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Effect of Sourdough on Shelf Life, Freshness and Sensory Characteristics of Egyptian Balady Bread

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Abstract The present study was aimed to improve the quality characteristics of Egyptian balady bread by using sourdough containing (2% Saccharomyces *cerevisiae*+ 1, 2 or 3% Lactobacillus *plantarum*). Microbial contents i.e. lactic acid bacteria, total bacterial count and yeasts, pH, organic acids and antimicrobial activity were evaluated during sourdough fermentation. Results showed an increase in organic acids, antimicrobial activity and reduction in pH during the preparation of different sourdough samples. These metabolites were increased by increasing lactic acid bacteria ratio in sourdough (3%> 2%> 1% *Lb. plantarum*). A significant reduction in total bacterial count and a significant increase in LAB and yeast count during fermentation period was recorded. Bread characteristics showed an extension of shelf life for 8 days for bread samples containing sourdough (2 or 3% *Lb. plantarum*) comparing to 3 days for control bread. Addition of 20% sourdough containing 2 or 3% *Lb. plantarum* to wheat flour dough also retarded staling rate by 19.98% and 19.30% after 3 days comparing to control sample was (42.84%). Improvements in sensory characteristics and acceptability of balady bread were also recorded. Accordingly, this could reduce the bread losses and consequently reduce the amount of wheat flour used.

Keywords: *sourdough, lactic acid bacteria, antimicrobial activity, organic acids, shelf life, staling and sensory evaluation*

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1. Introduction

Bread is generally viewed as a perishable commodity. It is one of the main staple foods consumed by humans. Its shelf life is limited by two main factors, i.e. staling and microbial spoilage (fungi spoilage and ropiness) [1] and [2].

A common trend of sourdough fermentation is the unique symbiosis of certain hetero and homo fermentative lactic acid bacteria with certain yeasts. The interaction of yeasts and lactobacilli is important for the metabolic activity of sourdough. Several yeasts have been found in sourdoughs but Saccharomyces *cerevisiae is* considered the dominant organism for bread leavening. The most relevant bacteria isolated from sourdough belong to the genus *Lactobacillus*. The rheology, flavor, nutritional and functional properties of sourdough based baked products greatly rely on the activity of these microorganisms. Lactic acid bacteria usually originate from either the flour, dough ingredients or the environment [3,4,5,6].

The use of the sourdough process as a form of leavening agent is one of the oldest biotechnological processes in food production that used for thousands of years and generally regarded as safe (GRAS). Traditional acidic sourdough is ancient way to improve flavor, texture and microbiological shelf life of bread used in Mediterranean countries [1].

Lactic acid bacteria to yeast ratio in sourdough are generally 100:1 [7]. Sourdough has been classified into three types, i.e. the first type produced with the traditional technique characterized by continuous daily refreshment to keep the microorganisms in an active state, Type II often use dough souring supplements during bread preparation and are characterized by long fermentation periods (from 2 up to 5 days) and fermentation temperature > 30°C to speed up the process. The third type is a dried product that increases the sourdough shelf life [8,9].

Most of the beneficial properties attributed to sourdough were determined to be due to the acidification activity of lactic acid bacteria (LAB) and the produced metabolites i.e. organic acids, exopolysaccharides (EPS) and enzymes. Sourdough LAB fermentation created an optimum pH for the activity of the endogenous enzymes (amylases and proteases)that improve the loaf volume; delaye starch retrogradation and bread firming, inhibit ropiness by spore-forming bacteria and enhance flavor [10-21].

Growth of *B. subtilis* and *Bacillus lichenformis* was inhibited by *Lb. plantarum VTT* in tests with wheat bread. Rope spoilage of wheat bread was inhibited by adding 20-30 g sourdough/ 100 g of wheat dough containing *Lb. plantarum* [12].

[22] Stated that, bacteriocin produced *by Lb. plantarum* has broad spectrum of inhibition against both pathogenic, food spoilage organisms and various lactic acid bacteria.

It has been reported that the antifungal activities of LAB might include reuterin, plantracin, hydroxyl fatty acids, proteinaceous compounds, cyclic peptides, 3-phenyllactic acid, caproic acid, diacetyl and hydrogen peroxide [17,23,24].

[25] Found a gradual increase in all organic acids concentrations during fermentation of sourdough starter (mixed culture of *Lb. plantarum* and *S. cerevisiae*). He observed that the rate of development of organic acids production was lower in yeast fermentation compared to other types of doughs containing LAB. The improved microbial shelf life of sourdough was initially attributed to the organic acids produced by LAB. Also, lactic acid bacteria had a fungistatic effect that attributed to acetic acid production [26].

The starter sourdoughs including LAB have greater antimicrobial activity against saprophytic microorganisms: *Bacillus subtilis, B. mesentericus*, Aspergillus *niger*, *Penicillium sp. and Rhizopus sp.*, but none of them inhibited the growth of the baker's yeasts Saccharomyces *cerevisiae*. It was established that addition of 10% of sourdough could prevent bacterial spoilage during bread making. While for prevention of mold spoilage the necessary amount of starter sourdough was from 15-20%.The application of the developed starters in wheat bread guaranteed longer shelf life with no adverse alterations in the features of the final bread [27,28].

The present study was carried out to evaluate the quality of balady bread as affected by the addition of sourdough containing *S. cerevisiae* and *Lb. plantarum.* In addition, identifying the produced metabolites (organic acids and other antimicrobial compounds) and their effect on shelf life, freshness and sensory characteristics of the produced balady bread was also evaluated.

2. Materials and Methods

2.1. Wheat Flour (*Triticumaestivum* L. Vulgare)

Wheat flour, 82% extraction was obtained from South Cairo Mills Company, Faysal, Giza, Egypt.

2.2. Microorganisms and Media

Pure cultures of Lactobacillus *plantarum* ATCC 14917., and Saccharomyces *cerevisiae* ATCC 4126 obtained from Microbiological Resource Center (Cairo – MIRCEN), Faculty of Agriculture , Ain Shams University, Cairo, Egypt were used.

The referenced microorganisms i.e. Aspergillus *niger* RCMB02317, Penicillium *italicum* RCMB 03924, Candida *albicans* RCMB o5031, Geotaricum *candidum* RCMB 05097 and Fusarium *oxysporum* RCMB 08213.Three Gram positive bacteria (Staphylococcus *aureus* RCMB 010028, Bacillus *subtilis* RCMB 010067 and Enterococcus *faecalis* RCMB 010068) and three Gram negative bacteria (Pseudomonas *aeruginosa* RCMB 010043, Escherichia *coli* RCMB 010052 and Salmonella *typhimurium* RCMB 010072), obtained from the Regional Center for Microbiology and Biotechnology, Al Azhar University, Cairo, Egypt, were also used. Strains were propagated as follows: *S. aureus, E. faecalis, E. coli* and *S. typhimurium* were cultured in a nutrient agar media at 37°C; *P. aeruginosa* cultured on the same medium at 28°C and *B. subtilis* were grown in (BHI) broth (Oxoid) at 30°C. All fungi were cultured on YM medium at 25°C.

2.3. The Proximate Chemical Analysis

Moisture, ash, crude fiber, lipids and crude protein (NX 5.71) and carbohydrates content of wheat flour was carried out according to [29].

2.4. Preparation of Sourdough Starters

Yeast strain (*Saccharomyces cerevisiae*) and (LAB) *Lactobacillus plantarum* were grown on Yeast Malt (YM) broth and MRS broth (Formula developed by Man. Rogosa and Sharpe to facilitate the growth of lactobacilli in general [30], respectively, at 30ºc for 24 h. The cells were harvested by centrifugation (Sigma 3K12, 5000 xg, 10 min) and washed twice with sterilized distilled water. Under these conditions 1g of either yeast or LAB pellet contained ≈ 10^6 and 10^9 cfu respectively, and theses were used for preparation of sourdough starters. Sourdough starter was prepared according to the methods described by [31] as follows:-Wheat flour (400 g) and various amounts of starter culture (w/w based on flour bases as (2% *S. cerevisiae* (S); 2% *S. cerevisiae*+ 1%*Lb. plantarum* (SL1); 2% *S. cerevisiae*+ 2% *Lb. plantarum* (SL2) and 2% *S. cerevisiae*+ 3% *Lb. plantarum* (SL3))were mixed with 200 ml sterilized tap water, for 5- 10 minutes, and left for 24 h. The prepared sourdough starters were added to bread dough at (20% w/w wheat flour), control bread were made with baker's yeast as a leavening agent without using sourdough starter.

2.5. Microbiological Analysis

Sourdough samples were analyzed at time points i.e. zero, 6, 12, and 24 h during fermentation period for total bacterial count (TBC), yeast and lactic acid bacteria (LAB) count). Total bacterial and fungal count of balady bread loaves were also determined during storage periodically i.e. zero, 1, 2, 3, 4, 5, 6, 7 and 8 days according to [32].

2.6. Sourdough Acidity and Organic Acids

Sourdough pH was determined by a pin electrode of a PH meter [5]. The organic acid contents of sourdough i.e. lactic, acetic, pyruvic, citric, mallic and formic were determined and quantified by HPLC apparatus (Hewlett Packard, series 1050) according to the method described by [33].

2.7. Determination of Sourdough Antimicrobial Activity

The ability of sourdough starter (*Lb. plantarum* strain) to produce antimicrobial metabolites was tested by an agar diffusion assay as described by [34] and [22], sourdough centrifugated at 6400 rpm for 15 min, the cells is removed. To eliminate the inhibitory effect of lactic acid and H_2O_2, the cell- free supernatant was neutralized with 1 M NaOH and treated with catalase enzyme (1mg ml $^{-1}$), followed by filtration through a cellulose acetate membrane filter with pore size 0.22 µm (ADVANTEC MFS, Inc., Japan). The resulting is referred to as a crude bacteriocin.

The referenced fungi were cultivated in an incubator at 25°C on yeast malt (YM) media for 3-7 days, whereas referenced bacteria were propagated for 3 days on optimal temperatures. Spores and cells were harvested from slants after growing to prepare inoculums containing ~ 10^5 spores/ cells ml^{-1} of fungi and ~ 10^5 cells ml^{-1} of bacteria. A 100µl of the indicator strain was poured into Petri dishes and overlaid with soft agar medium cooled to 45°C and mixed. Sourdough supernatant (50µl) was added to each well (6mm in diameter) punched in the cooled agar plates and incubated for 48 h at the optimal growth temperature. The antimicrobial activities were determined by measuring the inhibition zones (mm).

2.8. Balady Bread Making

Balady bread was prepared according to the method described [35].

2.9. Alkaline Water Retention Capacity (AWRC %)

The staling rate of balady bread was determined by alkaline water retention capacity method as described by [36].

2.10. Organoleptic Evaluation of Balady Bread

Fresh samples of balady bread loaves were organoleptically evaluated for i.e. crust color, crust characteristics, crumb color, grain and texture, flavor and taste and chewing as illustrated in Figure 1 according to [37].

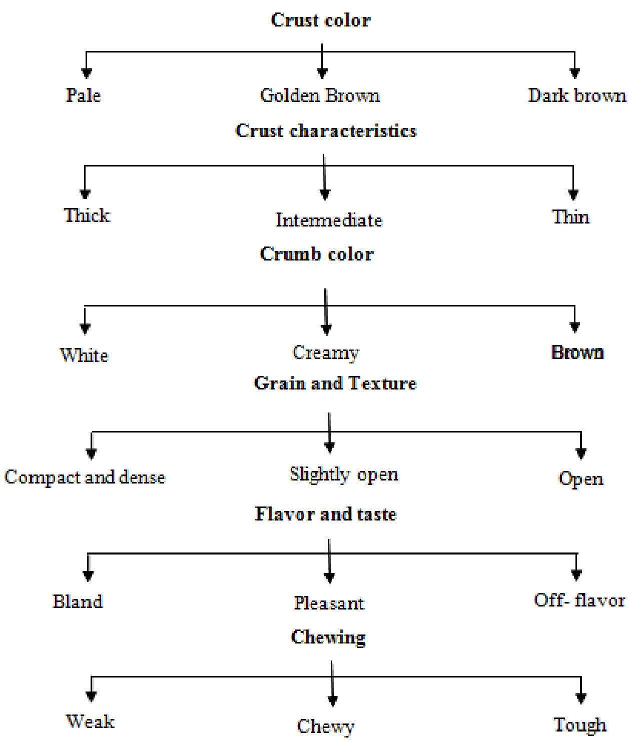

Figure 1. The score sheet used by the panelists for sensory evaluation of Egyptian balady bread [37]

2.11. Statistical Analysis

Data were handled by Analysis of Variance using General Liner Model (GLM) procedure according to the procedure reported by [38]. Means were separated using Duncan's test at a degree of significance (P≤ 0.05). Statistical analyses were made using the producer of the SAS soft ware system program [39].

3. Results and Discussions

Chemical composition of wheat flour show that it contained 12.1% moisture, 1.24% ash, 1.13% lipids, 11.59% crude protein, 1.18% crude fiber and 84.69% carbohydrates.

3.1. Sourdough Characteristics

3.1.1. TBC, Yeast and LAB Count

Results in (Table 1) show a highest significant reduction rate of (TBC) in sourdough containing 2% *S. cerevisiae*+ 3% *Lb. plantarum* (SL3). Such reduction in TBC could be due to the increase in the produced metabolites, such as organic acids and bacteriocins as well as the decrease in pH (Table 2) that inhibited the growth of the spoilage flora. The above mentioned results are similar with those reported by [40] and [25].

Table 1. Yeast, lactic acid bacteria, total bacterial counts (log $_{10}$cfu g^{-1}) during sourdough starter preparation

Treatments	Fermentation time (h)	Yeast	LAB	TBC
S	Zero	7.11 ^{h}	6.28^i	10.75 ^{a}
	6	7.22 ^{g}	6.75 k	10.72 ^{c}
	12	7.45 ^{f}	6.97 ^{g}	9.89 ^{e}
	24	8.15 ^{b}	7.20 ^{i}	9.15 ^{f}
SL1	Zero	7.12 ^{h}	8.39 ^{h}	10.92 ^{a}
	6	7.23 ^{g}	9.05 ^{g}	10.08 ^{d}
	12	7.63 ^{e}	9.90 ^{e}	9.07 ^{f}
	24	8.21 ^{b}	10.11 ^{d}	8.56 ^{h}
SL2	Zero	7.10 ^{h}	8.86 ^{g}	10.91 ^{ab}
	6	7.27 ^{g}	9.32 ^{f}	9.95 ^{de}
	12	7.73 ^{d}	10.32 ^{cd}	8.86 ^{g}
	24	8.33 ^{a}	10.91 ^{b}	7.91 ^{j}
SL3	Zero	7.11 ^{h}	9.01^g	10.98 ^{ab}
	6	7.57 ^{g}	10.72 ^{cd}	10.74 ^{bc}
	12	7.82 ^{c}	10.96 ^{c}	8.32 ^{i}
	24	8.63 ^{a}	11.64^a	6.22 ^{k}
LSD		0.0602	0.1719	0.1616

Means in same column with different letters are significantly different (P≤0.05). S (2% *S. cerevisiae*), SL1 (2% *S. cerevisiae*+ 1% *Lb. plantarum*), SL2 (2% *S. cerevisiae*+ 2% *Lb. plantarum*) and SL3 (2% *S. cerevisiae*+ 3% *Lb. plantarum*).

The yeast count in sourdough samples were significantly increased by increasing the fermentation period, and the yeast count also increased in the mixed culture of *S. cerevisiae* with *Lb. plantarum*. The highest number of yeasts was recorded in sourdough containing 3% *Lb. plantarum* (SL3). This indicates that the incorporation of *Lb. plantarum* enhanced the growth of *S. cerevisiae* during the fermentation of sourdough [40].

Results in (Table 1) also show that sourdough samples prepared by using only *S. cerevisiae* contained LAB as a normal flora but lower numbers than those for the other treatments. In sample (SL3) LAB counts were significantly increased from9.01 log cfu/ g at the beginning of the preparation to 11.64 log cfu/ g at the end of the preparation period [25,41,42].

3.1.2. Organic Acids and Acidity of Sourdough

Results in Table 2 show the different organic acids formed during the preparation of sourdough. In case of the (S) sample containing only *S. cerevisiae*, all organic acids i.e. citric, formic, acetic, malic, pyruvic and lactic were almost produced almost at the same concentrations. The highest values of all determined organic acids were found in sourdough samples SL 3 followed by SL 2 and SL 1. Addition of LAB to yeast caused a high increase in the concentration of lactic and acetic acids SL 3, this indicates that the dominant organic acids i.e. lactic and acetic produced during fermentation of sourdough in samples (SL 1, SL 2 and SL 3) were significantly higher than those produced in sample S. Therefore the addition of sourdough containing these metabolites to wheat flour dough would affect dough and final product characteristics (shelf life, freshness and sensory properties). The results are in line with [33,43].

Table 2. Organic acids and pH values during sourdough preparation

Starter	FT (h)	Organic acids mg/100g						
		Citric	Formic	Mallic	Acetic	Pyruvic	Lactic	pH
S	0	ND	ND	ND	ND	ND	ND	5.88^{a}
	6	2.709	3.758	4.740	4.667	0.931	10.334	5.81^{b}
	12	18.718	7.246	12.241	8.631	3.370	16.318	5.75^{c}
	24	43.767	27.199	24.732	20.826	6.423	36.097	4.53^{g}
SL 1	0	ND	ND	ND	ND	ND	ND	5.87^{a}
	6	3.721	5.427	8.920	34.617	2.731	65.732	5.09^{d}
	12	23.742	12.843	16.337	78.127	4.941	165.420	4.87^{e}
	24	51.564	33.725	31.172	184.220	11.024	278.71	4.13^{j}
SL 2	*0*	ND	ND	ND	ND	ND	ND	5.85^{ab}
	6	6.293	7.808	13.241	78.635	4.124	151.887	4.74^{f}
	12	38.460	24.224	27.304	145.410	8.039	365.411	4.33^{i}
	24	62.383	47.710	46.901	267.905	18.184	601.045	3.57^{l}
SL 3	0	ND	ND	ND	ND	ND	ND	5.89^{a}
	6	10.412	11.804	19.717	93.929	6.241	233.140	4.45^{n}
	12	52.162	38.920	37.634	207.218	10.792	402.736	4.05^{k}
	24	99.347	63.522	72.582	369.111	27.147	782.27	3.19^{m}

FT: Fermentation Time, ND: Not detected. S (2% *S. cerevisiae*), SL1 (2% *S. cerevisiae*+ 1% *Lb. plantarum*), SL2 (2% *S. cerevisiae*+ 2% *Lb. plantarum*) and SL3 (2% *S. cerevisiae*+ 3% *Lb. plantarum*).

In Table 2 it is also shown that the pH values were significantly decreased by increasing fermentation period of sourdough and the highest significant reduction were recorded at (SL3)after 24 h. The reduction in pH would be due to the great production of organic acids during preparation of sourdough by LAB as mentioned before. These results are in agreement with those mentioned by [5,12,14,33,44].

Table 3. Mean zone of inhibition in mm in sour dough samples ± standard deviation beyond well diameter (6mm) produced on a range of environmental and clinically pathogenic microorganisms

Sample / Tested microorganisms		S				SL 1				SL 2					SL 3		Standard antibiotic
FT (hr)	**zero**	**6hr**	**12hr**	**24hr**	**zero**	**6hr**	**12hr**	**24hr**	**zero**	**6hr**	**12hr**	**24hr**	**zero**	**6hr**	**12hr**	**24hr**	**Amphotricin**
FUNGI																	
A. niger	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	12.6±0.11	NA	15.6±0.10	17.4±0.16	20.2±0.2	22.3±0.58
p. italicum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	12.24±63	19.32± 0.72
G. candidam	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	13.2±0.72	15.6±0.27	NA	16.4±0.12	18.1±0.25	22.8±0.18	23.14± 0.58
F. oxysporum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	14.3±0.14	NA	13.8±0.16	16.1±0.31	20.6±0.23	18.32± 0.48
Gram positive bacteria																	Ampicillin
Staph. aureus	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	11.6±0.28	NA	12.6±0.18	15.2±0.34	19.3±0.16	22.36±0.44
B. subtilis	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	14.2±0.37	NA	11.7±0.35	13.4±0.25	17.1±0.19	24.25±0.58
E. .feacalis	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	13.3±0.12	NA	NA	11.7±0.63	16.5±0.36	21.25±0.58
Gram negative bacteria																	Gentamycin
Pseudo. aeruginosa	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	12.3±0.18	14.2±0.13	16.2±0.16	19.58±0.58
E. coli	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	12.6±0.20	NA	10.6±0.3	12.6±0.22	13.1±0.1	22.36±0.44
S. typhimurium	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	16.2±0.58	17.1±0.24	NA	18.1±0.15	19.6±0.31	22.4±0.29	23.25±0.58

Means of triplicate± SD. F T: Fermentation time ND: Not detected. S (2% *S. cerevisiae*), SL1 (2% *S. cerevisiae*+ 1% *Lb. plantarum*), SL2 (2% *S. cerevisiae*+ 2% *Lb. plantarum*) and SL3 (2% *S. cerevisiae*+ 3% *Lb. plantarum*).

3.1.3. The antimicrobial Effect of Sourdough

In Table 3 are presented the inhibition zones which are an indication for the antimicrobial effect of the metabolites (crude bacteriocin) produced by *Lb. plantarum* during sourdough fermentation on the investigated microorganisms (G+, G- and fungi). Results show that the metabolites produced in sourdough containing only (2% *S. cerevisiae*) or sourdough containing (2% *S. cerevisiae*+ 1% *Lb. plantarum*) had no effect on the tested microorganisms. However, increasing *Lb. plantarum* to 2% and 3% in sourdough resulted in antimicrobial effects on the tested microorganisms after 24 and 6 hrs of fermentation time, respectively. It was also found that *E. feacalis* was completely inhibited after 12 h of preparation time. This indicates that sourdough containing (*S. cerevisiae*+ 2 or 3% *Lb. plantarum*) had an antimicrobial activity that inhibited the tested microorganisms (G+, G- and fungi) compared to the dough containing only *S. cerevisiae*. The results showed that the produced metabolites (bacteriocins) could inhibit some of the spoilage microorganisms i.e. *B. subtilis* and *A. niger* (Previous results in Table 1) in wheat flour dough and might be in bread, which could consequently (this also would)affect the shelf life and other quality characteristics of the produced bread. LABs have been shown by many researches to possess both anti-bacterial and anti-fungal properties. Sourdough addition is an effective procedure to preserve wheat flour dough and bread from spoilage since it complies with the consumer request for additive-free products [4,23,27].

3.2. Balady Bread Characteristics

3.2.1. Shelf Life of Balady Bread

The bacterial and fungal counts in balady bread were determined at intervals i.e. zero, 1, 2, 3, 4, 5, 6, 7 and 8 days (Table 4 and Table 5), the results showed that the shelf life of balady bread was elongated from 3 days for control sample to 8 days for bread samples fermented by either SL 2 (2% *Lb. plantarum)* or SL 3 (3% *Lb. plantarum*). The results also showed that the highest values of bacterial and fungal counts were found in the control sample and bread fermented by sourdough starter containing only 2% *S. cerevisiae*. These counts were reduced as *Lb. plantarum* increased in sourdough.

Table 4. Log total bacterial count of balady bread

	Storage period (day)								
	zero	1	2	3	4	5	6	7	8
Cont.	2.04±0.04	2.81±0.055	3.53±0.041	5.81±0.01	7.86±0.035	9.61±0.06	-	-	-
20% S	2.0±0.06	2.32±0.04	2.97±0.047	3.86±0.07	5.92±0.06	7.57±0.04	-	-	-
20% SL 1	1.95±0.05	2.27±0.05	2.83±0.03	2.97±0.01	3.72±0.045	4.15±0.05	4.95±0.03	5.78±0.046	6.59±0.05
20% SL 2	1.93±0.04	2.08±0.035	2.63±0.06	2.79±0.031	2.90±0.03	3.59±0.04	3.86±0.035	4.53±0.01	4.96±0.04
20% SL 3	1.95±0.045	2.0±0.05	2.36±0.035	2.65±0.025	2.83±0.025	2.95±0.036	3.28±0.035	4.51±0.035	4.80±0.03

Means of triplicate± SD.

Table 5. Log total fungal count of balady bread

	Storage period (day)								
	zero	1	2	3	4	5	6	7	8
Cont.	1.84±0.02	3.81±0.01	5.73±0.02	7.96±0.02	9.59±0.02	9.98±0.02	-	-	-
20% S	1.83±0.02	2.92±0.02	4.57±0.02	5.72±0.01	7.82±0.02	9.90±0.026	-	-	-
20% SL 1	1.82±0.02	2.46±0.02	3.79±0.015	4.94±0.02	5.88±0.03	7.57±0.03	7.95±0.025	8.69±0.02	-
20% SL 2	1.82±0.02	2.13±0.02	2.90±0.02	3.497±0.03	4.27±0.03	4.88±0.03	5.42±0.02	5.42±0.02	6.72±0.02
20% SL 3	1.81±0.02	2.04±0.02	2.48±0.02	2.95±0.02	3.95±0.025	4.64±0.03	4.51±0.025	4.51±0.015	4.97±0.02

Means of triplicate± SD. S (2% *S. cerevisiae*) SL1 (2% *S. cerevisiae*+ 1% *Lb. plantarum*) SL2 (2% *S. cerevisiae*+ 2% *Lb. plantarum*) and SL3 (2% *S. cerevisiae*+ 3% *Lb. plantarum*).

Results also, showed that addition of 20% sourdough containing different combinations of (2 or 3% *Lb. plantarum*) with 2% *S. cerevisiae* to wheat flour dough were effective in inhibiting the growth of molds and bacteria in the produced bread. Mold spoilage of the control sample was noticed on the third day, it was delayed until the eighth day of storage without any indication for initiation of attack of mold spoilage in the case of use of sourdough containing either 2% or 3% *Lb. plantarum*. These concentrations of *Lb. plantarum* in the sourdough would consequently extend the shelf life of bread. This indicates that the production of organic acids (Table 2) and other metabolites compounds by *Lb. plantarum* during sourdough fermentation (Table 3) had an antimicrobial effect on the spoiled microorganisms. These results are in agreements with [45] who mentioned that, the bacteriocin associated with organic acids had antimicrobial effect. Also, [10] reported that, lactic acid bacteria produced a mixture of organic acids such as, lactic, acetic formic, caproic, propionic, butyric and n-valleric acting in a synergistic way and responsible for the antimold activity. The results are in line with [46] and [47] who found that using sourdough in bread making preventing mold and bacterial spoilage. [26] Stated that the onset of fungal growth was delayed for 7 days in bread started with *S. cerevisiae* and *Lb. plantarum* 21 B. using sourdough led to a positive effect on prolonged shelf-life. [12,48].

3.2.2. Freshness of Balady Bread

Table 6 is shown results of a gradual reduction in bread freshness as measured by AWRC% in all different samples during storage period. The lower reduction in freshness (Alkaline water retention capacity (AWRC)) values was noticed in SL 3 samples (comparing with control and other treatments. The incorporation of LAB with yeast during preparation of sour dough starter decreased the staling rate of balady bread as measured by AWRC ratio. This might be due to the presence of metabolites such as organic acids (Table 2) and antimicrobial compounds (Table 3)that have a positive effect on bread staling**.** These results are in line with [49] and [15] who reported that, the use of sourdough improved the structure of the gluten network and might alter water migration between starch, protein and bran particles during storage. Epoxypolysaccharides (EPS) acted as bread improvers, while, organic acids affected the protein and starch fractions of flour. Additionally, the drop in pH associated with acid production caused an increase in the enzymes activity of the flour, thus led to a reduction in staling and improved the textural qualities of bread [2,50].

Table 6. Alkaline water retention capacity (AWRC %) of sour balady bread during storage at room temperature (≈ 25ºC)

	Storage period (days)						
Short name	0	1	2	3	4	5	6
Cont.	317.36 [e]	250.65 [e]	219.05[e]	181.4[e]	-	-	-
20% S	331.06[d]	303.54[d]	266.73[d]	210.63 [d]	164.89 [d]	-	-
20% SL 1	346.71 [c]	328.59 [c]	298.25 [c]	277.05 [c]	234.93 [c]	202.41 [c]	-
20% SL 2	361.72 [b]	339.82 [b]	312.04 [b]	289.42 [b]	256.06 [b]	218.99 [b]	194.85 [b]
20% SL 3	377.95[a]	365.16 [a]	332.15 [a]	304.98 [a]	286.54 [a]	253.31 [a]	219.11 [a]
LSD	0.8906	0.5921	0.6085	0.5860	0.6279	0.5361	0.3490

Means in same column with different letters are significantly different (P≤0.05).

Table 7. Sensory evaluation of balady bread made from sour dough

Sample code	Crust color	Crust characteristics	Crumb color	Grain and texture	Flavor and Taste	Chewing
	Ideal Score					
	5	6	5.5	7.5	5	5
Cont.	4.33± 0.57 [bc]	5.33 ± 0.57 [a]	5.0 ± 0	5.67 ± 0.58 [b]	5 ± 0[b]	3.66 ± 0.58[b]
20% S	5.33 ± 0.57 [a]	5 ± 0[a]	5.0 ± 0	6.33 ± 0.58[b]	5.67 ± 0.58 [b]	6 ± 0.0 [a]
20% SL 1	3.67 ± 0.57 [c]	5 ± 0[a]	5.0 ± 0	8 ± 0.5[a]	5.83 ± 0.76 [b]	5.66 ± 0.58[a]
20% SL 2	4 ± 0 [c]	5 ± 0[a]	5.0 ± 0	8 ± 0[a]	5.83 ± 0.29 [b]	5 ± 1[a]
20% SL 3	5.0 [ab]	5 ± 0[a]	5.0 ± 0	7.830 ± 29[a]	7 ± 0.5[a]	5.33 ± 0.58[a]
LSD	0.8136	0.4697	0.0	0.8136	0.9096	1.1506

Means in same column with different letters are significantly different (P≤0.05). S (2% *S. cerevisiae*), SL1 (2% *S. cerevisiae*+ 1% *Lb. plantarum*), SL2 (2% *S. cerevisiae*+ 2% *Lb. plantarum*) and SL3 (2% *S. cerevisiae*+ 3% *Lb. plantarum*).

3.2.3. Sensory Characteristics of Balady Bread

Sensory characteristics of fresh balady bread samples fermented with 20% sour dough starter showed no significant differences (p> 0.05) in crust color, crust characteristics and crumb color between control sample and other samples. The addition of sourdough starter to wheat flour dough improved the grain and texture characteristics. A slight improvement in bread flavor and taste was noticed between control sample and those made from sour dough. Furthermore, sourdough samples scored higher sensory characteristics when compared to those without sourdough [19,40,48,51].

4. Conclusion

From the above results it might be concluded that the fermentation of wheat flour dough by using sourdough containing 2% *S. cerevisiae* and 1, 2 or 3% *Lb. plantarum* result in the formation of organic acids, that together with other compounds formed (bacteriocins) have antimicrobial activities also the pH decreases. It is probable that these effects are cause by the extra activities when *S. cerevisiae* and LAB are brought together in the fermented dough. The combination cause an increase in the effect of the total active metabolites than that present in the added sourdough starter. The metabolites added and formed in the dough were shown to greatly improve the final bread. The results also strengthen the reason to use of *Lb. plantarum* in food processing as a bio-preservative due to the broad inhibition spectrum found especially in bread making and bakery products.

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