Bread formulated with guava powder was enriched in phenolic and aroma compounds, and was highly acceptable by consumers

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Abstract Guava powder (GP) was used as source of aroma and phenolic compounds to fortify wheat bread 10% (GB10) and 20% (GB20), substituting for wheat flour. Phenolic compounds, antioxidant capacity, volatile compounds profile, and sensory acceptability of control bread (CB; without GP) and guava breads (GB) were evaluated. Incorporation of GP increased roughly 2-to-3-fold the phenolic compounds contents of bread. Ten phenolic compounds were identified in GB20, and quercetin-3-*O*-rutinoside was the major compound, while in CB, ferulic acid was the major among the six phenolic compounds in CB. Bread making seemed to promote the release of phenolic compounds from structural components. Breads incorporated with GP presented a richer volatile profile

than CB, especially due to the presence of terpenes. GB improved aroma profile of bread. GP added aroma compounds and phenolic antioxidants, and seemed to be an interesting approach to enhance bread bioactivity and acceptability.

Keywords *Psidium guajava* · Bread making · Antioxidant capacity · Sensory evaluation · Volatile compounds · Functional food

Introduction

Guava (*Psidium guajava* L.) is a succulent fruit native of the American continent and is widely cultivated in tropical and subtropical areas in the world. Guava presents high contents of lycopene, ascorbic acid, dietary fiber and phenolic compounds, which contribute to its high nutritional value and functional potential (Corrêa et al. 2014; Flores et al. 2015; Nunes et al. 2016). Additionally, guava presents high marketing potential due to its typical and pleasant aroma, and low market prices (Corrêa et al. 2014).

Recently, beneficial effects of guava bioactive compounds were extensively studied, and anti-inflammatory, anti-diabetic, antiviral and anticancer activities have been attributed to guava, which are partly associated to guava phenolic compounds (Gutiérrez et al. 2008; Owen et al. 2008). However, the high perishability of fruit limits commercialization and consumption. Guava is actually categorized as a subutilized crop, and there have been efforts to stimulate the development of new industrial products using this fruit (Williams and Haq 2000). In this sense, in a recent study, Nunes et al. (2016) obtained a guava powder by oven drying that presented high potential as an antioxidant-rich flavor enhancer food ingredient.

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Adding fruit powders to bread recipes have been shown as effective means to increase the phenolic contents and antioxidant capacity (Altunkaya et al. 2013; Bhol et al. 2016; Platat et al. 2015; Rahaie et al. 2014). Bread is one of the most popular staple foods in the world, highly consumed in most of the western part of the globe, presenting an annual worldwide consumption over 10^{10} kg (Cho and Peterson 2010). However, most of the bread consumed is made of refined wheat flour, ending up with high contents of starch and low contents of vitamins, minerals, fibers and phenolic compounds. The increasing prevalence of chronic health disorders, preventable by healthy food choices, has been increasing the demand for healthier foods and pressing the food industry to develop new healthier products, rich in bioactive compounds and functional appeal. In this context, adding guava powder to wheat bread might increase contents of bioactive compounds, while simultaneously improving aroma, but this remains to be tested.

Although guava powder addition to bread formulation was recently tested (Alves and Perrone 2015) the effects on bread volatile and phenolic compounds, and how it would reflect in consumer's acceptability were not reported elsewhere. Therefore, the aim of the present study was to evaluate the contents of soluble and insoluble phenolic compounds, antioxidant capacity, volatile compounds profile, and sensory acceptance of breads prepared by partially replacing wheat flour by guava powder, at two levels (10 and 20%).

Materials and methods

Materials, solvents, reagents and standards

Fluorescein, 2,4,6-Tris(2-pyridyl)-S-triazine (TPTZ), ($\pm$)-6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid (Trolox), 2,2'-azobis(2-methylpropionamide) dihydrochloride (AAPH), ferric chloride and all phenolic standards were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Iron (II) sulfate was purchased from Merck KGaA (Darmstadt, Germany). All solvents were HPLC grade from Tedia (Fairfield, OH, USA). HPLC grade water (Milli-Q system, Millipore, Bedford, MA, USA) was used throughout the experiments. A SPME fiber assembly composed of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μ m) was purchased from Supelco (St. Louis, MO, USA). All ingredients needed for bread making were acquired in local markets in Rio de Janeiro, Brazil.

Guava powder and bread samples

Guava powder was obtained as described by Nunes et al. (2016) and was vacuum-sealed and stored at -20°C until use. Control bread (CB) was prepared according to Alves and Perrone (2015) with modifications, in duplicate, in an automatic bread maker under conditions pre-established by manufacturer (BK2000B, Breadman[®], Middleton, WI). Ingredients were added in the following order: 171 mL of water, 12 g of sugar, 6 g of salt (NaCl), 12 g of unsalted butter, 300 g of white wheat flour, 3.7 g of carboxymethylcellulose, 3 g of bread improver and 3 g of dry yeast (*Saccharomyces cerevisiae*). Guava breads were prepared following the CB preparation steps, but partially substituting wheat flour by 10% (GB10) and 20% (GB20) of guava powder. Breads were cooled to ambient temperature, cut in small slices, ground in a laboratory mill (MF 10 Basic; IKA[®] Werke, Staufen, Germany), vacuum-sealed and stored at -20°C until analysis.

Soluble and insoluble phenolic compounds by HPLC–PDA

Extraction of soluble and insoluble phenolic compounds in control and guava breads was performed in duplicate, as previously described (Dinelli et al. 2011), with adaptations. For soluble phenolic compounds, 1 g of grounded samples was extracted for 10 min with 20 mL of chilled ethanol:water (80:20, v/v) and centrifuged ($2500\times g$, 5 min, 10°C , Sorvall ST 16R, Thermo Scientific[™], Osterode, Germany). The supernatant was collected and the residue re-extracted. Supernatants were combined, the solvent was evaporated in a rotary evaporator (R-215, Büchi[®], Flawil, Switzerland), the dried residue was reconstituted in 10 mL of water and stored at -20°C until analysis.

For analysis of insoluble phenolic compounds, the residue from soluble phenolic compounds extraction was subjected to alkaline and acid hydrolyses, as follows. For alkaline hydrolysis, the residue was incubated for 16 h, in the dark, with 12 mL of water and 5 mL of NaOH (10 M) at room temperature with orbital agitation (IKA KS 4000i control, Staufen, Germany). Subsequently, the pH was adjusted to 2.0 with concentrated HCl and the mixture was extracted with 15 mL ethyl acetate, centrifuged ($2500\times g$, 5 min, 10°C), and after aspirating the supernatant, the residue was re-extracted twice with ethyl acetate. Supernatants were combined, the solvent was evaporated in a rotary evaporator, the dried residue was reconstituted in 10 mL of methanol:water (80:20, v/v), and stored at -20°C until analysis.

For acid hydrolysis, the residue from alkaline hydrolysis was incubated with 2.5 mL of concentrated HCl at 85°C

for 30 min. Then, phenolic compounds were extracted as described previously, following the alkaline hydrolysis. All extracts were filtered through a 0.45 µm cellulose ester membrane filters (Millipore®, Brazil) prior to HPLC analysis. Results from alkaline and acid extracts were combined to represent insoluble phenolic compounds, and results of soluble and insoluble phenolics represent total phenolic compounds.

Phenolic compounds were analyzed by HPLC–PDA according to Nunes et al. (2016) using LC system (Shimadzu, Kyoto, Japan) that comprised a LC-20AT quaternary pump, a SIL-20AHT automatic injector, a SPD-M20A photo-diode array detector (PDA), a CBM-20A system controller and a DGU-20A5 degaser. Chromatographic separations were achieved using a reverse phase column C18 (5 µm, 250 mm × 4.6 mm, Kromasil®) and C18 guard column (5 µm, 10 mm × 3 mm, Kromasil®). The eluate was monitored for phenolic compounds by PDA from 190 to 370 nm and compounds were identified by comparison of their retention times and UV spectra with those of commercial standards. Quantification was performed by external standardization. Calibration curves ranging from 1.0 to 10.0 µg/mL were linear for all analytes ($R^2 > 0.98$, $p < 0.0009$, Limit of detection < 0.17 µg/mL, Limit of quantification < 0.53 µg/mL). Data were acquired by LC solution software (Shimadzu Corporation®, version 1.25, 2009). Results were expressed as mg of compound per 100 g on a dry weight basis (dwb).

The percentage recovery of phenolic compounds from guava powder and wheat flour incorporated to breads was calculated according to Rupasinghe et al. (2008), as follow:

recovery

$$= \frac{\text{mg of compound detected for g of bread (dry weight basis)}}{\text{mg of compound estimated for g of bread (dry weight basis)}} \times 100.$$

Antioxidant capacity

The antioxidant capacity (AC) was determined by FRAP (Ferric Reducing Antioxidant Powder) and ORAC (Oxygen Radical Antioxidant Capacity) assays on the extracts of soluble and insoluble phenolic compounds. The sum of AC in each extract corresponds to total antioxidant capacity (TAC) by each of these two assays. The FRAP assay was performed according to Benzie and Strain (1996) with slight modifications, as previously described (Nunes et al. 2016). Results were expressed as mmol of Fe^{2+} equivalents per 100 g on dwb. The ORAC assay was performed according to Zulueta et al. (2009) with slight modifications, as previously described (Nunes et al. 2016). Results were

expressed as mmol of Trolox equivalents (TE) per 100 g on dwb. Each extract was analyzed in duplicate.

Volatile compounds by HS–SPME–GC–MS

Volatiles from control bread and guava breads were analyzed by headspace solid-phase microextraction gas chromatography-mass spectrometry (HS–SPME–GC–MS), as described by Nunes et al. (2016) with modifications. Bread samples (5 g) were ground in a laboratory mill (MF 10 Basic; IKA®) and placed in a 20 mL vial. All vials were sealed with a 20 mm diameter aluminum seal with a PTFE septum (Supelco®). Samples were heated until equilibrium at 60 °C for 10 min.

For headspace extraction, the SPME fiber was exposed to the headspace of the sample for 25 min at 60 °C, and then was retracted and transferred immediately to the injection port of a Shimadzu GC-17A gas-chromatograph (Kyoto, Japan) for desorption at the injection port for 3 min at 260 °C. The GC was interfaced to a QP5050A mass spectrometer (MS) system and equipped with a capillary column (5% phenyl-methylpolysiloxane, 30 m, 0.32 mm i.d., 0.25 µm film; Quadrex 007-5, Quadrex Corp., USA). The temperatures of the injector and the interface were both 260 °C. Helium was used as carrier gas. The column oven was operated at 40 °C for 2 min, temperature programmed at 6 °C/min to 190 °C, held for 5 min, and then temperature programmed at 12 °C/min to 240 °C and held for 5 min. The MS was operated at 70 eV as ionization energy, in scan mode from 40 to 500 amu, and at 1 scan/s. The fiber was conditioned in the injection port at 260 °C for 40 min before the first daily analysis and for 15 min between each chromatographic run. This procedure was enough to guarantee no peaks in blank runs.

Chromatographic peaks were tentatively identified based on both mass spectra and linear retention index (RI). Mass spectra of volatile compounds were compared with those of the National Institute of Standards and Technology (NIST) mass spectral database. RI of each compound was calculated using *n*-alkanes (C_7 – C_{30}) as external references and compared with literature data. Compounds were tentatively identified when mass spectra similarity index (SI) was higher than 90% and RI differed less than 5% from literature data. Semi-quantitative analysis of relative contents of volatile compounds was assessed by area normalization. All analyses were performed in triplicate.

Sensory analysis

Thirty-four consumers (untrained panelists) between 19 and 50 years old were randomly recruited to participate in the sensory analysis of the control and GP incorporated

breads. All individuals reported to consume bread at least two times per week.

Samples were cut into equally sized slices (1.0 cm thick) and served in white dishes coded with three-digit random numbers and each sample was presented with a different number. The randomized order of the samples was presented once at a time to each panelist. Plain water was used for mouth rinsing before and after each sample testing. The panelists judged the acceptance of breads through the analysis of the attributes: overall aspect, aroma, crust color, crumb color, taste, texture and residual taste, using a nine-point hedonic scale (1 = disliked extremely; 9 = liked extremely).

In the following test, samples were presented together to conduct the preference ranking (1 = less preferred; 3 = most preferred), and the intents of both consumption and purchase of breads were assessed. The consumption intent was conducted using a seven-point scale (1 = would never eat; 7 = would always eat). Purchase intent was evaluated using a five-point scale (1 = certainly would not buy; 5 = certainly would buy).

Statistical analysis

Data are expressed as mean $\pm$ standard deviation. Analysis of variance (one-way ANOVA) followed by Tukey's multiple comparison post-test and *t*-tests were performed for comparing samples' mean values. Pearson correlation analysis was performed to evaluate associations between phenolic contents and antioxidant capacity. Statistical analyses were performed using GraphPad Prism software for Windows (version 5.04, GraphPad Software, San Diego, CA). Results were considered significant when $p < 0.05$.

Results and discussion

Soluble and insoluble phenolic compounds

Six phenolic compounds were identified in CB (4-hydroxyphenylacetic, 4-hydroxycinnamic, ferulic, rosmarinic and gallic acids and quercetin-3-*O*-rutinoside) (Table 1). In GB10 showed nine phenolic compounds and of which six were present in CB in addition to myricetin, naringenin and quercetin. GB20 showed the presence of ten phenolic compounds and of these phenolics were identified in GB10 in addition to syringic acid (Table 1). Recently, Nunes et al. (2016) reported that GP contained myricetin, naringenin and quercetin, observed in bread incorporated with GP.

Phenolic contents in CB (6.2 mg/100 g) were similar to those previously reported in breads made with white wheat

flour methods (3.4 mg/100 g to 11.1 mg/100 g) (Lu et al. 2014; Menga et al. 2010). The replacement of wheat flour by GP promoted a dose-dependent increase in phenolics contents guava breads (Table 1). Similar, increase in phenolic compounds in baking products incorporated with fruits powders was reported (Arun et al. 2015; Bhol et al. 2016; Platat et al. 2015; Rupasinghe et al. 2008).

Non-flavonoids accounted for 87% of total phenolic compounds in CB, being ferulic acid the major phenolic compound (37%), followed by 4-hydroxyphenylacetic acid (27%) and 4-hydroxycinnamic acid (13%). Ferulic acid was also prevalent in wheat flour (Supplementary Table 1). Previous reports also showed ferulic acid as the major phenolic compound in breads, with contents ranging from 3.4 mg/100 g to 8.2 mg/100 g, which were higher than that observed in the present study (2.3 mg/100 g) (Altunkaya et al. 2013; Lu et al. 2014). In both guava breads flavonoids accounted, on average, for 73% of total phenolics, being quercetin-3-*O*-rutinoside the major (56% on average), followed by ferulic acid (15% on average), quercetin (8% on average) and naringenin (8% on average). Both wheat flour and GP contributed to phenolics, ferulic acid arising from the former (Supplementary Table 1) and the later was the source of naringenin, quercetin and quercetin-3-*O*-rutinoside (Nunes et al. 2016).

In nature, a variable portion of phenolic compounds is bound to other plant components, altering solubility of these antioxidants and rendering their soluble and insoluble forms. Insoluble forms are linked to plant cell structural components through ester bonds (released by alkaline hydrolysis) or glycoside bonds (released by acid hydrolysis) (Acosta-Estrada et al. 2014). In the present study the contents of insoluble phenolics were determined using both alkaline and acid hydrolyses.

Phenolic compounds in breads were mainly present as insoluble forms (Fig. 1a) highlighting the importance to perform both alkaline and acid hydrolyses for a more accurate estimation of phenolic compounds contents in breads. Insoluble phenolics were highly prevalent in CB (80%) when compared to both guava breads (64% on average). These figures were related with the contents of insoluble phenolics observed in bread bulk ingredients, because bound phenolics were 75 and 96% of total in guava powder (Nunes et al. 2016) and in wheat flour, respectively (Supplementary Table 1). Non-flavonoids were also mostly found as insoluble forms in breads (86% on average), from which 83% on average were preferably linked by ester bonds (hydrolyzed by alkali) to structural constituents (Fig. 1b). This distribution agreed with published reports showing that non-flavonoids, especially hydroxycinnamic and hydroxybenzoic acids, were bound to cell wall components through ester bonds (Acosta-Estrada et al. 2014).

Table 1 Contents (mg/100 g) of soluble and insoluble phenolic compounds in control bread (CB), and in breads made from wheat flour substituted with guava powder at 10% (GB10) and 20% (GB20)

Compound	Soluble phenolics			Insoluble phenolics			Total phenolics (soluble + insoluble)		
	CB	GB10	GB20	CB	GB10	GB20	CB	GB10	GB20
Flavonols									
Myricetin	ND	ND	0.1 ± 0.0	ND	0.2 ± 0.0 ^a	0.4 ± 0.0 ^b	ND	0.2 ± 0.0 ^a	0.5 ± 0.0 ^b
Quercetin	ND	1.0 ± 0.1 ^a	1.5 ± 0.1 ^b	ND	ND	ND	ND	1.0 ± 0.1 ^a	1.5 ± 0.1 ^b
Quercetin-3- <i>O</i> -rutinoside	0.6 ± 0.0 ^a	2.9 ± 0.1 ^b	3.1 ± 0.1 ^b	0.2 ± 0.0 ^a	4.4 ± 0.0 ^b	8.2 ± 0.2 ^c	0.8 ± 0.0 ^a	7.3 ± 0.1 ^b	11.3 ± 0.3 ^c
Flavonone									
Naringenin	ND	0.7 ± 0.0 ^a	1.4 ± 0.1 ^b	ND	0.2 ± 0.0 ^a	0.3 ± 0.0 ^b	ND	0.9 ± 0.0 ^a	1.7 ± 0.1 ^b
<i>Total flavonoids</i>	0.6 ± 0.0 ^a	4.6 ± 0.2 ^b	6.1 ± 0.0 ^c	0.2 ± 0.0 ^a	4.8 ± 0.0 ^b	8.9 ± 0.2 ^c	0.8 ± 0.1 ^a	9.4 ± 0.2 ^b	15.0 ± 0.2 ^c
Hydroxybenzoic acids									
Gallic acid	0.2 ± 0.0 ^a	0.2 ± 0.0 ^{a,b}	0.3 ± 0.0 ^b	ND	0.2 ± 0.0 ^a	0.8 ± 0.0 ^b	0.2 ± 0.0 ^a	0.4 ± 0.0 ^b	1.1 ± 0.0 ^c
Syringic acid	ND	ND	ND	ND	ND	0.1 ± 0.0	ND	ND	0.1 ± 0.0
Hydroxycinnamic acids									
4-hydroxycinnamic acid	ND	ND	ND	0.8 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b	0.8 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b
Ferulic acid	0.1 ± 0.0	ND	ND	2.2 ± 0.1 ^a	2.4 ± 0.1 ^a	2.4 ± 0.0 ^a	2.3 ± 0.1 ^a	2.4 ± 0.1 ^a	2.4 ± 0.0 ^a
Rosmarinic acid	ND	ND	ND	0.4 ± 0.0 ^a	0.3 ± 0.0 ^b	0.3 ± 0.0 ^b	0.4 ± 0.0 ^a	0.3 ± 0.0 ^b	0.3 ± 0.0 ^b
Hydroxyphenylacetic acids									
4-Hydroxyphenylacetic acid	0.4 ± 0.0 ^a	0.4 ± 0.2 ^a	0.4 ± 0.0 ^a	1.3 ± 0.0 ^a	0.4 ± 0.0 ^b	0.2 ± 0.0 ^c	1.7 ± 0.0 ^a	0.8 ± 0.0 ^b	0.6 ± 0.0 ^c
Total non-flavonoids	0.7 ± 0.0 ^a	0.6 ± 0.0 ^a	0.7 ± 0.0 ^a	4.7 ± 0.1 ^a	3.4 ± 0.2 ^b	3.9 ± 0.0 ^b	5.4 ± 0.1 ^a	4.0 ± 0.2 ^b	4.6 ± 0.0 ^b
Total phenolic compounds	1.3 ± 0.0 ^a	5.2 ± 0.2 ^b	6.8 ± 0.0 ^c	4.9 ± 0.1 ^a	8.2 ± 0.2 ^b	12.8 ± 0.2 ^c	6.2 ± 0.1 ^a	13.4 ± 0.4 ^b	19.6 ± 0.3 ^c

Results are expressed in dry weight basis as means ± standard deviations of two replicates. Different superscript letters in the same row within each fraction indicate significant difference between mean values (One-way ANOVA followed by Tukey multiple comparison post hoc test, $p < 0.05$)

ND Not detected

Fig. 1 Distribution of phenolic compounds (a), non-flavonoids (b) and flavonoids (c) in soluble extract (white square box), alkaline extract (grey square box) and acid extract (black square box) of control bread (CB), and in breads made with guava powder substituting for wheat flour at two levels, 10% (GB10) and 20% (GB20)

In contrast, flavonoids in CB were mainly present as soluble forms (Fig. 1c). From those insoluble forms, on average 72%, were conjugated through glycosidic bonds, and released by acid hydrolysis (Fig. 1c). Flavonoids were usually bound by their C ring to cell wall components through β -glycosidic bonds (Acosta-Estrada et al. 2014). Interestingly, in wheat flour this class was found only as insoluble forms (Supplementary Table 1) and this reversal in chemical forms' solubility in CB may be explained by the action of hydrolytic enzymes from the wheat and/or *S. cerevisiae* (Đorđević et al. 2010; Katina et al. 2007). In both guava breads, flavonoids were equally distributed in soluble and insoluble forms (54% insoluble, on average). Considering the distribution of insoluble flavonoids in wheat flour (100%; Supplementary Table 1) and GP (63%) (Nunes et al. 2016) one would expect that contents of insoluble flavonoids in guava breads would be higher than that of soluble flavonoids, but this was not the case. Accordingly, these results reinforced the hypothesis of phenolics release from structural components during bread making. This release may have positively impacted the functional potential of guava breads, as soluble phenolics were more rapidly absorbed and distributed in the body than insoluble phenolics (Acosta-Estrada et al. 2014).

Based on the phenolic contents in wheat flour and guava powder, the recovery of the original phenolic compounds in guava breads was, on average, 105% (Supplementary

Table 2). Non-flavonoids and flavonoids were found, respectively, were found to be 32% lower and 33% higher than expected, indicating that flavonoids were more stable than non-flavonoids during bread making and/or insoluble flavonoids were released from cell structures. The higher stability of flavonoids compared to phenolic acids during baking has been reported previously (Rupasinghe et al. 2008). Loss of phenolic compounds during thermal processing may have been caused by oxidation, isomerization, and interaction with wheat proteins (Świeca et al. 2014). Additionally, phenolic compounds might have become undetected due to the incorporation into bread crust melanoidins, in which enzymatic treatment was necessary for their quantification (Alves and Perrone 2015). In fact, this previous report showed that naringenin was the major phenolic compound bound to bread crust melanoidins, which may have resulted into low recovery, 36% on average, in guava breads (Supplementary Table 2).

Of all phenolic compounds identified in guava breads only quercetin-3-*O*-rutinoside and 4-hydroxyphenylacetic acid presented recoveries higher than 100%. Other authors have been reported that the dough fermentation by *S. cerevisiae* promoted an increase in the contents of phenolic compounds due to the structural breakdown of cell wall, leading to the liberation of phenolic compounds and/or metabolism of phenolics by yeast (Đorđević et al. 2010; Hur et al. 2014; Katina et al. 2007). Moreover, Alves and

Table 2 Volatile fraction composition of breads made from wheat flour substituted with GP at 10% (GB10) and 20% (GB20)

Compounds ¹	Relative contents (% peak area)		
	RI ²	GB10	GB20
Alcohols			
2-Methyl-1-propanol	–	0.88 ± 0.08 ^a	0.49 ± 0.09 ^b
3-Methyl-1-butanol	833	2.70 ± 0.18 ^a	2.03 ± 0.46 ^a
2-Methyl-1-butanol	837	1.40 ± 0.10 ^a	0.72 ± 0.00 ^b
1-Pentanol	866	0.02 ± 0.00 ^a	0.01 ± 0.00 ^a
2,3-Butanediol	877	1.46 ± 0.05 ^a	1.78 ± 0.21 ^a
2-Furanmethanol	954	0.40 ± 0.04 ^a	0.27 ± 0.01 ^b
1-Hexanol	968	7.70 ± 1.05 ^a	0.97 ± 0.27 ^b
Ethanol, 2-butoxy-	1007	0.07 ± 0.01 ^a	0.17 ± 0.06 ^b
1-Octen-3-ol	1080	0.29 ± 0.05 ^a	0.22 ± 0.01 ^a
2-Phenylethanol	1226	5.08 ± 0.76 ^a	4.32 ± 0.14 ^a
Aldehydes			
Hexanal	900	1.02 ± 0.12 ^a	0.69 ± 0.02 ^b
Furfural	935	3.55 ± 0.01 ^a	3.46 ± 0.69 ^a
Heptanal	1002	0.18 ± 0.10 ^a	0.15 ± 0.02 ^a
(Z)-2-Heptenal	1060	0.22 ± 0.08 ^a	0.25 ± 0.06 ^a
Benzaldehyde	1071	3.19 ± 0.12 ^a	2.62 ± 0.07 ^b
Octanal	1106	4.14 ± 0.24 ^a	0.50 ± 0.06 ^b
(E)-2-Octenal	1163	0.25 ± 0.01 ^a	0.18 ± 0.04 ^b
Nonanal	1207	0.33 ± 0.16 ^a	0.27 ± 0.06 ^a
(E)-2-Nonenal	1266	0.27 ± 0.01 ^a	0.14 ± 0.00 ^b
2,4-Decadienal	1426	0.43 ± 0.06 ^a	0.15 ± 0.02 ^b
Ketones			
3-Hydroxy-2-butanone	808	8.75 ± 0.58 ^a	7.21 ± 1.26 ^a
2-Heptanone	991	0.92 ± 0.15 ^a	0.41 ± 0.07 ^b
5-Hepten-2-one, 6-methyl-	1088	2.62 ± 0.22 ^a	6.08 ± 1.84 ^b
2-Nonanone	1194	0.99 ± 0.17 ^a	0.45 ± 0.11 ^b
Pyranone	1258	1.09 ± 0.12 ^a	0.82 ± 0.16 ^a
Acids			
2-Methyl-propanoic acid	847	1.64 ± 0.28 ^a	1.03 ± 0.13 ^b
Butyric acid	873	0.58 ± 0.02 ^a	0.23 ± 0.07 ^b
3-Methyl-butanoic acid	930	1.23 ± 0.25 ^a	0.74 ± 0.22 ^a
Butanoic acid, 2-methyl-	940	0.34 ± 0.04 ^a	0.26 ± 0.01 ^b
Hexanoic acid	1067	2.18 ± 0.13 ^a	0.86 ± 0.29 ^b
Hexanoic acid, ethyl ester	1097	0.56 ± 0.07 ^a	0.21 ± 0.05 ^b
Octanoic acid, ethyl ester	1296	2.57 ± 0.55 ^a	0.70 ± 0.04 ^b
Decanoic acid, ethyl ester	1494	0.37 ± 0.12 ^a	0.08 ± 0.01 ^b
Terpenes			
Limonene	1141	4.99 ± 0.20 ^a	3.51 ± 0.03 ^b
(Z)-Ocimene	1150	0.43 ± 0.04 ^a	0.35 ± 0.02 ^a
Copaene	1509	0.36 ± 0.16 ^a	0.83 ± 0.11 ^b
β-Caryophyllene	1558	10.8 ± 0.87 ^a	16.1 ± 1.66 ^b
β-Gurjunene	1578	1.57 ± 0.02 ^a	2.60 ± 0.25 ^b
α-Humulene	1595	19.7 ± 2.52 ^a	29.2 ± 2.06 ^b
Aromadendrene	1610	0.12 ± 0.01 ^a	0.17 ± 0.06 ^a
β-Selinene	1634	0.53 ± 0.04 ^a	1.03 ± 0.01 ^b

Table 2 continued

Compounds ¹	Relative contents (% peak area)		
	RI ²	GB10	GB20
α-Selinene	1642	0.68 ± 0.06 ^a	1.39 ± 0.06 ^b
Caryophyllene oxide	1736	0.25 ± 0.01 ^a	0.42 ± 0.04 ^b
Other compounds			
Styrene	999	0.57 ± 0.08 ^a	4.10 ± 1.31 ^b
Furan-2-pentyl	1095	1.52 ± 0.02 ^a	0.61 ± 0.10 ^b
Dodecane	1301	0.63 ± 0.08 ^a	0.48 ± 0.15 ^b
Hexadecane	1501	0.30 ± 0.01 ^a	0.52 ± 0.03 ^b

Results are expressed as means ± standard deviations of three replicates

¹ Volatile compounds were tentatively identified based on both mass spectra (SI > 90%) and retention index (<5% difference from published data)

² Experimental retention index (RI) determined on 5% phenyl-methylpolysiloxane stationary phase. Different superscript letters in the same row indicate significant differences between mean values (*t* test, *p* < 0.05)

Perrone (2015) showed that 4-hydroxyphenylacetic acid was not incorporated into bread crust melanoidins, and this partially explained the higher recovery of this phenolic compound in guava breads (Supplementary Table 2).

Antioxidant capacity

AC of CB, GB10 and GB20 was evaluated by FRAP and ORAC assays in both soluble and insoluble extracts, which sum corresponds to the (TAC) (Fig. 2). The replacement of wheat flour by GP promoted a proportional increased on the TAC evaluated by FRAP assay (Fig. 2a). ORAC values were also higher in both guava breads (on average 0.58 mmol TE/100 g) than in CB (0.43 mmol TE/100 g) (Fig. 2b), but GB20 and GB10 did not show significant difference. Bread made with white wheat flour usually present low AC and the enrichment with natural sources of antioxidants, such as fruits, has been used to add nutritional and functional value to breads (Dziki et al. 2014; Rahaie et al. 2014). Other studies also have reported that the incorporation of fruit powders in bread promoted AC evaluated by FRAP and DPPH assays (Altunkaya et al. 2013; Bhol et al. 2016; Platat et al. 2015).

Considering AC of both soluble and insoluble phenolic extracts, FRAP values were higher in the insolubles extracts and corresponded, on average, to 58% of TAC in breads (Fig. 2a), consistently with the higher contents of insoluble forms of phenolic compounds (63% on average) (Fig. 1a). These results were also consistent with the contents of total insoluble phenolics (Table 1). However, no difference was observed for FRAP values in the extract of soluble phenolics between GB20 and GB10 despite the

Fig. 2 Antioxidant capacity of control bread (white square box), and in breads made with guava powder substituting for wheat flour at two levels, 10% (grey square box) and 20% (black square box) in soluble and insoluble fractions evaluated by FRAP (a) and ORAC (b) assays. Results are means $\pm$ standard deviations of two replicates. Values with different superscript letters in the same assay indicate significant difference between mean values (Oneway ANOVA followed by Tukey's multiple comparison post hoc test, $p < 0.05$)

former showed higher contents of soluble phenolics (Table 1). These results may be explained by the phenolics profile in soluble and insoluble extracts, as the AC of individual compounds varied widely (Rice-Evans et al. 1996). The contents of quercetin-3-*O*-rutinoside and gallic acid in soluble extract were same for GB10 and GB20, while insoluble extract of these were 46 and 75%, respectively, in GB20 (Table 1).

Likewise, ORAC values were higher in the insoluble phenolics extract and corresponded, on average, to 69% of TAC in breads (Fig. 2b). Therefore, as noted previously to TAC, ORAC values in the extract of insoluble phenolics were higher in both guava breads (on average 0.41 mmol TE/100 g) than CB (0.28 mmol TE/100 g). The soluble phenolics extract did not show any difference among all breads (Fig. 2b).

A strong positive correlation was observed between FRAP values and total phenolics content ($r = 0.95$, $p = 0.004$) and total flavonoids content ($r = 0.96$, $p = 0.003$). These results corroborate the expected

contribution of phenolic compounds to TAC of foods. Moreover, results suggested that flavonoids but not non-flavonoids contribute to the TAC of control and guava breads.

Volatile compounds

Concerning the sensory properties that define bread quality, consumers consider the aroma essential to acceptance. A total of forty-seven volatile compounds were identified in GB10 and GB20 (Table 2) and thirty-five in CB (Supplementary Table 3). For all volatile compounds, identification was based on chromatographic peak similarity index (SI) higher than 90%, and matching RI (with maximum error of 5%).

The major chemical classes of volatile compounds identified in CB were alcohols, aldehydes, ketones and acids, and this general profile (Supplementary Table 3) was in accordance reported earlier (Pico et al. 2015). In both guava breads these same classes observed in CB were identified in addition to terpenes (Fig. 3). It is well known that the characteristic flavor profile of bread was mainly formed during fermentation, baking and storage (Pico et al. 2015). However, other factors such as the recipe, may also affect its final aroma (Cho and Peterson 2010).

Compared to guava breads, CB presented a poor volatile profile (Supplementary Table 3), mainly due to the absence of terpenes (Fig. 3). Similarly, a richer volatile profile was observed in wheat breads supplemented with different contents of chestnut flour compared to unsupplemented controls (Dall'Asta et al. 2013). The major volatile compounds identified in CB were 2-phenylethanol, benzaldehyde, 3-methyl-1-butanol and 3-hydroxy-2-butanone, corresponding to 36.1% of total volatiles (Supplementary Table 3). From those, 2-phenylethanol, 3-methyl-1-butanol and 3-hydroxy-2-butanone were positively correlated with bread aroma while benzaldehyde was negatively correlated (Pico et al. 2015).

In wheat, breads volatile compounds are formed mainly by fermentation, lipid oxidation and Maillard reaction (Pico et al. 2015; Rehman et al. 2006). Volatile compounds characteristically from Maillard reaction in bread include benzaldehyde, furfural, 2-phenylethanol, 3-hydroxy-2-butanone and 3-methyl-butanoic acid. Interestingly, the relative contents of these compounds were higher in CB (33% of total volatiles) (Supplementary Table 3) than in GB10 (22%) and GB20 (18%) (Table 2). The substitution of wheat flour by guava powder reduced the contents of protein in both guava breads, which may explain the lower contents of these volatile compounds in GB10 and GB20, since proteins are reactants in Maillard reaction.

The main volatile compounds associated to fermentation (benzaldehyde, hexanoic acid, 3-methyl-butanol and

Table 3 Sensory profile of control bread (CB), and breads made from wheat flour substituted with GP at 10% (GB10) and 20% (GB20), ($n = 34$)¹

	CB	GB10	GB20
Attributes ²			
Overall aspect	7.4 ± 1.5 ^a	7.5 ± 1.1 ^a	7.2 ± 1.7 ^a
Aroma	6.3 ± 1.8 ^a	7.2 ± 1.7 ^{a,b}	7.8 ± 1.3 ^b
Crust color	7.8 ± 1.6 ^a	8.0 ± 1.0 ^a	7.4 ± 1.6 ^a
Crumb color	7.5 ± 1.6 ^a	7.4 ± 1.5 ^a	7.2 ± 1.4 ^a
Taste	6.6 ± 2.0 ^a	7.5 ± 1.6 ^a	7.6 ± 1.2 ^a
Texture	7.2 ± 2.0 ^a	8.1 ± 1.0 ^b	7.6 ± 1.5 ^a
Residual taste	6.7 ± 2.2 ^a	7.3 ± 1.4 ^a	7.5 ± 1.2 ^a
Preference ³	1.6 ± 0.8 ^a	2.1 ± 0.7 ^{a,b}	2.4 ± 0.8 ^b
Consumption intent ⁴	4.4 ± 2.0 ^a	4.9 ± 1.7 ^a	5.2 ± 1.4 ^a
Purchase intent ⁵	3.3 ± 1.4 ^a	3.7 ± 1.1 ^a	3.9 ± 1.1 ^{a#}

¹ Results are expressed as means ± standard deviations. Different superscript letters in the same row indicate significant difference (Friedman test followed by Dunn's multiple comparison post hoc test, $p < 0.05$)

² Nine-point scale (1 dislike extremely; 2 dislike moderately; 3 dislike regularly; 4 dislike slightly; 5 neither like nor dislike; 6 like slightly; 7 like regularly; 8 like moderately; 9 like extremely)

³ Preference ranking (1 less preferred; 2 neither the less nor the most preferred; 3 most preferred)

⁴ Seven-point scale (1 would never eat; 2 would eat very rarely; 3 would rarely eat; 4 would occasionally eat; 5 would frequently eat; 6 would eat very frequently; 7 would always eat)

⁵ Five-point scale (1 certainly would not buy; 2 probably would not buy; 3 maybe would buy, maybe would not buy; 4 probably would buy; 5 certainly would buy)

[#] compared to control bread, $p = 0.07$

Fig. 3 Relative contents (peak area %) of alcohols, aldehydes, ketones, acids and terpenes in control bread (white square box), and in breads made with guava powder substituting for wheat flour at two levels, 10% (grey square box) and 20% (black square box). Results are means ± standard deviations of three replicates. Values with different superscript letters in the same compound class indicate significant difference between mean values (Oneway ANOVA followed by Tukey's multiple comparison post hoc test, $p < 0.05$)

2-phenylethanol) were also higher in CB (37%) (Supplementary Table 3) when compared to GB10 (13%) and GB20 (10%) (Table 2). Guava breads probably presented lower contents of starch, from the reduced use of wheat

flour, which was the main source of fermentable sugars for *S. cerevisiae* fermentation. In this sense, results suggested a reduction in fermentation efficiency of dough in the presence of GP.

The incorporation of guava powder (GP) affected the volatiles profile in breads. Terpenes, which were found in high contents in GP (Nunes et al. 2016), were enhanced in guava breads, with a clear relation between quantity of added GP and contents of terpenes among bread volatiles (Fig. 3). α -Humulene, β -caryophyllene and limonene were the major volatile compounds in guava breads and together contributed to 49 and 35% of total volatiles in GB20 and GB10, respectively (Table 2). These compounds were also prevalent in guava powder (Nunes et al. 2016), being β -caryophyllene and limonene previously detected as the most odour-active compounds contributing to the overall guava aroma (Pino and Bent 2013). Although the final aroma of bread depends on a mixture of specific volatiles (Heenan et al. 2009), the occurrence of these odour-active compounds contributed with a guava aroma to both guava breads, as observed upon scenting the samples compared to the CB (data not shown). Interestingly, in the present study sensory analysis showed that GB20 was the most appreciated bread in terms of aroma when compared to CB, but was not different from GB10 (Table 3).

Conversely, some volatiles such as hexanal, benzaldehyde and 1-octen-3-ol are common bread off-flavors, mainly related to lipid degradation (Pico et al. 2015). Thus, breads presented more acceptable aroma when contents of these compounds were lowered. In fact, these off-flavor compounds were lower in GB20 (3.5%) than in GB10 (4.5%) and CB (15.1%) (Table 2; Supplementary Table 3), and this might explain at least partially the higher scores of aroma observed for GB20 (Table 3).

Sensory analysis

All attributes evaluated presented good average scores, being aroma and texture the sole attributes affected by substituting wheat flour by GP (Table 3). GB20 showed better acceptability in terms of aroma when compared to CB, being both similar to GB10 (Table 3). This result may be attributed to the pleasant and intense typical guava flavor, which was in accordance with volatile compounds profile. Other authors have also observed that breads made with 10% replacement of wheat flour by fruit powders showed similar score for aroma to control breads (Altunkaya et al. 2013).

It is known that the substitution of wheat flour by other ingredients in breads results in loss of texture (Salgado et al. 2011). Interestingly, in the present study GB10 showed higher score for texture than CB (7.2 ± 2.0) and GB20 (Table 3).

The preference ranking of bread samples showed that the panelists preferred GB20 over CB, but the former was not different from GB10 (Table 3). Both guava breads showed higher scores for consumption intent (5.1 on average). Although the purchase intent was similar in all samples, a marginal tendency ($p = 0.07$) for a higher score for GB20 when compared to CB was observed (Table 3). Similarly, the purchase intent of vegetable-enriched breads was not significantly different from control bread (Hobbs et al. 2014). Together, these results demonstrate that the guava breads have potential for commercialization.

Conclusion

In summary, the results of the present study demonstrated that the use of guava powder improved the phenolics and aroma compounds, with the incorporation of odour-active compounds related to guava flavor. These results associated with the good acceptability of guava breads by potential consumers indicate that the use of guava powder in breads can contribute to the development of new market food products with added nutritional and functional value, and desirable aroma.

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References

- Acosta-Estrada BA, Gutiérrez-Urbe J, Serna-Saldívar SO (2014) Bound phenolics in foods, a review. *Food Chem* 152:46–55
- Altunkaya A, Hedegaard RV, Brimer L, Gökmen V, Skibsted LH (2013) Antioxidant capacity versus chemical safety of wheat bread enriched with pomegranate peel powder. *Food and Function* 4:722–727
- Alves G, Perrone D (2015) Breads enriched with guava flour as a tool for studying the incorporation of phenolic compounds in bread melanoidins. *Food Chem* 185:65–74
- Arun KB, Persia F, Aswathy PS, Chandran J, Sajeev MS, Jayamurthy P, Nisha P (2015) Plantain peel - a potential source of antioxidant dietary fibre for developing functional cookies. *J Food Sci Technol* 52(10):6355–6364
- Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal Biochem* 239:70–76
- Bhol S, Lanka D, Bosco SJD (2016) Quality characteristics and antioxidant properties of breads incorporated with pomegranate whole fruit bagasse. *J Food Sci Technol* 53(3):1717–1721
- Cho IH, Peterson DG (2010) Chemistry of bread aroma: A review. *Food Sci Biotechnol* 19:575–582
- Corrêa RCG, Haminiuk CWI, Sora GTS, Bergamasco R, Vieira AMS (2014) Antioxidant and rheological properties of guava jam with added concentrated grape juice. *J Sci Food Agr* 94:46–152
- Dall'Asta C, Cirlini M, Morini E, Rinaldini M, Ganio T, Chiavaro E (2013) Effect of chestnut flour supplementation on physico-chemical properties and volatiles in bread making. *LWT-Food Sci Technol* 53:233–239
- Dinelli G, Segura-Carretero A, Silvestro RD, Marotti I, Arráez-Román D, Benedettelli S, Ghiselli L, Fernandez-Gutierrez A (2011) Profiles of phenolic compounds in modern and old common wheat varieties determined by liquid chromatography coupled with time-of-flight mass spectrometry. *J Chromatogr A* 1218:7670–7681
- Dorđević TM, Šiler-Marinković SS, Dimitrijević-Branković SI (2010) Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. *Food Chem* 119:957–963
- Dziki D, Różyło R, Gawlik-Dziki U, Świeca M (2014) Current trends in the enhancement of antioxidant activity of wheat bread by the addition of plant materials rich in phenolic compounds. *Trends Food Sci Technol* 40:1–14
- Flores G, Wua SB, Negrin A, Kenelly EJ (2015) Chemical composition and antioxidant activity of seven cultivars of guava (*Psidium guajava*) fruits. *Food Chem* 170:327–335
- Gutiérrez RMP, Mitchell S, Solis RV (2008) *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol* 117:1–27
- Heenan SP, Dufour JP, Hamid N, Harvey W, Delahunty CM (2009) Characterization of fresh bread flavour: relationships between sensory characteristics and volatile composition. *Food Chem* 116:249–257
- Hobbs DA, Ashouri A, George TW, Lovegrove JA, Methven L (2014) The consumer acceptance of novel vegetable-enriched bread products as a potential vehicle to increase vegetable consumption. *Food Res Int* 58:15–22
- Hur SJ, Lee SY, Kim Y-C, Choi I, Kim G-B (2014) Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chem* 160:346–356
- Katina K, Laitila A, Juvonen R, Liukkonen K-H, Kariluoto S, Piironen V, Landberg R, Åman P, Poutanen K (2007) Bran fermentation as means to enhance technological properties and bioactivity of rye. *Food Microbiol* 24:175–186
- Lu Y, Luthria D, Fuerst EP, Kiszonas AM, Yu L, Morris CF (2014) Effect of processing on phenolic composition of dough and bread fractions made from refined and whole wheat flour of three wheat varieties. *J Agr Food Chem* 62:10431–10436
- Menga V, Fares C, Troccoli A, Cattivelli L, Baiano A (2010) Effects of genotype, location and baking on the phenolic content and some antioxidant properties of cereal species. *Int J Food Sci Tech* 45:7–16
- Nunes JC, Lago MG, Castelo-Branco VN, Oliveira FR, Torres AG, Perrone D, Monteiro M (2016) Effect of drying method on volatile compounds, phenolic profile and antioxidant capacity of guava powders. *Food Chem* 197:881–890
- Owen PL, Martineau LC, Caves D, Haddad PS, Matainaho T, Johns T (2008) Consumption of guava (*Psidium guajava* L.) and noni (*Morinda citrifolia* L.) may protect betel quid-chewing Papua New Guineans against diabetes. *Asia Pac J Clin Nutr* 17:635–643
- Pico J, Bernal J, Gómez M (2015) Wheat bread aroma compounds in crumb and crust: a review. *Food Res Int* 75:200–215
- Pino JA, Bent L (2013) Odour-active compounds in guava (*Psidium guajava* L. cv. Red Suprema). *J Sci Food Agr* 93:3114–3120
- Platat C, Habib HM, Hashim IB, Kamal H, AlMaqbali F, Souka U, Ibrahim WH (2015) Production of functional pita bread using date seed powder. *J Food Sci Technol* 52(10):6375–6384
- Rahaie S, Gharibzadeh SMT, Razavi SH, Jafari SM (2014) Recent developments on new formulations based on nutrient-dense ingredients for the production of healthy-functional bread: a review. *J Food Sci Technol* 51(11):2896–2906

- Rehman S, Paterson A, Piggott JR (2006) Flavour in sourdough breads: a review. *Trends Food Sci Techn* 17:557–566
- Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Bio Med* 20:933–956
- Rupasinghe HPV, Wang L, Huber GM, Pitts NL (2008) Effect of baking on dietary fibre and phenolics of muffins incorporated with apple skin powder. *Food Chem* 107:1217–1224
- Salgado JM, Rodrigues BS, Donado-Pestana CM, Dias CTS, Morzelle MC (2011) Cupuassu (*Theobroma grandiflorum*) peel as potential source of dietary fiber and phytochemicals in whole-bread preparations. *Plant Food Hum Nutr* 66:384–390
- Świeca M, Sęczyk Ł, Gawlik-Dziki U, Dziki D (2014) Bread enriched with quinoa leaves—the influence of protein-phenolics interactions on the nutritional and antioxidant quality. *Food Chem* 162:54–62
- Williams JT, Haq N (2000) Global research on underutilised crops and assessment of current activities and proposals for enhanced cooperation. International Centre for Underutilised Crops, Southampton
- Zulueta A, Esteve MJ, Frígola A (2009) ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chem* 114:310–316