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Article

Influence of fermentation time on characteristics of sourdough bread

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Sourdough is used in the manufacture of numerous baked products. The microorganisms used in this preparation of sourdoughs included two strains from the *Lactobacillus paracasei* (1 and 2) and two strains from the *Saccharomyces cerevisiae* group (1 and 2). Samples of raw dough were analyzed for pH, titratable acidity and plate counts and samples of resulting bread were analyzed for pH, titratable DFLGLWDGVSHFLFYROXPH7KHVDPSOHVVHUHDDOOHGHYHUKEHWHHDGKRIIHUPHWDWLR After 10 hours of fermentation, the lowest values of pH were for dough with LC2 and bread with SC1. Titratable acidity values increased over time, with the highest levels of acidity were found in the dough and bread with yeasts. Lactic acid bacteria showed the highest microbial counts over time. With the exception of SC2, the greatest microbial increases occurred at 10 hours of fermentation. LC1 showed the lowest volume across all time points ($p < 0.05$). The largest volumes were found in breads after 6 KRXUVRIIHUPHWDWLR6MKRHGWKHEHVWVSHFLFYROXPHYDOXHVDFURVVDOOWLPHVWHVWHG

Uniterms: Sourdough. Bread. Lactic acid bacteria. Yeast. Linear regression.

2IHUPHWRDWXUDOpXVDGRRSURFHVVDPHWRGHYiULRVSURGXWRVGHSDLFDomR2VPLFURRUJDLPRV usados no preparo dos fermentos foram duas cepas de *Lactobacillus paracasei* (1 e 2) e duas cepas de *Saccharomyces cerevisiae* (1 e 2). Das amostras de massa crua, foi analisado o pH, a acidez titulável e a FRWDJHPHPSODFDVHGDVDPRVWUDVGRSmRIRLDDOLVDGRRS+DDFLGHWLWXOiYHOHRYROXPHHVSHFtFRV DPRVWUDVIRUDPDDOLVDGDVXPLWHUYDORGHKHWUHHKGHIHUPHWDomRDDORVPHRUHVYDORUHV GHS+IRUDPSDUDDPDVVDFUXDFRP/HRSmRFRP62VYDORUHVGHDFLGHWLWXOiYHODXPHWDUDPDR ORJRGRWHPSRDSUHVHWDGRRVPDLRUHVWHRUHVGHDFLGHDPDVVDHRSmRFRPOHYHGXUDVEDFWpULDV OiWLFDVDSUHVHWDUDPDVPDLRUHVFRWDJHVPLFUREL ROyJLFDVDRORJRGRWHPSRRPHFHomRGH6 RVPDLRUHVFUHVFLPHWRVPLFUREL DRVIRUDPFRPKGHIHUPHWDomR/DSUHVHWRXPHRUYROXPH HPWRGDVDVKRUDV2VPDLRUHVYROXPHVHFRWUDGRVRVSmHVIRUDPFRPKGHIHUPHWDomR 6DSUHVHWRXRVPHOKRUHVUHVXOWDGRVGHYROXPHHVSHFtFRGXUDWHWRGRRSHUtRGR

Unitermos: JHPPDomPSUDiDV ZHUHJHQ

INTRODUCTION

Due to increasing consumer demand for more natural, tastier and healthier food, the traditional process of sourdough bread production has enjoyed renewed success in recent years (Brümmer, Lorenz, 1991; Thiele *et al.*, 2002; Lopez *et al.*, 2003). Sourdough is employed in the manufacture of a number of baked products, such as breads, cakes and crackers (De Vuyst, Gänzle, 2005).

Gocmen *et al.* (2007) and Thiele *et al.* (2002) reported that the application of sourdough to wheat breads produced several effects, including leavening, acidification, improvement of the dough properties, DYRURIWKHEUHDGWHWXUHGHODHGUPHVVDGVWDO increased resistance to microbial spoilage and improved nutrient availability of cereals. It was suggested that all WKHVHEHHWVHUHDWWULEXWDEOHWRWKHDFLGOD (LAB) and yeasts naturally present in sourdough.

Moreover, it has been noted that when sourdough is added, there are changes in the fundamental rheological properties of wheat dough, making it soft, less elastic

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and therefore easily extendable (Clarke *et al.*, 2002). The impact of changes in the rheology of dough must be considered in order to choose an appropriate fermentation time and obtain good quality bread. The dough must contain a large volume of gas and also reserve gas retention for oven rise (Bloksma, 1990).

However, there are other factors to be taken into account, such as the type of flour, the sourdough fermentation conditions (pH and temperature) and the VHOHFWLRQ RI PLFURRUJDQLVPV WKDW WHUPLQDWH [illegible] metabolic properties (De Vuyst, Vancanneyt, 2007). In this VWXGB WKH [illegible] terms of pH, titratable acidity, microbiological counts and volume of dough and bread, was analyzed.

MATERIAL AND METHODS

Microorganisms

The microorganisms used included two strains from the *Lactobacillus paracasei* (1 and 2) and two strains from the *Saccharomyces cerevisiae* (1 and 2). The lactic acid bacteria from the *Lactobacillus paracasei* (LP1 - 9.4 log CFU/g and LP2 - 11.11 log CFU/g) and yeasts from *Saccharomyces cerevisiae 1* (SC1 - 8.18 log CFU/g and SC2 - 7.16 log CFU/g) were isolated from grape sourdough, phenotypically and genotypically characterized, freeze dried and applied in this study.

Sourdough preparation

Separate sourdoughs were prepared with each strain. The amount of microorganisms used was 1% (w/w [illegible] *et al.*, 2008) and fermented for 48 hours. Dough 1 (D1) was prepared by mixing 60 mL of tap [illegible] at 30 °C. After 24 hours' incubation, sourdough 1 (SD1) was obtained. Subsequently, 100 g of SD1 was mixed with 200 g of wheat flour and 100 mL of tap water to form dough 2 (D2). After 24 hours incubation, sourdough 2 (SD2) was produced, according to the procedure of Paramithiotis *et al.* [illegible]

Dough and bread analyses

Samples of raw dough were analyzed for pH, titratable acidity (TTA) and plate counts while samples of [illegible] volume. These analyses were carried out every 2 hours, between 4 and 10 hours of fermentation.

The pH value was recorded and acidity titrated using [illegible] determined by counting the number of viable cells in logarithms (log UFC/g) using the pour plate method (Silva *et al.*, 2010). Aseptically, 25 g of each dough was mixed with 225 mL of 0.1 g 100 g^{-1} of sterile peptone water in a [illegible] necessary dilutions of LAB and yeast were performed. LAB were grown on MRS Agar at 30 ºC/48 hours and yeasts were grown on PDA Agar with tartaric acid (10%) at 25 ºC/72 hours. All experiments were carried out in triplicate.

Bread making

The sourdoughs were applied in breads, whose [illegible] 20% sourdough (Katina *et al.*, 2006a; Paramithiotis *et al.*, 2005; Plessas *et al.*, 2011; Robert *et al.*, 2006), 60% water and 1.8% salt.

After mixing the ingredients, about 24 g of resultant dough was weighed out and fermented in a fermentation chamber (model CFC P-20, Perfecta, Curitiba, Brazil) at 30 ºC for 4, 6, 8 and 10 h (80% relative humidity) and [illegible] at 180 °C for 18 min.

Specific volume

Loaf volume was measured using the rapeseed replacement method described by Hallén *et al.* (2004). Each loaf was placed in a metal container with a known volume (V_C). The container was then topped up with rapeseed, the loaf removed and the volume of the rapeseed measured (V_R). Loaf volume (V_L) was then calculated and recorded according to Equation 1.

$$V_L\ (mL) = V_C - V_R \qquad \text{Eq.1}$$

After cooling for 1 h, the same loaves used for measuring volume were weighed on digital scales, W (g). [illegible] of bread was calculated according to Equation 2.

$$V_S\ (mL/\ g) = V_L / W \qquad \text{Eq.2}$$

Statistical analysis

Statistical analyses were performed using the Statistica® software version 8.0 (Statsoft Inc., Tulsa, OK, USA). The behavior of the raw dough and bread,

prepared using sourdough with LC1, LC2, SC1 and SC2, over the period between 4 and 10 hours of fermentation was studied by a simple linear regression model, given E[Y] = β_0 + β_1X + ε, where X is the fermentation time, expressed in hours. Differences between the means were established using one-way analysis of variance [illegible] 5% level of significance ($p < 0.05$) were considered [illegible]

RESULTS AND DISCUSSION

Dough and bread evaluation

The estimated equation of analysis of linear regression for pH, titratable acidity, and plate counts for raw dough, between 4 and 10 h of fermentation, are shown in Figure 1 (A, B and C) whereas pH, titratable acidity and specific volume for breads, between 4 and 10 h of fermentation, are shown in Figure 2 (A, B and C).

The pH values of dough and bread declined with longer fermentation time. The initial pH of dough and breads at 4 h of fermentation ranged from 4.51 to 4.73 ± 0.01 and 4.45 to 4.74 ± 0.01, respectively. At the end of the fermentation period of 10 h, the lowest pH values were for dough with LP2 (3.44 ± 0.04) and for bread with SC1 (2.99 ± 0.05). The analysis of regression indicated that the rates of decrease in pH of dough (Figure 1A) and of bread (Figure 2A), were statistically equal ($p > 0.05$).

Therefore, the null hypothesis (H_0) was not rejected by the F test at the significance level of 5%, where H_0 indicates that angular coefficients were equal. In the analysis of pH of dough, a higher coefficient of determination was observed for yeasts (SC2: 0.8832; SC1: 0.8723; LP2: 0.8416: LP1: 0.8094), while for the pH of breads LAB had the highest r^2 (LP2: 0.9512; LP1: 0.9162; SC1: 0.9061; SC2: 0.8831). Among many important effects, the drop in pH value caused by organic [illegible] dough (Wehrle, Arendt, 1998).

The titratable acidity values increased over time, with the highest levels of acidity found for dough and bread with yeast. SC2 showed the highest acidity of dough production at all-time points (5.08 ± 0.41; 5.52 ± 0.16; 5.68 ± 0.10; 6.51 ± 1.32), followed by SC1 (4.70 ± 0.13; 5.19 ± 0.49; 5.52 ± 0.01; 6.42 ± 0.13). Analyses of linear regression of increased acidity of the dough (Figure 1B) [illegible] significant difference between the linear and angular [illegible]

found in titratable acidity for SC1, followed by LP1; SC2 and LP2 ($r^2 = 0.9564$; 0.9459; 0.9225; 0.9080).

For the analysis of titratable acidity of bread (Figure 2B), a significant difference was noted between linear [illegible] coefficients of LP2/ SC2 and the linear coefficient of LP2/SC1 ($p < 0.05$). The highest coefficient of determination was LP1, followed by LP2; SC2 and SC1 ($r^2 = 0.9801$; 0.9196; 0.9138; 0.8296).

[illegible] of sourdough, is mainly used to improve quality, taste and [illegible] *et al.*, 2006a; Arendt *et al.*, 2007) and to delay staling (Katina *et al.*, 2006b; Plessas *et al.*, 2007). The use of salt has a marked effect on both LAB growth and acid production and can be used as a method of controlling overall bread acidity (Simonsom *et al.*, 2003).

LAB showed the highest counts over time. With the exception of SC2, the highest microbial growth occurred after 10 hours of fermentation, the highest value was for LP2 with 8.91 log CFU/g, followed by LP1 with 8.66 log CFU/g, SC1 with 8.03 log CFU/g. At the same time, SC2 showed a drop of 0.21 log CFU/g. At 6 hours of fermentation, LP2 obtained 8.66 log CFU/g and LP1 8.33 log CFU/g. Yeasts showed 7.18 log CFU/g for SC1 and 7.08 log CFU/g for SC2 (Figure 1C).

Plate counts of LAB and yeasts during spontaneous [illegible] Edema and Sanni (2008). LAB counts increased steadily from 4.62 log CFU/g at mixing (0 h) to 6.45 log CFU/g after 48 hours fermentation while yeast counts increased from 4.18 to 6.64 log CFU/g over the same period of fermentation.

For plate counts, the analysis of regression indicated that the angular and linear coefficients of LP1/LP2 were statistically the same ($p > 0.05$), as were the linear [illegible] revealed a statistical difference between treatments [illegible] for plate count was with SC1 at 0.9674, indicating that the adjustment of the regression explains 96.74% of total variation in Y, but only 3.26% for the residual variation [illegible]

[illegible] 2C) indicated that all the linear coefficients were [illegible] of determination (r^2) for yeasts were SC1 and SC2 (0.7975; [illegible] and LC2 showed the lowest volume across all hours tested ($p < 0.05$), where this occurred because they constitute facultative heterofermentative lactic bacteria.

Lactobacillus can be divided into three groups, [illegible] *Lactobacillus* thermophilic obligatory homofermentatives, that ferment only hexoses and lactic acid *(Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus helveticus*); *Lactobacillus*

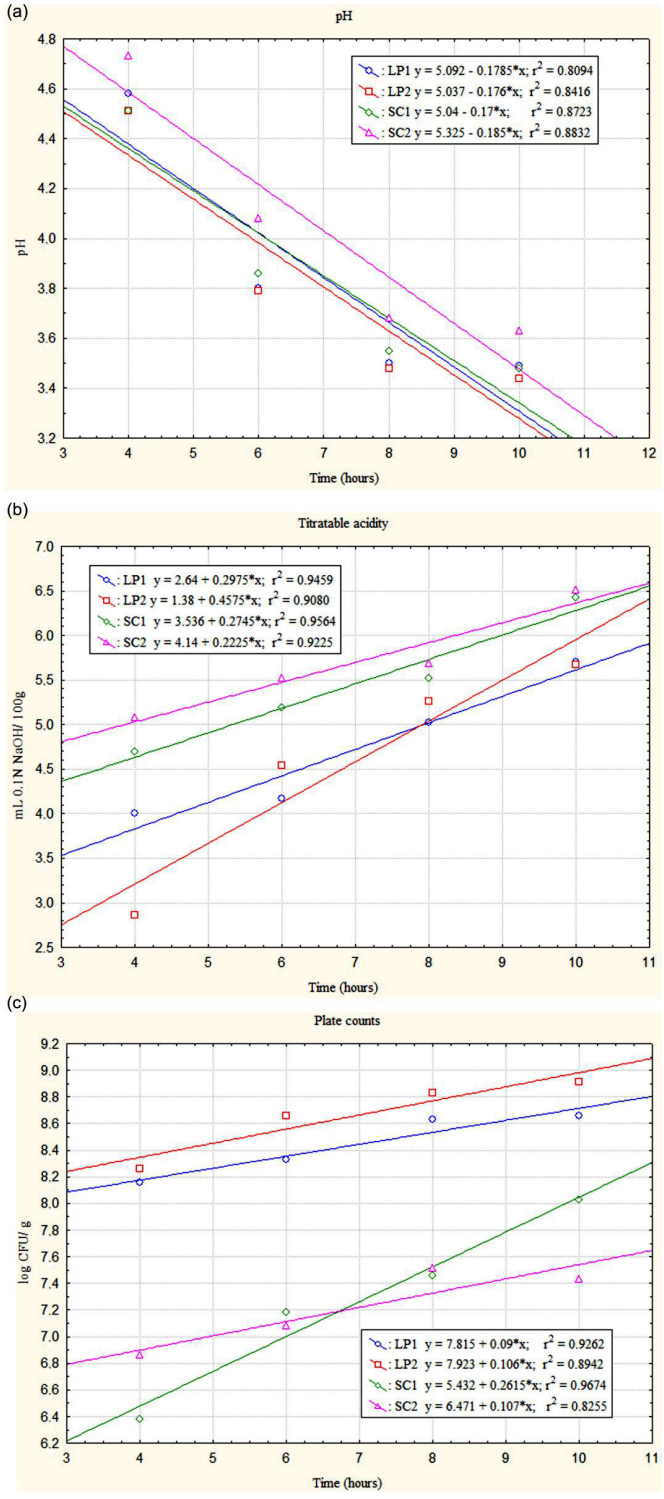

FIGURE 1 - Evolution of pH (a), titratable acidity, (b) and plate counts (c) of raw bread dough between 4 and 10 hours of fermentation.

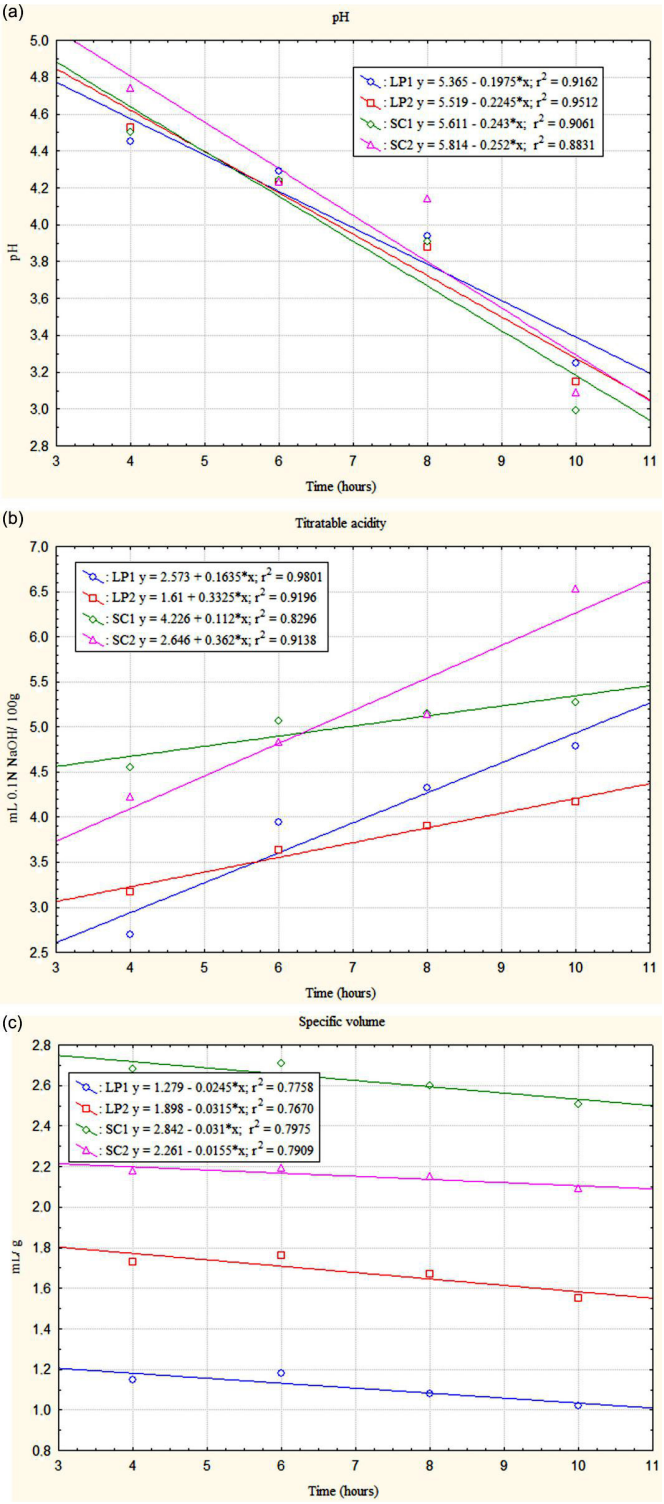

FIGURE 2 - Evolution of pH (a), titratable acidity (b) and specific volume (c) of bread between 4 and 10 hours of fermentation.

TABLE I 6HFLƒFYROXPHRIEUHDGVSURGXFHGIURPEDWWGRXSLHFHV

Samples	4 h	6 h	8 h	10 h
LP1	1.15 ± 0.03[a]	1.18 ± 0.04[a]	1.08 ± 0.04[a]	1.02 ± 0.03[a]
LP2	1.73 ± 0.15[b]	1.76 ± 0.10[a,b]	1.67 ± 0.07[b]	1.55 ± 0.08[b]
SC1	2.68 ± 0.08[c]	2.71 ± 0.22[c]	2.60 ± 0.10[c]	2.51 ± 0.17[c]
SC2	2.18 ± 0.03[d]	2.19 ± 0.37[b,c]	2.15 ± 0.09[d]	2.09 ± 0.07[d]

Results expressed as mean values of triplicates ± standard deviation.
[a-d]LIIHUHWVXSHUVFULSWOHWWHUVLWKHVDPHFROXPEHWHHVDPSOHVGHRWHVLJLFDWGLIIHUHFHV7XHVWHVWS

mesophilic facultative heterofermentatives, which are able to ferment other carbon sources beyond these hexoses, producing organic acids, CO_2, alcohol and H_2O_2 (*Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus plantarum*); and *Lactobacillus* mesophilic obligatory heterofermentatives that use principally hexoses and pentoses as carbon source, fermenting hexose and lactic acid, acetic acid, ethanol and CO_2 and pentoses to lactic acid and acetic acid (*Lactobacillus brevis*, *Lactobacillus fermentum*) (Fox *et al.*, 2000). The species *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus rhamnosus* belong to the "*Lactobacillus casei* Group" and are of great commercial value to the food industry (Ferrero *et al.*, 1996).

7KHKLKHVWVSHFLFYROXPHVLEUHDGHUHIRXG at 6 hours of fermentation (Table 1). SC1 showed better results across all times, followed by SC2. SC1 obtained at 6 hours of fermentation showed the best results, with 2.71 mL/ g ($p < 0.05$). *Saccharomyces cerevisiae* is used in the preparation of bread because it produces a dough with structure and has the ability to retain gas. After 6 KRXUVRIIHUPHWDWLRDLXHFHLYHUVHOSURSRUWLRDO to TTA increase, with a decrease in volume over time can be observed.

Sanz-Penella *et al.* (2012) used *Bifidobacteria pseudocatenulatum* as a starter in sourdough fermentation and reported that fermented dough with 20% sourdough LHOGHG77YDOXHVRIPO1D2+IJ and pH of 4.57 ± 0.11; while bread with LAB and yeast LHOGHG77RIPO1D2+IJDG pH of 4.96 ± 0.06. The sample volume increased with 20% sourdough content showed a significantly lower bread VSHFLFYROXPHWKDWKHFRWUROLWKPOJ Crowley *et al.* (2002) reported that bread specific YROXPHVLJLFDWOLEUHDGFRWDLLJVRXUGRXJK (3.40 ± 0.08 mL/g).

However, Collar *et al.* (1994) developed lower volume breads when using a high percentage of sourdough with *L. plantarum* and *L. brevis* DWDUWLHKHDFLLFDWLRQ of the sourdough and partial acidification of the bread dough have an impact on structure-forming components such as gluten and starch. During incubation of sourdough and dough fermentation, biochemical changes occur in the FDUERKGUDWHDGSURWHLFRPSRHWVRIIRXURLWR the action of microbial and endogenous enzymes (Rollan *et al.*, 2005). There is strong consensus with regard to the positive effects of the addition of sourdough on bread volume and crumb structure (Arendt *et al.*, 2007).

CONCLUSION

The pH values of the dough and bread declined with longer fermentation time. *Saccharomyces cerevisiae* 2 showed the highest production of acidity of dough over all times studied. LAB showed the highest microbiological counts at between 4 and 10 hours. *Lactobacillus paracasei* VKRHGWKHORHVWVSHFLFYROXPHIRUDOOKRXUVWL ($p < 0.05$). The best fermentation time of breads found in this study was 6 hours, evidenced by the largest volumes. The two strains exhibited different characteristics in the analyses performed, despite being of the same species.

ACKNOWLEDGEMENTS

7KHDXWKRUVZLVKWRWKDQNWKH&RQVHOKR1DFLRQDO GHHVHQYROYLPHQWR&LHQWtƒFRH7HFQROyJLFR&13T %UD]LOIRUWKHLUƒQDQFLDOVXSSRUW

REFERENCES

$5(1'7(.$HWDO(IIHFWRI sourdough on the texture of bread. *Food Microbiol.*, v.24, n.2, p.165-174, 2007.

BLOKSMA, A.H. Rheology of the breadmaking process *Cereal Foods World*, v.35, n.2, p.228-236, 1990.

%5h00(5-0/25(1=(XURSHDGHYHORSPHWVL wheat sourdoughs. *Cereal Foods World*, v.36, n.3, p.310-314, 1991.

single strain and traditional mixed strain starter cultures on rheological properties of wheat dough and on bread quality. *Cereal Chem.*, v.79, n.5, p.640-647, 2002.

properties and breadmaking potential of wheat dough. *J. Food Sci.*, v.59, n.3, p.629-633, 1994.

E.K. The effect of storage time on textural and crumb grain characteristics of sourdough wheat bread. *Eur. Food Res. Technol.*, v.214, n.6, p.489-496, 2002.

on sourdough: from fundamentals to applications. *Trends Food Sci. Tech.*, v.16, n.1, p.2-3, 2005.

identification of sourdough lactic acid bacteria. *Food Microbiol.,* v.24, n.2, p.120-127, 2007.

starter cultures for sour maize bread. *Food Microbiol.,* v.25, n.4, p.616-625, 2008.

VESCOVO, M. Molecular characterization of *Lactobacillus casei* strains. *FEMS Microbiol. Lett.*, v.140, n.2-3, p.215-219, 1996.

T.P. *Fundamentals of cheese science.* Gaithersburg: Aspen Publishiers, 2000. 587 p.

patterns, dough rheology and bread properties. *Eur. Food Res. Technol.*, v.225, n.5-6, p.821-830, 2007.

of fermented/ germinated cowpea flour addition on the *J. Food Eng.,* v.63, n.2, p.177-184, 2004.

BRASIL. Ministério da Saúde. Instituto Adolfo Lutz. Métodos 2004. 1018 p.

Optimization of sourdough process for improved sensory profile and texture of wheat bread. *LWT – Food Sci. Technol.*, v.39, n.10, p.1189-1202, 2006a.

sourdough and enzymes on staling of high-fibre wheat bread. *LWT – Food Sci. Technol.*, v.39, n.5, p.479-491, 2006b.

C.; RÉMÉSY, C. Making bread with sourdough improves in rats. *Nutrition*, v.19, n.6, p.524-530, 2003.

PARAMITHIOTIS, S.; CHOULIARAS, Y.; TSAKALIDOU, E.; cultures for the production of wheat sourdough bread using a traditional three-stage procedure. *Process Biochem.,* v.40, n.8, p.2813-2819, 2005.

PLESSAS, S.; ALEXOPOULOS, A.; BEKATOROU, aroma volatile composition during storage. *Food Chem.,* v.124, n.2, p.627-633, 2011.

immobilized cell biocatalysts in baking. *Process Biochem.,* v.42, n.8, p.1244-1249, 2007.

Kluyveromyces marxianus, Lactobacillus delbrueckii ssp. *bulgaricus* and *L. helveticus* for sourdough bread making. *Food Chem*., v.106, n.3, p.985-990, 2008.

ROBERT, H.; GABRIEL, V.; LEFEBVRE, D.; RABIER, P.; behaviour of *Lactobacillus plantarum* and Leuconostoc starters during a complete wheat sourdough breadmaking process. *LWT – Food Sci. Technol.,* v.39, n.3, p.256-265, 2006.

52//1 (1(/,6 0 2%%(77,0 (
9(=)3URWHRO\WLFDFWLYLW\DQGUHGXFWLRQRIJOLDGL
like fractions by sourdough lactobacilli. *J. Appl. Microbiol.*, v.99, n.6, p.1495-1502, 2005.

61=3(1(//+0707<2502626+526
0SSOLFDWLRQRIELGREDFWHULDDVVWDUWHUFXOWX
wheat sourdough breadmaking. *Food Biol. Technol.*, v.5, n.6, p.2370-2380, 2012.

6,/9 1 -8148(,5 9 6,/9(,5 1)
71,:,0+6172651)620(655
Manual de métodos de análise microbiológica de alimentos e água. 6mR3DXOR9DUHOHS

*,***Q*

RKHWRKUGKSDUDPHWHUVWRWHPSHDWXUHDQG-
sucrose variations. *Food Microbiol.*, v.20, n.2, p.193-199, 2003.

7+,(/(1=/(092(/5)RWULEXWLRQRI
sourdough lactobacilli, yeast and cereal enzymes to the generation of amino acids in dough relevant for bread DYRXU. *Cereal Chem.*, v.79, n.1, p.45-51, 2002.

:(+5/(5(17(5)5KHRORJLFDOFKDQJHVLQZKHDW
sourdough during controlled and spontaneous fermentation. *Cereal Chem.*, v.75, n.6, p.882-886, 1998.

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