

VOLATILES FORMATION BY SINGLE STRAIN STARTERS OF INDIGENOUSLY ISOLATED LACTIC ACID BACTERIA IN SOURDOUGH

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Volatile compounds in fermented sourdoughs were formed by using starter cultures [*Lactobacillus brevis* (St-I), *Lactobacillus fermentum* (St-II) and *Lactobacillus plantarum* (St-III)] along with control starter culture (*Saccharomyces cerevisiae*). Sourdough samples were obtained by fermenting the dough with starter cultures at different fermentation times (12, 18 and 24 hrs). Furthermore, the fermented samples were analyzed for pH, total titratable acidity (TTA), organic acids, sugars and volatile compounds. From the results, it is noticed that the pH decreased as a function of fermentation time in all starter cultures and maximum pH drop was observed in St-II after 24 h of fermentation followed by St-I at the same time interval. However, least drop in pH was recorded in the control starter culture. Moreover, highest TTA (10.30%) was produced by St-I after 24 h of fermentation followed by the same culture at 18 h of fermentation (9.90%). Conversely, the control samples yielded lowest TTA. In case of organic acids and sugars after evaluating by HPLC depicted that St-I gave more concentrations of sugar fractions (maltose and fructose) and St-II produce higher concentrations of organic acids, all sugar contents and organic acids increased with passage of time. Sourdough samples were analyzed for volatile compounds by GC-MS through headspace analysis. Sourdough produced by the addition of starter cultures yielded 1-hexanol, hexanal, octanal and ethyl acetate in general whereas, diacetyl was produced in St-III ferments only.

Keywords: Starter culture, wheat flour, sourdough, fermentation, volatile compounds, GC-MS.

INTRODUCTION

Fermentation of bread is done through commercial baker's yeast, but it does not produce reasonable amounts of volatiles, organic acids, which are required to increase the shelf-life of bread. So, there is need for innovations in bread production to enhance its shelf-life and consumer's attraction. Sourdough is a traditional and classical bread making process, which is effective in improving the taste, aroma, texture and shelf life of bread (Elhariry *et al.*, 2011). A mixture of water and flour fermented with lactic acid bacteria (LAB) and yeast is called sourdough (Banu *et al.*, 2011) which affects the properties of bread in different ways. Sourdough is comprised of yeast and range of lactic acid bacteria (LAB) that produce sour taste of the end product (Ravyts and Vuyst, 2011). The skill of controlled fermentation and use of precise starter cultures in sourdough fermentation has become the trend. Sourdough metabolic activity is controlled by connections between lactobacilli and yeasts (Poutanen *et al.*, 2009). Dough rheology and sensory profile is affected by LAB of sourdough which in turn affect the bread flavor and volume (Arendt *et al.*, 2007). Bacterial proteolysis during sourdough fermentation results in the production of typical sourdough flavors of baked breads as compared to the chemically leavened breads (Hansen *et al.*, 1989). LAB have been

reported to produce a number of organic acids and volatile compounds which contribute greatly to the bread flavor and bread shelf life. The lactic acid fermentation by the use of LAB improves the sensorial value which is mainly dependent on the amount of lactic acid, acetic acid and several aromatic compounds. Incorporation of fructose as hydrogen acceptor and use of hetero-fermentative starter cultures increase acetic acid content in sourdoughs. When acetic acid content increases, it has positive effect on the aroma of bread and also has antimicrobial function against molds and rope producing bacillus which results in enhanced shelf life of the final product (Rosenquist and Hansen, 1998). LAB and yeast fermentation of dough produce bread flavor in baking process which contains a range of flavoring compound by thermal reactions and lipid oxidation (Hansen and Schieberle, 2005; Pozo-Bayonet *et al.*, 2006). LAB produce organic acids (*i.e.* caproic acid, lactic acid, formic acid, acetic acid and phenyl lactic acid) that act as preservatives, improve bread flavor and shelf-life. Organic acids also help to give protection against mold spoilage and rope-forming bacteria (Corsetti *et al.*, 2005). Different LAB strains isolated from sourdoughs show the ability to metabolize various sugars *e.g.* galactose, sucrose, fructose, etc. (Tieking *et al.*, 2003). The exopolysaccharides (EPS) such as fructans and glucans, also exhibit positive impact on the shelf life and volume of bread.

EPS also have a significant role to improve texture of the sourdough and the corresponding bread (Tieking *et al.*, 2003). Amino acids both as precursors for flavoring compounds or free amino acids are of vital importance in contributing flavor to the final bread (Ganzle *et al.*, 2007, 2008). The level of amino acids in dough may be affected by the proteolytic strains of LAB, but cereal proteases play a major role in degradation of proteins in sourdoughs (Thiele *et al.*, 2004). Lactic acid bacteria (LAB) play a vital role in the development of flavor and aroma of the end fermented product. They cause acidification of the food by lowering pH which results in pungent lactic acid taste, and produce aromatic compounds due to proteolytic and lipolytic activities (Yvon and Rijnen, 2001; Van Kranenburg *et al.*, 2002). Almost all of the available sugar is completely converted into lactic acid by LAB (homofermentative) through pyruvate pathway yielding energy and balancing the redox. Volatile substances produced by LAB are contributor of the typical flavor to the sourdough and other fermented products (Kleerebezem *et al.*, 2000).

Mostly white bread is produced by using yeasted preferments. Shelf life and quality of products can be increase by utilizing LAB starter culture. Novel fermented foods such as sourdough bread with longer shelf life and superior quality can be produced by LAB present in naturally fermented sourdoughs (Saeed *et al.*, 2009). The aim of present study is the evaluation of different volatile compounds produced in sourdoughs fermented with starter cultures [*Lactobacillus brevis* (St-I), *Lactobacillus fermentum* (St-II) and *Lactobacillus plantarum* (St-III)].

MATERIALS AND METHODS

Procurement of raw material: The white wheat flour was procured from the local market of Faisalabad, Pakistan. The main characteristics of the flour were moisture 12.6%, crude protein 10.34% and ash content 0.60%. Twenty wheat flour dough samples were collected from different bakeries located in vicinity of Faisalabad city. The samples were drawn in sterilized screw capped bottles and preserved under refrigerated conditions at 4°C for further studies.

Isolation and purification of starter culture: The representative colonies, showing catalase negative and Gram positive, were randomly picked from higher dilution (10^{-6}) of De Man Rogosa Sharpe (MRS) agar plates and transferred into 10 ml test tubes containing sterile MRS broth. Presumptive lactobacilli were selected on the basis of morphological, Gram's staining reaction and the catalase test, and the representative colonies from MRS agar were transferred to the Acetate agar plates. The isolates were purified by successive streaking on the Rogosa agar and MRS agar media before being subjected to characterization. The pure bacterial isolates were inoculated into MRS broth,

incubated for 24 h at 30°C, centrifuged (Sigma, 3K30, Germany) at 3000 rpm for 15 min and the supernatant was decanted. The cell pellets were resuspended in sterile MRS broth containing 10% (v/v) glycerol. The suspension was aseptically transferred into sterile cryotubes containing acid-washed glass beads and stored at -80 °C until required for identification (Harrigan and McCance, 1990).

The pure isolates of lactobacilli obtained from MRS agar and broth medium were identified and characterized by applying sugar fermentation, biochemical and enzymatic activity tests according to the procedure described by Cappuccino and (1996). The growth of the lactobacilli at different NaCl concentrations, different pH and temperatures was also observed according to the differential scheme proposed by Wood and Holzapel (1995).

Inoculum preparation: The starter cultures of pure bacterial strains of the genus *Lactobacillus* i.e. *L. brevis*, *L. fermentum*, (heterofermentative) and *L. plantarum*, (homofermentative) isolated from spontaneously fermented wheat sourdoughs were used. Actively growing single cultures of lactic acid bacteria were inoculated (inoculum level 1.0% v/v) into Erlenmeyer flasks containing 100 ml of MRS broth, and inoculated for 24h at 30°C. Biomass was collected by centrifugation ($5000 \times g$, 15 min, 4°C) and suspending into 50 ml of sterile saline. This cell suspension that contained 10^8 CFU/ml of lactic acid bacteria, was used as starter culture in sourdough preparation (Settanni *et al.*, 2013).

Sourdough preparation: A sponge was prepared from flour (200 g), water (200 ml) and starter culture was mixed thoroughly and incubated for 20 h at 30°C. The sourdough was prepared from flour (200 g), water (200 ml) and fermented sponge (70 g), incubated for 24 h at 30°C. Sourdoughs were prepared from each starter culture using the same conditions as mentioned above and incorporated at 20 g/100 g level (base flour) in corresponding bread dough. Leavening was ensured by addition of a small amount of baker's yeast (0.2g/100g) during the bread dough preparation. The bread dough containing baker's yeast alone in the same amount was included in the test series as a control. Besides *Saccharomyce scerevisiae* (control), *Lactobacillus brevis* (St-I), *Lactobacillus fermentum* (St-II) and *Lactobacillus plantarum* (St-III) were used as starter culture in four treatments and each treatment was subjected to 12, 18 and 24 hrs of fermentation.

pH and Total titrable acidity (TTA): Sourdough sample (10 g) was mixed with sterile distilled water (100 ml). The pH was recorded using pH-meter (inoLab pH 720). For determination of total titratable acidity (TTA) resultant mixture was titrated against 0.1 N NaOH until the pH was reached at 8.5. The results were expressed as percent lactic acid (Pyler, 1988).

Organic acids and sugars: 10g of sample was homogenized with 90ml distilled water. Five ml of 1 mol/L HClO₄ solution was added to a 10 ml aliquot of the homogenate. The mixture was centrifuged for 15min at $4000 \times g$ at 15°C, the supernatant

was neutralized (pH 7.0±0.1) with 2 mol/L KOH and the volume was adjusted to 25 ml with distilled water. After 30 minutes precipitation on ice, the solution was filtered on 0.45mm cellulose filter (Millipore). Organic acids were determined through HPLC (Perkin Elmer 200 series) under the following conditions: mobile phase H₂SO₄ (0.05 N), flow rate 0.3 ml/min (Gerez *et al.*, 2013). Sugars were also determined by HPLC under the following conditions: mobile phase H₂SO₄ (0.001 N), flow rate 0.7 ml/min (Lefebvre *et al.*, 2002).

Essential amino acids profile: 0.1g of samples was taken and 10 ml of 6N HCl was added to each test tube. The test tubes were evacuated by nitrogen, sealed and placed in oven at 110°C for 22h. After specified time test tubes were allowed to cool at room temperature and hydrolysate (so formed) was evaporated under vacuum at 60°C to remove excess HCl. The hydrolysates were dissolved in 5ml of 6N HCl, centrifuged at 1000rpm and filtered to remove the visible sediments. A known volume (20µL) of the supernatant was injected into amino acid analyzer for the estimation of amino acid profile of each sample. Amino acids were quantified by liquid chromatography following the method given by Dong and Gant (1985). The column was an amino acid analyzer (L-8500 A, Hitachi) with a column oven temperature of 63°C, flow rate was 0.4ml/min and detection was made using a fluorescence detector.

Volatile compounds: GC-MS was used to determine volatile compounds in sourdough samples through headspace analysis. Sampling was done by solid phase micro-extraction (SPME) technique (DVB/CAR/PDMS fiber). In 20 mL sealed glass vial, sample (0.25 g) and 20% (w/v) NaCl (10 ml) solution were transferred and after that content was stirred at temperature 50°C for 5.5 min. The fiber was then exposed to the headspace for 60 min. Desorption of volatiles took place in injector port of the GC at 230°C in split less mode for 70 min. Volatiles were separated on a Gas Chromatograph (GC 6890 Agilent, USA) fixed with a Mass Spectrometer (MS 5973N Agilent, USA) (70 eV ionization energy; 29-400 m/z mass range). Helium was used as carrier gas with 2 ml/ min flow rate with constant held oven temperature (35°C) for 5 min and then temperature increased to 60°C, 200°C, and 250°C at rates of 2, 5 and 25°C /min, respectively. Retention times and obtained data was compared with those of standard compounds and with data of libraries (Mallouchos *et al.*,

2002) for identification of volatiles compounds. The content of compounds was expressed as relative peak areas (peak area of each compound / total area) ×100. In order to have better comparisons amongst the volatiles which were produced at much lower concentration than ethanol, ethanol area was excluded from the total area (Damiani *et al.*, 1996).

Statistical Analysis: The samples for each parameter were run in triplicate and the data obtained was subjected to statistical analysis using Cohart.V.6.1 (Costat, 2003). Two factor factorial design was applied and level of significance was determined. Means were further compared through Duncan's multiple range test (DMRt) following the procedures as described by Steel *et al.* (1997).

RESULTS AND DISCUSSION

Acidification characteristics of wheat sourdoughs: The pH of wheat sourdoughs ranged from 3.53 to 5.74 among the treatments during different fermentation time (Table 1). Minimum pH (3.53) was recorded after 24 h of fermentation time while maximum (5.74) was observed after 12 h of fermentation time due to formation of organic acids by the action of selective strain.

The total titratable acidity (TTA) of sourdoughs prepared from different starters (Table 1) showed that St-I possessed the highest TTA (10.30 %) followed by St-III and St-II which contained TTA 9.60 % and 8.80 % respectively. The control dough samples showed the lowest content of TTA as compared to other treatments. The outcomes of current research are found inside of the extents given by Katina (2004), who studied the wheat sourdoughs produced by different starters, flour types and fermentation conditions and found the TTA values ranging from 1.4 to 19.1.

Organic acids of sourdough: The lactic acid content was not produced in the control sourdough and among other starter cultures the lactic acid ranged between 0.25 to 0.51 g/100g during different fermentation times. The sourdoughs prepared from St-III yielded significantly the highest lactic acid content (0.47 g/100g) followed by the sourdoughs produced from St-I and St-II but the latter two were at par with respect to lactic acid production (Table 2). The interaction between the starter cultures and fermentation time showed significantly maximum lactic acid (0.51 g/100g) production by the starter

Table 1. Effect of starter cultures and fermentation time on pH and TTA of wheat sourdoughs

Treatments	pH			Acidity		
	12hrs	18hrs	24hrs	12hrs	18hrs	24hrs
<i>Saccharomyces cerevisiae</i> (Control)	5.74±0.11a	5.61±0.11b	5.32±0.11c	2.40±0.05g	2.60±0.05g	2.90±0.06g
<i>Lactobacillus brevis</i>	4.06±0.08de	3.75±0.08efg	3.60±0.07fg	8.20±0.16de	9.90±0.20ab	10.30±0.21a
<i>Lactobacillus fermentum</i>	4.12±0.08d	3.80±0.08d-g	3.53±0.07g	7.00±0.14f	8.50±0.17cde	8.80±0.18cd
<i>Lactobacillus plantarum</i>	3.94±0.08def	3.77±0.08d-g	3.71±0.07efg	7.90±0.16e	9.00±0.18c	9.60±0.19b

Means carrying same letters within a column or row do not differ significantly (P < 0.05).

Table 2. Effect of starter cultures and fermentation time on organic acids of sourdoughs

Treatments	Acetic acid (g/100g)			Lactic acid (g/100g)		
	12hrs	18hrs	24hrs	12hrs	18hrs	24hrs
<i>Saccharomyces cerevisiae</i> (Control)	0.00±0.00 ^e	0.00±0.00 ^e	0.00±0.00 ^e	0.00±0.00 ^g	0.00±0.00 ^g	0.00±0.00 ^g
<i>Lactobacillus brevis</i>	0.07±0.01 ^d	0.10±0.04 ^b	0.14±0.07 ^a	0.25±0.09 ^f	0.32±0.07 ^e	0.35±0.04 ^d
<i>Lactobacillus fermentum</i>	0.08±0.01 ^c	0.10±0.01 ^b	0.10±0.03 ^b	0.27±0.08 ^f	0.31±0.09 ^e	0.33±0.02 ^{de}
<i>Lactobacillus plantarum</i>	0.00±0.00 ^e	0.00±0.00 ^e	0.00±0.00 ^e	0.43±0.07 ^c	0.48±0.12 ^b	0.51±0.09 ^a

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$).

Table 3. Effect of starter culture and fermentation time on sugar contents of sourdoughs.

Treatments	Glucose (g/100g)			Maltose (g/100g)			Fructose (g/100g)		
	12hrs	18hrs	24hrs	12hrs	18hrs	24hrs	12hrs	18hrs	24hrs
<i>S. cerevisiae</i> (Control)	0.35±0.01 ^b	0.42±0.19 ^a	0.43±0.19 ^a	1.70±0.21 ^g	2.00±0.06 ^{ef}	2.10±0.15 ^{de}	0.11±0.01 ^h	0.16±0.01 ^g	0.17±0.01 ^{fg}
<i>L. brevis</i>	0.15±0.01 ^d	0.16±0.12 ^{cd}	0.15±0.13 ^d	1.90±0.11 ^f	2.30±0.07 ^{bc}	2.50±0.11 ^a	0.33±0.08 ^c	0.39±0.11 ^{ab}	0.41±0.13 ^a
<i>L. fermentum</i>	0.16±0.01 ^{cd}	0.15±0.13 ^d	0.16±0.13 ^{cd}	1.70±0.15 ^g	1.90±0.06 ^f	2.20±0.21 ^{cd}	0.29±0.06 ^d	0.34±0.06 ^c	0.37±0.08 ^b
<i>L. plantarum</i>	0.18±0.01 ^c	0.17±0.12 ^{cd}	0.16±0.13 ^{cd}	1.60±0.13 ^g	2.10±0.16 ^{de}	2.40±0.18 ^{ab}	0.21±0.04 ^e	0.18±0.01 ^{fg}	0.19±0.04 ^{ef}

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$).

Table 4. Effect of starter cultures and fermentation time on amino acids of sour dough.

Treatments	Methionine			Lysine			Leucine		
	12hrs	18hrs	24hrs	12hrs	18hrs	24hrs	12hrs	18hrs	24hrs
<i>S. cerevisiae</i> (Control)	0.04±0.0 ^g	0.05±0.0 ^f	0.05±0.0 ^f	0.19±0.2 ^d	0.20±0.2 ^d	0.22±0.1 ^c	0.32±0.09 ^g	0.35±0.1 ^g	0.40±0.6 ^f
<i>L. brevis</i>	0.08±0.1 ^e	0.10±0.1 ^c	0.14±0.1 ^a	0.24±0.5 ^c	0.26±0.6 ^b	0.33±0.9 ^a	0.46±0.9 ^{de}	0.68±0.2 ^b	0.81±0.9 ^a
<i>L. fermentum</i>	0.10±0.2 ^c	0.12±0.2 ^b	0.12±0.3 ^b	0.24±0.6 ^c	0.27±0.9 ^b	0.28±0.8 ^b	0.42±0.7 ^{ef}	0.50±0.3 ^d	0.77±0.9 ^a
<i>L. plantarum</i>	0.09±0.1 ^d	0.12±0.1 ^b	0.12±0.2 ^b	0.22±0.7 ^c	0.23±0.1 ^c	0.28±0.1 ^b	0.41±0.01 ^f	0.62±0.2 ^c	0.68±0.8 ^b
	Isoleucine			Histidine			Valine		
	12hrs	18hrs	24hrs	12 hrs	18 hrs	24hrs	12hrs	18hrs	24hrs
<i>S. cerevisiae</i> (Control)	0.22±0.01 ^g	0.25±0.04 ^f	0.26±0.01 ^f	0.09±0.01 ^b	0.10±0.01 ^{gh}	0.10±0.01 ^{gh}	0.16±0.01 ^g	0.21±0.02 ^f	0.24±0.02 ^e
<i>L. brevis</i>	0.31±0.08 ^e	0.34±0.03 ^{cd}	0.42±0.06 ^a	0.14±0.02 ^d	0.19±0.05 ^b	0.22±0.06 ^a	0.25±0.03 ^e	0.35±0.07 ^c	0.47±0.12 ^a
<i>L. fermentum</i>	0.33±0.11 ^{cd}	0.35±0.06 ^{bc}	0.40±0.08 ^a	0.12±0.02 ^{ef}	0.16±0.03 ^c	0.18±0.02 ^b	0.26±0.04 ^e	0.31±0.05 ^d	0.43±0.09 ^b
<i>L. plantarum</i>	0.26±0.07 ^f	0.32±0.05 ^{de}	0.37±0.04 ^b	0.11±0.01 ^{fg}	0.13±0.02 ^{de}	0.16±0.03 ^c	0.31±0.07 ^d	0.43±0.09 ^b	0.45±0.11 ^b
	Threonine								
	12hrs	18hrs	24hrs						
<i>S. cerevisiae</i> (Control)	0.18±0.01 ^g	0.20±0.01 ^g	0.25±0.02 ^f						
<i>L. brevis</i>	0.31±0.08 ^e	0.36±0.05 ^d	0.48±0.12 ^a						
<i>L. fermentum</i>	0.26±0.06 ^f	0.40±0.08 ^c	0.43±0.09 ^b						
<i>L. plantarum</i>	0.29±0.07 ^e	0.37±0.09 ^d	0.42±0.04 ^c						

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$).

culture-1 after 24 hours of fermentation time followed by same culture after 18 hours fermentation time (0.48 g/100g). The lactic acid content found in sourdoughs is similar to the study reported by Robert *et al.* (2005), who found the acidification properties, metabolic activity and technological performance of the wheat sourdough bread produced by using the freeze dried starters and found that lactic acid production was in the range of 0.25 to 0.55 g/100g.

The acetic acid content of sourdoughs showed that the St-I (*Lb. brevis*) produced significantly the highest acetic acid content (0.10 g/100g) followed by St-II (*Lb. fermentum*) (0.09 g/100g) (Table 2). The acetic acid was not produced by the control (*S. cerevisiae*) and St-III (*Lb. plantarum*). The interaction between the starter culture and fermentation time showed significantly maximum production of acetic acid (0.14 g/100g) by Starter culture-I after 24 hours of fermentation time followed by the same culture after 18 hours of fermentation time (0.10 g/100g). The results of the present

study are in agreement of Katina (2004), who reported that acetic acid content of the wheat sourdoughs lies in the range of 0.0003 to 0.25 g/100g depending upon the flour type, starter and fermentation conditions used.

Sugars of sourdough: The results for the maltose content of sourdoughs prepared from different starter cultures given in Table 3 showed that the glucose, maltose and fructose content ranged from 0.15 to 0.43 g/100g, 1.60 to 2.50 g/100g and 0.11 to 0.41 g/100g among different starter cultures. The results also indicated that significantly the highest amount of maltose, fructose contents were exhibited by the St-I (*Lb. brevis*) while control (*S. cerevisiae*) showed the highest value of glucose. During fermentation time, maltose contents increased in sourdoughs of all treatments, fructose content increased only in doughs having St-I and St-II and glucose contents remained almost constant in all treatments. The aggregate values of glucose, sucrose, fructose and maltose varied from 1.55 to 1.85% contingent on the variation among

starch hydrolysis, amount of flour microbial proteins and microbial utilization (Martinez Anaya, 1996).

Amino acids of sourdough: The content of essential amino acids of sourdoughs produced by the addition of different starter cultures varied significantly. The highest amount of amino acids *i.e.* 0.14 g/100g methionine, 0.33 g/100g lysine, 0.81 g/100g leucine, 0.42 g/100g isoleucine, 0.22 g/100g histidine, 0.47 g/100g valine, 0.14 g/100 g methionine, 0.48 g/100g threonine were observed in sourdoughs produced from St-I after 24th h of fermentation (Table 4). The amino acids content of sourdoughs increased significantly by increase in the fermentation time. The results further indicated that production of methionine, lysine and valine did not differ significantly between St-II and St-III after 24 hours of fermentation time. Similarly, St-I and St-II did not show significant variation for the production of leucine and isoleucine content.

Volatile compounds of wheat sourdoughs: 1-propanol and 2-methyl-1-propanol content of sourdoughs prepared from different starter cultures and fermentation time varied from 4.6-7.2% and 10.3-15.7% respectively (Fig. 1, 2).

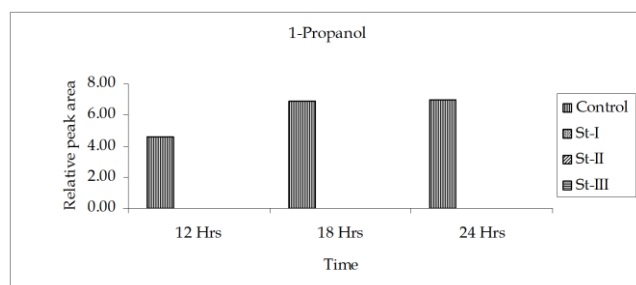


Figure 1. Content of 1-propanol in wheat sourdoughs fermented with different LAB starter cultures during different fermentation times. Relative peak area = (peak area of each compound/total area) \times 100; Control= *Saccharomyces cerevisiae*; St-I = *Lactobacillus brevis*; St-II = *Lactobacillus fermentum*; St-III = *Lactobacillus plantarum*.

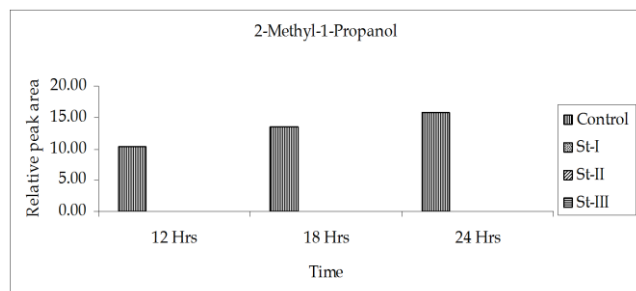


Figure 2. Content of 2-methyl-1-propanol in wheat sourdoughs fermented with different LAB starter cultures during different fermentation times. Relative peak area = (peak area of each compound/total area) \times 100; Control= *Saccharomyces cerevisiae*; St-I = *Lactobacillus brevis*; St-II = *Lactobacillus fermentum*; St-III = *Lactobacillus plantarum*.

The content of 2-methyl-1-butanol and 3-methyl-1-butanol in the sourdoughs varied from 5.1 to 6.7% and 12.0 to 15.3% (Fig. 3, 4), respectively. 1-hexanol and hexanal content of sourdoughs prepared from different starters varied from 2.9 to 9.4% and 2.5 to 16% (Fig. 5, 6).

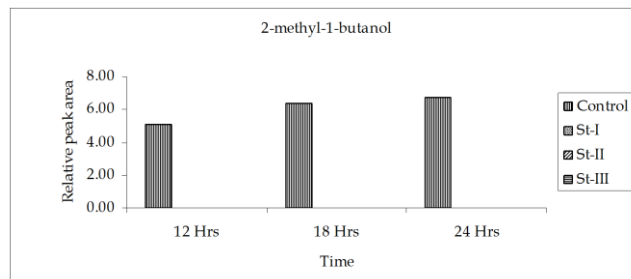


Figure 3. Content of 2-methyl-1-butanol in wheat sourdoughs fermented with different LAB starter cultures during different fermentation times. Relative peak area = (peak area of each compound/total area) \times 100; Control= *Saccharomyces cerevisiae*; St-I = *Lactobacillus brevis*; St-II = *Lactobacillus fermentum*; St-III = *Lactobacillus plantarum*.

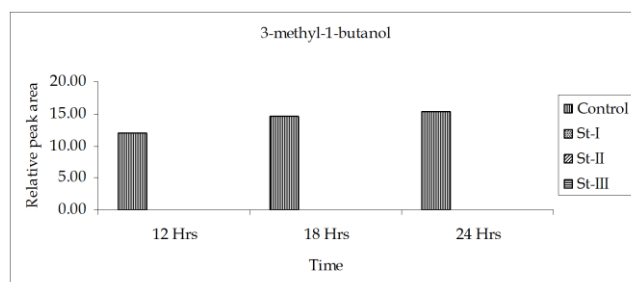


Figure 4. Content of 3-methyl-1-butanol in wheat sourdoughs fermented with different LAB starter cultures during different fermentation times. Relative peak area = (peak area of each compound/total area) \times 100; Control= *Saccharomyces cerevisiae*; St-I = *Lactobacillus brevis*; St-II = *Lactobacillus fermentum*; St-III = *Lactobacillus plantarum*.

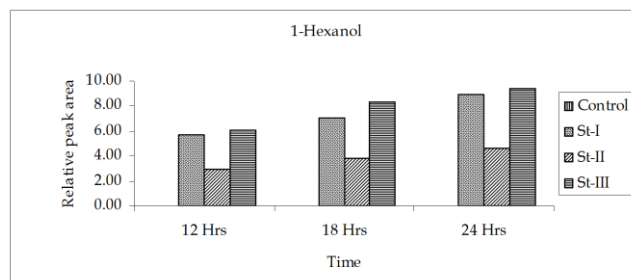


Figure 5. Content of 1-hexanol in wheat sourdoughs fermented with different LAB starter cultures during different fermentation times. Relative peak area = (peak area of each compound/total area) \times 100; Control= *Saccharomyces cerevisiae*; St-I = *Lactobacillus brevis*; St-II = *Lactobacillus fermentum*; St-III = *Lactobacillus plantarum*.

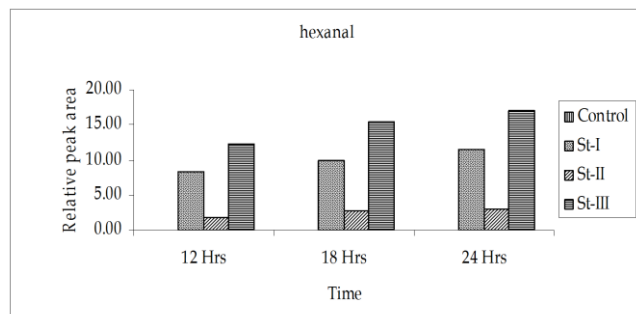


Figure 6. Content of hexanal in wheat sourdoughs fermented with different LAB starter cultures during different fermentation times. Relative peak area = (peak area of each compound/total area) \times 100; Control= *Saccharomyces cerevisiae*; St-I = *Lactobacillus brevis*; St-II = *Lactobacillus fermentum*; St-III = *Lactobacillus plantarum*.

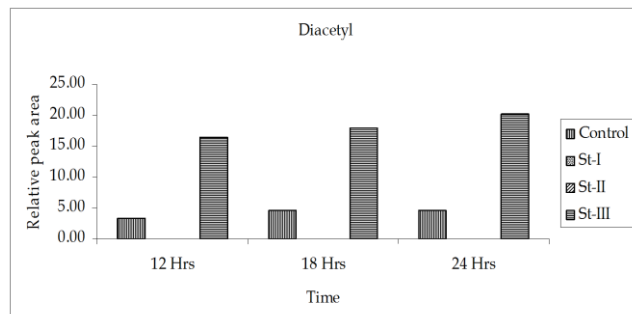


Figure 9. Content of diacetyl in wheat sourdoughs fermented with different LAB starter cultures during different fermentation times. Relative peak area = (peak area of each compound/total area) \times 100; Control= *Saccharomyces cerevisiae*; St-I = *Lactobacillus brevis*; St-II = *Lactobacillus fermentum*; St-III = *Lactobacillus plantarum*.

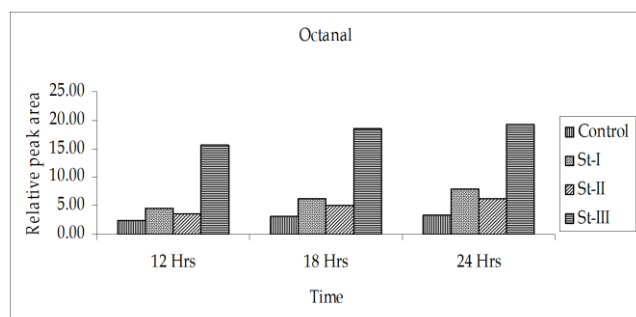


Figure 7. Content of octanal in wheat sourdoughs fermented with different LAB starter cultures during different fermentation times. Relative peak area = (peak area of each compound/total area) \times 100; Control= *Saccharomyces cerevisiae*; St-I = *Lactobacillus brevis*; St-II = *Lactobacillus fermentum*; St-III = *Lactobacillus plantarum*.

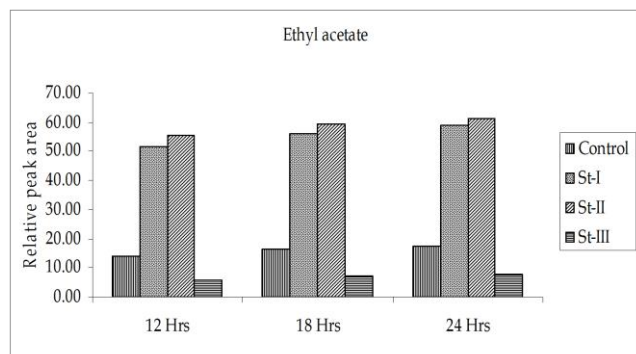


Figure 8. Content of ethyl acetate in wheat sourdoughs fermented with different LAB starter cultures during different fermentation times. Relative peak area = (peak area of each compound/total area) \times 100; Control= *Saccharomyces cerevisiae*; St-I = *Lactobacillus brevis*; St-II = *Lactobacillus fermentum*; St-III = *Lactobacillus plantarum*.

DISCUSSION

The pH of bread dough will vary depending on the rate of addition of sour dough. Mixing behavior of the doughs is greatly influenced by its acidity and can directly be related to pH and stability of the dough *i.e.* less mixing time required for dough having low pH and having less stability as compared to those having high pH (Hoseney, 1994). Structure forming components of the dough such as starch, arabinoxylans and gluten are exaggerated by partial acidification of bread and acidification of sourdough. It has been hypothesized that starch is hydrolyzed mildly in sourdoughs while gluten get expansion (Barber *et al.*, 1992). The TTA relies on flour utilized: as the lactobacilli ferment to pH 3.6, flours with high buffering limit, *e.g.* entire flours have a higher TTA than white flours with a low buffering limit. Since in present study straight grade flour was used, this produced lower TTA than whole meal and low grade flour. The present study suggested that LAB starter cultures produced significantly more acid as compare to the control in which *S. cerevisiae* was used. It may be concluded that LAB starter cultures used in present study have the ability to produce more acids as compare to control.

Mathewson (2000) has reported that maltose content expanded somewhat from 1.5 to 2.5 g/100g and credited this expansion to the hydrolytic activity of grain amylases on starch division. The glucose production did not show relationship with homo or hetero fermentative metabolism as it did not vary between different LAB strains. The results of the present study regarding sugars (maltose, glucose and fructose) are in conformity with the studies of Robert (2005) who reported similar findings regarding the behavior of homo and hetero fermentative starter cultures for the wheat sourdough bread making process. The results are also in close agreement with the Lefebvre *et al.* (2002) who found that the fructose content during sourdough fermentation increased due to metabolism by lactic acid bacteria and also observed

difference in the behavior of homo and hetero fermentative starter cultures for the fructose content. The present study suggested that the heterofermentative cultures showed better ability to produce fructose content as compare to homofermentative starter culture. Since the control comprised of yeast which exhibited the lowest production of fructose during different fermentation periods.

The acidification and the production of lactic acid and acetic acid have very important role regarding the preservation against the spoilage microorganisms provided that the raw material and the pre-fermentation conditions are optimal (Ganzle, 2008). The lactic acid production depends upon the metabolic activity, technological performance and acidification properties of the wheat sourdoughs. The acetic acid content of the wheat sourdoughs depends upon starter and fermentation conditions used. Rheological properties of sourdoughs are greatly affected by the organic acids especially lactic acids and acetic acids produced by lactobacilli (Arendt *et al.*, 2007). The lactic acid can give rise to elastic gluten structure while acetic acid contributes to harder and shorter gluten (Corsetti *et al.*, 2005). The inhibitory effect of organic acids produced during the fermentation also ensures microbial safety of the product (Erbaş *et al.*, 2006). The present results suggest that starter cultures produce significant amount of acetic and lactic acids in sourdough which besides other roles may also act as preservative in end product.

The results revealed that higher amount of essential amino acids was found in sourdoughs containing LAB starter cultures as compared to the control. LAB fermented sourdoughs have higher proteolytic activity so significant production of amino acids was observed as compared to yeast preferments (Thiele *et al.*, 2002). The optimum (acidic) conditions created by LAB have an positive impact on activity of cereal proteinases during sourdough fermentation due to high acidity (Vermeulen *et al.*, 2006). The breakdown of proteins during sourdough fermentation also affects the flavor and texture of sourdough. LAB release small peptides and free amino acids during fermentation that have a direct effect on growth of microorganisms and dough acidification characteristics. Lactobacilli and pediococci have the ability of utilizing gluten as a nitrogen source thereby increasing the essential amino acids content in gluten based medium (Gerez *et al.*, 2006). During fermentation of white flour with yeast, decrease in concentration of amino acids has been observed (Damiani *et al.*, 1996; Thiele *et al.*, 2004). It has been reported that among LAB, lactobacilli possess the greater cell bond proteinase actions (Christensen *et al.*, 1999). It is known that cereals are deficient in certain amino acids. Lysine is one of the most limiting amino acid in wheat. The LAB starter culture in the present study produced significant amount of essential amino acids especially lysine which can help to improve the level of amino acids in end product.

Both 1-propanol and 2-methyl-1-propanol were only produced by control yeast culture while no production of 1-propanol and 2-methyl-1-propanol content was observed in the sourdoughs prepared from LAB starter cultures. Damiani *et al.* (1996) found that 1-propanol was only produced in the range of 0 to 7.9% by the yeast and no production of 1-propanol content was observed in sourdoughs containing LAB. Hansen and Hansen (1994) reported that 2-methyl-1-propanol was the characteristic volatile compound of fermentative yeast. They also witnessed the production of volatile compounds in wheat sourdoughs and reported that without using yeast, concentration of the 1-hexanol was the dominating alcohol. Lund *et al.* (1989) reported that the content of hexanal was very high in sourdoughs fermented with a non-commercial homofermentative culture. Damiani *et al.* (1996) have observed that the content of octanal was mainly produced in sourdoughs fermented with lactic acid bacteria especially homofermentative starter culture (*L. plantarum* as in present study). The results for the ethyl acetate content in the present investigation are closely in agreement with the earlier findings of Damiani *et al.* (1996) and Lund *et al.* (1989), who found that ethyl acetate was higher in wheat sourdoughs fermented with heterofermentative LAB. El-Gendy *et al.* (1983) and Torner *et al.* (1992) both observed that the diacetyl content is generated by both LAB and yeast. Similarly Damiani *et al.* (1996) and Lund *et al.* (1989) reported that the content of diacetyl was higher in sourdoughs manufactured with homofermentative as compared to heterofermentative cultures. Thus it may be inferred from the present study that the sourdoughs made from LAB starter cultures St-I (heterofermentative) and St-III (homofermentative) gave higher production of volatile compounds and can be incorporated in bread preparation.

Conclusions: The present study has given a detailed depiction of bioactive components produced in wheat sourdoughs with different starter cultures and fermentation time. It has also been revealed how the production of bioactives in wheat sourdough is affected by predominant micro-organisms, *L. plantarum*, *L. brevis* and *L. fermentum*. These three types of LAB starter cultures have the ability to impart unique characteristics in sourdough through fermentation. Therefore, sourdough technology should be used on commercial scale in the production of bakery products to improve shelf life and quality; satisfying the ever increasing demand of the consumers for specialty products.

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