Evaluation of sourdoughs for the production of bread using spontaneous fermentation technique

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ABSTRACT. This study developed three sourdoughs using sugarcane, apple and grape as substrate. The fermentative activity and physicochemical, microbiological and sensory characteristics of the sourdoughs and breads were evaluated. Among the breads that were prepared using different sourdoughs, that which was made from grape sourdough presented high pH (4.30) and less acidity (2.99 mL 0.1N 100 g^{-1}). The grape sourdough was selected for research because it presented the highest fermentation volume and because it was the most preferred in the sensory analysis. The aerobic lactic acid bacteria count at 30°C was 7.52 log CFU g^{-1} and yeast count was 7.62 log CFU g^{-1}. After one year of cultivation, the aerobic lactic acid bacteria increased by 1.34 logarithmic cycles at a temperature of 30°C and the yeasts were reduced by 0.61 logarithmic cycles. The results of this study indicate that the use of different substrates in the preparation of sourdoughs provides breads with different sensory characteristics. Although it is an old process, the application of the spontaneous fermentation technique in bread making is still much used in view of the demand for products with the specific characteristics of this type of fermentation.

Keywords: fermentative activity, yeast, lactic acid bacteria, sensory analysis.

Avaliação do fermento natural na produção de pães aplicando a técnica da fermentação espontânea

RESUMO. Neste estudo foram desenvolvidos três fermentos naturais usando como substrato: cana-de-açúcar, maçã e uva. A atividade fermentativa e as características físico-químicas, microbiológicas e sensoriais do fermento natural e dos pães foram avaliadas. Dentre os pães preparados com os diferentes fermentos naturais, o elaborado com fermento de uva apresentou maior pH, com 4,30 e menor acidez, correspondendo a 2,99 mL NaOH 0,1N 100 g^{-1}. O fermento natural de uva foi o selecionado para esta pesquisa por apresentar o maior volume na fermentação e por ter obtido a preferência na análise sensorial. A contagem de bactéria lática aeróbia a 30°C foi de 7,52 log UFC g^{-1} e a de leveduras foi de 7,62 log UFC g^{-1}. Após um ano de cultivo, as bactérias láticas aeróbias aumentaram 1,34 ciclos logarítmicos na temperatura de 30°C, e as leveduras reduziram 0,61 ciclos logarítmicos. Os resultados deste estudo indicam o uso de diferentes substratos na preparação do fermento natural, fornecendo características sensoriais diferentes. Apesar de ser um processo antigo, a aplicação da técnica da fermentação espontânea em pães ainda é muito utilizada, tendo em vista a demanda por produtos com as características específicas deste tipo de fermentação.

Palavras-chave: atividade fermentativa, levedura, bactéria lática, análise sensorial.

Introduction

Bread is consumed in large quantities throughout the world in different types and forms depending on cultural habits. Flat breads are the oldest and most popular product worldwide. It is estimated that over 1.8 billion people consume various kinds of flat breads globally. Bread products and their production techniques differ widely around the world (CHAVAN; CHAVAN, 2011). Among cereal flours, wheat flour is the only flour that can form a three-dimensional viscoelastic dough when mixed with cold water. The characterization of rheological properties of dough is effective in predicting processing behavior and in controlling the quality of food products (SONG; ZHENG, 2007).

Several starter cultures have been applied in sourdough bread making, targeting an increase in bread shelf-life and the improvement of sensorial qualities (PLESSAS et al., 2011b). Traditional sourdough fermentation is a long process and the resulting bread has, in general, a specific texture and a typical flavor which are mainly related to the particular technological conditions applied (DECOCK; CAPPELLE, 2005).

In general, sourdoughs can be obtained from different methods, either through spontaneous fermentation or with selected starter strains. Depending on the technological parameters applied, sourdoughs have been classified into three types. Type I sourdoughs are traditionally prepared at ambient temperature (30ºC) and are continuously propagated by back-slopping. Type II sourdoughs are industrially obtained and the fermentation is carried out at a higher temperature (above 30ºC) over a long period. Type III sourdoughs are generally initiated by selected starter cultures and are dried before use (CORSETTI; SETTANNI, 2007; DE VUYST; NEYSENS, 2005; HAMMES et al., 2005).

Sourdough is a mixture of flour and water that affects bread properties in different ways. Besides yeasts, it contains a wide diversity of lactic acid bacteria (LAB) that produce lactic acid and/or acetic acid, which results in a sour taste in the final product (DE VUYST; NEYSENS, 2005). The metabolic activities of *Lactobacillus* during sourdough fermentation improve dough properties, bread texture and flavor; retard the the process of bread becoming stale; and prevent the bread developing mold and bacterial spoilage (DE VALDEZ et al., 2010; GEREZ et al., 2009). Sourdough fermentation further improves these nutritional features and enhances the textural and sensory properties of breads containing bran fractions (RIZZELLO et al., 2012). Physicochemical and biochemical characteristics, which arise from this diversity, typically result in good baked products (ARENDT et al., 2007; DE VUYST; NEYSENS, 2005). Optimum temperatures for the growth of *Lactobacillus* are 30 to 40ºC depending on the strain, and 25 to 27ºC, depending on the yeasts (CHAVAN; CHAVAN, 2011). The objective of this study was to develop and analyze sourdoughs obtained by spontaneous fermentation for application in breads, with the aim of producing products with different sensory characteristics to those currently produced in Brazil.

Material and methods

Wheat flour characterization

The moisture analysis of wheat flour followed AACC (1999) 44-15.02 and ash content was analyzed according to AACC (1999) 08-12. The quality of wheat flour was determined by AACC (1999) 54-21 using a farinograph (model 8 101, Brabender OHG, Duisburg, Germany). The viscoelastic characteristics were determined by AACC (1999) 54-30A using an alveo-consistograph (model NG, Chopin, Villeneuve la Garenne, France). Falling number (model 2000,

Perten Instruments, Huddinge, Sweden) was determined by AACC (1999) 56-81B. Wet and dry gluten percent and gluten index were obtained by Glutomatic (model 220, Perten Instruments, Huddinge, Sweden) following the methodology of AACC (1999) 38-12.

Sourdough preparation

Three sourdoughs were developed using sugarcane, apple and grape as substrate. These ingredients were chosen because they are commonly used in the preparation of sourdough in Brazil. A five stage procedure (one initial fermentation + four daily replenishments) was used. Each substrate was mixed with water and spontaneously fermented for 3 days at 25°C (IF). Thereafter, the first mixing was carried out with the addition of 100% initial fermentation (IF), 100% wheat flour, 10% rye flour and 90% water, standing for 24 hours at room temperature (pre-fermentation). Following intervals of 24 hours, the second, third and fourth mixture were performed using 100% of pre-fermentation, 50% wheat flour, 10% of rye flour and 40% water. After each daily alimentation the sourdoughs were stored for 8 hours at 25°C, and the rest of the time at 5°C. The sourdough was refreshed regularly throughout a period of one year.

Evaluation of leavening activity

After the completion of the sourdough, a portion of sugarcane, apple and grape sourdough (10 g) was fermented in a graduated tube and the leavening power was determined. Maximum leavening at 25, 30, and 35°C for 13 hours was calculated, starting with 10 mL volume.

Bread making

The sourdoughs were tested in breads whose formulation consisted of 100% wheat flour, 20% sourdough (KATINA et al., 2006; PARAMITHIOTIS et al., 2005; PLESSAS et al., 2011a; ROBERT et al., 2006), 60% water and 1.8% salt. After mixing the ingredients, the breads were fermented at 30°C for 5 hours (80% relative humidity) and then cooked with steam at 180°C for 30 min.

Physicochemical and microbiological properties of sourdough and bread

The sourdough and bread samples were analyzed for pH (model Q-400ª, Quimis, Diadema, Brazil) and titratable acidity. The pH value was recorded and the acidity was titrated using 0.1N NaOH (IAL, 2004). The TTA was expressed in mL 0.1 N NaOH 100 g^{-1}. The bread samples were analyzed for moisture, ash, fat (IAL, 2004) and protein (AOAC, 2005). The bread

samples were analyzed for coliforms at 45°C, *Salmonella* sp., *Staphylococcus aureus*, *Bacillus cereus*, moulds and yeasts (APHA, 2001).

Sensory analysis

Bread samples were judged by 50 random untrained testers. The testers were invited to classify the bread samples in order of preference. The least preferred sample scored 3 and the most preferred sample scored 1, based on overall impression. Samples were served on white plastic dishes and were coded with random three-digit numbers. Mineral water was provided to rinse the mouth between evaluations.

Preference-ranking test was performed according to the method described by Meilgaard et al. (2007) to evaluate the bread samples and the sourdough with sugarcane, apple and grape. Protocol number 876/10 was approved by the Human Research Ethics Committee at the Federal University of Santa Catarina. Microbiological analyses were performed for the security of the testers during sensory analysis. The objective of this test was to identify the best sourdough and from that starting point select LAB and yeasts of interest in the preparation of breads.

Microbiological counting of lactic acid bacteria and yeast

The counting of LAB and yeast was performed using the pour plate method. From the sourdough with the highest score in the sensory test, 25 g was aseptically withdrawn and mixed with 225 mL of 0.1 g 100 g^{-1} of peptone water in Bag Mixer (model P, Interscience, St. Nom, France). The subsequent necessary dilutions were carried out from this dilution. The cultivation of LAB was carried out in MRS agar (Difco, Sparks, USA) in conditions of aerobiosis and anaerobiosis, with incubation at 30°C for 48h and yeasts were grown in PDA Agar (Himedia, Mumbai, India) and tartaric acid 10% at 25°C for 72h. LAB were maintained as stock culture in tubes with MRS agar and yeast with PDA agar and tartaric acid 10% at 5°C and peaked monthly. The morphology of the lactic bacteria was observed as well as Gram coloration and catalase test. The yeast colonies were subjected to microscopic tests for the presence of hyphae or pseudohyphae.

Statistical analysis

The significant difference between the averages was calculated using analysis of variance (ANOVA) and calculated by Tukey's test ($p < 0.05$) using the Statistica program, version 8.0 (STATISTICS, 2007). All tests were performed in triplicate and the data expressed as mean ± standard deviation. The results of the preference-ranking test were analyzed by non-parametric Friedman's test (MEILGAARD et al., 2007) using the Bioestat 5.0 program ((AYRES et al., 2007).

Results and discussion

Flour characteristics

The wheat flour presented an average content of 14.1 ± 0.10% moisture, 0.48 ± 0.02% ash, 9.5 ± 0.10% dry gluten and 355 ± 2.00 s of falling number. The data from the flour analysis are presented in Table 1. The sample analysis in this study presented a mean value of water absorption of 60.8 ± 0.21% and 2.1 ± 0.14 min. of development of the dough. The results were close to those obtained by Indrani and Rao (2007), which were 0.45% ash, 10.1% dry gluten, falling number of 374 s and 57.5% farinograph water absorption. The stability of dough is measured by the time interval during which it maintains the greatest consistency, and the value obtained in this research was 3.6 ± 0.64 min. The flour showed a low tolerance index to mixing (MTI), of 58 ± 2.83 BU, which characterizes it as capable of forming a strong dough. Tenacity (P) and extensibility (L) obtained was of 104 ± 2.12 and 57 ± 0.71 mm, respectively. The relation of P L^{-1} of the sample was 1.84 ± 0.06. This relation indicates flour with average tenacity, ideal for breads.

Stoenescu and Ionescu (2011) have observed that the main quality indices of flour without additives are a wet gluten content of 26.2% and a falling number of 478 s. The high value of the falling number recommends the addition of α-amylase to improve baking properties. The dough prepared from Romanian wheat flour had a dough elasticity of 92 mm and extensibility of 42 mm. The Romanian wheat flour had good baking properties with a falling number ranging from 250 to 300 s and optimum values of dough elasticity for bread flour, 60-70 mm.

Sourdoughs and bread characteristics

The leavening activities are presented in Table 2. The grape and apple had similar results, that were higher than sugarcane, at 25 and 35°C during the first and second hour of growth. In general, the sugarcane sourdough had a slower fermentation. Lactic acid bacteria developed well at this temperature, resulting in increased acidity in the bread.

The sourdough that presented the highest volume was grape, at 25°C. Its high point of fermentation was 5 hours, with 36 ± 3.08 mL. Statistical analysis was performed and showed that there was significant difference between the averages of nine treatments in a significance level of $p < 0.05$. The average of the grape sourdough during 13 hours of fermentation at 25°C was 28.62 mL, superior and significantly different from the others.

Table 1. Rheological and physicochemical characteristics of flours.

Farinography		Alveo-consistography		Physicochemical	
WA (%)	60.8 ± 0.21	P (mm)	104 ± 2.12	moisture (% w w^{-1})	14.10 ± 0.10
AT (min.)	0.98 ± 0.03	L (mm)	57 ± 0.71	ash (% w w^{-1})	0.48 ± 0.02
TD (min.)	2.1 ± 0.14	P L^{-1}	1.84 ± 0.06	wet gluten (% w w^{-1})	27.93 ± 0.55
ST (min.)	3.6 ± 0.64	W (cm^2 x 10^{-4} J)	241 ± 2.12	dry gluten (% w w^{-1})	9.50 ± 0.10
MTI (BU)	58.0 ± 2.83			gluten index (% w w^{-1})	89.70 ± 2.29
				falling number (s)	355 ± 2.00

Results as mean ± standard deviation (n = 3).Farinographic properties: WA, water absorption (to the dry basis at 14% moisture); AT, arrival time; DT, development time; ST, stability; MTI, mixing tolerance index. Alveo-consistograph properties: P, tenacity; L, extensibility; P/ L relation tenacity and extensibility; W, dough deformation energy.

Table 2. Evaluation of leavening activity of sourdoughs at 25, 30, and 35ºC, expressed as volume (mL).

Temperature	25ºC			30ºC			35ºC		
Hour	Sugarcane	Apple	Grape	Sugarcane	Apple	Grape	Sugarcane	Apple	Grape
0	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00
1	12.8 ± 0.84	15.6 ± 1.14	15.8 ± 0.84	13.8 ± 1.30	16.2 ± 0.84	12.8 ± 1.30	14.8 ± 0.84	16.8 ± 1.30	16.25 ± 0.96
2	14.2 ± 1.10	22.4 ± 1.52	22.8 ± 2.39	16.6 ± 1.82	21.8 ± 0.84	16.4 ± 2.41	17.6 ± 0.55	22.2 ± 2.59	19.25 ± 1.71
3	16.6 ± 1.34	26.6 ± 2.51	25.0 ± 2.12	19.8 ± 2.77	24.0 ± 1.00	19.2 ± 3.03	20.8 ± 2.28	29.40 ± 3.65	25.0 ± 0.83
4	17.8 ± 1.64	30.0 ± 1.22	32.8 ± 1.79	24.8 ± 1.79	32.6 ± 0.55	34.2 ± 2.49	23.2 ± 1.92	35.0 ± 1.73	34.5 ± 1.73
5	22.8 ± 1.92	31.4 ± 0.89	36.0 ± 3.08	28.6 ± 2.61	31.2 ± 1.30	31.8 ± 2.86	26.2 ± 2.59	32.2 ± 2.59	26.0 ± 0.82
6	27.0 ± 2.00	32.2 ± 0.84	35.4 ± 2.30	29.6 ± 2.97	30.6 ± 1.34	30.6 ± 1.52	26.8 ± 3.11	30.0 ± 3.74	27.25 ± 1.71
7	28.2 ± 1.92	31.2 ± 0.84	31.8 ± 2.17	30.2 ± 2.95	30.4 ± 1.95	28.6 ± 1.67	28.0 ± 3.39	28.2 ± 1.10	26.0 ± 1.63
8	28.6 ± 2.41	30.8 ± 1.64	29.8 ± 0.84	29.4 ± 3.36	27.0 ± 3.08	26.2 ± 1.79	27.8 ± 3.27	18.8 ± 1.92	20.25 ± 3.86
9	29.0 ± 2.00	30.8 ± 0.84	30.4 ± 0.55	26.2 ± 1.10	27.2 ± 1.64	26.4 ± 2.70	28.2 ± 3.42	12.6 ± 3.58	17.0 ± 3.56
10	27.8 ± 1.30	24.4 ± 3.56	30.4 ± 2.07	26.2 ± 1.30	26.4 ± 1.67	24.6 ± 2.07	26.8 ± 2.77	10.0 ± 0.00	15.25 ± 3.59
11	27.6 ± 0.89	27.0 ± 2.61	30.0 ± 3.39	26.0 ± 2.35	26.4 ± 2.79	22.6 ± 1.95	25.6 ± 1.95	10.0 ± 0.00	13.0 ± 4.24
12	26.0 ± 1.22	21.8 ± 1.28	26.2 ± 1.30	25.2 ± 1.79	21.6 ± 2.30	21.0 ± 1.58	24.4 ± 2.70	10.0 ± 0.00	13.0 ± 4.24
13	22.2 ± 1.48	20.0 ± 0.71	25.6 ± 1.34	21.4 ± 1.67	20.6 ± 1.34	20.4 ± 1.14	22.2 ± 1.48	10.0 ± 0.00	12.5 ± 3.32
Means in 13h	23,12 [ab]	26,48 [ab]	28,62 [b]	24,29 [ab]	25,85 [ab]	24,22 [ab]	24,03 [ab]	20,40 [a]	20,40 [a]

Results as mean ± standard deviation (n = 3). [a–b] Different superscript letters between sourdoughs denote significant differences (tukey's test, p < 0.05).

The treatments of sugarcane and apple at 25°C, sugarcane, apple and grape at 30°C, and sugarcane at 35°C were considered equal to each other; the average did not significantly differ between them. The treatments of apple and grape at 35°C differed from the rest and were the least successful treatment, with the lowest volumes.

The results of the analyses of sourdoughs and breads are shown in Table 3. The pH values of the sourdoughs were similar, ranging from 4.22 ± 0.02 to 4.37 ± 0.01. The bread with grape sourdough had the highest level with 4.30 ± 0.02 and the lowest acidity with 2.99 ± 0.02 mL NaOH 100 g^{-1}. The sourdough and bread with sugarcane had the lowest pH and the highest titratable acidity (TTA). The pH values obtained were 4.11, 6.01, 5.21, and 4.75 for sourdough, standard bread, bread containing 20 % sourdough and bread containing 40% sourdough, respectively. Values corresponding to total titratable acidity were 12.35, 2.6, 5.2, and 8.18 mL NaOH 100 g^{-1}, respectively. As expected, increasing the level of sourdough in the bread resulted in decreasing pH values and consequently higher TTA values (CROWLEY et al., 2002). The pH of a ripe sourdough varies with the nature of the process and the starter culture used but for wheat sourdoughs it ranges from 3.5 to 4.3 (COLLAR et al., 1994; THIELE et al., 2002).

Paramithiotis et al. (2005), using 20% of sourdough, found pH that ranged from 3.57 to 3.85 and TTA ranging from 10.1 to 12.5 mL 100 g^{-1}. The lactic acid concentrations were higher in all the breads containing 50% sourdough when compared with breads made with 30% sourdough (PLESSAS et al., 2008).

Table 3. Physicochemical and sensory characterization of sourdoughs and breads.

	Sourdough		
	Sugarcane	Apple	Grape
pH	4.22 ± 0.02	4.37 ± 0.01	4.36 ± 0.02
Total titratable acidity (mL 0.1 N)	11.56 ± 0.10	5.55 ± 0.34	7.54 ± 0.64
	Bread		
	Sugarcane	Apple	Grape
pH	4.04 ± 0.01	4.16 ± 0.05	4.30 ± 0.02
Total titratable acidity (mL 0.1 N)	5.44 ± 0.15	4.35 ± 0.06	2.99 ± 0.02
Moisture (% w w^{-1})	36.27 ± 0.19	37.07 ± 0.06	38.6 ± 0.10
Ash (% w w^{-1})	1.46 ± 0.01	1.58 ± 0.01	1.55 ± 0.04
Fat (% w w^{-1})	1.17 ± 0.06	1.32 ± 0.08	1.22 ± 0.05
Protein (% w w^{-1})	8.89 ± 0.05	8.84 ± 0.02	8.46 ± 0.09
Sensory analysis	84[a]	105[ab]	116[b]

Results as mean ± standard deviation (n = 3). [a–b] Different superscript letters indicate a significant difference between the treatments (Tukey's test, p < 0.05).

The bread with grape sourdough had a high moisture content, with 38.6 ± 0.10%. The bread with sugarcane had a low content of moisture, ash and fat. The protein contents of the breads ranged from 8.46 ± 0.09 for grape and 8.89 ± 0.05% for sugarcane. Microbiological analyses of coliforms at 45°C, *Salmonella* sp., *Staphylococcus aureus*, *Bacillus cereus*, molds and yeasts of bread made with sourdough presented satisfactory results and were able to be subjected to sensory analysis.

According to the Friedman test, there were significant differences (p < 0.05) between the samples of breads containing sourdough of sugarcane and grape. The best scores of the preference-ranking test were found in breads containing sourdough of grape, which was the sample chosen for selection of lactic acid bacteria and yeasts for future application in breads. The testers reported that the bread with sugarcane presented a very acidic taste, while that of grape had low acidity.

Microbiological characteristics

Plate counts were carried out for grape sourdough and the results are presented in Table 4. This sourdough presented similar plate count values at the different temperatures and there was no predominance of one type of microorganism. The aerobic LAB count at 30°C was 7.52 ± 0.07 log CFU g^{-1}, anaerobic LAB was 7.55 ± 0.18 log CFU g^{-1}, and yeasts was 7.62 ± 0.29 log CFU g^{-1}. After one year of cultivation of the grape sourdough plate counting was carried out. Aerobic LAB increased by 1.34 logarithms cycle at a temperature of 30°C and yeasts were reduced by 0.61 logarithms cycle.

Table 4. Plate counts of LAB and yeast of sourdough of grape at 25, 30, and 35°C (log CFU g^{-1}).

yeast	Lactic acid bacteria			
aerobic	aerobic		anaerobic	
25°C	30°C	35°C	30°C	35°C
7.62 ± 0.29	7.52 ± 0.07	7.61 ± 0.05	7.55 ± 0.18	7.45 ± 0.21
		after 1 year		
7.01 ± 0.16	8.86 ± 0.11	8.97 ± 0.08	8.42 ± 0.11	8.51 ± 0.16

Results as mean ± standard deviation.

The LAB microbiota was predominant (8 log CFU g^{-1}) and was principally represented by members of the *Lactobacillus* genus. Yeasts are generally counted at lower levels (7 log CFU g^{-1}) and can even be absent. Yeasts principally belong to the *Saccharomyces* and *Candida* genera (VERA et al., 2012). Saeed et al. (2009) researched fifteen sourdough samples in Pakistan and found that the LAB count ranged from 4.8 to 7.84 log CFU g^{-1} and the yeast from 3.8 to 7.9 log CFU g^{-1}. Plate counts of LAB and yeasts during spontaneous fermentation of maize flour were studied by Edema and Sanni (2008). LAB counts increased steadily from 4.62 log CFU g^{-1} at mixing (0h) to 6.45 log CFU g^{-1} after 48 hours fermentation, while yeast counts increased from 4.18 to 6.64 log CFU g^{-1} within the same period of fermentation.

Sourdough is mainly dominated by heterofermentative LAB (DE VUYST; NEYSENS, 2005). Endo et al. (2011) reported that carbohydrates have a big impact on the isolation of a variety of LAB in fermented food. Scheirlinck et al. (2009) have indicated that specific strains of LAB persist in artisan doughs over a period of years and circulate in the bakery environment. Furthermore, the air is a potential carrier of LAB in artisan bakery environments.

Even if an apparent stability is observed as a result of the implantation of a specific microbiota, sourdoughs still continue to evolve slowly. Consequently, every sourdough can be considered as unique. For instance, type I sourdoughs are frequently dominated *by Lactobacillus sanfranciscensis*, *L. brevis* and *L. plantarum* whereas *L. fermentum*, *L. panis* and *L. amylovorus* are more typical of type II sourdoughs. Physicochemical and biochemical characteristics, which arise from this diversity, typically result in good baked products (ARENDT et al., 2007; DE VUYST; NEYSENS, 2005).

Yeast cells metabolize fermentable sugars (glucose, fructose, sucrose and maltose) under anaerobic conditions, producing carbon dioxide (CO_2) as a residual product, which acts as a leavening agent and enhances dough volume (CHAVAN; JANA, 2008; GIANNOU et al., 2003). Rosenquist and Hansen (2000) reported that *S. cerevisiae* was the only yeast species isolated from the sourdoughs in their study.

Starter cultures provide important advantages when compared to spontaneous fermentations without starter cultures. Generally, spontaneous fermentations take a long time (about 96 hours) to complete, in order to lower the pH to a sufficient level and improve the structural and sensory characteristics of the product. The initiation of the process can take a relatively long time (24-48 hours) and there is also the risk that contaminating microorganisms will compete with the desirable microorganisms (HOLZAPFEL, 2002). Adding a starter culture notably reduced fermentation times, with a fast decrease in pH and a noticeable increase in acidity, when compared with the controls. This helps to reduce the risk of the growth of contaminating microorganisms and also contributes to a greater control of the aroma, texture and flavor of the final product (HOLZAPFEL, 2002; LEROY; DE VUYST, 2004).

Conclusion

The results of this study indicate that the use of different substrates in the preparation of sourdoughs provides breads with different sensory characteristics. The grape sourdough was selected for research due to its high volume in fermentation and because it was the most preferred in sensory analysis. Although the application of the spontaneous fermentation technique in bread making is an old process, it is still much used nowadays in view of the demand for products with different tastes.

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