

## **Sensory evaluation and staling of bread produced by mixed starter of *saccharomyces cerevisiae* and *L.plantarum***

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### **ABSTRACT**

Impact of processed conditions and type of starter cultivars on characteristics of wheat dough bread by using mixed starter cultivars of *Sacch. cerevisiae* and *L. plantarum* was estimated. *L. plantarum* seemed to be more effective in combination with *Sacch. cerevisiae* on the dough volume. Dough produced by starter containing *L. plantarum* characterized with lower PH and higher total titratable acidity (TTA) and moisture content, in comparison with control treatment. Highly significant differences in aroma and crumb texture were recorded with bread produced by starter culture containing *L. plantarum*. The obtained results also revealed significant differences in bread firmness which reflected the staling and its rate among the tested treatments after 1, 3, 5 and 6 days during storage time at room temperature. Data also confirmed a processing technique using *L. planturium* mixed with *Sacch.cerevisia* to enhance organoleptic properties of produced bread.

### **INTRODUCTION**

Cereal fermentation is one of the oldest biotechnological processes, dating back to ancient Egypt, where both beer and bread were produced by using yeasts and lactic acid bacteria (LAB), Clarke and Arendt, (2005). Starters composed of specific individual LAB, or mixed with yeasts, became available a few years ago allowing the production of a full sourdough in one-stage process. Such commercial starters improve the control of the sourdough production while ensuring reliable quality in bread production Corsetti *et al.*, (2001).

The modern biotechnology of baked goods largely uses sourdough as a natural leavening agent because of many advantages over baker's yeast, e.g. in the development of the characteristic flavor and resulting in a final product with high sensory quality Hansen and Hansen,( 1996).

Use of lactic acid bacteria and yeast strains as a part of the sourdough formulation resulted in improved crust properties and greater anti-mold activities. At the same time, the favorite sensorial attributes that are specific to sourdough bread is preserved Najafi *et al.*, (2012).

Valeria *et al.*, (2013) reported the inhibitory activity of *L. plantarum* strains tested on used indicator fungi similar to that obtained when using calcium propionate as preservative agent for baking industry.

Osama *et al.*, (2013) confirmed capability of *L. plantarum* for improving bread quality; good flavor and natural antifungal substances which could be inhibit molds growth.

Ojokoh *et al.*, (2013) studied the effect of fermentation process on the chemical composition, anti-nutrient content, pH, titratable acidity, and microbiological changes of bread fruit and cowpea blends.

Niloofer *et al.*, (2013) reported that mixture cultivars of *Saccharomyces cerevisiae* and *Lactobacillus p.* (ATCC43332) had significant effect ( $p < 0.05$ ) on shelf life of soy bread in comparison with control sample.

The aim of the current study was to evaluate the impact of *Lactobacillus plantarum* in improving the quality and increasing shelf life of bread.

## MATERIALS AND METHODS

### Materials:-

#### Flour:

The wheat flour used throughout the current study for the preparation of sourdough and bread samples was commercial-type 72 % extraction rate which is specific for bread making in Egypt. The used wheat flour was obtained from North Cairo Flour Mills Company. Which characterized with moisture content, 13.2 %; mineral, 0.55 %; proteins, 12.85 %; fiber, 0.5 %; fat, 0.99 %; carbohydrates, 85 %. Sugar, active yeast and salt were purchased from local market, Giza-Egypt.

#### Starter cultures and microbiological media

Two strains of *Saccharomyces cerevisiae* (Y-66 and Y-64) were used as starter cultures for dough fermentation. Yeast strains were provided from Agricultural Res. Center, Giza, Egypt. One strain of *Lactobacillus p.* (L.NRRL) was used as starter culture to initial sourdough fermentation.

Yeast strains were propagated on nutrient broth medium Difco, (1985) and incubated at 30 oC for 48 hrs. The fresh starter culture was obtained by centrifugation (4000 xg for 15 min.) collection of the cells pellets (fresh yeast) was done. *Lactobacillus p.* was propagated on MRs medium (agar) and incubated at 37 oC for 48 hrs. The fresh starter culture was obtained by centrifugation (8000 xg for 15 min.), as described earlier Katina *et al.*, (2004).

#### Sourdough fermentation

Fifteen sourdough fermentation runs were handout. Fermentation mixture contained 1 Kg flour, 600 ml water, 10 gm sugar, 15 gm salt and 10 gm of fresh yeast. Starter cultures of *L. plantarum* were added at rate of 10, 20, 40 and 50 % on fresh weight yeast basis as shown in Table (1). The inoculated sourdoughs were incubated at 28 oC for 30 min and relative humidity of 76 %, and then the dough were divided into 100 gm loaves and molded mechanically. The loaves were proofed in pans (60 min at 35 oC, RH 75 %). Water activity was performed. For shelf-life evaluation, loaves were

stored in plastic bags at 25 oC for six-days. Texture profile analysis (TPA) was estimated after 1, 3, 5 and 6-days. Fifteen bread groups were performed for sensory evaluation and texture analysis.

**Table (1) Sourdough fermentation runs**

<b>code</b>	<b>Fermentation sourdough mixture</b>
T <sub>1</sub>	<i>Sac.66+other ingredient (control 1)</i>
T <sub>2</sub>	<i>Sac.66+10%L.p+ other ingredient</i>
T <sub>3</sub>	<i>Sac.66+20%L.p+ other ingredient</i>
T <sub>4</sub>	<i>Sac.66+40%L.p+ other ingredient</i>
T <sub>5</sub>	<i>Sac.66+50%L.p+ other ingredient</i>
T <sub>6</sub>	<i>Sac.64+ other ingredient (control 2)</i>
T <sub>7</sub>	<i>Sac.64+10%L.p+ other ingredient</i>
T <sub>8</sub>	<i>Sac.64+20%L.p+ other ingredient</i>
T <sub>9</sub>	<i>Sac.64+40%L.p+ other ingredient</i>
T <sub>10</sub>	<i>Sac.64+50%L.p+ other ingredient</i>
T <sub>11</sub>	<i>Sac.66+64+ other ingredient (control 3)</i>
T <sub>12</sub>	<i>Sac.66+64+10%L.p+ other ingredient</i>
T <sub>13</sub>	<i>Sac.66+64+20%L.p+ other ingredient</i>
T <sub>14</sub>	<i>Sac.66+64+40%L.p+ other ingredient</i>
T <sub>15</sub>	<i>Sac.66+64+50%L.p+ other ingredient</i>

*(LP) Lactobacillus plantarum*

### **Sensory evaluation**

Descriptive analysis was used to determine the sensory profiles of the bread samples. The control for the bread samples was wheat bread without used *Lactobacillus p* Sensory quality was evaluated by 10 trained panelists with proven skills according to Guarda *et al.*, (2004). From each sample, half of the bread slices were served on white, odorless, disposable plates. Samples were scored for appearance, odor, Taste, texture and overall quality using a scale from 0 (unfit) to 5 (excellent)]. Samples with scores of <2 were regarded as unacceptable for sale, and with scores of < 1.5 unacceptable for human consumption. Panelists were also asked to describe any defects noticed in sensory quality.

### **Texture analysis**

Crumb firmness (N) was measured for 6-days (1, 3, 5 and 6 days) to assess the potential shelf-life of the breads. Bread crumb firmness (N) during storage was determined according to (Bourne, 1978) Instrumental texture evaluation of crust and crumb was performed using a TA.XT2 Texture Analyzer equipped with a 25 kg load cell (Stable Micro Systems, Goldalming, UK) and Texture Expert for Windows software (version 1.22) for data analysis

### **Analytical methods**

Determination of moisture, protein, ash, fiber and fat were determined using the method of **AACC, (2000)**.Carbohydrate were calculated by difference.

### **Determination of pH and total titratable acidity (TTA)**

For the determination of pH, 10.00g of each sample ground bread or dough was blended with 100 ml, boiled/distilled water then, stirred by magnetic stirrer for 20 min. at room temperature. Then, pH was measured using a pH meter (Hanna Instruments, model 211)

The same sample was used for measuring TTA, which was titrated against NaOH solutions (0.1N) to a final pH value of 6.5 using phenolphthalein solution (1.0g /100ml) as indicator. TTA is defined as the amount of NaOH titrated the acidity (in ml) and expressed as the mean value of duplicate Najafi et al.,(2012).

#### **Antifungal activity**

Spoilage fungal growth on the produced breads was detected according to the method described by Delnobile et al., (2003) and Sidhu et al., (1997).The bread was considered unacceptable if the bread showed visible growth of molds.

#### **Dough – raising capacity:-**

Cylinder test was used to determine the fermentation capacity according to the method described by Fernandes et al., (1985).

#### **Statistical analysis**

Means and standard Error (SEM) were calculated with SPSS statistical software (Version 19.0, SPSS Inc., Chicago, IL, USA). **SPSS (2000)** was used to perform one-way analysis of variance (ANOVA) and least significant difference test (LSD) at a 95% confidence level ( $p > 0.05$ ) to identify differences of evaluated parameters both among bread types during storage. Statistical analyses were carried out by SPSS10 program. Data were expressed as means  $\pm$  SEM and the Statistical analysis was performed using one-way analysis of variance followed by Duncan's tests.

## **RESULTS AND DISCUSSION**

Changes in the dough fermentation of the samples prepared using different starters are shown in Table(2) .The control treatments ( $T_1, T_6$  and  $T_{11}$ ) recorded an increase percentages in the dough volume being 170, 171.43 and 153.84 %, respectively, while all the tested treatments recorded increases ranging from 141.38 to 185.19 %.  $T_5$  showed the highest increase in the dough volume. However,  $T_8$  showed the lowest one when compared to control treatment.  $T_{12}, T_{13}$  and  $T_{15}$  also showed minimum increases in volume (148.15, 151.85, and 151.85 respectively). The obtained results revealed that LAB seemed to be more effective in combination with *Sacch. cerevisiae* on the dough volume. These results are in agreement with those reported by Palacios et al., (2006), who demonstrated the role of LAB on the yeast leavening and  $CO_2$  production in the dough. Lactic acid bacteria (LAB) contributes mostly to the process of dough acidification, while yeasts are primarily responsible for the leavening.

#### **Effect of some starters and LAB on pH and T.T.A**

PH values and TTA of dough and bread samples produced by using different starter cultures of *L. plantarum* and *Sacch. cerevisiae* are shown in Table (3). The control treatment showed higher pH and lower TTA values.

The obtained results also revealed that sourdough inoculated with different starters of *L.plantarum* and *Sacch. cerevisiae* characterized with low pH and high TTA values comparing with the control treatments. Similar results were reported by Ravyts and Vuyst, (2011) and Glosan *et al.*, (2013), who found decreasing pH value of sourdough produced with different starters cultures of LAB. Data also revealed that bread produced by using *L. plantarum* scored high pH value and lower TTA in comparison with fermented dough. Similar results were reported by Dall Bello *et al.*, (2007).

**Table (2): Impact of some starter cultures of *Sacch. cerevisiae* and *L. plantarum* on fermentation time**

Treatment	Time(min)													
	Zero	15	30	45	60	75	90	105	120	135	150	165	180	
T1	27	29	32	40	45	54	60	63	65	68	69	72	73	
T2	26	27	30	36	43	51	57	59	61	63	64	67	68	
T3	28	30	35	45	51	63	69	71	73	74	75	76	76	
T4	30	31	33	42	50	58	64	68	71	73	74	75	77	
T5	27	29	32	40	43	57	66	69	73	76	76	77	77	
T6	28	30	31	40	52	60	64	67	70	71	74	74	76	
T7	28	29	31	39	52	58	62	64	68	71	72	74	75	
T8	29	30	32	38	48	56	60	64	66	69	69	70	70	
T9	30	31	32	38	48	56	60	64	69	72	74	74	75	
T10	27	27	28	31	38	45	52	57	60	64	64	66	68	
T11	26	27	29	35	40	49	54	55	58	63	65	66	66	
T12	27	28	30	38	42	50	55	58	60	65	67	67	68	
T13	27	27	29	34	46	47	53	56	60	64	66	66	67	
T14	26	27	29	34	40	49	54	56	58	63	66	66	67	
T15	27	28	30	35	40	48	53	53	58	63	66	66	67	

**Table(3):Influence of starter cultures of *L.plantarum* and *Sacch. cerevisiae* on pH and TTA (ml) values of bread dough and bread loaf**

Treatment	Bread Dough		Bread Dough	
	pH	TTA (ml)	pH	TTA (ml)
T1	5.29	1.30	5.7	2.94
T2	5.15	1.55	5.65	2.95
T3	4.91	1.85	5.64	3.05
T4	4.85	1.95	5.61	3.06
T5	4.81	2.05	5.20	4.94
T6	5.37	1.35	5.67	2.94
T7	5.19	1.55	5.63	3.18
T8	4.96	1.80	5.50	3.60
T9	4.91	1.85	5.45	3.65
T10	4.86	2.00	5.40	3.66
T11	5.30	1.30	5.63	3.29
T12	5.20	1.95	5.57	3.65
T13	5.00	1.75	5.45	3.76
T14	4.95	1.85	5.37	4.35

T15	4.85	2.00	5.30	4.71
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TTA : Total titratable acidity

### Moisture

Bread produced by using different starter cultures of *L. plantarum* and *Sacch. cerevisiae* exhibited a highly significant differences in moisture content during storage time compared to control treatments (T<sub>1</sub>, T<sub>6</sub> and T<sub>11</sub>) which, exhibited significant lower moisture content (Table 4). The moisture content of foods is usually used as an indicator of food quality. It is important to measure the moisture content in breads because of its potential impact on the sensory, physical, and microbial properties of the bread Gallagher *et al.*, (2003).

Bread produced by using mixed starters cultures of *L. plantarum* and *Sacch. cerevisiae* Exhibited significant higher moisture content during storage time in comparison with control treatments (T<sub>1</sub>, T<sub>6</sub> and T<sub>11</sub>), which exhibited lower moisture content. Therefore, bread produced by mixed starter cultures (*Sacch.c +L. .plantarum*) contained moisture content ranging from 37.12 to 41.3 % after the first day of storage. The corresponding Table (4) after six days of storage were ranged from 25.2 to 30.28 %. The moisture content of control treatments (T<sub>1</sub>, T<sub>6</sub> and T<sub>11</sub>) after one to six-day of storage were ranged from 36.47 to 36.87 and from 22.32 to 22.99 %, respectively. In general the obtained results revealed that moisture content of bread produced either using mixed starter culture or yeast alone was decreased during storage time. This could be usually thin with a greater surface area, which facilitate more moisture loss during baking, cooling and storage Sidhu *et al.*, (1997). Similar results were reported by Quail, (1996), who estimated that arabic flat bread staling is due to in part, to moisture loss.

**Table(4):Influence of starter cultures of *L. plantarum* and *Sacch. cerevisiae* on Moisture (%) of bread during storage**

Treatment	Storage time at 25c (days)					
	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	6 <sup>th</sup> day
T1	36.63±07	34.83 ±.14 <sup>l</sup>	32.71 ±.11 <sup>k</sup>	27.61 ±.13 <sup>k</sup>	25.31 ±.17 <sup>h</sup>	22.43 ±.09 <sup>h</sup>
T2	37.42±.3 <sup>g</sup>	36.60±.03 <sup>f</sup>	35.58 ±.09 <sup>g</sup>	30.49 ±.15 <sup>g</sup>	27.12 ±.07 <sup>g</sup>	24.23 ±.05 <sup>f</sup>
T3	38.41±.41 <sup>e</sup>	38.12±.06 <sup>e</sup>	37.07±.04 <sup>e</sup>	33.29±.05 <sup>d</sup>	30.11±.06 <sup>e</sup>	27.30±.11 <sup>d</sup>
T4	39.71c±.6 <sup>c</sup>	39.33±.17 <sup>c</sup>	38.74±.05 <sup>b</sup>	34.74±.05 <sup>c</sup>	32.66±.60 <sup>cd</sup>	30.10±.17 <sup>d</sup>
T5	40.52±.17 <sup>a</sup>	40.15±.03 <sup>a</sup>	39.57±.08 <sup>a</sup>	36.51±.07 <sup>a</sup>	34.17±.02 <sup>a</sup>	32.32±.03 <sup>a</sup>
T6	36.87±.02 <sup>hi</sup>	34.69±.09 <sup>l</sup>	31.63±.18 <sup>l</sup>	28.10±.06 <sup>l</sup>	24.18±.94 <sup>l</sup>	22.99±.02 <sup>g</sup>
T7	37.12±.04 <sup>hg</sup>	36.02±.02 <sup>g</sup>	35.08±.60 <sup>h</sup>	29.97±.09 <sup>h</sup>	27.47±.05 <sup>g</sup>	25.20±.01 <sup>e</sup>
T8	38.50±.03 <sup>e</sup>	38.05±.03 <sup>e</sup>	36.96±.03 <sup>e</sup>	33.43±.12 <sup>d</sup>	30.18±.02 <sup>e</sup>	27.60±.032 <sup>c</sup>
T9	40.25±.04 <sup>b</sup>	39.88±.01 <sup>b</sup>	37.49±.02 <sup>d</sup>	34.65±.03 <sup>c</sup>	32.02±.04 <sup>d</sup>	30.31±.11 <sup>b</sup>
T10	41.30±.12 <sup>a</sup>	39.98±.02 <sup>ab</sup>	38.26±.5 <sup>c</sup>	35.31±.10 <sup>b</sup>	33.85±.03 <sup>ab</sup>	30.28±.05 <sup>b</sup>
T11	36.47±.04 <sup>l</sup>	34.60±.05 <sup>l</sup>	31.75±.13 <sup>l</sup>	27..73±.06 <sup>k</sup>	24.42±.12 <sup>l</sup>	22.32±.32 <sup>h</sup>
T12	37.27±.16 <sup>hg</sup>	35.55±.08 <sup>h</sup>	34.00±.06 <sup>l</sup>	30.16±.08 <sup>h</sup>	27.50±.02 <sup>g</sup>	24.35±.03 <sup>f</sup>
T13	37.77±.11 <sup>f</sup>	36.83±.14 <sup>f</sup>	35.63±.06 <sup>g</sup>	31.59±.59 <sup>f</sup>	28.57±.03 <sup>f</sup>	25.25±.08 <sup>e</sup>
T14	39.21±.21 <sup>a</sup>	38.38±.06 <sup>d</sup>	36.39±.10 <sup>a</sup>	32.61±.06 <sup>e</sup>	30.69±.46 <sup>e</sup>	27.59±.13 <sup>c</sup>
T15	41.03±.09 <sup>a</sup>	40.19±.01 <sup>a</sup>	38.76±.03 <sup>b</sup>	35.49±.11 <sup>a</sup>	33.20±.05 <sup>ab</sup>	30.19±.10 <sup>b</sup>

Values with different letters are significantly different (p ≤0.05)

### Sensory evaluation

Sensory evaluation of the breads was ascertained with ten trained panelists. Parameters with the greatest influence on bread quality and acceptance by consumers particularly appearance, odor, taste, texture and overall quality; therefore, sensory quality of stored products and overall quality was the main subject of this investigation.

The obtained results of sensory evaluation of bread samples are shown in (Table 5). Results didn't show significant differences between the appearance of samples and controls (T<sub>1</sub>, T<sub>6</sub>, T<sub>11</sub>). However, bread samples made with *L. plantarum* (T<sub>5</sub>, T<sub>10</sub> and T<sub>15</sub>) showed high significant differences ( $p > 0.05$ ) in odor and texture.

**Table(5): Sensory evaluation of bread produced by using mixed starter of *L. plantarum* and *Sacch. cerevicae***

Treatment	Appearance	Odor	Taste	Texture	Overall quality
T1	4.90±0.06 <sup>a</sup>	4.00±.12 <sup>d</sup>	4.30±.06 <sup>d</sup>	4.00±.06 <sup>c</sup>	4.2±.06 <sup>e</sup>
T2	4.90 ±.0. <sup>a</sup>	4.40±.06 <sup>c</sup>	4.45±.00 <sup>cd</sup>	4.50±.00 <sup>b</sup>	4.75±.06 <sup>d</sup>
T3	5.00±.00 <sup>a</sup>	4.50±.00 <sup>c</sup>	5.00±.00 <sup>a</sup>	4.50±.00 <sup>b</sup>	4.70±.00 <sup>c</sup>
T4	4.90±.07 <sup>a</sup>	4.86±0.12 <sup>ab</sup>	4.73±.12 <sup>ab</sup>	4.50±.00 <sup>b</sup>	4.49±.00 <sup>c</sup>
T5	4.90±.06 <sup>a</sup>	5.00±.00 <sup>a</sup>	4.50±.08 <sup>cd</sup>	5.00±.00 <sup>a</sup>	4.90±.00 <sup>a</sup>
T6	4.90±.11 <sup>a</sup>	4.10±.06 <sup>d</sup>	4.20±.15 <sup>d</sup>	4.00±.00 <sup>c</sup>	4.25±.06 <sup>e</sup>
T7	4.90±.00 <sup>a</sup>	4.50±0.12 <sup>c</sup>	4.20±.08 <sup>d</sup>	4.50±.00 <sup>b</sup>	4.60±.06 <sup>d</sup>
T8	5.00±.00 <sup>a</sup>	4.50±.00 <sup>c</sup>	5.00±.00 <sup>a</sup>	4.50±.00 <sup>b</sup>	4.50±.00 <sup>c</sup>
T9	5.00±.00 <sup>a</sup>	4.80±.08 <sup>ab</sup>	4.75±.16 <sup>ab</sup>	4.50±.00 <sup>b</sup>	4.59±.00 <sup>c</sup>
T10	5.00±.00 <sup>a</sup>	5.00±.00 <sup>a</sup>	4.20±.08 <sup>d</sup>	5.00±.00 <sup>a</sup>	4.90±.00 <sup>a</sup>
T11	4.90±.11 <sup>a</sup>	4.00±0.13 <sup>d</sup>	4.25±.00 <sup>d</sup>	4.00±.00 <sup>c</sup>	4.15±.06 <sup>e</sup>
T12	5.00±.00 <sup>a</sup>	4.50±.00 <sup>c</sup>	4.50±.00 <sup>cd</sup>	4.50±.00 <sup>b</sup>	4.00±.00 <sup>c</sup>
T13	5.00±.00 <sup>a</sup>	4.50±.00 <sup>c</sup>	5.00±.00 <sup>a</sup>	4.50±.00 <sup>b</sup>	4.00±.00 <sup>ab</sup>
T14	5.0007 <sup>a</sup>	4.85±.00 <sup>ab</sup>	4.72±0.12 <sup>ab</sup>	4.50±.00 <sup>b</sup>	4.07±.00 <sup>ab</sup>
T15	5.0007 <sup>a</sup>	5.00±.00 <sup>a</sup>	4.72±0.07 <sup>ab</sup>	5.00±.00 <sup>a</sup>	5.00±.00 <sup>a</sup>

Values with different letters are significantly different ( $p \leq 0.05$ )

Regarding the tastes of the bread samples, sour or acidity taste was observed in samples T<sub>5</sub>, T<sub>10</sub> and T<sub>15</sub>. The maximum taste score was found with T<sub>3</sub>, T<sub>8</sub> and T<sub>13</sub>. Najafi *et al.*, (2012) used mixed sourdough starters having obligate homo-, facultative and obligate hetero-fermentative LAB mixed with *Sacch. cerevicae* which induced significant enhanced intensity of bread taste in comparison with *Sacch. cerevicae* fermentation. These results are in agreement with Rehman (2006), and Hansen (2004) who found that bread fermented with *L. plantarum* had an unpleasant metallic sour taste, but when the sourdough was also supplemented with *S. cerevisiae*, the bread acquired a more aromatic bread flavor. The most desirable sensory characteristics are obtained at pH 4.0–5.5. Thus, mixed cultures with both Lactic acid bacteria and yeast are recommended for an aromatic and pleasant sourdough bread flavor.

#### **Inhibitory activity of sourdough fermentation on fungi spoilage**

Sourdough fermentation using mixed starter cultures was studied as a result of consumer's requirement for bread free of chemical preservation. Therefore, inhibitory activity of mixed starter cultures of *L. plantarum* and *Sacch. cerevisiae* against fungal spoilage of flat bread was estimated as shown in Table (6). The obtained results revealed that the best treatments

(T<sub>4</sub>, T<sub>5</sub>, T<sub>9</sub>, T<sub>10</sub>, T<sub>14</sub> and T<sub>15</sub>) were obtained with mixed starter cultures of *L. plantarum* at level of 40 and 50 % on yeast fresh weight basis. These results indicated that *L. plantarum* with selected characteristic could be used for management of problems caused by spoilage fungi during dough fermentation. Similar results were reported by *Martinez-Anaya et al., (1993); and Cossignani et al., (1996).*, who reported capability of sourdough LAB to inhibit or delay microbial spoilage of bread and improving its shelf-life. The obtained results also revealed the capability of mixed starter cultures at *L. plantarum* level of 40 and 50 % to inhibit fungal growth. However, it caused an increase of bread acidity which have negative influence on antifungal cyclic dipeptides has been shown with *L.plantarum* Ström et al., (2002).

**Table (6): Inhibitory activity of fermented sourdough fermentation on fungal spoilage of bread**

Treatment	1 <sup>st</sup> day	3 <sup>rd</sup> day	5 <sup>th</sup> day	6 <sup>th</sup> day
T1	—	+	++	+++
T2	—	+	++	+++
T3	—	-	+	++
T4	—	-	-	+
T5	—	-	-	+
T6	—	+	++	+++
T7	—	+	++	+++
T8	—	-	-	+
T9	—	-	-	+
T10	—	-	-	+
T11	—	+	++	+++
T12	—	+	++	+++
T13	—	-	+	++
T14	—	-	-	+
T15	—	-	-	+

**NOTE: Inhibition levels of growth of indicator fungi:**

“—” no inhibition around the filter disc;

“+” inhibition with weak, almost undetectable zone;

“++” inhibition with detectable zone (diameters of 1 - 2 cm);

“+++” strong inhibition with clear but irregular zone (diameters of 2 - 4 cm);

### Staling

The results obtained are presented in Table (7). The dependent variable, firmness(N), increased throughout the time of storage, with values from 28.71N (after 24 h) up to 40.15N (after 6 days storage) Table( 7). The data revealed a changes in crumb firmness (N) over time differed with a significant differences due to the interaction between starch level and time of storage.

Significant differences among mixed starter cultures were found over time as shown in Table (7). The obtained results indicated that the combined mixture of *Sacch. cerevisiae*-Y66 and *L. plantarum* at 50 % level (T<sub>5</sub>) resulted in the lowest firmness (N) value ( $p>0.05$ ). A rapid initial increase after 3 to 5-days was observed with all used starter cultures; however, data revealed that firmness (N) of bread (T<sub>5</sub>, T<sub>10</sub> and T<sub>15</sub>) almost didn't change from first to third day. Treatments comprise *L.plantaurum* scored increasing of firmness up to 6-days. In addition, the initial soft bread produced by *Sacch. Cerevisiae* –Y64



(T<sub>6</sub>) increased in firmness and scored the highest compression force (40.15 N), which differed (p>0.05) from all other treatments. Similar results were reported by Axford *et al.*, (1968); and Stöllman and Lundgren, (1987) and confirming by Corsetti and Settanni (2007) who repeated that specific strain of LAB in sourdough bread making may delay firmness and staling.

**Table (7): Effect of mixed starter cultures of *L. plantarum* and *Sacch. cerevisiae* on bread firmness(N)**

Treatment	Firmness(N)			
	1 <sup>st</sup> day	3 <sup>rd</sup> day	5 <sup>th</sup> day	6 <sup>th</sup> day
T1	26.54±0.13 <sup>d</sup>	28.99±0.275 <sup>e</sup>	34.55±0.029 <sup>i</sup>	37.74±0.14 <sup>k</sup>
T2	26.45±0.09 <sup>d</sup>	28.20±0.049 <sup>e</sup>	33.59±0.189 <sup>n</sup>	36.36±0.03 <sup>k</sup>
T3	24.45±0.01 <sup>c</sup>	24.83±0.069 <sup>d</sup>	28.92±0.256 <sup>e</sup>	30.57±0.07 <sup>g</sup>
T4	21.95±0.21 <sup>b</sup>	22.81±0.01b <sup>c</sup>	26.98±0.226 <sup>cd</sup>	28.28±0.044 <sup>e</sup>
T5	19.97±.276 <sup>a</sup>	20.46±0.19 <sup>a</sup>	24.78±0.148 <sup>b</sup>	25.68±0.012 <sup>c</sup>
T6	28.71±0.00 <sup>g</sup>	30.42±0.03 <sup>g</sup>	36.92±0.195 <sup>i</sup>	40.15±0.05 <sup>m</sup>
T7	28.43 ± 0.36 <sup>g</sup>	29.87± 0.059 <sup>g</sup>	32.26± 0.347 <sup>g</sup>	34.77± 0.015 <sup>i</sup>
T8	26.42 ± 0.19 <sup>d</sup>	27.14± 0.087 <sup>e</sup>	30.10± 0.330 <sup>f</sup>	32.37±0.067 <sup>h</sup>
T9	24.11±0.143 <sup>c</sup>	24.65±0.104 <sup>d</sup>	25.75±0.440 <sup>b</sup>	27.33±0.045 <sup>d</sup>
T10	21.83 ± 0.24 <sup>b</sup>	22.16± 0.398 <sup>b</sup>	23.41± 0.153 <sup>a</sup>	25.03±0.019 <sup>b</sup>
T11	28.13±0.035 <sup>f</sup>	31.49±0.956 <sup>n</sup>	35.57±0.033 <sup>k</sup>	38.69±0.021 <sup>l</sup>
T11	27.28±0.275 <sup>e</sup>	28.21±0.038 <sup>e</sup>	30.43±0.032 <sup>f</sup>	33.30±0.116 <sup>i</sup>
T13	26.12±0.058 <sup>d</sup>	27.01±0.058 <sup>e</sup>	28.21±0.163 <sup>d</sup>	29.41±0.019 <sup>f</sup>
T14	24.64±0.006 <sup>c</sup>	25.51±0.184 <sup>d</sup>	26.48±0.039 <sup>c</sup>	27.33±0.044 <sup>d</sup>
T15	22.35±0.200 <sup>b</sup>	23.08±0.200 <sup>c</sup>	23.78±0.148 <sup>a</sup>	24.57±0.200 <sup>a</sup>

Values with different letters are significantly different (p ≤ 0.05)

Crowley *et al.* (2002) also found that the addition of 20% sourdough to wheat bread reduced crumb firmness and slowed down firming in comparison with breads made with no or larger addition of sourdough (40% flour basis).

Valerio *et al.* (2008) demonstrated that the sourdough *L. plantarum* and its metabolites such as lactic and acetic acids, which act like calcium propionate (0.3 %), prolonged the Bacillus free shelf-life to 7 days at 30°C.

#### Rate of staling

The staling rate of bread samples (calculated from the differences of firmness from 3,5,6 and 1day of storage), was showed in (Fig. 1).

Data of staling rate of bread samples revealed that replacement of *S. cerevisiae* with *L. plantarum* at 50% level had the a retardation effect on the staling rate of the resulting bread.

Hansen, (2004) stated that the rate of starch retro gradation was not influenced if the acidification was rather low, whereas a standard sourdough fermented by *L. plantarum* 13 and *S. cerevisiae* 141 was able to retard the staling rate. Shadi *et al.*, (2010) added *L. plantarum* to bread dough formulation at 0,5,10,15% concentration. They found that bread with,15% sourdough had lowest staling rate. From the obtained data, it could be

concluded that *L. plantarum* is a suitable starter for wheat sourdough and bread production.

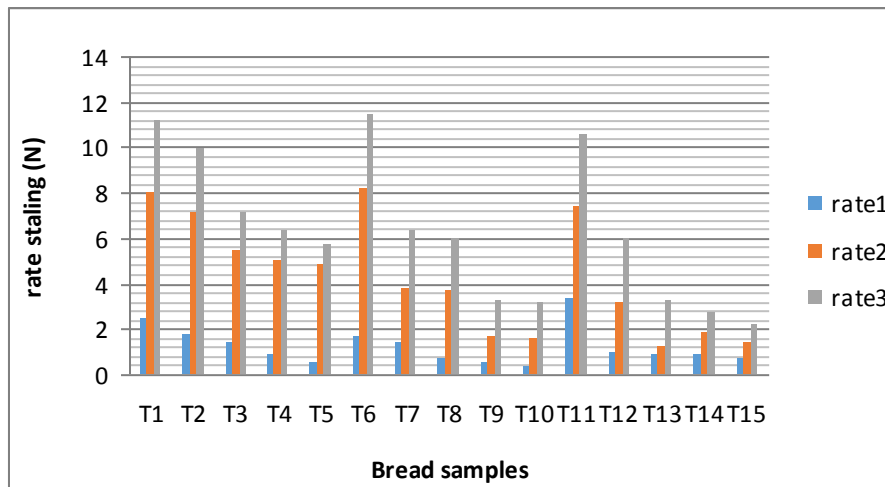


Fig.(1) Staling rate (N) of breads prepared by replacing yeast with *Lactobacillus plantarum* at different rates rate1 (difference between 3rd and first day),rate2 (difference between 5rd and first day),rate3(difference between 6rd and first day).

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التقييم الحسى وظاهرة البيات للخبز المنتج باستخدام خليط من البكتريا  
*Saccharomyces* ( والخميرة ) و *Lactobacillus plantarum*  
( *cerevisiae* )

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اجريت هذه الدراسة لتقييم خواص العجين وا لخبز المنتج باستخدام مخلوط من  
البكتريا والخميرة . ووضحت النتائج ان الخلط بين البكتريا والخميرة اكثر تأثير على حجم  
العجين و الخبز المنتج من حيث خفض درجة ال pH و ارتفاع الحموضه الكليه مقارنة  
بالكنترول ( خميره بدون بكتريا ) . وقد أظهرت النتائج وجود فروق معنويه عاليه فى النكهه  
والطعم . كما أكدت النتائج وجود فروق معنويه ايضا فى الطزاجه بعد يوم وثلاثه و خمسة  
وسته أيام من التخزين وهذه النتائج جاءت مؤكده مع التقييم الحسى.

قام بتحكيم البحث

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