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Stability, sensory attributes and acceptance of panettones elaborated with *Lactobacillus fermentum* IAL 4541 and *Wickerhamomyces anomallus* IAL 4533

Raquel Facco Stefanello[a], Elizabeth Harumi Nabeshima[b], Aline de Oliveira Garcia[b], Rosane Teresinha Heck[a], Marcelo Valle Garcia[a], Leadir Lucy Martins Fries[a], Marina Venturini Copetti[a,*]

[a] Federal University of Santa Maria, Center of Rural Sciences, Department of Technology and Food Science, Avenida Roraima 1000, University City, Camobi, Santa Maria, RS, CEP 97105-900, Brazil
[b] Food Technology Institute, ITAL, C.P. 139, Campinas, SP, CEP 13078-170, Brazil

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ABSTRACT

This work aimed to elaborate sourdough panettones with *Lactobacillus fermentum* (LF) and *Wickerhamomyces anomallus* (WA) evaluating their microbiological stability, physical and chemical characteristics as well as the impact of these microorganisms on sensory aspects and consumer acceptance. For characterization, panettones were elaborated by long fermentation, using two selected microorganisms, LF and WA in different proportions; and control panettones were formulated using commercial yeast with and without preservative. For sensory analyses, LF, WA, LF/WA (1:1), control with calcium propionate and commercial panettones were compared. Regarding the pH values and total titratable acidity (TTA), a similar behavior was observed among panettones elaborated with the selected strains (LF, WA and mixtures). On the other hand, panettones elaborated with commercial yeast showed a higher pH and lower TTA. Until the 112nd day of storage, the water activity (aw) was similar among all panettones, but in the following analyses, it dropped in all panettones. Panettones elaborated with sourdough maintained better their softness during the storage, when compared with the controls. Regarding microbial stability, control panettones with and without preservative became moldy faster; while sourdough panettones (WA and LF/WA) remained stable throughout all the monitored storage. Sensory evaluation by CATA allowed distinguishing between sourdough and commercial yeast panettones. Desirable characteristics such as nice aroma, pleasant taste and uniform color were checked more often for LF and WA elaborated panettones, whereas yeast flavor was checked more often for the Commercial. Moreover, according to the descriptors used, panettones were grouped into 3 groups: LF/WA, LF + WA and controls. The panettones elaborated with the specific microorganisms of this study were well-accepted sensorially, proving to be very competitive with respect to control and commercial panettone. So, the use of selected microorganisms as a starter for sourdough is a promising alternative for producing panettones with good technological quality, microbiological stability, sensorially differentiate and well accepted by consumers; and, additionally, with the appeal of no added preservative.

1. Introduction

Fungal deterioration is the main problem regarding the shelf life of bread and baked goods. Chemical preservatives such as propionic, sorbic, and benzoic acid and its derivative salts are still widely used, but consumers are looking for more natural products. Several studies (Crowley & Mahony, 2013; Hassan, Zhou, & Bullerman, 2015; Valerio, Di Biase, Lattanzio, & Lavermicocca, 2016) have focused on the antifungal activity of compounds from natural sources and on biopreservation as effective alternatives to chemical preservatives. Recent studies (Hassan et al., 2015; Valerio et al., 2016) proposed a combined effect of sourdough fermentation, which extended the shelf life of bread.

The use of selected lactic acid bacteria and yeasts as starter cultures

Abbreviations: CATA, Check-All-That-Apply; IAL, Instituto Adolfo Lutz; YM, Yeast extract-mannitol; MRS, Man Rogosa Sharpe; OD, Optical density; CFU, Colony forming unit; D1-D7, Doughs (number 1 to 7); SD1-SD7, Sourdough (number 1 to 7); Aw, Water activity; TTA, Total titratable acidity; B.O.D, Biochemical Oxygen Demand; PDA, Potato Dextrose Agar

* Corresponding author.
E-mail address: mvc@smail.ufsm.br (M. Venturini Copetti).

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for industrial bread making, resembling the traditional sourdough fermentation, has been extensively explored during these last years (Salvucci, LeBlanc, & Pérez, 2016; Stefanovic, Fitzgerald, & Mcauliffe, 2017). Studies have shown that the use of *Lactobacillus fermentum* from brewing sourdough in bread is beneficial for controlling fungal deterioration in bread, which may extend the shelf life of these products (Fazeli et al., 2004). Species of *Wickerhamomyces anomallus* (formerly *Pichia anomala*) are known to control a range of postharvest fungi decreasing sporulation and mycotoxin production (Melin, Schnürer, & Håkansson, 2011).

The microbiological complexity of sourdough fermentation has the potential to exploit the technological, nutritional, functional and sensory features of wheat flours (Coda, Di Cagno, Gobbetti, & Rizzello, 2014). In particular, sourdough fermentation improves dough workability, bread structure and organoleptic and nutritional properties of flours (Rizzello, Calasso, Campanella, De Angelis, & Gobbetti, 2014). Adequate starter cultures are needed to exploit the potential of particular flour matrices. Thus, the use of sourdough in wheat bread retained their importance in contemporary bread production and is undergoing a revival of interest (Gänzle, 2014) as a means to improve the quality and flavor of bread (Najafi, Pourfarzad, Zahedi, Ahmadian-Kouchaksaraie, & Khodaparast, 2016). Mediterranean countries maintained the use of sourdough as a leavening agent for specialty products with unmatched quality, like panettone and colomba, sweetbreads consumed mainly at Christmas and Easter, respectively (Gänzle, 2014). Panettones are products with an upward trend, because of the sensoriality of this product, which appeals to different age groups and social classes (De Cristo et al., 2018), and has expanded for several countries besides Italy (Lattanzi et al., 2013). During the industrial production of panettone, the main challenges are both to produce dough with a capacity to hold fruits and raisins during proofing and baking and to obtain products that keep their quality during storage (Benejam, Steffolani, & León, 2009).

Panettones are products with a relatively long shelf life — they may last for months — and their quality is maintained during storage through the use of various additives such as oxidizing agents, emulsifiers and enzymes (Benejam et al., 2009). However, for increased the healthness, many studies on reformulation of bakery products, such as panettones, have been studied (Benejam et al., 2009; De Cristo et al., 2018), especially through the use of sourdough with selected strains with specific effects, as a way to reduce or eliminate chemical additive content, as well as to improve flavor and aroma (Chaván & Chaván, 2011; Ryan, Dal Bello, & Arendt, 2008).

Sensory analysis is an important tool to assist in developing new products, quality assurance and consumer satisfaction assessments (ASTM, 2014). Because of the limitations of some methods of measuring perceptions, methods such as Check-All-That-Apply (CATA) or "Check All That Matches" have been developed to quickly obtain product profiles through consumer perceptions, instead of trained teams. The evaluation consists of presenting a list of attributes and asking consumers to indicate words and phrases that adequately describe sensory attributes, emotional and/or hedonic responses, purchase intentions, product positioning, or other terms that consumers may associate with the sample (Meyners & Castura, 2014).

The main objective of this work was to evaluate the effect *of* L. *fermentum* and *W. anomallus* (formerly *Pichia anomala*) on the microbiological, physical, and chemical characteristics, as well as the impact of these strains on the sensorial aspects of long fermentation panettones. In this study, seven types of panettone, produced in a pilot plant with different yeasts, were compared. The seven sponges (mother sponge) differed in the type of sourdough used taking into account the microorganisms tested.

2. Material and methods

2.1. Microorganisms

The strains of microorganisms used throughout this study were *Lactobacillus fermentum* IAL 4541 and *Wickerhamomyces anomallus* IAL 4533, selected for their antifungal activities (Coda et al., 2011; Fazeli et al., 2004). The strains were isolated from lyophilized natural yeast produced from a blend of refined wheat flour and whole wheat flour for 14 days (Stefanello et al., 2018) and kept lyophilized by the Collection Nucleus of the Adolfo Lutz Institute (IAL).

2.1.1. Inoculum preparation

Actively growing single strains of L. *fermentum* IAL 4541 and *W. anomalus* IAL 4533 were inoculated (inoculum level 1.0%, *v*/v) into Erlenmeyer flasks containing 100 mL of YM or MRS broth, and incubated for 24 h at 25 and 30 °C, respectively (Paramithiotis, Chouliaras, Tsakalidou, & Kalantzopoulos, 2005). Twelve-hour cells were harvested (5000 x *g*, 15 min, 4 °C), washed twice with distilled sterile water and resuspended in distilled water to an optical density at 620 nm (OD_{620nm}) corresponding to about 10^{10} colony forming unit (CFU) per mL of L. *fermentum* IAL 4541 and 10^8 CFU/mL of *W. anomalus* IAL 4533. These inoculums were used as a starter culture in sourdough preparation.

2.2. Sourdough preparation

To prepare the sourdough, a seven-stage technique derived from a traditional procedure (Paramithiotis et al., 2005) and adjusted to this experiment was applied. Dough 1 (D1) was prepared by mixing the 50 mL of the starter culture with 100 g of wheat flour (Renata®, Selmi mill). After 24 h incubation at 28 °C, sourdough 1 (SD1) was formed.It was followed by successive mixing and incubation until reach the final sourdough 7 (SD7) on day 7 (Fig. 1).

2.3. Panettone formulation

From the sourdoughs produced (*L. fermentum* IAL 4541 and *W. anomalus* IAL 4533) a proportion of the microorganism was established in each treatment (Table 1). Thus, the treatments were composed of: LF: 100% *L. fermentum* IAL 4541 and 0% *W. anomalus* IAL 4533; 75 LF / 25 WA: 75% *L. fermentum* IAL 4541 + 25% *W. anomalus* IAL 4533; LF / WA: 50% *L. fermentum* IAL 4541 + 50% *W. anomalus* IAL 4533; 25 LF / 75 WA: 25% *L. fermentum* IAL 4541 + 75% *W. anomalus* IAL 4533; WA: 0% *L. fermentum* IAL 4541 and 100% *W. anomalus* IAL 4533; CP: 100% commercial yeast +0.5% calcium propionate; Control: 100% commercial yeast. (See Figs. 1–5.)

For panettone preparation, the ingredients were separated, weighed in a semi-analytical balance and subjected to semi-industrial process

Table 1
Description of the treatments with the % used of the *Lactobacillus fermentum* strains IAL 4541 (LF) and *Wickerhamomyces anomalus* IAL 4533 (WA) in the sourdough preparation. Control trials using the commercial yeast *Saccharomyces cerevisiae* with (CP) and without calcium propionate (Control) as an artificial preservative.

TREATMENTS	*L. fermentum* IAL 4541	*W. anomalus* IAL 4533	Commercial yeast *S. cerevisiae*	Calcium propionate
LF	100%	–	–	–
75LF/25WA	75%	25%	–	–
LF/WA	50%	50%	–	–
25LF/75 WA	25%	75%	–	–
WA	–	100%	–	–
CP	–	–	3%	0.5%
Control	–	–	3%	–

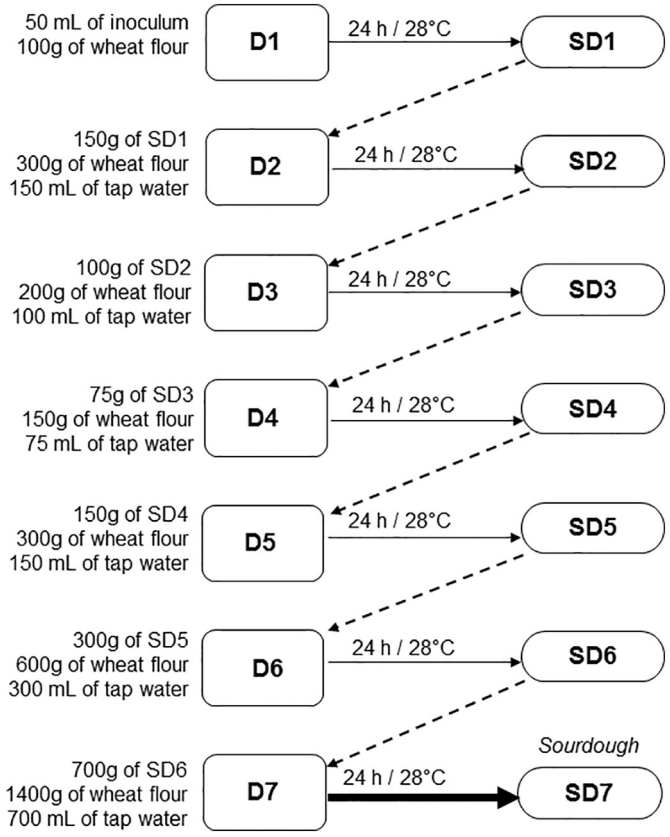

Fig. 1. Sourdough preparation. D = Dough; SD = Sourdough.

using a vertical spiral kneading trough with two speeds (A30 model, Indústria Máquinas para Panificação Progresso, Santa Teresinha, PR, Brazil) and turbo-electric oven (Vipinho 0448, Perfecta, Curitiba/PR, Brazil). The panettones were prepared using a previously tested, conventional formulation (Table 2), and the mixing process was the same for all panettones.

The ingredients in the sponge phase were mixed for 9 min at slow speed (190 rpm hook and 50 rpm bowl) and 2 min at high speed (380 rpm hook and 100 rpm), while the mass phase mixture took 7 min at slow speed and 4 min at fast speed for gluten network to form. The masses were divided in portions of 550 g each and were left to rest in the climatic Evolution proofer (Super Freezer, Poços de Caldas, MG, Brazil) until they doubled in size at a controlled temperature of 32 °C / 80%, relative humidity. Afterwards they were supplied in oven Vipinho 0448 (Perfecta, Curitiba/PR, Brazil) at 180 °C for 50 min. Fig. 2 describes the panettone flowchart.

2.4. Physicochemical analysis

2.4.1. Instrumental firmness analysis

The firmness of the panettones was determined by the texturometer SMS, model TA-XT2i (Godalming/Surrey, UK), according to the method 74–09.01 (AACCI, 2010). For the analysis, the probe P36R and the platform HPD 90 were used in the following operating conditions: compression force measurement, pre-test speed: 1.0 mm/s, test speed: 1, 7 mm/s, post-test speed: 10.0 mm s, penetration distance 40%. Ten readings of each sample were performed. The readings were performed every 28 days for 140 days (1, 28, 84, and 140° day).

2.4.2. Specific volume

The specific volume of the panettones, 2 h after the cooking, was evaluated by the rapeseed method according to AACCI 10.05.01 (AACCI, 2010) using the Vondel Mill brand volume meter (Model MDMV 03 / MVP 1300, Series 60, Vondel Indústria e Comércio de Máquinas e Componentes Ltda., São José dos Pinhais, PR, Brazil). The specific volume was calculated by the ratio between volume and weight of the panettones (cm^3/g). The analyses were performed in quadruplicate.

2.4.3. Color analysis

The color analysis of the panettones was performed on a Konica Minolta CR450 colorimeter at room temperature under the following conditions: illuminant D65, observation angle: 10° for the analysis of crumb color. The results were expressed in the CIELab system. The colorimeters measure color using three parameters: L*, ranging from 100 (white) to zero (black); b*, ranging from blue (negative) to yellow (positive); and a*, that varies from green (negative) to red (positive). The results were the average of three readings.

2.4.4. Moisture analysis

The moisture analysis of the panettones was performed in two phases. In the first phase, method 62–05.01 (AACCI, 2010), the panettone slices were chopped and homogenized (crumb and crust), weighed 10 g sample in tared aluminum capsules, heated to an oven at 70 °C / 5 h, cooled and weighed. In a second step, method 44–15.02 (AACCI, 2010), the dry residue was ground in a gral, weighed 2 g of sample in a tared aluminum dish, heated to 130 °C / 1 h, cooled and weighed. This analysis was performed with 3 replicates.

2.4.5. Water activity (aw)

For Aw, the panettone slices were chopped and homogenized, and carried out the direct measurement in an AquaLab 4TEV water activity analyzer, at a temperature of 25 °C. The water activity was monitored for 140 days, with readings performed on days 1, 14, 28, 42, 56, 70, 84, 98, 112, and 140.

2.5. pH and total titratable acidity (TTA)

The pH and TTA were determined on 10 g of panettone homogenized with 90 mL of deionized water at room temperature (Gamel, Abdel-aal, & Tosh, 2015) at days 1, 28, 56, 84, 112, and 140. The pH was measured using a pH meter (Digimed®, DM-22, SP, Brazil) and TTA was determined by the standard method 02–31.01 (AACCI, 2010) using 0.1 M NaOH (expressed in mL of NaOH) and phenolphthalein as indicator (pH 8.3). These analyses were carried out during 14 days.

2.6. Study of shelf-life and microbiological analyses of panettones

The panettones were stored in a chamber type B.O.D (Biochemical Oxygen Demand) at 28 °C/75% RH and monitored for up to 140 days after manufacture. The microbiological counts, expressed in CFU/g, of molds and yeasts, were determined on days 28, 98, 112, and 126 after manufacture. Approximately 10 g of sample were 10-fold diluted with 9 g/L NaCl solution [NaCl (Merck, Darmstadt, Germany), 0.85%, m/v; peptone (Oxoid, Basingstoke, Hampshire, United Kingdom), 0.1%, m/v] and homogenized in a Lab Blender Stomacher (Seward Medical, London, UK) for 2 min. Decimal dilutions were performed and plated onto Potato Dextrose Agar (PDA) Agar (Himedia, Mumbai, India) and tartaric acid 10% under aerobic conditions at 25 °C for 72 h (Aplevicz et al., 2014).

2.7. Sensory evaluation

The sensory evaluation was performed for the LF, LF/WA, WA, CP, and commercial panettone (PCOM). PCOM was purchased locally. This study was approved by the Research Ethics Committee of the Federal University of Santa Maria (RS, Brazil) (CAAE: 46155415.6.0000.5346). A three-digit code was assigned to the samples, which were evaluated by each consumer in a monadic order, following a balanced design

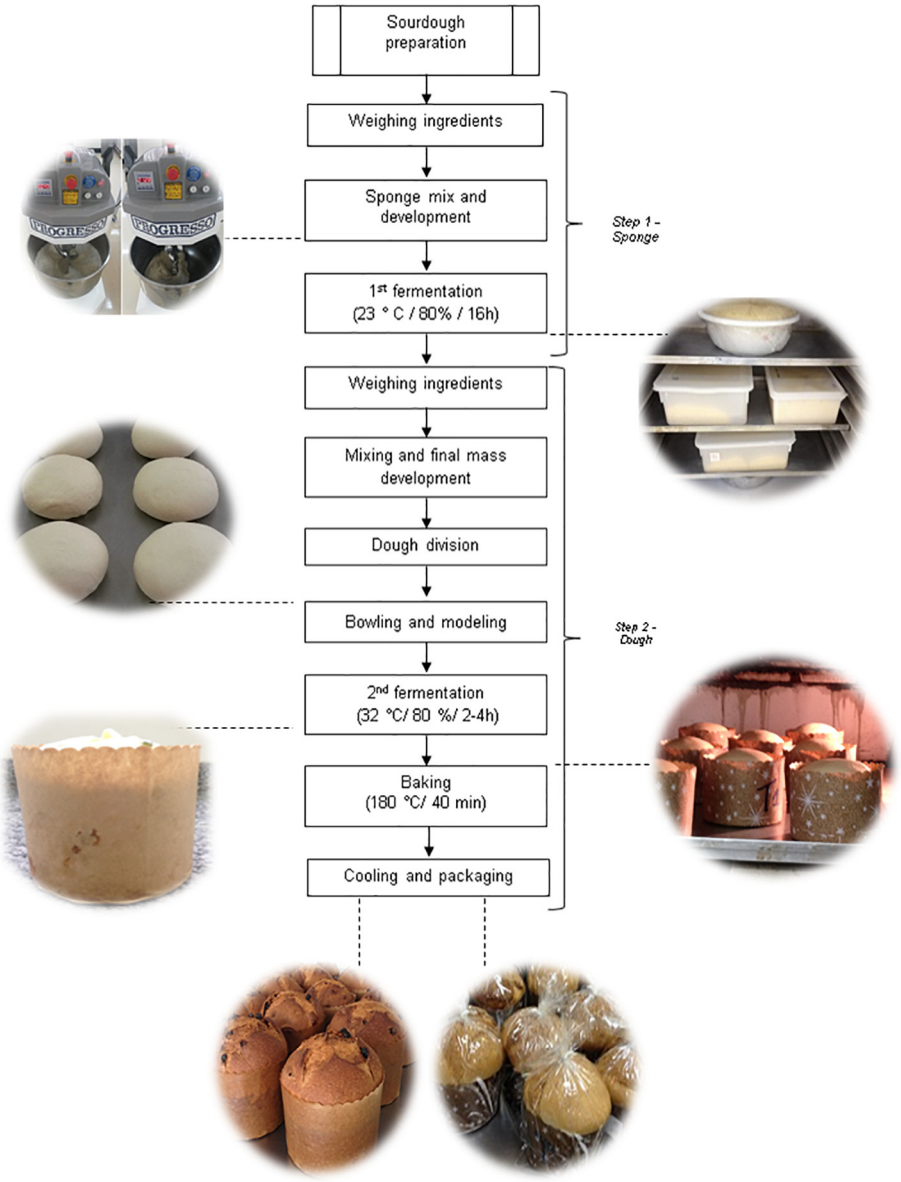

Fig. 2. Preparation of panettones.

(Ares et al., 2016). The tests were performed in individual booths with fluorescent lighting according to Heck et al. (2017). Panettones were cut into 7 × 5 cm and water at room temperature was provided to consumers for palate cleansing.

Participants were recruited from the Federal University University of Santa Maria (UFSM) among students, staff, and visitors, through invitations via social networks, emails, and posters. They were selected according to their habit of panettone consumption, interest in the study, and availability to participate in the sensory tests. One hundred and forty consumers of panettones (66% female and 34% male, aged from 17 to 60 years) participated in the sensory tests.

2.7.1. Check-all-that-apply (CATA) evaluation

The CATA questions were composed of 23 sensory terms divided into two groups: defects (Mustiness aroma, Holes in the core, Remaining taste, Pasta devoid of flavor,Excessively aromatic,Alcohol aroma, Bitter taste, Raw pasta, Few candied fruits and raisins, Yeast flavor, Aroma of preservative, Crumbly and dry crumb and qualities (Alveoli elongated and of uniform size, Nice aroma, Uniform color, Candy, Candied fruits and raisins in the proper amount, Soft taste, Sweet aroma, Proper texture, Soft, Pleasant taste, Moist crumb).

Sensorial terms used in the CATA test were suggested by a small group of experienced professionals who worked with sensory analysis of panetones and were selected based on previous research (Bianchini, 2004) and preliminary studies (Ares & Jaeger, 2013, 2015; Jaeger et al., 2015). The use of a trained panel for the choice of sensory terms in some cases may be interpreted as a disadvantage because of the high cost and delay in having the results. Another important aspect is that the attributes assigned by a trained panel can be quite distant from the reality and the understanding of the general consumers (Cruz et al., 2013).

Participants were asked to select how many terms they considered appropriate to describe the samples in a questionnaire containing the sensorial attributes according to methodology proposed by Torres et al. (2017). To avoid tendencies, the position of the terms in the questionnaire was balanced according to a methodology recommended by Ares and Jaeger (2013).

2.7.2. Acceptance tests

Consumers were instructed to evaluate the panettones samples with

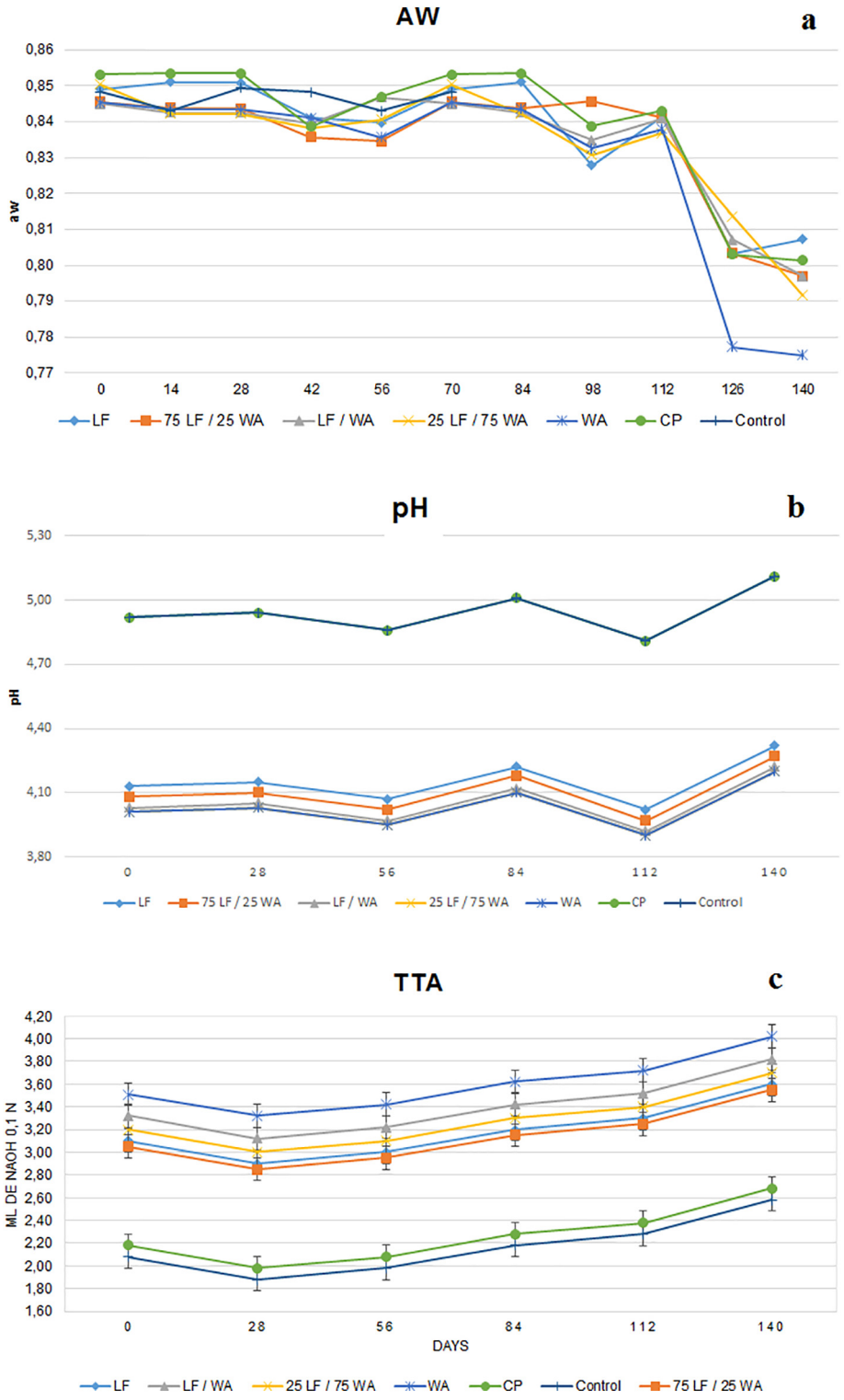

Fig. 3. Water activity - Aw (a), pH (b) and titratable total acidity - TTA (c) of the panettones over 140 days of storage.

respect to the degree of liking of the color, odor, flavor, softness and moisture using a 9-point hybrid hedonic scale (1 = disliked immensely, 9 = liked immensely). This hedonic testing had been used previously by Dantas et al. (2016). To purchase intent it was used a 5-point scale (5 = definitely buy, 3 = may buy or may not buy, 1 = definitely would not buy) (Meilgaard, Civille, & Carr, 2006).

2.8. Statistical analyzes

The whole experiment was repeated three times. Data (except sensory evaluation) were analyzed by analysis of variance (ANOVA) using a general linear model considering the treatments as a fixed effect and the replicates as a random effect (n = 3). Tukey's test was used at the 5% level of significance. Pearson's test was applied for correlation analysis using a statistical program (StatSoft, 2007). Cochran's Q test, equivalent to multiple McNemar pairwise comparisons, and Analysis of

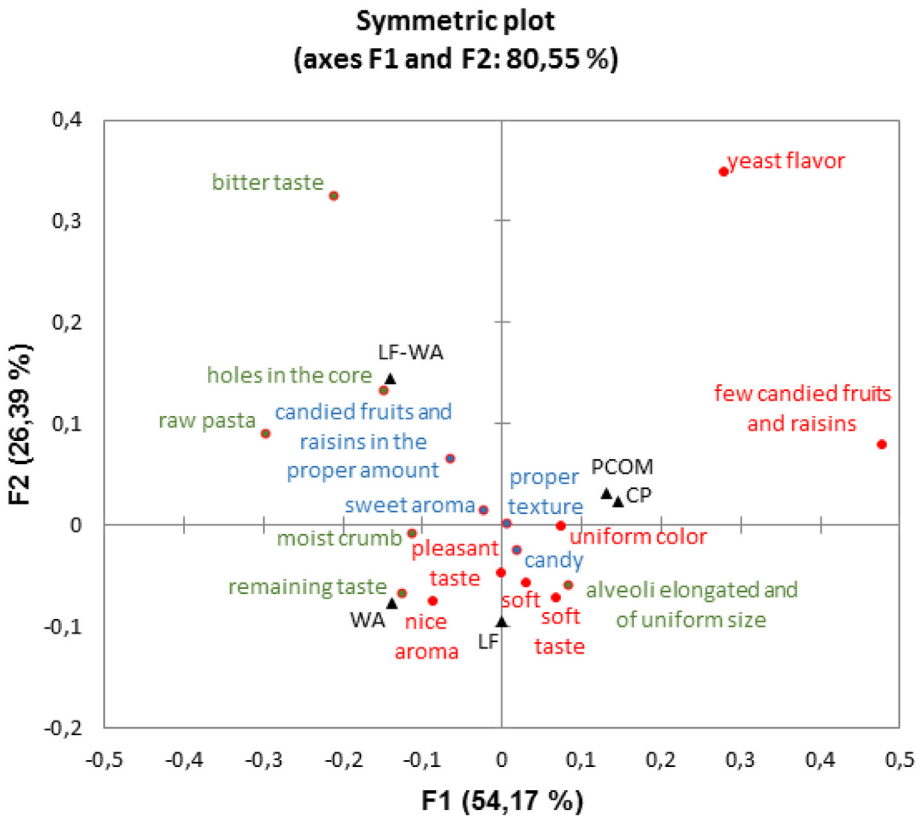

Fig. 4. Correspondence Analysis of panettones made with *Lactobacillus fermentum* IAL 4541 (LF), *Wickerhamomyces anomalus* IAL 4533 (WA) and commercial yeast *Saccharomyces cerevisiae* with (CP) and without calcium propionate (Control) as artificial preservative.

Correspondence, considering the chi-square distance were used to analyze data from CATA questionnaire (Vidal, Tárrega, Antúnez, Ares & Jaeger, 2015), calculated on the matrix containing the frequency of use of each term for each sample. Multidimensional Alignment (MDA) for analysis of CATA test was carried out according to Esmerino et al. (2017). For the consumer test, a two-way ANOVA (consumers x samples) followed by Tukey's test at 5% significance level ($p < .05$) was carried out.

Correlation analyses were carried out with the software R environment, version 3.2.4 (R Development Core Team, 2016). Pearson's correlation coefficients (r) were calculated to identify possible associations between the attributes of the different elaborated panettones and the buy intention. Interpretation of values was performed according to Pearson's coefficient (r) which are: very weak $0.000 \leq r \geq 0.200$; weak $0.201 \leq r \geq 0.400$; moderate $0.401 \leq r \geq 0.600$; strong $0.601 \leq r \geq 0.800$ and very strong $0.801 \leq r \geq 1000$ (Christmann & Badgett, 2009).

3. Results and discussion

3.1. Specific volume, color, moisture, and firmness of panettones

In bakery products, attributes such as texture, specific volume, color and moisture are fundamental characteristics appreciated by consumers (Valcárcel-Yamani & Lannes, 2013). The results of instrumental firmness are presented in Table 3, with values ranging from 3.27 to 6.87 N over 140 days, both values belonging to the control sample with calcium propionate. For the remaining treatments, the firmness, in general, the variation over the storage time was small. The softness of panettones made with LF and 75LF / 25WA did not show significant differences over time, whereas, the panettones made with commercial yeast with and without calcium propionate (CP and Control) showed the greatest softness among all the treatments. However, from day 84, the treatments that demonstrated the highest averages were the CP and Control. At the end of the analysis period (140 days), the best treatments among the panettones made with sourdough were with the proportions of 50% *L. fermentum* IAL 4541 and *W. anomalus* IAL 4533

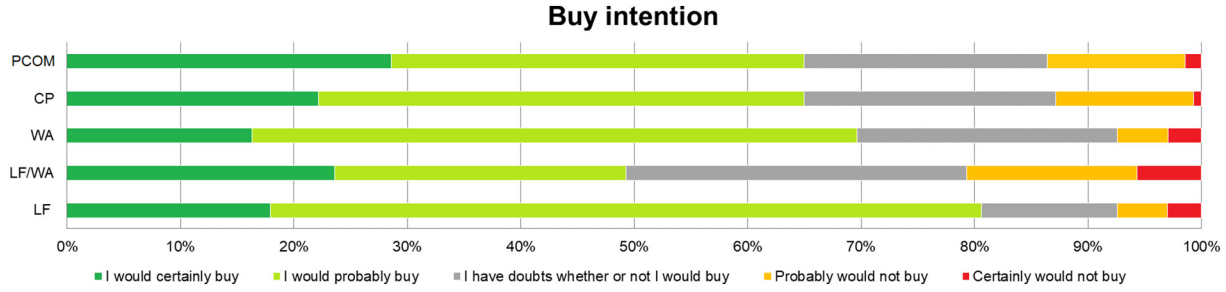

Fig. 5. Results of the Buy intention of panettones made with *Lactobacillus fermentum* IAL 4541, *Wickerhamomyces anomalus* IAL 4533 and commercial yeast *Saccharomyces cerevisiae* with calcium propionate (CP) as artificial preservative and commercial panettone.

Table 2
Panettone formulations.

Ingredients	*Step 1 - Sponge % (b.f.)***
Wheat flour	76.0
Enzima Spring	0.045
Ascorbic acid	0.005
Datem	0.5
Monoglycerides	2.0
Gluten	3.5
Pasteurized Gems	9.5
Dried malt	1.0
Sourdough *	0 e 30.0
Commercial Sponge **	0 e 30.0
Water	38.0
Sugar	20.5
	Step 2 - Dough % (b.f.)
Wheat flour	24.0
Calcium propionate *	0.0–0.5
Margarine	15.0
Sugar	17.5
Ascorbic acid	0.008
Salt	1.0
Sorbitol	0.15
Panettone Aroma	0.35
Fruit Blend	50.0

* Sourdough and Calcium propionate as per Table 1. ** Commercial sponge prepared with 3% commercial yeast *Saccharomyces cerevisiae*, 60% water and 40% wheat flour 15 min before preparation of CP and Control panettones (Table 1). *** b.f. = base flour, i.e., the weight of the ingredients was calculated based on the total weight of the wheat flour.

and 25% *L. fermentum* IAL 4541 and 75% of *W. anomalus* IAL 4533.

Through the Pearson correlation analysis it was possible to observe that firmness at 1 day correlated negatively with the specific volume ($r = -0.91$), moisture ($r = -0.91$), lightness ($r = -0.91$), and b* ($r = -0.91$). The results are consistent with other studies (Benejam et al., 2009; Spier, Rapacci, Dutcosky, Tedrus, & Souza, 2007), which explain these correlation due to the panettones with lesser firmness or softer are obtained from bakery products with better crumb structure or more aerated, in consequence resulting in crumb with greater lightness and high humidity. The lightness is explained because the larger or more porous alveoli reflect more light. While the crumb moist and softness is related with the freshness of a product.

In products with complex formulations such as panettone, the texture is affected by the fat and sugar content, and the addition of xylanase counteracted to produce softer panettones when compared with the control (Benejam et al., 2009). The panettones added of xylanase from the Benejam et al. (2009) study presented minimum values of 8 Newton for the instrumental texture after six days of storage at room temperature; on the other hand, the panettones from our study made with L. *fermentum* IAL 4541 and *W. anomallus* IAL 4533 were softner along all one hundred and forty days of storage at room temperature, presenting values between 5.04 and 6.09 (Table 3).

Valcárcel-Yamani, Lannes, and Da (2013) analyzed nine trade panettone brands and obtained values of instrumental firmness ranging from 2.14 to 7.55 N. Considering that in this study the panettones were purchased and evaluated with a time of estimated from 30 to 90 days.

The results of specific volume, moisture and color (L*, a* and b*) are described in Table 4. Regarding the specific volume, the values obtained were similar, with variation ranging from 2.88 to 3.25 cm^3/g, and the lowest values were obtained in the 100% LF and in the control without calcium propionate (Control). These results are probably due to higher CO_2 production during the fermentation phase (Gerardo-rodríguez et al., 2017) and subsequent expansion of these gases during the cooking, which were imprisoned inside the gluten network. The treatments 75LF / 25WA, 25LF / 75WA, LF / WA and WA did not differ statistically ($p > .05$) between each other and with CP treatment. The treatment with 100% *L. fermentum* IAL 4541 (LF) had the lowest

Table 4
Specific volume, moisture and instrumental color of panettones made with the strains of *Lactobacillus fermentum* IAL 4541 (LF) and *Wickerhamomyces anomalus* IAL 4533 (WA) and panettones using the commercial yeast *Saccharomyces cerevisiae* with (CP) and without calcium propionate (Control) as an artificial preservative.

Treatments	Specific volume (g/cm^3)***	Moisture (g/100 g)	Instrumental color		
			L_*	a_*	b_*
LF	2.88^{c}	25.31^{a}	76.90^{bc}	-0.25^{b}	23.12^{d}
75LF/25WA	3.15^{ab}	24.56^{ab}	76.60^{c}	0.002^{a}	22.76^{d}
LF/WA	3.25^{a}	23.91^{b}	77.03^{bc}	-0.15^{ab}	22.93^{d}
25LF/75WA	3.10^{ab}	24.50^{ab}	77.23^{b}	-0.11^{ab}	23.01^{d}
WA	3.21^{ab}	24.93^{ab}	77.21^{b}	-0.02^{a}	23.67^{c}
CP	3.06^{abc}	25.36^{a}	77.79^{a}	-0.15^{ab}	24.39^{b}
Control	3.04^{bc}	24.95^{ab}	74.90^{d}	-0.06^{a}	25.18^{a}
SEM	0.063	0.421	0.173	0.059	0.146
Sig	**	*	**	**	**

a Mean values in the same column not followed by a common letter differ significantly ($p < .05$). SEM: standard error of the mean.
Sig.: significance.
*** $P < .001$.
** $P < .01$.
* $P < .05$.

Table 3
Monitoring the instrumental firmness of panettones during 140 days of storage.

Treatments	Firmness Instrumental (N)							
	0 day	28 day*	56 day	84 day	112 day	140 day	SEM	Sig
LF	6.41^{Ba}	6.45^{Ba}	12.73^{Aa}	6.71^{Ba}	5.29^{Bab}	5.54^{Ba}	0.595	***
75LF/25WA	5.63^{Bb}	5.84^{Ba}	10.18^{Ab}	6.46^{Bab}	5.71^{Bab}	5.54^{Ba}	0.450	***
LF/WA	4.28^{Ccd}	5.76^{Ba}	10.94^{Ab}	5.89^{Bb}	4.68^{BCb}	4.84^{BCa}	0.470	***
25LF/75WA	4.54^{Dc}	5.97^{Ba}	9.55^{Aab}	5.01^{CDc}	4.92^{CDab}	5.04^{Ca}	0.157	***
WA	4.40^{Cc}	5.72^{BCa}	8.42^{Ac}	6.29^{Bab}	4.93^{BCab}	4.26^{Ca}	0.418	***
CP	3.27^{Be}	6.33^{Aa}	5.90^{Ad}	6.87^{Aa}	5.51^{Aab}	6.63^{Aa}	0.513	***
Control	3.69^{Dde}	3.51^{Db}	5.63^{Bd}	5.26^{Bc}	6.68^{Aa}	4.64^{Ca}	0.183	***
SEM	0.209	0.261	0.507	0.203	0.570	0.767		
Sig	***	***	***	***	**	n.s.		

A Mean values in the same row not followed by a common capital letter differ significantly ($P < .05$). NS: not significant. [a] Mean values in the same column not followed by a common lowercase letter differ significantly ($P < .05$). SEM: standard error of the mean. Sig.: significance.
*** $P < .001$.
** $P < .01$.
* $P < .05$.

specific volume (2.88 cm^3/g). The control treatment, although elaborated only with commercial yeast, did not produce a good result of specific volume, being below the expectations. The panettones elaborated with the strain *W. anomalus* IAL 4533 presented the largest volumes. This can be explained by the fact that this yeast adapts easily in a sourdough ecosystem, thus producing more CO_2 (Heide-Marie, Moons, Huret, Vrancken, & De Vuyst, 2011). In the Valcárcel-Yamani et al. (2013) study, the nine brands of commercial panettone presented values from 3.18 to 5.13 cm^3/g.

Based on the values obtained for the lightness (L*) represented in Table 4, all treatments showed values of L* above 70, which represents good sensory acceptability, according to Purlis (2011). CP treatment was the clearest, although the variation between treatments was small (74.90 to 77.79) (Table 4). According to one study reporting the effect of sourdough addition on mass (Torrieri, Pepe, Ventorino, Masi, & Cavella, 2014), we observe a correlation between the treatments using acid mass. However, the treatment that had its color influenced in the fermentation was the Control (L*: 74.90). Although the Control treatment was made only with commercial yeast, it presented the darkest color of the crumb disagreeing with Andrade (2017) study that obtained the highest values of L* for samples made with dry commercial yeast. The presence of enough sugar in the formulation of the panettones accelerates the reaction of Maillard, leading to a progressive darkening of the crust and crumb (Benejam et al., 2009; Valcárcel-Yamani et al., 2013). With respect to the negative values of a*, we can infer that the samples of panettones showed slightly reddish tones and based on the positive values of b*, the samples approach more of the yellow tint (Prati, Moretti, & Cardello, 2005).

Comparing the mean values of the moisture content of panettones (Table 4) with a study by Valcárcel-Yamani et al. (2013) on Brazilian trademarks, the results of this work ranged from 26.4 to 23.1%. The moisture values for the panettones produced with sourdough (LF, 75LF / 25WA, LF / WA, 25LF / 75WA, WA) varied from 23.90 to 25.31 and for panettones made with commercial yeast (CP and Control), there was a slight increase in this parameter (24.95 and 25.36).

3.2. Water activity, pH, and titratable total acidity

Fig. 3 shows the water activity results - Aw (a), pH (b) and titratable total acidity - TTA (c). The water activity suffered a sudden drop in values from day 112. Taking a general evaluation from the beginning of the monitoring until the 140 days, during the first 28 days the treatment using calcium propionate as a preservative (CP) presented the highest values of Aw. After 42 days, the observed mean values varied widely between the treatments using sourdough. The treatment using commercial yeast (Control) obtained the lowest value for this parameter, with a marked drop after 112 days of storage.

In relation to the pH values (Fig. 3 - b) monitored during the 140 days, we observe very similar behavior with the treatments from the mixture of the selected strains (LF; 75LF / 25WA; LF / WA; 25LF / 75WA; WA) obtaining pH values between 3.90 and 4.34. Panettones made with commercial yeast (Control and CP) showed higher pH values ranging from 4.79 to 5.13.

This separation in groups (natural yeast group and commercial yeast group) was also verified for TTA as shown in Fig. 3 - c. TTA values were higher, as expected, for 100 LF, 75LF / 25WA, LF / WA, 25LF / 75WA and 100 WA treatments assuming values (minimum and maximum) of 2.8 and 4.1, respectively, during the monitoring period (values expressed in mL of 0.1 N NaOH). In contrast, the lowest values of TTA 1.9 to 2.7 (in mL of 0.1 N NaOH) were found in panettones obtained with commercial yeast (Control) and the addition of calcium propionate (CP), as shown in Fig. 3 - c. According to the results presented in Fig. 3–c, the titratable total acidity is within the maximum range established by the legislation, that is, 6 mL.

Table 5
Microbiological counts of molds and yeasts of panettones made with *Lactobacillus fermentum* IAL 4541 (LF), *Wickerhamomyces anomalus* IAL 4533 (WA) and commercial yeast *Saccharomyces cerevisiae* with (CP) and without calcium propionate (Control) as an artificial preservative during 140 days of storage.

Treatments	Microbial counts[a]			
	After 28 days	After 98 days	After 112 days	After 126 days
LF	< 1	3114	*	*
75LF/25WA	< 1	< 1	2.301	*
LF/WA	< 1	< 1	< 1	2.199
25LF/75WA	< 1	< 1	2.176	*
WA	< 1	< 1	< 1	2.438
CP	< 1	< 1	3.000	*
Control	1.477	*	*	*

[a] (Log CFU/ml).
* Moldy. < 1: < 1 log CFU/mL.

3.3. Shelf life and microbiological analyses of panettones

Table 5 provides data describing the microbiological growth of molds and yeasts in panettones. Microbiological counts of molds and yeasts were expressed in Log CFU/mL. The control panettones (Control) reached only 56 days of shelf life, while the LF / WA and WA treatments remained microbiologically stable throughout the monitoring period (> 126 days). Treatment with the addition of calcium propionate (CP) preservative becomes moldy after 112 days.

Comparing with the results of the water activity (Fig. 3 - a) it is possible to say that during the whole experiment, panettones presented high aw values (0.77) and therefore were susceptible to fungal deterioration (Zanqui et al., 2014). So, the extended shelf life of sourdough panettones could be attributed to a wide variety of organic acids and low molecular weight metabolites produced by microorganisms during the fermentation process (Hassan et al., 2015).

3.4. Check-all-that-apply (CATA)

The attributes mustiness aroma, pasta devoid of flavor, crumbly and dry crumb, excessively aromatic, alcohol aroma and aroma of preservative were checked for < 15% of the consumers and were not considered for these analyses. Table 6 shows the CATA data by the frequency of citations and the Cochran Q test, where significant differences were observed between the samples. Considering the frequency of each attribute, shown in Table 6, it was possible to obtain a description of the samples, where the LF-WA treatment was characterized by the nice aroma, uniform color, soft taste, and softness. Similar results were obtained by Meyners, Castura, and Carr (2013) when they studied whole-grain bread using the CATA test methodology.

The task of understanding how consumers perceive food products is difficult and challenging for industry researchers and should not be underestimated (Fonseca et al., 2016). CATA is a fast methodology, which represents consumer perception. Cadena et al. (2014) concluded that this methodology obtained information similar to the classic descriptive analysis with trained evaluators.

As shown in Fig. 4, sweet aroma was positively correlated with the acceptance of a product (LF-WA) and yeast flavor was negatively related to the Comercial sample and CP samples of panetones. However, for many other sensory attributes, each consumer has a level of particular preference for a particular food. In a study it was found that products with high intensity of positive attributes or with less intensity compared to another product will lose consumer acceptance (Fonseca et al., 2016).

Fig. 4 shows the Correspondence Analysis (CA). This CA explained 80.55% of the total variance, with 54.17% and 26.39% in the first and second dimensions, respectively. These analyses indicate that the

Table 6
CATA data by citation frequency and Cochran's Q test, equivalent to multiple McNemar pairwise comparisons.

Attributes	*p*-values	Panettones				
		CP	LF	LF/WA	PCOM	WA
Alveoli elongated and of uniform size	0.215	0.100[a]	0.136[a]	0.086[a]	0.171[a]	0.143[a]
Nice aroma	0.000	0.579[ab]	0.750[b]	0.557[a]	0.486[a]	0.743[b]
Holes in the core	0.342	0.121[a]	0.129[a]	0.193[a]	0.157[a]	0.186[a]
Uniform color	0.018	0.671[ab]	0.707[b]	0.529[a]	0.657[ab]	0.586[ab]
Remaining Taste	0.198	0.143[a]	0.236[a]	0.179[a]	0.150[a]	0.214[a]
Candy	0.541	0.321[a]	0.321[a]	0.257[a]	0.314[a]	0.350[a]
Candied fruits and raisins in the proper amount	0.401	0.564[a]	0.593[a]	0.643[a]	0.557[a]	0.643[a]
Soft Taste	0.006	0.471[ab]	0.564[b]	0.343[a]	0.464[ab]	0.421[ab]
Sweet aroma	0.592	0.307[a]	0.400[a]	0.343[a]	0.364[a]	0.357[a]
Proper texture	0.648	0.486[a]	0.521[a]	0.436[a]	0.464[a]	0.500[a]
Bitter taste	0.011	0.100[a]	0.121[a]	0.214[a]	0.093[a]	0.093[a]
Raw pasta	0.055	0.093[a]	0.093[a]	0.150[a]	0.071[a]	0.164[a]
Soft	0.001	0.486[ab]	0.650[b]	0.436[a]	0.621[b]	0.586[ab]
Few candied fruits and raisins	0.000	0.286[b]	0.171[ab]	0.093[a]	0.243[b]	0.071[a]
Pleasant taste	0.007	0.521[a]	0.693[b]	0.486[a]	0.550[ab]	0.579[ab]
Yeast flavor	0.006	0.114[ab]	0.057[ab]	0.107[ab]	0.157[b]	0.043[a]
Moist crumb	0.073	0.107[a]	0.200[a]	0.186[a]	0.221[a]	0.229[a]

a Mean values in the same column not followed by a common letter differ significantly ($p < .05$). CP: 100% *S.cerevisiae* + 0.5% calcium propionate; LF: 100% *L. fermentum*; LF/ WA: 50% *L. fermentum* + 50% *W. anomalus*; PCOM: commercial panettone; WA: 100% *W. anomalus*.

characteristics of candy, candied fruits and raisins in the proper amount, sweet aroma and proper texture were checked by > 25% of the consumers for all the samples. The characteristics perceived as different ($p < 0,05$) by the consumers are described below: the aromas of LF and WA were nicer than the aromas of LF/WA and PCOM sample; the color of LF was more uniform than the color of LF/WA; the taste of LF was considered softer than the taste of LF/WA; the LF and PCOM samples were considered softer than LF/WA; the CP and PCOM samples had fewer candied fruits and raisins than LF/WA; the taste of LF was more pleasant than the taste of LF/WA and CP; the yeast flavor of the PCOM sample was more intense than the WA. The others attributes were mentioned for < 25% of the consumers and did not differ the samples. These results suggest that the application of CATA as a sensorial evaluation method was able to clearly express the main categories responsible for the characterization of different panettones formulated with sourdough according to the consumers' percept.

According to Esmerino et al. (2017), Multidimensional Alignment (MDA) can constitute a practical and quantitative way to express the associations between attributes and products and therefore, to evaluate the data of descriptive analyses such as CATA. Thus, MDA was applied to determine the cosine values between vector pairs (product vector × descriptors vectors) originating in the CA of CATA questions. The descriptors that were correlated (positively or negatively) with the samples using the first two dimensions of the CA bidimensional map values are highlighted in Table 7.

3.5. Acceptance tests

The results of the acceptance test performed after 1 day of manufacture are presented in Table 8. The results on color, odor, flavor, softness and moisture attributes show that panettones were well accepted. On average, consumers referred to the global sensory characteristics of the product as “moderately liked” in the scale used. Regarding the color, it can be observed that the panettone made with LF / WA presented the lowest mean (7.25) and PCOM the best mean (7.71). However, for the odor attribute, it was possible to note that the

Table 7
Table containing the values of the cosine between vectors pairs (product vector vs sensory terms used to describe the samples vector) obtained by Correspondence Analysis (CA) for samples of five panettones elaborated with different micro-organisms in CATA questions.

Attributes	Panettones				
	CP	LF	LF/WA	PCOM	WA
Alveoli elongated and of uniform size	0.67	0.57	-1.00[b]	0.49	−0.44
Mustiness aroma	0.99[a]	−0.39	−0.48	1.00[a]	−0.99[b]
Nice aroma	−0.79[b]	0.77[a]	0.01	−0.91[b]	0.93[a]
Holes in the core	−0.68	−0.56	1.00[a]	−0.50	0.45
Uniform color	0.94[a]	0.12	−0.85[b]	0.84[a]	−0.81[b]
Remaining taste	−0.94[b]	0.54	0.32	−0.99[b]	1.00[a]
Candy	0.76[b]	0.47	−0.98[b]	0.59	−0.54
Pasta devoid of flavor	0.91[a]	−0.61	−0.24	0.98[a]	−0.99[a]
Candied fruits and raisins in the proper amount	−0.67	−0.58	1.00[a]	−0.48	0.43
Crumbly and dry crumb	0.94[a]	0.12	−0.85[b]	0.84[a]	−0.81[b]
Excessively aromatic	−0.82[b]	−0.38	0.96[a]	−0.67	0.62
Soft taste	0.58	0.66	−1.00[b]	0.39	−0.33
Sweet aroma	−0.98[b]	0.00	0.78[a]	−0.90[b]	0.88[a]
Alcohol aroma	0.16	−1.00[b]	0.67	0.37	−0.43
Proper texture	0.55	0.69	−1.00[b]	0.35	−0.30
Bitter taste	−0.50	−0.73[b]	0.99[a]	−0.29	0.24
Raw pasta	−0.91[b]	−0.19	0.89[a]	−0.80[b]	0.76[a]
Soft	0.14	0.93[a]	−0.86[b]	−0.08	0.14
Few candied fruits and raisins	1.00[a]	−0.19	−0.65	0.97[a]	−0.95[b]
Pleasant taste	−0.29	1.00[a]	−0.57	−0.49	0.54
Yeast flavor	0.72[a]	−0.83[b]	0.09	0.86[a]	−0.89[b]
Moist crumb	−0.98[b]	0.02	0.77[a]	−0.91[b]	0.88[a]
Aroma of preservative	0.70[a]	−0.85[b]	0.12	0.85[a]	−0.88[b]

CP: 100% *S.cerevisiae* + 0.5% calcium propionate; LF: 100% *L. fermentum*; LF/ WA: 50% *L. fermentum* + 50% *W. anomalus*; PCOM: commercial panettone; WA: 100% *W. anomalus*.
a Values indicate a high positive correlation of the sensory attribute with the respective sample.
[b] Values indicate a high negative correlation of sensory attribute with the respective sample.

Table 8
Results of the acceptance test of panettones made with *Lactobacillus fermentum* IAL 4541, *Wickerhamomyces anomalus* IAL 4533 and commercial yeast *Saccharomyces cerevisiae* with calcium propionate (CP) as an artificial preservative and commercial panettone.

Treatments	Color	Odor	Flavor	Softness	Moisture	Buy intention
LF	7.49[ab]	7.46[a]	7.45[a]	7.74[ab]	7.42[a]	3.88[a]
LF/WA	7.25[b]	7.24[ab]	6.99[a]	6.95[c]	6.71[b]	3.46[b]
WA	7.47[ab]	7.32[ab]	7.31[a]	7.43[b]	7.27[a]	3.76[ab]
CP	7.66[ab]	6.92[b]	7.18[a]	7.29[bc]	7.01[ab]	3.74[ab]
PCOM	7.71[a]	7.04[ab]	7.16[a]	7.94[a]	7.52[a]	3.78[ab]
SEM	0.151	0.180	0.187	0.172	0.191	0.117
Sig	**	**	n.s.	***	***	**

CP: 100% *S.cerevisiae* + 0.5% calcium propionate; LF: 100% *L. fermentum*; LF/ WA: 50% *L. fermentum* + 50% *W. anomalus*; PCOM: commercial panettone; WA: 100% *W. anomalus*.
[a] Mean values in the same column not followed by a common letter differ significantly ($p < .05$). SEM: standard error of the mean. Sig.: significance; n.s. (not significant). *** $p < .001$; ** $p < .01$; * $p < .05$.

sourdough panettones obtained the best evaluations in comparison with the CP and PCOM ones. Fermented sourdough panettones have more odor than traditionally processed panettones (with commercial yeast) showing an increase in the concentration of odor and taste precursor compounds (Torrieri et al., 2014).

Analyzing Table 8, it can be observed that for all formulations the evaluations were close to 7, indicating a good acceptability in relation to the flavor, and the differences found between the means, for the attributes analyzed, were not significant at a level of 5% by the ANOVA

variance test, demonstrating the acceptability of the tasters in relation to all the samples. Thus, it is possible to add sourdough to the panettone without displeasing consumers' palate, since it received notes that are equivalent to "liked moderately" in the scale used. Good evaluations for the odor and flavor attributes may be related to the properties of exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) (Torrieri et al., 2014). These EPSs act as bio-thickeners or hydrocolloids that stabilize the rheological properties of the dough improving taste, odor and texture and ensure a long shelf life (Tieking & Gänzle, 2005). It is known that the effect of probiotic bacteria on taste depends mainly on the species and lineages added and also on the metabolic activity of the strains during production and storage of food (Dantas et al., 2016). A study by Dantas et al. (2016) using a specific bacterium (*Lactobacillus casei*) showed poor acceptance that may be linked to the off-favors compounds masking the aroma released in Minas Frescal cheese.

On the other hand, for the softness attribute, there was a statistical difference ($p < .05$) and the best means were for the commercial treatment (7.94) and for the LF (7.74). In general, the other treatments received scores close to 7, which means good acceptability by consumers. The LF treatment presented the best score (7.42) for moisture (Table 8) as well as one of the highest moisture (25.31) described in Table 4. Based on the statistical analysis performed, it was observed that the treatments presented similar averages, where only the LF/WA treatment was shown to be relatively inferior to the others. Although the treatments had significant differences ($p < .05$), the means indicated for the moisture attribute were "moderately liked" in the scale used.

From the results presented in Fig. 4 it can be verified that the consumers presented a positive attitude regarding the Purchase intention of the evaluated panettones. A total of 80% of consumers "probably or certainly" would buy the LF panettone, 68.6% the WA and 49.3% the LF / WA. These values for both CP and PCOM were 65.7%.

Results of correlation analyses are showed in Table 9. A very strong positive correlation was found between flavor and the buy intention of LF/WA panettone; it was also strong for the LF and WA elaborated panettones, but was weak or very weak to CP and PCOM. Softness was also strong correlated with buy intention of both LF an LF/WA panettones; this parameter was only weak to moderate for WA and PCOM and very weak for CP.

Unlike bread, panettone is a product that maintains its quality during storage and can be consumed after a long period, up to 6 months. During storage, there are transformations in the product, called "apparent maturation," which help to accentuate the flavor of the product and, in this sense, favor the sensorial quality of the final product (Valcárcel-Yamani & Lannes, 2013). In general, people always seek or accept food prepared from ingredients traditionally established and close to their eating habits, since food behavior is influenced by environmental, biological, ecological, and sociocultural factors. However, sensory and cultural characteristics, such as taste, satisfaction, and convenience, may also interfere with the choice of food and even increase the nutritional ratios for the choice of a particular food (Barker, Thompson, & McClean, 1995).

Table 9
Correlation coefficients (r) of attributes versus the buy intention of panettones elaborated with *Lactobacillus fermentum* IAL 4541, *Wickerhamomyces anomalus* IAL 4533 and commercial yeast *Saccharomyces cerevisiae* with calcium propionate as preservative, and commercial panettone.

Attributes	Buy intention				
	CP	LF	LF/WA	PCOM	WA
COLOR	0.11	0.51	0.55	0.08	0.39
ODOR	0.12	0.60	0.22	0.20	0.41
FLAVOR	0.16	0.68	0.81	0.34	0.66
SOFTNESS	−0.08	0.64	0.64	0.39	0.44
MOISTURE	−0.05	0.47	0.70	0.11	0.24

CP: 100% *S.cerevisiae* + 0.5% calcium propionate; LF: 100% *L. fermentum*; LF/WA: 50% *L. fermentum* + 50% *W. anomalus*; PCOM: commercial panettone; WA: 100% *W. anomalus*.

Panettones of good technological quality and a good level of acceptance were produced in this work. The products developed met the legislation in the parameters of humidity and acidity. Regarding the analysis of instrumental firmness, the panettones elaborated with sourdough had their softness less affected during the storage if compared with panettones elaborated with commercial yeast, indicating a technological advantage of the use of these strains as starters. The physical and chemical analyses met the standard available in the literature. Microbiological analysis of panettones for molds and yeasts makes it clear that the use of L. *fermentum* IAL 4541 and *W. anomalus* IAL 4533 provides an increase in the shelf life of the products. The species used as sourdough are often described in the literature as microorganisms with antifungal properties, and the treatments LF / WA and WA proved this capacity because they are the only panettones that not became moldy after 126 days of monitoring. These results show the economic advantage of using sourdough in panettones to increase shelf life without the addition of artificial preservatives, thereby reducing fungal spoilage losses. In addition, the use of sourdough in the formulating panettones also presented satisfactory results from the sensorial point of view. Consumers perceived differences in some attributes, especially with regard to odor. In addition, more natural and preservative-free panettones are more attractive to consumers than those traditionally marketed.

Based on the results obtained in the acceptance test, it is possible to conclude that tasters had a good acceptance of the taste attribute. The use of sourdough in the preparation of panettones from selected strains and without artificial preservatives is shown as a more natural and a promising alternative because it was well-accepted sensorially with values equivalent to "moderately liked." The panettones where proving to be very competitive with respect to the control and PCOM. Among the treatments, panettone made with LF / WA showed better physical, chemical, and microbiological characteristics, although PCOM presented the best marks in the acceptance test. According to the CATA test, there was a clear separation between the samples showing that the LF and WA panettones differ from the LF / WA, and the CP and PCOM differ from the others. Desirable characteristics such as nice aroma, pleasant taste and uniform color were checked more often for LF and WA samples, whereas yeast flavor was checked more often for PCOM.

4. Conclusion

Panettones of good technological quality and good level of acceptance were produced in this work. The use of L. *fermentum* IAL 4541 and *W. anomalus* IAL 4533 as the starter of sourdough panettones generated a product that remained soft for a longer time and presented increased shelf life when compared with panettones elaborated with commercial yeast. The sourdough panettones were also well-accepted sensorially, demonstrating to be very competitive with traditional panettones. Consumers perceived differences in some attributes, especially with regard to odor. Desirable characteristics such as nice aroma, pleasant taste and uniform color were checked more often for sourdough samples, whereas yeast flavor was checked more often for commercial samples. In addition, more natural and preservative-free panettones are more attractive to consumers than those traditionally marketed.

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Conflicts of interest

None.

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