

Bread with flour obtained from green banana with its peel as partial substitute for wheat flour: Physical, chemical and microbiological characteristics and acceptance

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Abstract

The effect of the addition of green banana (with its peel) flour (GBF) as a partial substitute (10% or 20%) for wheat flour on the physical, chemical and microbiological characteristics and on the acceptance of breads was evaluated. Breads with GBF had lower protein content (8.9-10.8 g/100g), but higher water (30.6-32.7 g/100g), ash (\approx 2.9 g/100g) and fiber (12.9-17 g/100g) contents than the traditional bread. The addition of GBF resulted in a decreased specific volume, darkening of the crumb and increase in water activity of the breads. Higher hardness and acidity values were only observed in the breads with 20% of substitution. Regarding the acceptance, breads with GBF had similar purchase intent and were as accepted (taste, texture and general) as the traditional bread, with only a slight loss of acceptance in the appearance. The microbiological characteristics of the products with GBF were similar to those of the traditional bread. It can be concluded that the use of up to 20% of GBF as a substitute for wheat flour in bread, although provided changes in physical and chemical characteristics, resulted in products with improved nutritional properties without loss on the acceptance or shelf life.

Keywords

Bread

Green banana

Fiber

Musa sapientum

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Introduction

Banana is one of the most cultivated fruit crops in the tropical and subtropical climate countries, being the fourth most important agricultural commodity in the world, after corn, rice and wheat (Subbaiah *et al.*, 2013; Sarawong *et al.*, 2014). Brazil is the 5th largest producer in the world, and banana is the second most produced fruit crop in the country, behind orange (Fao, 2012). However, of the total production, approximately 40% is lost only in the post-harvest period (Wang *et al.*, 2012; Subbaiah *et al.*, 2013).

One way to reduce the waste of this fruit is to encourage the use of banana in green stage, since its shelf life becomes longer and it is more easily transported (Daramola and Osanyinlusi, 2006; Bezerra *et al.*, 2013). Furthermore, the green banana presents higher nutritional value than that of ripe banana with 14.5 g/100g of fiber, 17.5 g/100g of resistant starch and is a source of antioxidant polyphenols (Fasolin *et al.*, 2007; Ovando-Martinez *et al.*, 2009). However, the green banana has a high degree of astringency, which precludes their consumption in a fresh form (Aurore *et al.*, 2009).

The green banana flour (GBF) has no taste and

odor and, therefore, the products keep their original taste and aroma after the addition of GBF (Zandonadi, 2009). However, due to the greater amount of fiber, the GBF can negatively influence the quality of the breads (Noort *et al.*, 2010).

The banana peel represents 47-50% of the weight of the fruit, but, to date, does not have industrial applications, being discarded or occasionally used as animal feed or as fertilizer (Ghorade *et al.*, 2011; Fatemeh *et al.*, 2012). The use of banana peels in food products can improve the nutritional quality of them, since they still have high contents of vitamins, minerals and fiber, and can also reduce the accumulation of waste (Garzón *et al.*, 2011).

Many studies have been conducted with mixed flours applied to the bakery industry, using chickpeas, flax, oats, cassava, malt and pumpkin as raw material. Few studies have evaluated the effect of the addition of green banana flour to bread (Pacheco-Delahaye and Testa, 2005; Juarez-Garcia *et al.*, 2006; Zuleta *et al.*, 2012), but only one (Garzón *et al.*, 2011) used green banana with its peel as raw material, but did not study the real effect of the addition on the quality of the breads.

Therefore, the aim of this study was to evaluate

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Table 1. Ingredients used for obtaining one bread

| Ingredients (g) | Formulation | | | | | |
|--------------------|-------------|---------|---------|---------|---------|---------|
| | Traditional | 10% GBF | 20% GBF | 30% GBF | 40% GBF | 50% GBF |
| Wheat flour | 250 | 225 | 200 | 175 | 150 | 125 |
| Green Banana Flour | 0 | 25 | 50 | 75 | 100 | 125 |
| Yeast | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| Salt | 5 | 5 | 5 | 5 | 5 | 5 |
| Margarine | 10 | 10 | 10 | 10 | 10 | 10 |
| Flour improver | 1 | 1 | 1 | 1 | 1 | 1 |
| Sugar | 20 | 20 | 20 | 20 | 20 | 20 |
| Egg | 50 | 50 | 50 | 50 | 50 | 50 |
| Water | * | * | * | * | * | * |

* Variable amount in accordance with the mass characteristics (133 to 143 mL)

the effect of the addition of green banana (with its peel) flour as partial substitute for wheat flour on the physical, chemical and microbiological characteristics and on the acceptance of breads.

Material and Methods

Material

The bananas used in the study were from 'Prata' (*Musa sapientum*) cultivar and they were in totally green maturity stage (Grade II of ripening classification) (Aurore et al., 2009). They were obtained from the Maringá Distribution Center (CEASA) of the city of Maringá, Paraná, Brazil. The bananas were 10 to 12 cm long and had no defects and / or injury. Wheat flour (Bunge®), sugar (Alto Alegre®), salt (Moc®), flour improver (Itaquara®), egg, margarine (Coamo®), water and fresh yeast (Reforpan®) were also used in the experiment.

Green banana flour (GBF) preparation

The bananas were washed in running water, sanitized (6 mL/L Pury Vitta® fruit and vegetable disinfectant with 0.96 g/100g active chlorine) and cut into 5 mm slices using stainless steel knives (Tribess et al., 2009). Then, the slices were immersed in a 1 g/100 mL citric acid solution for 5 minutes (Pan et al., 2008), placed in trays and kiln dried at 50°C until they have constant weight (approximately 72 hours) (Ovando-Martinez et al., 2009).

The bananas obtained after drying were ground in a rotor mill with 3 mm mesh screen and at room temperature. Then, the flour was placed in glass jars of 500 g capacity and subjected to analysis and / or

used in the preparation of the breads.

Preparation of the breads

The breads were processed in a commercial bakery, located in Ivaíporã, Paraná, Brazil, in the production conditions usually used in it. The formulations of the breads with GBF were developed by a modification of the traditional formulation, using different levels of green banana flour addition (0 – 50%) in substitution for part of the wheat flour (Table 1). The traditional formulation was based on the recipe used on the bakery and on data obtained in the literature (Gutkoski and Jacobsen Neto, 2002; Piekarski, 2009). Preliminary tests were conducted in order to define the maximum quantity of green banana flour that could be used in the production of the breads. More than 50% of substitution of the wheat flour by green banana flour resulted in products with poor technological and sensory properties, limiting the GBF utilization by 50%.

To obtain the breads, the ingredients (wheat flour, green banana flour, fresh yeast, salt, margarine, flour improver, sugar and egg) were mixed in an industrial mixing machine (Lieme®) with capacity of 5 kg and stem type hook. The mixture was held at speed 3 for 10 minutes. Water, at a temperature of 8 to 10°C, was used to obtain dough with a final temperature of 26 to 28°C.

After mixing, the dough was rolled into a cylinder (G. Paniz®) to enhance the development of the gluten, manually divided, hand-rounded and allowed to rest for 15 minutes. Then, the breads were mechanically molded, placed in a baking pan and proofed for 120 min at 30°C. Then, the breads

were baked in an oven (Progás®) at 180°C for 30 minutes. Once baked, breads were allowed to cool down for 30 min, removed from the pans, packed in polyethylene bags and kept at room temperature for further assessment.

Only the breads with specific volume greater than 4 L/kg were selected to continue the study because lower values than that negatively affect the quality of the breads (Sluimer, 2005). Therefore, only the breads with 0 (Traditional), 10 and 20% of GBF were evaluated.

Evaluation of chemical composition and caloric value of the wheat flour, the green banana flour and the breads

The chemical composition was determined using the methodologies proposed by the AOAC (2004): moisture in a vacuum oven at 70°C for 24 h, ash by burning in a muffle furnace at 550°C, lipids by the solvent extraction method (Sohxlet method), protein by the Kjeldahl method, fibers by the enzymatic method and carbohydrates by difference. The caloric value was made based on the chemical composition, using the Atwater conversion factors: 4 kcal/g for protein, 4 kcal/g for carbohydrates, and 9 kcal/g for lipids (Merril and Watt, 1973). The chemical composition of the flours and the breads was determined at the 1st day of storage of the products.

Physical and chemical characteristics of the flours and breads

The pH was determined using a digital potentiometer (Hanna-Instruments HI 3221®) previously calibrated with phosphate buffers (Synth®) at pH 4.0 and 7.0. The titratable acidity was measured according to AOAC (2004), and expressed as mL of NaOH for the flours and % of acetic acid for the breads. Ten grams of the samples were diluted with sufficient water to total 100 mL of solution. The solutions were then titrated with 0.1N NaOH solution until the pH 8.3 was reached.

A colorimeter (Minolta®, model CR400) was used for the assessment of color parameters values. The colorimeter provided directly the parameters L^* (luminosity), a^* (red-green component) and b^* (yellow-blue component).

The water activity was determined using Aqua Lab CX-2 (Decagon Devices®), as recommended by the manufacturer. The hardness of the breads was analyzed by the compression test of the crumb. A texturometer (Stable Micro Systems®) fitted with a 10 kg load cell and a cylindrical probe with 36 mm diameter was used. The parameters of the test were: pre-test speed: 1 mm/s, test speed, 1.7 mm/s, post-

test speed: 10 mm/s, compression depth: 40%, and slices thickness: 25 mm (Hadradev *et al.*, 2014).

The specific volume (L/Kg) of the breads was calculated by the ratio between the apparent volume of the baked bread and their mass. The bread mass was determined by the average value of a direct measurement of three breads, using a semi-analytical balance. The apparent volume was determined by the flax seed displacement method, where it determines the volume of flax seed that is necessary to cover the sample to a predetermined standard point in a beaker of known volume (Skrbic and Filipcev, 2008). The physical and chemical evaluations were conducted in the 1st day of storage of the products.

Microbiological and sensory evaluations of the breads

Total coliforms, thermotolerant coliforms, Salmonella, and yeasts and molds were determined as described by AOAC (2004). The analyses were done on days 1, 3, 6 and 7 of the storage at room temperature of the products.

For the sensory evaluation 70 judges were recruited, among students, professors and staff of the Federal Institute of Paraná - Campus Ivaiporã, who were bread consumers. The judges answered a questionnaire in order to characterize the population of the study.

Sensory analyzes were performed on cabins on the first day of storage of the products. The breads, on napkins with 3-digit random numbers, were evaluated in a randomized order and in monadic form under white light. The acceptance (appearance, flavor, texture and overall impression) of the formulations of bread was evaluated by the consumers of the products through a hedonic 9-point scale (9 - like extremely, 1 - dislike extremely). The purchase intent was assessed using a 5-point scale (5 - definitely would buy; 1 - definitely would not buy) (Stone and Sidel, 2004).

Statistical analysis

The complete experiment was repeated twice in a completely randomized design. The data from chemical composition analysis and physical and chemical characteristics were subjected to analysis of variance (ANOVA) and Tukey's comparison of the means test ($p \leq 0.05$). For the acceptability and purchase intent the design was a randomized complete block design where treatments were the formulations and blocks the judges. The data were submitted to a two-way ANOVA (formulations and judges), an F test and a Tukey test for comparison of means ($p \leq 0.05$). Statistical analyses were conducted

in SAS (version 9.1.3, SAS Institute Inc., Cary, NC).

Results and Discussion

Chemical composition and physical and chemical characteristics of wheat flour and green banana flour (GBF)

The results of the chemical composition and physical and chemical characteristics of wheat flour and green banana flour are shown in Table 2. Regarding the chemical composition, the green banana flour had higher levels ($p \leq 0.05$) of ash (5.5 g/100g), carbohydrates (81.4 g/100g) and total dietary fiber (14.73 g/100g); lower moisture (7.0 g/100g), protein content (5.0 g/100g) and calories (296.46 kcal/100g); and similar lipid contents (1.1 g/100g) when compared to the wheat flour.

Furthermore the GBF presented acid pH (5.4), titratable acidity of 1.03 mL of NaOH and water activity of 0.34, interesting features of the technological point of view when compared to wheat flour. Water activities lower than 0.6 do not allow the development of micro-organisms, including xerophilic molds and osmophilic yeasts (Troller, 1980). The color data for the GBF indicated that it had a brownish color ($L^* = 52.50$, $a^* = 9.89$ and $b^* = 17.18$), while the wheat flour was yellowish white ($L^* = 92.5$; $a^* = 0.30$ and $b^* = 7.59$).

Chemical composition of the breads

The results of the chemical composition of the formulated breads are presented in Table 3. By gradually replacing part of the wheat flour for GBF, a decrease ($p \leq 0.05$) in the protein content was found, what was expected, since this component is present in lower quantities in GBF (5.0 g/100g, Table 2) than in wheat flour (10.0 g/100g, Table 2). From a technological point of view this may be undesirable, since there are reduced amount of gluten and weakening and thinning of the present proteins, with consequent impairment of important properties for the production of breads (Gallagher *et al.*, 2003). An increase ($p \leq 0.05$) occurred on moisture, ash and fiber contents with the addition of GBF. The lipid content remained constant, while the carbohydrates decreased in the breads with 20% of GBF.

Increased moisture content with the addition of the GBF is related to the increased capacity of the fibers to absorb moisture from the media, and to the fact that more water had to be added to make the "mass point" during the preparation of the bread. The higher moisture content favors the development of the enzyme activity and the fermentation of the mass, contributes to the mass temperature control,

and results in breads with softer texture. However, the microorganisms could be favored, affecting the stability and shelf life of the products (Juarez-Garcia *et al.*, 2006).

The fiber content increased 26% on the bread with 10% of GBF and 67% on the bread with 20% GBF when compared to the traditional bread. Furthermore, a reduction in caloric value of 8.59 and 15.4%, respectively, was observed. The presence of dietary fiber in food is of great interest in health, as numerous studies have linking their role in the reduction of the risk of some diseases, such as diverticulitis, colon cancer, obesity, heart problems and diabetes (Kaczmarczyk *et al.*, 2012; Mudgil and Barak, 2013).

The increase in ash content is related to the higher concentration of minerals in the GBF, especially potassium, calcium, phosphorus and magnesium (Fasolin *et al.*, 2007; Choo and Aziz, 2010). The ash content is considered an important measure of quality of wheat flours. The ash content alone is not related to the final quality of the bread, but gives an indication of the degree of extraction of these components from the grains (Choo and Aziz, 2010).

Regarding the carbohydrate content, a reduction in bread with 20% GBF was observed. However, it is important to note that in the green banana flour the great part of the starch in carbohydrate fraction is in the form of resistant starch, which increases the interest in this flour as a functional ingredient (Zuleta *et al.*, 2012).

Maintaining the lipid content in breads made with GBF when compared to traditional bread is important because the fat in baked goods acts as an important lubricant of the mass, caloric enricher, enhancer of the flavor and color, and favors the gas retention, contributing to higher volume and softness in breads (Pareyt *et al.*, 2011). This maintenance was possible due to the use of raw materials in a complete form (pulp and peel). In fact, according to Juarez-Garcia *et al.* (2006), the use of banana peels in food products results in increased lipid content. The lipid components present in the peels are mainly carotenoids. The results of chemical composition indicate that breads with GBF have superior nutritional value (ash and fiber) than the traditional bread, but with a decrease in the protein content.

Physical and chemical evaluation of the formulated breads

The results of the physical and chemical characteristics of the breads are shown in Table 4. Breads containing GBF had lower specific volume and more brownish crumb color ($p \leq 0.05$) than the

Table 2. Chemical composition (wet basis, g/100 g), caloric value (Kcal/100 g) and physical and chemical characteristics of green banana flour and wheat flour

| Parameter | Green Banana Flour | Wheat Flour |
|----------------------------------|----------------------------|----------------------------|
| Moisture | 7.00 ± 0.17 ^b | 12.00 ± 0.43 ^a |
| Protein | 5.00 ± 0.34 ^b | 10.0 ± 0.22 ^a |
| Lipids | 1.10 ± 0.12 ^a | 1.00 ± 0.09 ^a |
| Ash | 5.50 ± 0.47 ^a | 0.71 ± 0.05 ^b |
| Carbohydrate* | 81.40 ± 0.66 ^a | 76.29 ± 0.70 ^b |
| Fiber | 14.73 ± 0.34 ^a | 2.00 ± 0.15 ^b |
| Caloric value | 296.46 ± 0.66 ^b | 330.00 ± 0.70 ^a |
| pH | 5.40 ± 0.01 ^b | 6.4 ± 0.03 ^a |
| Titrateable acidity (mL of NaOH) | 1.03 ± 0.01 ^b | 1.30 ± 0.05 ^a |
| L* | 52.50 ± 1.25 ^b | 92.5 ± 3.05 ^a |
| a* | 9.89 ± 0.21 ^a | 0.30 ± 0.01 ^b |
| b* | 17.18 ± 0.27 ^a | 7.59 ± 0.15 ^b |
| Water activity | 0.34 ± 0.01 ^b | 0.60 ± 0.04 ^a |

Same letters in the same line do not differ at $p = 0.05$

*including fiber

traditional bread. The specific volume is a parameter often used to evaluate the quality of breads, and specific volumes below a certain value (4 L/kg) adversely affect the quality of the bread (Sluimer, 2005). The specific volumes of the breads in the present study (4.14- 6.99 L/kg) were higher than the recommended values.

The specific volume of breads is directly related to the ability of the mass to retain the gas produced during the fermentation period, which in turn is inherent to the gluten present in wheat flour (Noort *et al.*, 2010). The fibers presented in the GBF compromise the gluten structure, resulting in changes in the viscoelastic properties of the dough. Thus, the dough cannot form a network capable of expanding, give a form to the bread and efficiently retain the gases formed during the fermentation process, resulting in breads with lower volume (Polaki *et al.*, 2010).

The darker coloration of the crumb of the breads with GBF is related to the use of the peels, and a brown color formation at the edge during the drying period in the kiln. In general, the baker's flour should be white, however, products considered wholegrains, have naturally darker color (reddish brown). Consumers already associate the brown color to healthier products (Kantor *et al.*, 2001).

The addition of green banana flour in

concentrations of 10% did not change ($p > 0.05$) the acidity (pH and titrateable acidity) and the hardness of the breads. At higher concentrations (20%), these parameters were increased ($p \leq 0.05$), indicating that the breads became harder and more acid. As the studied formulations contained the same ingredients and in the same proportions, the higher hardness of the samples with GBF (20%) could be related to the low mass development and consequently to their more closed structure and lower specific volume (Noort *et al.*, 2010). The higher acidity could assist the activity of the yeasts during fermentation as these microorganisms prefer acidic conditions for their development, with optimal pH around 4.5 (Battcock and Azam-Ali, 1998).

By the results of the physical and chemical characteristics it can be observed that partial substitution of wheat flour by GBF, especially in quantities of 20%, reduces the potential of bread making quality, resulting in lower volume, increased hardness and darkening of the crumb. However, more favorable acidity values were obtained.

Sensory evaluation of the breads

The acceptance of the breads was determined using the hedonic scale test, developed with the participation of 70 potential consumers. The panel

Table 3. Chemical composition (g/100 g) (dry basis) and caloric value (Kcal/100 g) of the breads

| Parameter | Traditional Bread | 10% GBF Bread | 20% GBF Bread |
|-----------------|---------------------------|---------------------------|---------------------------|
| Moisture | 26.45 ± 2.41 ^b | 30.64 ± 0.77 ^a | 32.71 ± 0.95 ^a |
| Protein | 13.74 ± 1.55 ^a | 10.83 ± 0.85 ^b | 8.91 ± 0.37 ^c |
| Lipids | 4.30 ± 0.05 ^a | 4.27 ± 0.03 ^a | 4.27 ± 0.06 ^a |
| Ash | 2.78 ± 0.09 ^b | 2.90 ± 0.06 ^a | 2.92 ± 0.07 ^a |
| Carbohydrate* | 68.95 ± 1.58 ^a | 69.15 ± 0.81 ^a | 66.87 ± 0.27 ^b |
| Fiber | 10.20 ± 0.70 ^c | 12.86 ± 0.83 ^b | 17.03 ± 0.92 ^a |
| Caloric Value** | 271.86 ^a | 248.52 ^b | 229.89 ^c |

Same letters in the same line do not differ at p = 0.05

*Including fiber content

**Calculated from the values on a wet basis

Table 4. Physical and chemical characteristics, acceptance and purchase intent of the breads

| Parameter | Traditional Bread | 10% GBF Bread | 20% GBF Bread |
|-------------------------------------|---------------------------|---------------------------|---------------------------|
| pH | 5.90 ± 0.04 ^a | 5.83 ± 0.02 ^a | 5.69 ± 0.05 ^b |
| Titrateable acidity (% acetic acid) | 0.26 ± 0.02 ^b | 0.27 ± 0.01 ^b | 0.31 ± 0.00 ^a |
| Water activity | 0.89 ± 0.01 ^b | 0.93 ± 0.01 ^a | 0.93 ± 0.01 ^a |
| Hardness (N) | 3.96 ± 0.38 ^b | 4.10 ± 0.36 ^b | 7.95 ± 0.79 ^a |
| Specific volume (L/kg) | 6.99 ± 0.32 ^a | 5.46 ± 0.31 ^b | 4.14 ± 0.12 ^c |
| L* (crumb) | 80.19 ± 3.19 ^a | 59.80 ± 1.30 ^b | 47.35 ± 1.75 ^c |
| a* (crumb) | 3.11 ± 0.10 ^c | 6.47 ± 0.55 ^b | 9.19 ± 0.51 ^a |
| b* (crumb) | 8.94 ± 0.98 ^c | 13.24 ± 1.27 ^b | 15.04 ± 0.43 ^a |
| L* (crust) | 49.71 ± 1.91 ^a | 42.47 ± 0.89 ^b | 35.04 ± 1.12 ^c |
| a* (crust) | 20.08 ± 0.19 ^a | 18.14 ± 0.45 ^b | 15.88 ± 0.58 ^c |
| b* (crust) | 32.10 ± 1.35 ^a | 25.87 ± 0.62 ^b | 17.71 ± 1.47 ^c |
| Appearance | 8.24 ± 1.25 ^a | 7.89 ± 1.39 ^{ab} | 7.69 ± 1.27 ^b |
| Flavor | 7.93 ± 1.09 ^a | 7.96 ± 1.24 ^a | 7.73 ± 1.41 ^a |
| Texture | 7.91 ± 1.15 ^a | 8.00 ± 1.53 ^a | 7.79 ± 1.34 ^a |
| Overall Impression | 8.17 ± 1.02 ^a | 8.10 ± 1.02 ^a | 7.97 ± 0.98 ^a |
| Purchase intent | 4.47 ± 0.83 ^a | 4.33 ± 0.90 ^a | 4.29 ± 0.86 ^a |

Same letters in the same line do not differ at p = 0.05

Hedonic Values (appearance, flavor, texture and overall impression): 1- dislike extremely; 9- like extremely; Purchase Intent: 1- definitely would not buy; 5- definitely would buy.

consisted of 53% of men and 47% women, with ages ranging from 15 to over 50 years, prevailing the age between 15 and 25 years (73%).

Table 4 also shows the results of acceptance (appearance, taste, texture and overall impression) and purchase intent of the breads. The acceptance in terms of the appearance, flavor, texture and overall impression of the breads was between 7 and 8 on a

hedonic 9-point scale, indicating that the consumers liked from “moderately” to “very” the products. Regarding the purchase intent, the results were found near 4 in a 5-point scale for all formulations tested, indicating that consumers would most likely buy the breads.

The acceptance for taste, texture and overall impression and the purchase intent were similar (p

Table 5. Microbiological evaluation of the breads

| Microbiological characteristic | Storage time | | | |
|----------------------------------|--------------|-------------------|-------------------|-------------------|
| | (days) | Formulation | | |
| | | Traditional Bread | 10% GBF Bread | 20% GBF Bread |
| Total coliforms (MNP/g) | 1 | 9.2 | < 3 | < 3 |
| | 3 | < 3 | < 3 | < 3 |
| | 6 | < 3 | < 3 | < 3 |
| | 7 | < 3 | < 3 | < 3 |
| Thermotolerant coliforms (MNP/g) | 1 | < 3 | < 3 | < 3 |
| | 3 | < 3 | < 3 | < 3 |
| | 6 | < 3 | < 3 | < 3 |
| | 7 | < 3 | < 3 | < 3 |
| Yeasts and molds (CFU/g) | 1 | 5.8×10^2 | 1.4×10^3 | 1.0×10^3 |
| | 3 | 1.3×10^3 | 1.2×10^3 | 1.5×10^3 |
| | 6 | 3.0×10^3 | 2.0×10^3 | 1.0×10^3 |
| | 7 | 5.8×10^3 | 5.3×10^3 | 5.0×10^3 |
| <i>Salmonella</i> spp. | 1 | Absent | Absent | Absent |
| | 3 | Absent | Absent | Absent |
| | 6 | Absent | Absent | Absent |
| | 7 | Absent | Absent | Absent |

> 0.05) for the breads added GBF when compared to the traditional bread. Regarding the appearance, the bread with 20% GBF was less accepted ($p \leq 0.05$) than the traditional bread, with no difference ($p > 0.05$) among the other breads. Possibly the most brownish color of the crumb of the GBF (Table 4) decreased the acceptance of the products by consumers, who prefer bread with a lighter color. It is noteworthy; however, that even if there were differences in the acceptance for the appearance, it may be considered appropriate, as consumers indicated they liked very much the appearance of the assessed breads (values close to 8).

Therefore, although there have been physical and chemical changes with the addition of the GBF to breads (lower specific volume, crumb darker coloration, acidification of the product, etc.), these characteristics did not affect the consumer's desire to consume or buy the products prepared. Thus, it is possible to replace 20% of wheat flour for GBF in bread with no loss in the acceptance of the products (flavor, texture and overall impression).

Microbiological evaluation

Table 5 shows the results of the microbiological evaluation of the breads. Breads with GBF, as well as the traditional bread, showed number of coliforms lower than 3 MNP/g and absence of *Salmonella* spp.

during 7 days of storage, which is in accordance with the Brazilian legislation (Brasil, 2001).

A gradual increase in mold and yeast counts over the storage period was verified. On the sixth day, the mold and yeast counts were lower than 2×10^3 CFU/g for formulations with GBF, without being observed the presence of molds on the surface of the bread. In the seventh day, the counts exceeded 5×10^3 CFU/g, and there was presence of molds on the surface. The same observation was done in the traditional bread. Consumption of breads containing molds in the surface is not recommended, since some of these microorganisms can produce mycotoxins, which can cause serious damage to the human body (Inmetro, 2006). Therefore, the recommended shelf life for the breads with GBF, based on the microbiological results, was 6 days.

There was no difference in the shelf life of breads made with GBF and the traditional bread, indicating that although the moisture and water activity of the bread with GBF have been higher, they did not affect the microbiological stability of the products. This may be related to the higher acidity of the products with GBF because in more acidic conditions there is a less development of spoilage microorganisms. This result is interesting from the marketing point of view, since lower shelf lives hinder the marketing of highly perishable products, such as bread.

Conclusion

It can be concluded that the use of up to 20% of green banana flour in substitution for wheat flour in bread results in improved nutritional characteristics (higher ash and fibers contents) without losing acceptance in flavor, texture and overall impression. However, there is a decrease in the color acceptance with GBF addition due to the increment of dark-brown color of the breadcrumbs. Color and specific volume changes are characteristic of wholegrain products. The addition of GBF had no influence on the microbiological stability of the breads; therefore, the recommended shelf life is of 6 days.

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