorganisms, being the *B. bifidum* stimulated only from the second week of incubation. The best condition to this bacteria was founded when it was used a smaller GBP concentration (3%), this phenomenon probably occurred because yogurts prepared with higher GBP concentration presented lager acid lactic accumulation, that can be unfavorable to the *B. bifidum* (Zarcachenco & Massaguer-Roig, 2004).

Both the *L. acidophilus* and the *B. bifidum* presented higher counts in the yogurts enriched with GBP when compared to the counts of the control yogurt (CO), mainly after seven days of storage; therefore, the GBP added to the yogurt presented a potential prebiotic effect.

The values observed in this work are comparable to those obtained by Cunha et al. (2008), when carrying out the physicochemical, microbiologic and rheological analyses of lactic beverages and fermented milks containing added probiotics observed values greater than 10^6 CFU/mL of probiotics. Silva (2007) showed values around 8.3×10^8 CFU/mL of viable cells of *L. acidophilus* and 3.1×10^6 CFU/mL of viable cells of *Bifidobacterium* sp.

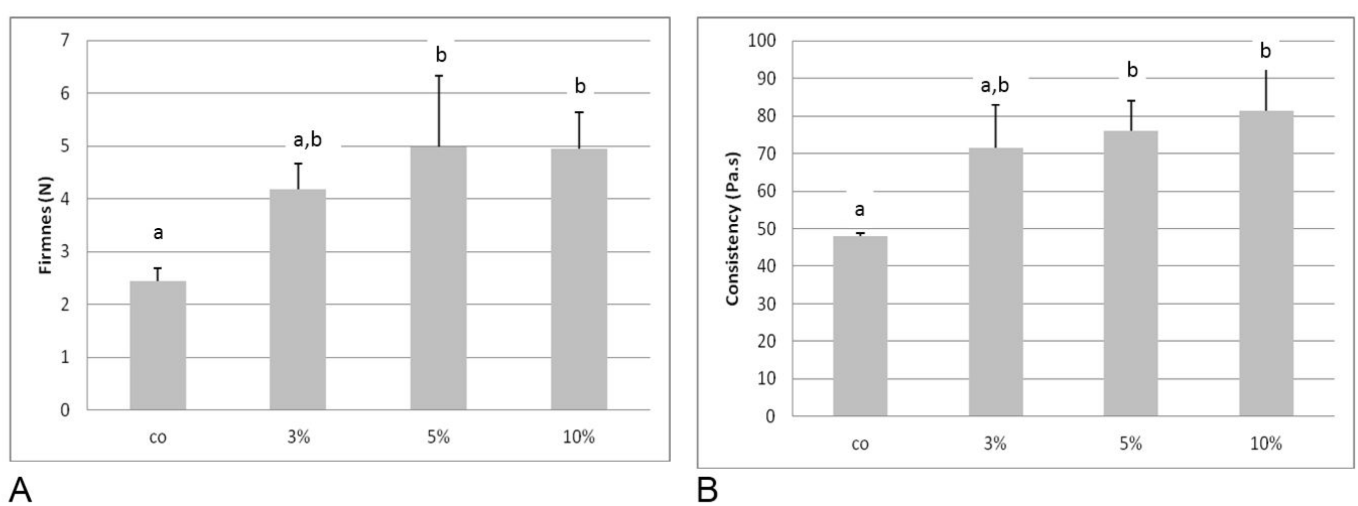

Figure 1. Firmness (A) and Consistency (B) of yogurt with different concentrations of GBP. Different letters mean statistical differences by Tukey's test ($p < 0.05$).

Table 4. Counting of probiotics (*B. bifidum and L. acidophilus*) in control yogurt (CO), and with different concentrations of GBP (3%, 5% and 10%; w/v) in the 1[st] and 7[th] days (Log CFU/mL).

Yogurts	1[st] day		7[th] day	
	B. bifidum	*L. acidophilus*	*B. bifidum*	*L. acidophilus*
3%	0	6.6* ± 0.04	6.1* ± 0.38	7.4* ± 0.04
5%	0	5.5* ± 0.47	5.3* ± 0.27	6.5 ± 0.46
10%	0	6.7* ± 0.11	5.3* ± 0.27	6.9* ± 0.32
CO	0	0	2.5 ± 0.04	5.7 ± 0.16

*$p < 0.05$ *vs* control (Student *t*-test).

The current Brazilian legislation (Brasil, 2008) sets a value within the range of 10^8 to 10^9 CFU of probiotics in products ready-to-eat, according to the recommendation of the manufacturer. Thus, the consumption of 200mL of yogurt enriched with GBP in any concentration would be enough to supply this probiotics recommendation. However, according to the Identity and Quality Standard (IQS) of fermented milks, yogurts containing *Bifidobacterium* must have a minimum counting of 10^6 CFU/mL, being apt only the formulated product with 3% of GBP after seven days of storage (Brasil, 2000a).

4 Conclusions

This work has shown that the application of the GBP in yogurts presents a potential prebiotic effect without affecting the sensorial characteristic and the most important macronutrients and ash. The effect observed on texture profile may be positive to produce new dairy products. However, new investigations must be performed to evaluate the prebiotic effect of the GBP in longer storage period.

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