



Guava, orange and passion fruit by-products: Characterization and its impacts on kinetics of acidification and properties of probiotic fermented products

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ABSTRACT

This study aimed at characterizing guava, orange and passion fruit by-products and investigating the effect of adding these fruit by-products to probiotic fermented goat milk and cereal-based fermented products. Fruit by-products showed total fiber content, phenolic compounds and antioxidant activity varying from, respectively, 58.20–89.80%, 253.14–420.89 mg GAE/100 g, and 11.38–17.37 μmol TE/g. Most carotenoids were represented by β-carotene, which ranged from 7.91 to 56.07 μg/g. The presence of fruit by-products did not affect the fermentation time of fermented oat beverage and fermented goat milk; however, a significant increase (ranging from 0.28 to 0.91 h) in fermentation time of fermented rice beverages was observed after the addition of by-products. Fruit by-products also resulted in an increase in acidification throughout storage; however, they did not affect the counts of probiotic bacteria. A decrease in probiotic survival during *in vitro* gastrointestinal simulation was observed in all treatments. Nonetheless, the presence of orange and passion fruit by-products enhanced the resistance of the probiotics to simulated gastrointestinal conditions and resulted in population 2 log CFU/mL higher than the control treatment. Fruit by-products can be considered relevant sources of bioactive compounds useful in raising the functional attributes of probiotic fermented products.

1. Introduction

The intake of functional food has been increasing worldwide due to the consumers' awareness of the association between diet and health. Functional food products are those which, in addition to basic nutrition, offer health benefits to the consumer and may play a role in reducing the risk of certain diseases (Aboufazi, Shori, & Baba, 2016). Fruit is widely known for its functional potential and, more recently, fruit by-products have gained attention due to their higher nutritional contents in comparison to their respective edible portion (Can-Cauich et al., 2017). It is noteworthy that the use of by-products also contributes to reduce the economic and environmental problems caused by the discard of waste by fruit processing industries (O'Shea, Arendt, & Gallagher, 2012).

Fruit by-products can be successfully incorporated into a variety of food products, among them, fermented milk or plant-based fermented

products stand out. These products are often produced using probiotic strains, making them one of the most lucrative and important categories of functional food (Bansal, Mangal, Sharma, & Gupta, 2016). Probiotics have been used by industry for decades and, currently, are defined as live microorganisms which, when ingested in adequate amounts, confer beneficial effects on the consumer's health (Hill et al., 2014).

The addition of fruit by-products to probiotic fermented food is desirable since they may protect the probiotics from adverse conditions found in the human gastrointestinal tract and may also present prebiotic potential, acting in synergy with probiotics in the human intestines after their ingestion. Although it is known that fruit by-products are important sources of nutrients, their prebiotic potential has just started to be explored (Sah, Vasiljevic, McKechnie, & Donkor, 2016a). Moreover, fruit by-products are rich in dietary fiber and bioactive compounds (O'Shea et al., 2015) which can help improve the overall functional characteristics of fermented products. Among the fruit by-

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products with a potential application to fermented products are orange, guava and passion fruit. These fruits are cultured in large scale in Brazil (Instituto Brasileiro de Geografia e Estatística – IBGE, 2016) and their industrial processing produces tons of by-products, thus reinforcing the importance of their practical application.

Several studies have already evaluated the influence of adding fruit by-products on the characteristics of fermented milk products (Chouchouli et al., 2013; Espírito-Santo et al., 2012; Frumento et al., 2013; Sah, Vasiljevic, McKechnie, & Donkor, 2015; Sah et al., 2016a; Santos et al., 2017). However, according to our knowledge, the effect of adding fruit by-products to cereal-based probiotic fermented beverages has not been evaluated yet. In recent years, due to the growth of vegetarianism, lactose intolerance and allergy to milk proteins, there has been an increase in research into plant-based probiotic fermented beverages (Martins et al., 2013; Shori, 2016), which demonstrates the importance of evaluating this type of matrix in studies with probiotic strains.

In this context, the objectives of this study were to characterize guava, orange and passion fruit by-products and to investigate the effects of adding 1% of these fruit by-products to probiotic cereal-based fermented beverage and probiotic fermented goat milk, considering the following parameters: fermentation kinetics, post-acidification, probiotic viability and its survival under simulated gastrointestinal (GI) tract conditions.

2. Materials and methods

2.1. Preparation of fruit by-products

Guava, orange and passion fruit by-products were donated by fruit processing companies located in Brazil. The by-products were collected immediately after industrial processing, frozen and transported to the laboratory. In the laboratory, the samples were kept at -18°C and subsequently treated according to Espírito-Santo et al. (2012), with modifications. The material was dried in an oven with air circulation (MA037, Marconi, São Paulo, Brazil) at 60°C until constant weight, grounded to obtain a fine powder using a processor (TM 31, Vorwerk, Wuppertal, Germany), which was standardized to particle size $< 42\ \mu\text{m}$ to facilitate the mixture of these by-products with goat milk and cereal extracts. The powders were vacuum packed and stored under refrigeration until irradiation, which was carried out at the Nuclear and Energy Research Institute (IPEN, São Paulo, Brazil). The samples were treated with a 5 kGy dose and stored at 4°C .

2.2. Microbiological safety and chemical characterization of fruit by-products

The populations of yeasts and molds, coliforms, *Bacillus cereus* and *Salmonella* sp were estimated according to Compendium of Methods for the Microbiological Examination of Foods (APHA, 2001), after the sample irradiation.

The chemical composition of fruit by-products was determined regarding moisture, proteins, ashes, lipids (IAL, 2008), soluble and insoluble fiber (AOAC, 1999). The total dietary fiber was obtained through the addition of the insoluble and soluble fractions. The available carbohydrates were determined by difference, calculated as the percentage difference between 100 and the sum of moisture, protein, lipid ash, and total dietary fiber percentages. Water activity was determined in a TH-500 digital hygrometer (Novasina, Snack, Switzerland).

β -carotene and lycopene were extracted and quantified spectrophotometrically according to the method described by Rodríguez-Amaya and Kimura (2004). Phenolic compounds were assessed according to Folin–Ciocalteu (Macoris, De Marchi, Janzanti, & Monteiro, 2012; Waterhouse, 2014) and the results were expressed as milligrams of gallic acid equivalents (mg GAE) per 100 g of dry basis sample.

Antioxidant activity of fruit by-products was determined using the DPPH method and the results were expressed as micromole Trolox equivalent ($\mu\text{mol TE}$) per g of dry basis sample (Rufinole et al., 2007). Trolox [(\pm)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, 97%] and DPPH (2,2-Diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich (St. Louis, USA) and Folin–Ciocalteu and gallic acid ($\text{C}_7\text{H}_6\text{O}_5$, P.A. ACS) in turn were obtained from Dinâmica (Diadema, Brazil). The analyses were carried out in triplicate, except for carotenoids and fibers, which were determined in duplicate.

2.3. Preparation of fermented products

Oat and rice extracts (BioV, Jasmine Foods, Curitiba, Brazil) and goat milk (Caprilat, Castro, Brazil) were used to prepare the fermented oat beverage (FOB), fermented rice beverage (FRB) and fermented goat milk (FGM), respectively. Each substrate was divided into four treatments: three were added with 1% fruit by-products (guava, orange and passion fruit) and one of them was not supplemented (control). Additionally, oat and rice extracts were added with 1% food grade lactose (Daxia, São Paulo, Brazil). Then, the bases were heated to 90°C and maintained at this temperature for 10 min. After the heat treatment, the bases were transferred to sterile flasks and kept at 4°C for 24 h, until fermentation. Three independent fermentations for each treatment were performed on different days.

To prepare the inoculum, 1.0 g of *Lactobacillus casei* Lc-1 (Chr. Hansen, Valinhos, Brazil) and 0.15 g of *Streptococcus thermophilus* TA040 (Danisco, Sassenage, France) were suspended in 50 mL of sterile reconstituted milk (10 g/100 g of total solids), homogenized and incubated at 42°C for 30 min. This procedure provided an inoculum with a population of 10^8 – 10^9 CFU/mL. Then, *L. casei* (2%) and *S. thermophilus* (0.4%) were inoculated in 500 mL of fermentation bases, giving counts of approximately 10^6 CFU/mL for *L. casei* and 10^7 CFU/mL for *S. thermophilus*.

The fermentation was carried out at 42°C until pH 4.6 was reached; a CINAC system (Cynétique d'acidification, Alliance Instruments, Frepillon, France) was used in order to evaluate the acidification kinetics (Casarotti & Penna, 2015). The following parameters were considered: V_{max} , acidification rate (dpH/dt) expressed as 10^{-3} pH units/min; $t_{V_{\text{max}}}$, time (h) at which the V_{max} was reached; $\text{pH}_{V_{\text{max}}}$, pH of the medium when the maximum V_{max} was reached; $t_{\text{pH}5.0}$, time (h) to reach pH 5.0; $t_{\text{pH}4.6}$, time (h) to reach pH 4.6. After fermentation, the products were cooled to 4°C in an ice-water bath, sterile packaged in plastic containers and stored at 4°C until the moment of analyses. The products were characterized after 1, 14 and 28 days of storage.

2.4. Titratable acidity, pH and bacterial counts

Titratable acidity was measured by titration using 0.1 mol/L NaOH solution and phenolphthalein as indicator (IAL, 2008). The pH value was determined using a digital potentiometer. The results were expressed as the means of three replicates.

S. thermophilus colonies were enumerated in M17 agar (Himedia, Mumbai, India), whereas those of *L. casei* in MRS agar (Difco, Becton Dickinson Co., Sparks, MD, USA) with 0.2 lithium chloride and 0.3 g/L sodium propionate (Sigma-Aldrich), according to IDF (1997) and Vinderola and Reinheimer (1999), respectively. Plates were incubated under aerobic conditions at 37°C for 72 h. Bacterial counts of each treatment were carried out in duplicate.

2.5. Survival of probiotic strains under simulated GI tract conditions

Viability of strains under GI tract simulated conditions was tested employing an *in vitro* model of subsequent exposure to gastric and enteric conditions, divided into three phases with a total length of 360 min. The assay was performed according to the protocol of Buriti, Castro, and Saad (2010), with modifications described by Casarotti and

Penna (2015). At the beginning, 10 mL aliquots of fermented products were diluted in duplicate in 90 mL of sterile saline 0.5% (w/v). From this initial dilution, 10 mL were distributed in three flasks, corresponding to the gastric, enteric phase I and enteric phase II, totaling six flasks for each fermented product. To simulate the gastric phase, the pH was adjusted to 2.0–2.2 using 0.5 M HCl and pepsin (from porcine stomach mucosa, Sigma-Aldrich, 3 g/L) solution. The flasks were incubated for 120 min at 37 °C, and after this period an alkaline solution containing bile (bovine bile, Sigma-Aldrich, 10 g/L) and pancreatin (from porcine pancreas, Sigma-Aldrich, 1 g/L) was added to increase the pH to 4.3–5.2, leading to the simulation of the enteric phase 1. Flasks were incubated again at 37 °C for 120 min. To simulate the enteric phase 2, the pH value was increased to 7.0–7.3 using the same alkaline solution, and the bile and pancreatin concentrations were adjusted (10 g/L and 1 g/L, respectively, in the final mixture). Flasks were incubated once again at 37 °C for another 120 min. Aliquots were taken from the flasks before the beginning of the *in vitro* assay and the end of each stage, i.e., after 120 (gastric phase), 240 (enteric phase 1) and 360 min (enteric phase 2). The aliquots were used to evaluate the viability of *L. casei* Lc-1 strain, as described previously.

2.6. Statistical analysis

The results were submitted to one-way analysis of variance followed by Tukey test at $p < 0.05$ to compare means. Statistical analysis was performed using Statistica 12 (StatSoft Inc. Tulsa, OK, USA).

3. Results and discussion

3.1. Characterization of fruit by-products

The microbiological evaluation of by-products showed absence of *Salmonella* sp., fecal coliforms, yeasts and molds and *Bacillus cereus* (results not shown). This outcome can be explained by the irradiation of samples and by their water activity values close to 0.3, which is favorable to microbiological stability of food systems and inhibits the growth of bacteria, molds and yeasts (Jay, 2000). The chemical composition of fruit by-products is quite different; protein and lipid contents of passion fruit are higher than guava and orange by-products. The composition of orange and passion fruit by-products (Table 1) is

Table 1
Chemical composition and water activity of fruit by-products.

Parameters	Guava	Orange	Passion fruit
Moisture (%)	3.97 ± 0.12 ^B	7.37 ± 0.21 ^A	7.13 ± 0.06 ^A
Proteins (%)	2.07 ± 0.06 ^C	5.23 ± 0.15 ^B	12.60 ± 0.10 ^A
Lipids (%)	1.20 ± 0.00 ^C	2.07 ± 0.06 ^B	7.97 ± 0.23 ^A
Ash (%)	0.83 ± 0.06 ^C	2.73 ± 0.06 ^B	7.33 ± 0.15 ^A
Carbohydrates ^a (%)	2.13	24.40	0.77
Total dietary fiber (%)	89.80 ± 0.14 ^A	58.20 ± 0.28 ^C	64.20 ± 0.28 ^B
Insoluble fibers (%)	86.10 ± 0.00 ^A	46.90 ± 0.14 ^B	44.80 ± 0.14 ^C
Soluble fibers (%)	3.70 ± 0.14 ^C	11.30 ± 0.14 ^B	19.40 ± 0.14 ^A
Carotenoids (µg β-carotene/g)	7.91 ± 0.03 ^B	39.14 ± 0.03 ^B	56.07 ± 0.03 ^A
Carotenoids (µg lycopene/g)	4.77 ± 0.02 ^C	18.51 ± 0.02 ^B	28.57 ± 0.03 ^A
Total phenolic compounds (mg GAE/100 g)	253.14 ± 21.22 ^B	420.89 ± 6.40 ^A	384.44 ± 22.50 ^A
Antioxidant activity (µmol TE/g)	.1737 ± 0.58 ^A	11.38 ± 1.50 ^C	13.40 ± 0.42 ^B
Water activity	0.164 ± 0.003 ^B	0.284 ± 0.006 ^A	0.302 ± 0.011 ^A

Different capital letters in the same row denote a significant difference ($p < 0.05$) among fruit by-products.

^a Obtained from 100 – (moisture + ash + lipids + proteins + total dietary fiber).

similar to that found by Macagnan et al. (2015) and Espirito-Santo et al. (2012), respectively, especially regarding total dietary fiber. Additionally, guava had higher total dietary and insoluble fiber contents, while passion fruit by-products presented the highest content of soluble fibers. The levels of total dietary fiber, both insoluble and soluble, found for guava by-products are close to those obtained by Amaya-Cruz et al. (2015). The high levels of dietary fibers are indicative of the prebiotic potential of fruit by-products.

Phenolic compounds and carotenoids were measured for by-products because they may offer numerous positive health effects, such as reducing the risk of cancer, cardiovascular disease, and cell damage caused by reactive oxygen species (Can-Cauich et al., 2017). Orange and passion fruit by-products presented high levels of phenolic compounds, which are among the most active natural antioxidants and also have antimicrobial activity (Casquete et al., 2015). The results obtained in this study for orange and passion fruit were higher than those reported by Casquete et al. (2015) and Nascimento, Mulet, Ascheri, de Carvalho, and Cárcel (2016), respectively. On the other hand, Amaya-Cruz et al. (2015) and Bertagnolli, Silveira, Fogaça, Umann, and Penna (2014) found higher values for phenolics in guava by-products. According to the classification proposed by Vasco, Ruales, and Kamal-Eldin (2008), all three by-products were classified as fruits with medium phenolic contents (100–500 mg GAE/100 g).

β-carotene and lycopene concentrations in orange and passion fruit by-products were also higher than in guava by-products. The concentration of lycopene was lower than the one found for β-carotene. This carotenoid has gained attention in the last decades due to its possible effect against cancer, mainly prostate cancer, and cardiovascular diseases (Barros, Ferreira, & Genovese, 2012). Bertagnolli et al. (2014) and Silva et al. (2014) found lower values for guava and passion fruit by-products.

Considering antioxidant activity using the DPPH method, the highest value was obtained for guava by-products. This method is based on the elimination of the free radical 1,1-diphenyl-2-picrylhydrazyl and it measures antioxidants action by scavenging free radicals (Dastmalchi et al., 2008). The methods usually employed to analyze the antioxidant activity only measure phenolic compounds in which the phenolic groups are free, i.e., not glycosylated or ester linked, thus the antioxidant capacity may be underestimated comparing fruits, in which interactions with other phenolic compounds or other compounds occur. Therefore, the antioxidant capacity is mostly influenced by the type of phenolic compounds present in the matrix, compared to the amount of these substances (Vasco et al., 2008). In this sense, a substance with the highest concentration of phenolic compounds will not necessarily have the highest antioxidant activity.

In general, the results indicate that fruit by-products are a potential source of bioactive compounds, such as fibers, phenolics, and carotenoids, which might encourage their utilization by food industries for the development of new added-value products.

3.2. Kinetics of acidification

The acidification profiles of control fermented products and fermented products with guava, orange and passion fruit by-products were characterized using the parameters V_{max} , t_{Vmax} , pH_{Vmax} , $t_{pH5.0}$ and $t_{pH4.6}$ (Table 2). The acidification curves of oat, rice and goat milk fermented products are shown in Fig. 1.

The fruit by-products had a distinguished influence on the fermentation kinetics when compared to the control treatment (Table 2). For FOB treatments, passion fruit had a negative effect on V_{max} and guava and passion fruit by-products resulted in lower pH_{Vmax} and in higher t_{Vmax} values ($p < 0.05$). Conversely, the presence of fruit by-products did not cause a significant difference on $t_{pH5.0}$ and $t_{pH4.6}$ ($p > 0.05$). Considering FRB treatments, orange and passion fruit led to a decrease in V_{max} and only passion fruit led to an increase in $t_{pH4.6}$ ($p < 0.05$). However, for this substrate, the fruit by-products had no influence on

Table 2Kinetics parameters of acidification obtained during fermentation of fermented oat beverages (FOB), fermented rice beverages (FRB) and fermented goat milk (FGM).^a

Substrate	Treatment ^b	V_{\max} (10^{-3} upH/min)	$t_{V_{\max}}$ (h)	$pH_{V_{\max}}$	$t_{pH5.0}$ (h)	$t_{pH4.6}$ (h)
FOB	C	36.56 ± 3.19^a	1.50 ± 0.14^b	5.87 ± 0.39^a	2.36 ± 0.13^a	3.77 ± 0.24^a
	G	29.62 ± 0.5^a	2.49 ± 0.48^a	5.09 ± 0.20^b	2.64 ± 0.23^a	4.14 ± 0.53^a
	O	31.69 ± 2.26^a	1.91 ± 0.25^{ab}	5.28 ± 0.05^{ab}	2.24 ± 0.32^a	3.20 ± 0.41^a
	PF	20.86 ± 2.23^b	2.40 ± 0.42^a	5.06 ± 0.13^b	2.48 ± 0.32^a	3.32 ± 0.18^a
FRB	C	27.64 ± 2.23^a	0.95 ± 0.46^a	5.71 ± 0.32^a	1.54 ± 0.31^a	2.35 ± 0.32^b
	G	24.56 ± 0.91^{ab}	1.45 ± 0.22^a	5.46 ± 0.19^a	1.80 ± 0.13^a	2.72 ± 0.21^{ab}
	O	21.96 ± 2.32^b	1.09 ± 0.34^a	5.57 ± 0.15^a	1.78 ± 0.42^a	2.63 ± 0.44^{ab}
	PF	20.95 ± 2.16^b	1.23 ± 0.13^a	5.51 ± 0.13^a	1.78 ± 0.14^a	3.26 ± 0.25^a
FGM	C	15.53 ± 0.58^a	1.63 ± 0.08^a	5.85 ± 0.11^a	3.09 ± 0.38^a	6.29 ± 1.15^a
	G	13.47 ± 0.56^b	1.45 ± 0.24^a	5.81 ± 0.04^{ab}	2.95 ± 0.13^a	6.08 ± 0.10^a
	O	11.89 ± 0.21^c	1.56 ± 0.08^a	5.81 ± 0.01^{ab}	3.27 ± 0.06^a	6.41 ± 0.27^a
	PF	10.25 ± 0.36^d	1.61 ± 0.16^a	5.69 ± 0.01^b	3.46 ± 0.19^a	6.75 ± 0.58^a

^a Values are expressed as mean \pm SD ($n = 3$). For each substrate, different lowercase letters in the same column denote a significant difference ($p < 0.05$) among treatments.

^b Abbreviations are: C = control fermented product, without addition of fruit by-product; G = fermented product with addition of guava by-product; O = fermented product with addition of orange by-product; PF = fermented product with addition of passion fruit by-product; V_{\max} = maximum rate of acidification; $t_{V_{\max}}$ = time required to reach V_{\max} ; $pH_{V_{\max}}$ = pH in V_{\max} ; $t_{pH5.0}$ = time required to reach pH 5.0; $t_{pH4.6}$ = time required to reach pH 4.6 (end of fermentation).

$t_{V_{\max}}$, $pH_{V_{\max}}$ and $t_{pH5.0}$ ($p > 0.05$). A similar behavior was observed for FGM treatments, in which all the fruit by-products also resulted in lower V_{\max} and only passion fruit by-products were responsible for lower $pH_{V_{\max}}$ values ($p < 0.05$). Fruit by-products had no impact on the other parameters evaluated ($p > 0.05$) for this substrate.

Contrary to our expectations, fruit by-products had no effect on the time required to complete fermentation ($t_{pH4.6}$) in FOB and FGM ($p > 0.05$) and a negative effect on FRB treatments. It was expected that fruit by-products would stimulate the growth of microorganisms, due to the presence of fructose and dietary fibers in these ingredients, which would increase the amount of available carbohydrates and stimulate acid production by bacteria (Frumento et al., 2013; Sah, Vasiljevic, McKechnie, & Donkor, 2016b). Our results could be attributed to the presence of phenolic compounds in fruit by-products, which could adversely affect the fermentation time. Orange and passion fruit by-products showed similar phenolic compound contents (Table 1) and, on the other hand, only the later had a negative effect on the fermentation time for FRB. In this sense, this phenomenon depends on the type of phenolic compound and not only on its amount.

The substrates showed different initial pH values (Fig. 1), which could impact on microbial growth. It is noteworthy that the fermentation time of cereal-based fermented beverages was at least 2 h shorter than the time obtained for FGM. This difference is due to the higher buffering capacity of milk in relation to vegetable extracts (Wang et al., 2009). Although they are rich in polysaccharides, which could lead to longer fermentation time, cereals are considered good media for bacterial fermentation because they are rich in minerals, vitamins and other growth factors required by microorganisms (Bianchi et al., 2014).

This feature becomes even more important in the fermentation by probiotic microorganisms which are usually fastidious.

3.3. Acidification and bacterial viability during storage

The presence of fruit by-products, especially orange and passion fruit, resulted in lower pH and higher titratable acidity values ($p < 0.05$) during all the analysis period, the most accentuated difference being on the 28th day of storage (Tables 3 and 4). Supplementation with orange and passion fruit by-products may have increased the acid production capacity of these bacteria, although during fermentation this ingredient has stimulated the microorganisms only in FOB.

In FRB products, pH values were below 4 after 28 days of storage, which may be undesirable from a sensory standpoint (Bernat, Cháfer, Chiralt, & González-Martínez, 2014). The titratable acidity of FOB and FRB was lower than the one observed for FGM. This means that oat and rice extracts have a lower buffering capacity than goat milk.

The addition of fruit by-products has a significant effect ($p < 0.05$) on the viability of the probiotic strain, for all studied matrices (Table 5). Nevertheless, the effect has little relevance from the microbiological point of view, since the difference between the control treatment and the treatment with by-products was less than 1 log cycle CFU/mL. The most notable effect was observed for FRB containing passion fruit by-products, which had a population of 0.72 log CFU/mL higher than the control treatment.

The use of fruit by-products to enrich fermented products has been proven to be a successful strategy to increase the viability of probiotics

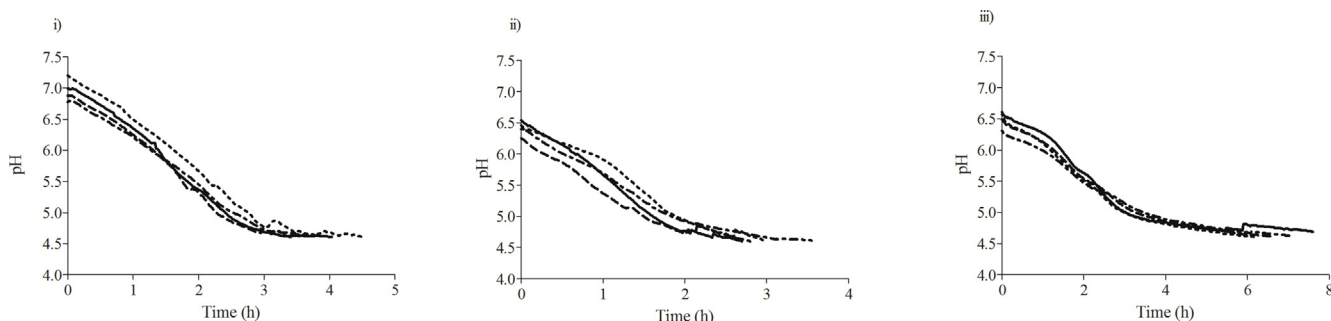


Fig. 1. Acidification curves obtained during fermentation at 37 °C using oat, rice or goat milk substrates (i, ii and iii, respectively) without (solid line) the addition of 1% guava, orange and passion fruit by-products (short-dashed, long-dashed and short/long-dashed lines, respectively).

Table 3

pH values for fermented oat beverages (FOB), fermented rice beverages (FRB) and fermented goat milk (FGM) during storage.^a

Substrate	Treatment ^b	Storage day		
		1	14	28
FOB	C	4.61 ± 0.03 ^{Aa}	4.31 ± 0.04 ^{Ba}	4.12 ± 0.03 ^{Ca}
	G	4.61 ± 0.03 ^{Aa}	4.32 ± 0.02 ^{Ba}	4.09 ± 0.06 ^{Cab}
	O	4.60 ± 0.03 ^{Aa}	4.22 ± 0.03 ^{Bb}	4.09 ± 0.07 ^{Cab}
	PF	4.57 ± 0.04 ^{Aab}	4.24 ± 0.03 ^{Bb}	4.07 ± 0.06 ^{Cabc}
FRB	C	4.53 ± 0.02 ^{Ae}	4.19 ± 0.06 ^{Bbc}	3.76 ± 0.03 ^{Cd}
	G	4.60 ± 0.02 ^{Aa}	4.12 ± 0.01 ^{Bde}	4.01 ± 0.02 ^{Ccd}
	O	4.52 ± 0.06 ^{Abcd}	3.97 ± 0.06 ^{Bf}	3.84 ± 0.02 ^{Ce}
	PF	4.50 ± 0.07 ^{Acd}	4.05 ± 0.03 ^{Be}	3.86 ± 0.04 ^{Ce}
FGM	C	4.56 ± 0.04 ^{Aabc}	4.13 ± 0.04 ^{Bcd}	4.07 ± 0.04 ^{Cabc}
	G	4.56 ± 0.04 ^{Aabc}	4.18 ± 0.03 ^{Bbc}	4.08 ± 0.02 ^{Cab}
	O	4.48 ± 0.07 ^{Ad}	4.10 ± 0.04 ^{Bde}	4.04 ± 0.04 ^{Cbc}
	PF	4.50 ± 0.02 ^{Acd}	4.13 ± 0.05 ^{Bcd}	4.05 ± 0.05 ^{Cbc}

^a Values are expressed as mean ± SD (n = 9). For each treatment, different capital letters in the same row denote a significant difference ($p < 0.05$) among days of storage. For each storage period, different lowercase letters in the same column denote a significant difference ($p < 0.05$) among treatments of all substrates.

^b Abbreviations are: C = control fermented product, without addition of fruit by-product; G = fermented product with addition of guava by-product; O = fermented product with addition of orange by-product; PF = fermented product with addition of passion fruit by-product.

Table 4

Titratable acidity (% acid lactic/100 g) of fermented oat beverage (FOB), fermented rice beverage (FRB) and fermented goat milk (FGM) during storage.^a

Substrate	Treatment ^b	Storage day		
		1	14	28
FOB	C	0.14 ± 0.02 ^{Cef}	0.22 ± 0.02 ^{Be}	0.27 ± 0.04 ^{Ae}
	G	0.16 ± 0.01 ^{Cde}	0.24 ± 0.01 ^{Bde}	0.29 ± 0.03 ^{Ade}
	O	0.17 ± 0.03 ^{Ccd}	0.26 ± 0.03 ^{Bd}	0.34 ± 0.01 ^{Acd}
	PF	0.20 ± 0.03 ^{Cc}	0.30 ± 0.05 ^{Bc}	0.38 ± 0.03 ^{Ac}
FRB	C	0.10 ± 0.00 ^{Cgh}	0.16 ± 0.02 ^{Bf}	0.20 ± 0.04 ^{Af}
	G	0.09 ± 0.01 ^{Ch}	0.12 ± 0.02 ^{Bg}	0.16 ± 0.01 ^{Af}
	O	0.12 ± 0.01 ^{Cfg}	0.22 ± 0.02 ^{Be}	0.30 ± 0.02 ^{Ade}
	PF	0.18 ± 0.01 ^{Ccd}	0.26 ± 0.06 ^{Bd}	0.32 ± 0.04 ^{Ad}
FGM	C	0.70 ± 0.05 ^{Cb}	0.88 ± 0.02 ^{Bb}	0.96 ± 0.06 ^{Ab}
	G	0.71 ± 0.02 ^{Cb}	0.90 ± 0.02 ^{Bb}	0.98 ± 0.03 ^{Ab}
	O	0.74 ± 0.01 ^{Ca}	0.96 ± 0.02 ^{Ba}	1.03 ± 0.02 ^{Aa}
	PF	0.77 ± 0.00 ^{Ca}	0.96 ± 0.01 ^{Ba}	1.06 ± 0.04 ^{Aa}

^a Values are expressed as mean ± SD (n = 9). For each treatment, different capital letters in the same row denote a significant difference ($p < 0.05$) among days of storage. For each storage period, different lowercase letters in the same column denote a significant difference ($p < 0.05$) among treatments of all substrates.

^b Abbreviations are: C = control fermented product, without addition of fruit by-product; G = fermented product with addition of guava by-product; O = fermented product with addition of orange by-product; PF = fermented product with addition of passion fruit by-product.

during storage. This effect can be attributed to the buffering capacity of the fibers present in by-products and to the considerable amount of growth promoting nutrients in the medium due to the addition of by-products (Espírito-Santo et al., 2012; Frumento et al., 2013).

All substrates used for the preparation of products supported the growth of the probiotic strain, confirming that other sources can be used as probiotics vehicles. These substrates can be used as alternatives to individuals who are lactose intolerant or allergic to milk proteins. Several studies have shown that different probiotic strains are able to survive in cereal-based fermented products (Bernat, Chafer, Gonzalez-Martinez, Rodriguez-Garcia, & Chiralt, 2015; Rathore, Salmeron, & Pandiella, 2012; Salmeron, Thomas, & Pandiella, 2015).

The population of *S. thermophilus* remained stable during the

Table 5

L. casei Lc-1 counts (log CFU/mL) in fermented oat beverage (FOB), fermented rice beverage (FRB) and fermented goat milk (FGM) during storage.^a

Substrate	Treatment ^b	Storage day		
		1	14	28
FOB	C	8.60 ± 0.16 ^{Aab}	8.52 ± 0.27 ^{Aa}	8.40 ± 0.29 ^{Aab}
	G	8.51 ± 0.36 ^{Aabc}	8.49 ± 0.03 ^{Aa}	8.47 ± 0.13 ^{Aab}
	O	8.55 ± 0.31 ^{Aabc}	8.66 ± 0.46 ^{Aa}	8.51 ± 0.27 ^{Aa}
	PF	8.78 ± 0.22 ^{Aa}	8.70 ± 0.49 ^{Aa}	8.61 ± 0.20 ^{Aa}
FRB	C	8.19 ± 0.14 ^{Ac}	7.99 ± 0.52 ^{Ab}	8.01 ± 0.63 ^{Ab}
	G	8.33 ± 0.05 ^{Bbc}	8.41 ± 0.02 ^{ABab}	8.49 ± 0.09 ^{Aa}
	O	8.37 ± 0.13 ^{Abc}	8.35 ± 0.04 ^{ABab}	8.43 ± 0.10 ^{Aab}
	PF	8.65 ± 0.20 ^{Aab}	8.71 ± 0.18 ^{Aa}	8.73 ± 0.31 ^{Aa}
FGM	C	8.33 ± 0.08 ^{Bbc}	8.54 ± 0.04 ^{Aa}	8.52 ± 0.06 ^{Aa}
	G	8.47 ± 0.12 ^{Aabc}	8.55 ± 0.17 ^{Aa}	8.57 ± 0.19 ^{Aa}
	O	8.62 ± 0.08 ^{Aab}	8.72 ± 0.07 ^{Aa}	8.70 ± 0.08 ^{Aa}
	PF	8.77 ± 0.26 ^{Aa}	8.71 ± 0.12 ^{Aa}	8.73 ± 0.06 ^{Aa}

^a Values are expressed as mean ± SD (n = 6). For each treatment, different capital letters in the same row denote a significant difference ($p < 0.05$) among days of storage. For each storage period, different lowercase letters in the same column denote a significant difference ($p < 0.05$) among treatments of all substrates.

^b Abbreviations are: C = control fermented product, without addition of fruit by-product; G = fermented product with addition of guava by-product; O = fermented product with addition of orange by-product; PF = fermented product with addition of passion fruit by-product.

Table 6

S. thermophilus counts (log CFU/mL) in fermented oat beverage (FOB), fermented rice beverage (FRB) and fermented goat milk (FGM) during storage.^a

Substrate	Treatment ^b	Storage day		
		1	14	28
FOB	C	9.03 ± 0.30 ^{Aab}	8.55 ± 0.31 ^{Babc}	8.58 ± 0.23 ^{Abcde}
	G	8.96 ± 0.26 ^{Aab}	8.72 ± 0.06 ^{Babc}	8.73 ± 0.10 ^{ABabc}
	O	8.66 ± 0.28 ^{Abcd}	8.88 ± 0.28 ^{Aabc}	8.77 ± 0.13 ^{Abc}
	PF	8.82 ± 0.22 ^{Aabc}	8.91 ± 0.31 ^{Aab}	8.75 ± 0.24 ^{Abc}
FRB	C	8.29 ± 0.22 ^{Ad}	8.04 ± 0.34 ^{Ad}	8.30 ± 0.23 ^{Ae}
	G	8.52 ± 0.06 ^{Acd}	8.50 ± 0.11 ^{Abc}	8.49 ± 0.07 ^{Acd}
	O	8.47 ± 0.19 ^{Acd}	8.46 ± 0.05 ^{Acd}	8.41 ± 0.08 ^{Ade}
	PF	8.71 ± 0.22 ^{Aabc}	8.69 ± 0.17 ^{Aabc}	8.72 ± 0.26 ^{Abcd}
FGM	C	8.95 ± 0.08 ^{Aab}	8.76 ± 0.29 ^{Aabc}	8.70 ± 0.07 ^{Abcd}
	G	8.73 ± 0.04 ^{Aabc}	8.90 ± 0.09 ^{Aabc}	8.74 ± 0.09 ^{Abc}
	O	8.98 ± 0.07 ^{Aab}	8.93 ± 0.08 ^{Aab}	8.91 ± 0.08 ^{Aa}
	PF	9.08 ± 0.19 ^{Aa}	8.96 ± 0.05 ^{ABa}	8.83 ± 0.13 ^{Bab}

^a Values are expressed as mean ± SD (n = 6). For each treatment, different capital letters in the same row denote a significant difference ($p < 0.05$) among days of storage. For each storage period, different lowercase letters in the same column denote a significant difference ($p < 0.05$) among treatments of all substrates.

^b Abbreviations are: C = control fermented product, without addition of fruit by-product; G = fermented product with addition of guava by-product; O = fermented product with addition of orange by-product; PF = fermented product with addition of passion fruit by-product.

product storage (Table 6) and the treatments containing fruit by-products had higher counts compared to the control treatment ($p < 0.05$). Furthermore, it was observed that FGM and FOB resulted in higher counts ($p < 0.05$) in relation to FRB.

3.4. *In vitro* resistance of probiotic strain to GI tract conditions

In general, the presence of fruit by-products led to an increased resistance of the probiotic strain in *in vitro* tests for all substrates used (Figs. 2–4). On the 1st day of storage, FRB and FGM treatments containing orange and passion fruit by-products showed a population at least 1 log cycle superior compared to the respective control treatments at the end of the assay (Fig. 2). After 14 days of storage, only FGM and

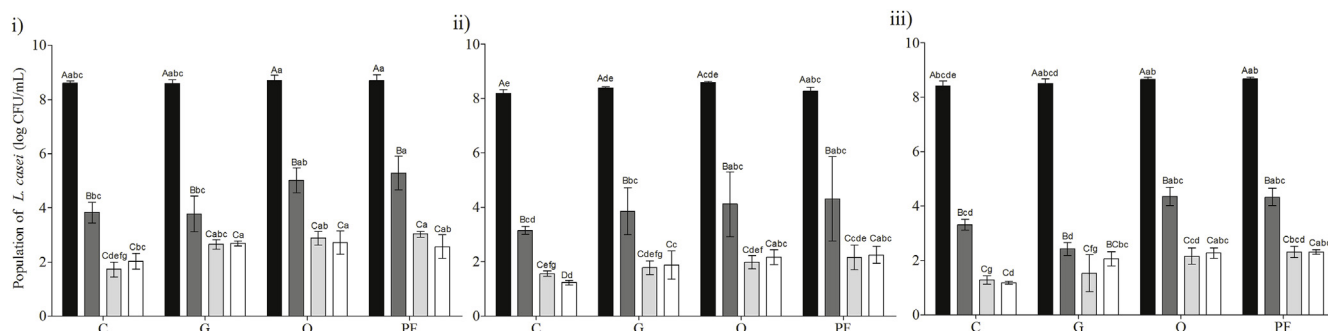


Fig. 2. Survival of *L. casei* Lc-1 (log CFU/mL) in fermented oat beverages, fermented rice beverages and fermented goat milk (i, ii and iii, respectively), before (■) and during exposure to simulated gastric conditions, for 120 min (■, pH 2.0–2.2) and enteric conditions, for 240 (■, pH 4.3–5.2) and 360 (□, pH 7.0–7.3) min, on the 1st day of storage. For the same sampling period of *in vitro* assay, different lowercase letters denote significant differences ($p < 0,05$) among treatments of all substrates. For each treatment, different capital letters denote significant differences ($p < 0,05$) among different sampling periods of the *in vitro* assay ($n = 6$). C = fermented product control, without addition of fruit by-product; G = fermented product with addition of guava by-product; O = fermented product with addition of orange by-product; PF = fermented product with addition of passion fruit by-product.

FRB supplemented with orange by-products were able to increase by 1 log cycle the surviving population of the strain at the end of the assay in comparison to the control treatment (Fig. 3). On the 28th day of storage, the positive effect of fruit by-products on *L. casei* resistance under GI tract conditions was noted for the treatments containing orange and passion fruit by-products for all types of fermented products (Fig. 4). FRB supplemented with orange was the treatment that resulted in the best survival of probiotic strains at the end of the assay, on the last day of storage, which is an important feature for a probiotic product (Fig. 4).

The higher survival rate in the presence of fruit by-products is due to the physical protection provided by these ingredients, which have in their composition fibers resistant to digestive enzymes. The beneficial effect of other ingredients with high fiber contents, such as lychee and inulin (Kingwatee, Apichartsrangkoon, Chaikham, Pankasemsuk, & Changrue, 2014), inulin (Souza, Gioielli, & Saad, 2017) and açai pulp (Costa, Ooki, Vieira, Bedani, & Saad, 2017) on the population of probiotics after passing through the simulated conditions of the GI tract has been demonstrated.

There was a significant ($p < 0.05$) decrease in the population of *L. casei* Lc-1 after 360 min of assay under simulated GI tract conditions on all analyses days (Figs. 2–4). The type of substrate used for fermentation also affected significantly ($p < 0.05$) the survival of the strain, although to a lesser extent than the presence of fruit by-products. Considering only the control treatments (without adding by-products) FOB resulted in a higher population on the 1st and 14th of fermentation (Figs. 2 and 3). Furthermore, on the 28th of storage, the highest

survival rate was noted for FRB (Fig. 4).

4. Conclusion

The phytochemical contents of fruit by-products indicate that they have high levels of phenolic compounds, carotenoids and fibers, besides a high antioxidant activity, indicating their potential to be used as an added-value ingredient in health-enhancing food products. All types of substrates used supported the development of the bacteria during fermentation, as well as the maintenance of the population during storage of the product. Even though the inclusion of by-products did not have a significant effect on the probiotic population, it resulted in an increased tolerance of the probiotics to simulated GI tract conditions, especially orange and passion fruit by-products. Further studies evaluating the symbiotic fermented beverages on gut health, using dynamic or *in vivo* models, could be carried out to verify whether it modulates the microbiota composition and metabolites production. The supplementation with fruit by-products was demonstrated to be a viable alternative to producing probiotic fermented products, granting them a higher appeal towards consumers. These results encourage the application of waste from fruit industries, which help add value to such residues and reduce the environmental impact caused by their disposal.

Declarations of interest

None.

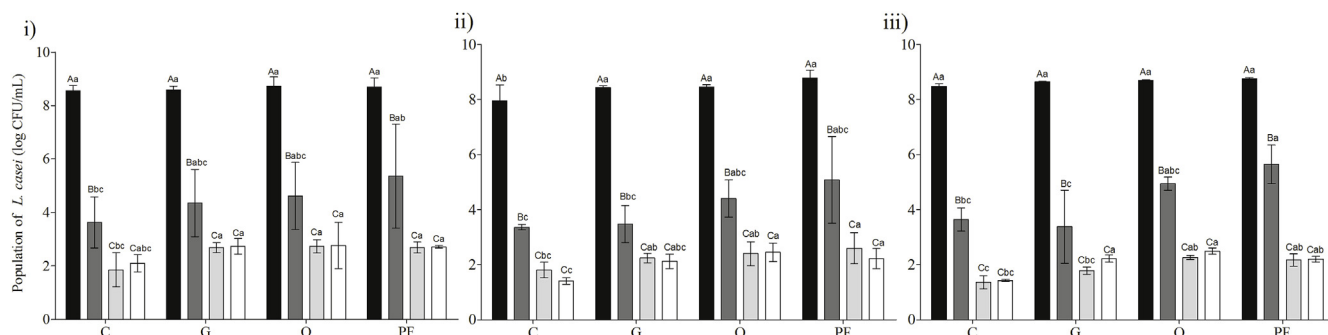


Fig. 3. Survival of *L. casei* Lc-1 (log CFU/mL) in fermented oat beverages, fermented rice beverages and fermented goat milk (i, ii and iii, respectively), before (■) and during exposure to simulated gastric conditions, for 120 min (■, pH 2.0–2.2) and enteric conditions, for 240 (■, pH 4.3–5.2) and 360 (□, pH 7.0–7.3) min, on the 14th day of storage. For the same sampling period of *in vitro* assay, different lowercase letters denote significant differences ($p < 0,05$) among treatments of all substrates. For each treatment, different capital letters denote significant differences ($p < 0,05$) among different sampling periods of the *in vitro* assay ($n = 6$). C = fermented product control, without addition of fruit by-product; G = fermented product with addition of guava by-product; O = fermented product with addition of orange by-product; PF = fermented product with addition of passion fruit by-product.

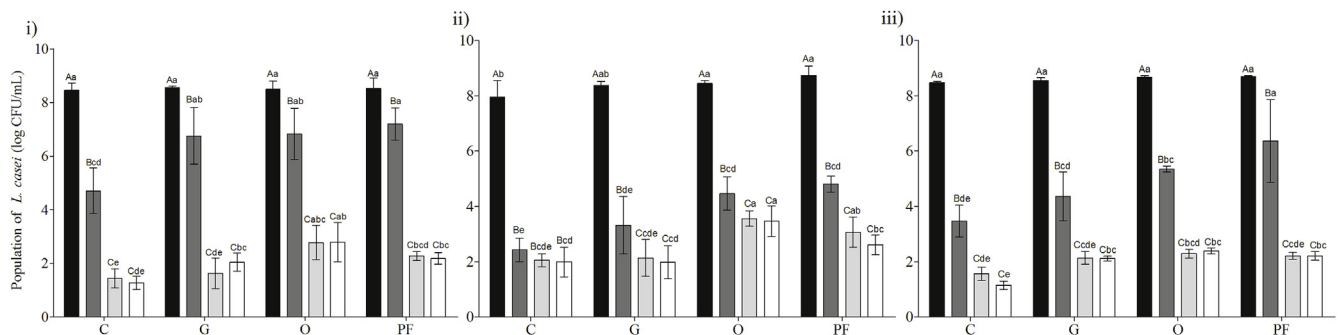


Fig. 4. Survival of *L. casei* Lc-1 (log CFU/mL) in fermented oat beverages, fermented rice beverages and fermented goat milk (i, ii and iii, respectively), before (■) and during exposure to simulated gastric conditions, for 120 min (■, pH 2.0–2.2) and enteric conditions, for 240 (■, pH 4.3–5.2) and 360 (□, pH 7.0–7.3) min, on the 28th day of storage. For the same sampling period of *in vitro* assay, different lowercase letters denote significant differences ($p < 0,05$) among treatments of all substrates. For each treatment, different capital letters denote significant differences ($p < 0,05$) among different sampling periods of the *in vitro* assay ($n = 6$). C = fermented product control, without addition of fruit by-product; G = fermented product with addition of guava by-product; O = fermented product with addition of orange by-product; PF = fermented product with addition of passion fruit by-product.

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