The Impact of *Lactobacillus plantarum*, *Paracasei*, *Casei–Casei*, and *Sanfranciscensis* on Reducing Acrylamide in Wheat Bread

F. Dastmalchi¹, S. H. Razavi²∗, M. Labbafi², and M. Faraji¹

ABSTRACT

Acrylamide as a possible carcinogenic compound is known to produce in heated carbohydrate-rich food such as bread. In this study, the effect of the fermentation process by four Lactic Acid Bacteria (LAB) and yeast on an industrial scale, was studied on acrylamide reduction in bread. Results showed that the flour specifications and the kind of microorganisms in the fermentation process are important factors for acrylamide formation in bread. Acrylamide content in control bread which is fermented by yeast, containing the highest amount of reducing saccharides was found to be the highest (239.12 µg kg⁻¹). Fermentation by LAB and yeast reduced acrylamide formation. Fermented bread with *Lactobacillus paracasei* showed the lowest amount of acrylamide (131.06 µg kg⁻¹) due to its lower pH of sourdough (3.51) and glucose content (5.44 mg g⁻¹). Bread leavened with lactic acid bacteria starters had the softest texture to yeast starter. The addition of sourdough starters with mean pH 3.56 decreased the pH of bread, causing enhancement of the texture and sensory properties, as well as reduction of acrylamide. The sourdough bread, especially fermented bread by *L. paracasei* had the lowest amount of acrylamide and softest texture during three days.

Keywords: Acrylamide, Fermentation, Lactic acid bacteria, Wheat bread.

INTRODUCTION

Acrylamide is widely used as a monomer or intermediate in organic synthesis, and polyacrylamide has uses in the paper, textile and cosmetics industries, as well as the treatment of wastewater (Charoenpanich, 2013). Acrylamide, however, is known to be a neurotoxic, genotoxic and probably carcinogenic compound (IARC, 1994). Exposure to acrylamide reasons harm to the nervous system in humans and animals (Lopachin and Lehning, 1994), and acrylamide is discussed as a reproductive contaminant with mutagenic and carcinogenic attributes in some researches (Friedman, 2003; Rahangadale et al., 2012; Sadat Mousavian et al., 2015). In April 2002, scientists of the Swedish National Food Administration, in aggregation with Stockholm University, informed that carbohydrate rich foods heated or fried at high temperatures, such as fried potato products, french fries, bread, coffee, biscuits, and other cereal products, contained relatively high levels of acrylamide (Swedish National Food Administration, 2002). These results encouraged many researchers to study on the analysis method, formation mechanism and preventing plans on acrylamide. At present it is generally accepted that acrylamide is formed during Millard reaction between the amino acid asparagine and reducing sugars at temperatures above 120°C (Amrein et al., 2004; Bemiller and Huber, 2008; Mottram et al., 2002; Stadler et al., 2002). A few strategies have been planned to decrease

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Acrylamide through food processing, such as decreasing the contents of precursors (Capuano et al., 2010; Muttucumaru et al., 2008; Viklund et al., 2008), interrupting formation reactions by adding other compounds (Capuano and Fogliano 2011; Curtis et al., 2009), and adjusting process parameters (Amrein et al., 2006; Grantby et al., 2008; Morales et al., 2009).

Among the food mentioned above, bread is one of the most consumed products worldwide. It is a good source of energy, protein, Dietary Fibre (DF), minerals, vitamins and many alternative bioactive compounds (Bartkiene et al., 2013). Fermentation processes by Lactic Acid Bacteria (LAB) and yeast could reduce the acrylamide content of bread (Fink et al., 2006; Fredriksson et al., 2004).

Sourdough is an ancient method to improve texture and flavour of bread, it also increases the shelf life of bakery products. Applications of sourdough that contain lactobacilli strains in bread production have grown in the recent years because of the consumers switching to more natural products containing less chemical preservers. Lactobacilli strains can be obligate homofermentative, obligate heterofermentative or facultative heterofermentative strains (Katina, 2005).

Therefore, the aim of this study was to evaluate the impact of L. plantarum, L. casei-casei, L. paracasei as obligate homofermentative strains, L. sanfranciscensis as an obligate heterofermentative strain and yeast on reducing acrylamide content in bulk wheat bread. For this purpose, different formulations of bread were made and acrylamide, sugar, and some physicochemical specifications were determined after baking. The relationship between variables was assessed with acrylamide.

MATERIALS AND METHODS

Chemicals

All chemical substances and solvents utilized in the tests were of analytical and had HPLC grade purity and were purchased from Merck Chemical Company (Darmstadt, Germany) and Sigma-Aldrich Company (St. Louis, MO, USA).

Ingredients

Wheat flour obtained from an area mill (Karaj, Iran) was used for bread preparation. The dried instant yeast (Iran Maye Co., Iran) and other ingredients were purchased from local markets.

Lactic Acid Bacteria Strains

The LAB strains were L. plantarum sub sp. Plantarum (DSM 20179), L. casei-casei (DSM 20011), L. paracasei (DSM 20207), and L. sanfranciscensis (DSM 20663), which were obtained from the culture collection of the food microbiology laboratory of faculty of Agricultural Engineering and Technology, Tehran University. The LAB strains were previously selected based on their ability for improvement of dough properties, bread texture and flavour, crumb firmness, staling process retardation, bread inhibits from mould and bacterial spoilage and overall acceptability (Gamel et al., 2015; Katina, 2005; Martinez-Anaya, 2003).

The strains were stored at -70°C and propagated at 37°C for 24 hours (h) in MRS broth (de Man, Rogosa and Sharpe) until 10^9 CFU g^-1, before the experiment. For bacteria cell number, 10 ml of above activated bacteria were homogenised with 90 mL of sterile saline solution (0.9 %). Serial dilutions (10^-4–10^-8) of this suspension were made, spread onto MRS agar and incubated under anaerobic conditions at 35°C for 72 hours. The bacteria cell number was calculated and expressed as log CFU g^-1.

Sourdough Preparation

Stationary phase cells of the LAB strains, as described above, were harvested by
centrifugation 8,000×g, for 10 minutes (min), at 4°C. Then they were washed twice with sterile 0.9% saline solution, suspended in milk, and incubated at 37°C for 24 hours. The cell suspension (L. plantarum, L. casei-casei, L. paracasei, and L. sanfranciscensis) was added to the substrates containing “wheat flour and water” and was incubated at 37°C for 24 hours, to obtain 10⁷ CFU g⁻¹ in the dough. Four different sourdoughs by L. plantarum, L. casei-casei, L. paracasei, and L. sanfranciscensis were prepared.

### Bread Making Procedure

Flour specifications and bread dough formulations are shown in Tables 1 and 2. Ingredients were blended in a mixer and as much as % 50 tap water was slowly added, whereas mixing continues at low speed for 3 minutes, followed by 5 minutes at medium speed till dough shaped. The dough was divided into 50 g portions and formed as the round roll. Fermentation and proofing were done at 29±0.5°C for 90 minutes, continuously. Then, the dough was baked on an industrial scale in a commercial local bakery (Karaj, Iran) at 190°C for 20 minutes. The loaves were allowed to cool at room temperature and packaged by polyethylene bags and stored at 25°C.

### Preparation of Test Samples

Bread was sliced, and dried in an oven at 30-40°C. The drying temperature was set relatively low to avoid acrylamide formation throughout the drying procedure. Then bread samples were ground and homogenized by using a mixer (National-Model Mj-176NR-Japan).

### Determination of Moisture, Wet gluten, Ash, Protein, pH and Acidity


Protein content of flour was determined based on American Association of Cereal Chemists (AACC) Method No. 46-12 (AACC, 2000).

pH of flour, sourdough and bread was measured by preparing a 10% solution in water (10 g of sample and 100 ml distilled water). The measurement was done employing a pH meter (Metrohm Model 632), based on Association of Official Analytical Chemists (AOAC) Method No. 943.02 (AOAC, 1995).

The Total Titratable Acidity (TTA) was

### Table 1. Physicochemical specifications of flour.

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>Gluten (%)</th>
<th>Gluten Index</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>11.2±0.0</td>
<td>0.58±0.0</td>
<td>11.3±0.0</td>
<td>29.6±0.0</td>
<td>72±0.0</td>
<td>5.6±0.0</td>
</tr>
</tbody>
</table>

a Results are mean±SD of three determinations on dry weight (dw) basis

### Table 2. Formulations of dough for control bread and bread with different sourdough.

<table>
<thead>
<tr>
<th></th>
<th>Control bread</th>
<th>Bread with different sourdough</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour (g)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sourdough (g)</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Instant dry yeast (g)</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Bread improver (g)</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Vegetable oil (g)</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
measured by the amount of 0.1N NaOH required to neutralize solution according to AOAC Method No. 947.05 (AOAC, 1995).

**Extraction and Analysis of Acrylamide in Bread**

Acrylamide content was determined by gas chromatography equipped with a mass spectrometry detector (GC-MS) (A 7890A GC system from Agilent Technologies, Palo Alto, CA, USA, with a triple-axis detector coupled with a 5975C inert MSD network mass selective detector) according to Lim and Shin (2013) and Ghasemzadeh-Mohammadi et al. (2012) with minor modifications. The planned technique highly and sensitively determines ultra-trace levels of acrylamide concentration in bread after derivatization with xanthydrol and Dispersive Liquid–Liquid MicroExtraction (DLLME).

In order to perform the acrylamide primary extraction from the matrix, 12 mL of mixing solution containing potassium hydroxide (1M), and ethyl alcohol (25:75), were added to 1 g of the sample. The sample was hydrolyzed through microwaving (Delonghi type MW 602) at 500 MHz for 2 min. After cooling, the compounds were centrifuged at 4,000×g for 5 minutes (Heltich Rotorfix 32A). Then the pH of aqueous phase was adjusted to 6.5 by adding concentrated hydrochloric acid. Finally, to precipitate the proteins and carbohydrates, 2 mL of Carrez solutions I and II (50:50) were added to the vessel, which was then centrifuged again at 4,000xg for 5 minutes. Ten mL of filtered sample, 30 µL of 0.5M xanthydrol solution (by ethyl alcohol), 80 µL of HCl (6.0M), and 2 µL of acrylamide-d3 (1.0 mg L⁻¹ in methanol) were shaken for 30 minutes at 300 rpm using a mechanical shaker. The derivatization reaction was conducted at ambient temperature for 30 min in the dark, and then the solution was neutralized with KOH (6.0M). The pH of the solution was adjusted to 9.0 with 0.2 g of NaHCO₃/K₂CO₃ (3:1, w/w). Then DLLME procedure was applied to extraction of xanthyl-acrylamide.

Briefly, a solution consisting of 100 µL tetrachloroethylene as the extracting solvent, 600 µL of acetone as the disperser solvent was added rapidly into the xanthyl-acrylamide solution. The mixture was gently shaken and centrifuged at 4,000xg for 10 minutes. The dispersed fine particles of the extraction phase were sedimented at the bottom of the vessel and about 1.5 µL of the sedimented phase was injected directly into the GC–MS using a microsyringe.

**Reducing Saccharides Analysis**

Fructose, glucose and maltose were separated and quantified by Ultra-High Pressure Liquid Chromatography (UHPLC) (Knauer, platin blue, Germany) using an instrument equipped with a Refractive Index (RI) detector (Knauer, smart line, 2300) according to AACC Method No. 80-04 (AACC, 2000).

**Free Amino Acids Analysis**

Flour free amino acids were extracted and determined by derivatization according to Butikofer and Ardo (1999). O-Phthaldialdehyde (OPA) and FluorenylMethyl Chloroformate (FMOC) amino acids were measured by HPLC (Knauer, Germany) equipped by fluorescence detector. A Hypersil ODS 250×4 mm column with a 20×4 mm pre-column was used.

**Sensory Evaluation**

Sensory evaluation was analysed based on the modified AACC Method No. 74-30 (AACC, 2000). A panel of 10 specialists was used to evaluate the sensory characteristics of the bread. They were asked to evaluate the overall quality (colour, odour, taste and chewiness) of each sample.
concerning general properties. The ranking scale ranged from 0 (unacceptable) to 5 (ideal). The average of the panellist scores was calculated.

**Bread Firmness Measurements**

Bread firmness during storage was determined based on the modified AACC Method No.74-09 (AACC, 2000), as a maximum force (40% compression), required to compress loaf bread by a preset distance. Crumb firmness was measured on days 1, 2, and 3 after baking to evaluate the shelf life of the bread. The thickness of each bread slice was 2.5 cm, and edges of the slice were cut off before measurement.

**Statistical Analysis**

All analysis was conducted independently on dry weight (dw) basis in triplicate. Statistical analysis of the data was performed through an Analysis of Variance (ANOVA) using SPSS software package (IBM, SPSS, Statics, and Version 22.0). Duncan’s multiple range test was used to determine any significant difference among the samples at a 95% confidence level.

**Method Validation of Acrylamide Analysis**

**Linearity**

Nine aqueous standards containing the analytes in study in concentrations ranging from 15 to 600 µg L\(^{-1}\) were submitted to the whole analytical procedure. The method showed large dynamic linear range with good correlation of determination (\(R^2\)) higher than 0.9988.

**Limit Of Detection (LOD) and Limit Of Quantification (LOQ)**

The detection limits of the method were determined by successive analyses of sample extracts with decreasing amounts of the compounds until a 3:1 signal-to-noise ratio was reached. Based on obtained results, \(LOD\) was 5.0 µg L\(^{-1}\). The quantification limit was established as the lowest concentration assayed with acceptable accuracy and precision which corresponds to the lowest calibration level of the calibration curve (15 µg L\(^{-1}\)).

**Accuracy and Enrichment factor**

Accuracy of the method was tested by spike bread samples at known level of 50 µg L\(^{-1}\), extraction, analysis, and determination of the recovery for analyte in triplet. Good results were obtained, with average recoveries ranging from 90.0 to 101.6% with \(RSD\%\) less than 10%. It is interesting to note that, samples did not require matrix matched calibration curves to compensate for difficulties in measuring peak area for AA at low concentration, making more rapid analysis possible.

The Enrichment Factor (EF) is defined as the ratio of concentration of acrylamide between the extracted organic phase and the initial concentration of the analyte in the aqueous sample. For these three replicate extractions were performed at the optimal conditions of sample containing 50 µg L\(^{-1}\) of acrylamide and the EF obtained was 180.

**Precision**

Inter-day and intra-day Relative Standard Deviation (\(RSD\%\)) of the method were determined by analyzing six independent solutions in six and one day in reagent water spiked with acrylamide at 10 and 100 µg L\(^{-1}\) levels, respectively. Results showed that the intra-day and inter-day \(RSD\%\) are less than 10% for both of 10 and 100 µg L\(^{-1}\) levels. So, the optimized method guarantees appropriate measurement of acrylamide.
RESULTS AND DISCUSSION

Influence of Sourdough Addition on Acrylamide Formation in Bread

Significant differences were observed in acrylamide content between the bread fermented by yeast and different added LAB in sourdough that are shown in Table 3 (P<0.05). Control bread, which had the highest concentration of glucose and fructose in comparison to bread prepared with sourdough showed the highest acrylamide content. The lowest acrylamide amount was formed in bread with L. paracasei that contained the lower amount of reducing sugar content in comparison with the other, except bread with L. casei-casei in this experiment. This may suggest that the presence of the highest amount of reducing saccharides in bread causes formation of the maximum amount of acrylamide in bread. Fermentation by LAB causes the pH in dough to drop due to release of acid and carbon dioxide. Acrylamide formation in bread decreased by reducing pH value with microorganisms. Bartkiene et al. (2013) showed that the fermentation with a commercial strain L. casei has a higher impact on acrylamide reduction in bread samples compared to L. sakei, Pediococcusacidilactici and Pediococcuspentosaceus strains that were confirmed by our results.

Acrylamide–precursors Relationship in Bread

During the baking of bread, starch and sucrose could be hydrolysed to form reducing saccharides that can participate in Maillard reaction (Capuano and Fogliano, 2011). Mottram et al. (2002) reported that asparagine was the main amino acid that reacted with reducing sugars to produce acrylamide. The highest amount of acrylamide was produced when the mole ratio of asparagine/glucose was 1:1 and the

<table>
<thead>
<tr>
<th>Samples</th>
<th>LAB strain (in sourdough)</th>
<th>Acrylamide (µg kg⁻¹)</th>
<th>Acidity (%)</th>
<th>Moisture (%)</th>
<th>pH of bread</th>
<th>pH of sourdough</th>
<th>Scores of sensory evaluation</th>
<th>Score of evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>L. plantarum</td>
<td>16.6±6.2±9.10</td>
<td>2.24±0.02 a</td>
<td>23.20±0.01 a</td>
<td>5.02±0.02 a</td>
<td>3.60±0.10 a</td>
<td>4.60±0.00 b</td>
<td>4.0±0.00 a</td>
</tr>
<tr>
<td>Bread</td>
<td>L. paracasei</td>
<td>131.06±8.29</td>
<td>2.25±0.02 c</td>
<td>21.90±0.02 b</td>
<td>4.95±0.02 c</td>
<td>3.51±0.12 c</td>
<td>4.70±0.05 a</td>
<td>4.60±0.00 a</td>
</tr>
<tr>
<td>Bread</td>
<td>L. casei-casei</td>
<td>219.11±5.31</td>
<td>2.01±0.03 b</td>
<td>22.61±0.02 b</td>
<td>5.02±0.01 c</td>
<td>3.60±0.12 a</td>
<td>4.60±0.03 b</td>
<td>4.0±0.00 c</td>
</tr>
<tr>
<td>Bread</td>
<td>L. sanfranciscensis</td>
<td>229.34±8.76</td>
<td>2.00±0.03 b</td>
<td>21.81±0.01 b</td>
<td>5.12±0.01 b</td>
<td>3.53±0.10 a</td>
<td>4.0±0.00 a</td>
<td></td>
</tr>
</tbody>
</table>

Different letters in each column indicate significant differences between the values (P<0.05) by Duncan’s multiple-comparison test. Results are means±SD of three determinations on the basis.

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temperature was 160°C (Mottram et al., 2002).

Milling will significantly differentiate the asparagine and reducing sugar levels within the different milling fractions. Fredriksson et al. (2004) reported that free asparagine contents are lower in sifted wheat flour [0.14–0.17 g kg\(^{-1}\) dry weight (dw)] compared to whole wheat flour [0.5 g kg\(^{-1}\) dw]. In the present study, because of the low amount of free asparagine in the wheat flour (0.08±0.005 g kg\(^{-1}\)), the relationship between acrylamide and asparagine was not considered. In this case, sugar seems to be the most important ingredient in the dough formula for acrylamide creation in bakery products because the free asparagine is relatively low in wheat flour (Hamlet et al., 2005; Keramat et al., 2011; Surdyk et al., 2004).

The obtained results in this study, demonstrated that maltose, glucose and fructose levels (2.36, 2.77, 8.39 mg g\(^{-1}\)) were lower in wheat flour than in bread (Table 4). Wheat flour was 73%-80% less total reducing sugar than in bread. Wheat flour contains different levels of nutrients and enzymes as amylases, decarboxylases, proteases, and transaminases related to the bran and outer layers of the grain. The extraction level of flour is one of the most important elements influencing the nutrients and enzymes content of flour (Azizi et al., 2006). However, these factors will stimulate the growth and biochemical activity of the sourdough microflora, increasing the acids and flavour compound synthesis (Brummer and Lorenz, 1991; De Vuyst and Neysens, 2005).

Martinez-Anaya (2003) has reported that the endogenous α-amylase activity starts during mixing and increases primary maltose levels by ten to fifteen fold. During the fermentation process, glucoamylases enzyme activity causes a release of glucose, that favours the growth of homofermentative LAB. The preferred use of fructose is observed in most heterofermentative LAB (Ganzle et al., 2007).

The obligate heterofermentative Lactobacilli as L. sanfranciscens is also show maltose phosphorylase activity, utilize only maltose, and release glucose, which is assimilated by the yeast(Ganzle et al., 2007). Eventually, starch and sucrose of wheat flour were hydrolysed to reducing sugars (glucose and fructose) that were partly utilized after fermentation by LAB and yeast. We found that the amount of remaining sugar in the dough after fermentation and cooking, shows microbial activity.

The obtained results showed that the content of glucose and fructose was higher in control bread than in bread fermented with LAB (Table 4). These results are in agreement with those reported by other authors suggesting that reducing sugar is an important factor for determining acrylamide formation in bread (Amrein et al., 2004; Hamlet et al., 2005; Keramat et al., 2011, Surdyk et al., 2004).

### Table 4. Reducing sugar content (mg g\(^{-1}\) dw basis) in flour, control bread and treated bread. *

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added strain in sourdough</th>
<th>Maltose</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Total reducing sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>wheat flour</td>
<td>-</td>
<td>2.36±0.11 d</td>
<td>2.77±0.09 f</td>
<td>8.39±0.05 f</td>
<td>13.52±0.07 e</td>
</tr>
<tr>
<td>Bread</td>
<td>L. plantarum</td>
<td>10.12±0.08 b</td>
<td>7.66±0.20 b</td>
<td>37.74±0.20 d</td>
<td>56.36±0.31 b</td>
</tr>
<tr>
<td>Bread</td>
<td>L. paracasei</td>
<td>9.59±0.33 c</td>
<td>5.44±0.38 d</td>
<td>39.47±0.22 b</td>
<td>54.51±0.65 c</td>
</tr>
<tr>
<td>Bread</td>
<td>L. casei – casei</td>
<td>9.48±0.13 c</td>
<td>5.64±0.11 d</td>
<td>35.91±0.09 e</td>
<td>51.06±0.08 d</td>
</tr>
<tr>
<td>Bread</td>
<td>L. sanfranciscens</td>
<td>10.78±0.50 a</td>
<td>6.75±0.19 c</td>
<td>38.91±0.20 c</td>
<td>56.45±0.53 b</td>
</tr>
<tr>
<td>Control Bread</td>
<td>-</td>
<td>9.51±0.10 c</td>
<td>12.14±0.12 a</td>
<td>45.70±0.10 a</td>
<td>67.35±0.10 a</td>
</tr>
</tbody>
</table>

* Different letters in each column indicate significant differences between the values (p<0.05) by Duncan’s multiple-comparison test. Results are mean±SD of three determinations on dw basis.
On the other hand, a survey has shown that baker’s yeast can selectively consume added asparagine to the dough and can reduce acrylamide up to 80% for a proof time of 1 hour (Hamlet et al., 2005). Granby et al. (2008) showed that in wheat bread, 12% of the asparagine firstly present in the flour (0.14 g kg\(^{-1}\)) remained after yeast fermentation and baking, also Fredriksson et al. (2004), showed that > 80% of the asparagine was utilized after 2 hours of fermentation with yeast. Surdyk et al. (2004) studied the effect of asparagine and fructose on acrylamide concentration in wheat bread fermented by yeast. Added asparagine intensely increased the content of acrylamide in crusts, but added fructose did not affect the acrylamide.

In the present study, baker’s yeast, like LAB, cannot adequately reduce acrylamide, due to low levels of asparagine in wheat flour. It is thought that an imbalance between asparagine and reducing sugars in the flour is the one of the reasons.

**Sugars Utilization, Acid Synthesis and Acrylamide Production in Bread**

Acid production was proportional to reducing sugar, especially glucose catabolism. All strains demonstrated similar maximum acid production. The sourdough process depends on several factors including the composition of microflora, flour specifications, fermentation circumstances and enzymatic activities. The amount of fermentable carbohydrates is the main factor for regulating acidification (Martinez-Anaya, 2003).

Investigations have shown that extended yeast fermentation and pH reduction can individually reduce acrylamide formation in baked cereal products (Fredriksson et al. 2004; Hamlet et al., 2005).

Our results showed that bread fermented by *L. paracasei* was superior in acid production rates and lower in pH value and glucose, thus acrylamide formation was 45% lower than control bread (Tables 3 and 4).

Pearson correlation was used to analyse the relationship between the dependent variables. Glucose, fructose and total reducing saccharides of bread influenced the pH value in the bread (\(r = 0.807\), \(r = 0.699\), \(r = 0.788\)), also acrylamide contents of bread, correlated with pH and acidity (\(r = 0.842\), \(r = -0.953\) respectively). These correlations were significant at the 0.01 level. It shows a decrease in the activity of microorganisms and reducing saccharides consumption and thus lower production of organic acids. Therefore, the higher pH of bread followed by the remaining reducing sugars in dough and bread also acrylamide content of bread which is a result of lower consumption by the microorganisms. Comparison of the means of acrylamide, acidity, pH and total reducing sugars of bread was shown in Figure 1. These results are in agreement with other authors that low pH values by microorganism sources in the dough inhibits the formation of the Schiff base by protonation of the amine group of amino acid, therefore decreasing the acrylamide content in bread. The primary step in acrylamide formation in Maillard reaction is the formation of the Schiff base which will change to form 3-aminopropionamide and a potent precursor of acrylamide (Bemiller and Huber, 2008; Granvogl et al., 2004).

**Effect of Sourdough on Bread Properties**

The overall sensory evaluations and firmness of the bread are shown in Table 3 and Figure 2.

The bread produced from sourdough had higher scores of sensory evaluation and lower firmness compared with the control bread during three days. On the first day, the firmness of bread fermented by sourdough of *L.paracasei* also *L.sanfranciensis* was 64 and 69% respectively, lower than the control bread. Although the stalling process in bread fermented with sourdough is more desirable in all cases than the control bread, the bread fermented by *L.paracasei* had the softest
Figure 1. Compare means of acrylamide, acidity, pH, and total reducing sugars of bread. Different letters in each legends indicate significant differences between the values (P< 0.05) by Duncan’s multiple-comparison test. Results are mean±2 SD (error bars) of three determinations.

Figure 2. Compare means of bread firmness on first, 2nd and 3rd day after baking. Different letters in each legends indicate significant differences between the values (P< 0.05) by Duncan’s multiple-comparison test. Results are mean±2 SD (error bars) of three determinations.

Note: Abbreviation used in Figures: Pl= L. Plantarum; Pca= L. paracasei, Ca-Ca= L. casei- casei, San= L. sanfranciscensis

texture until three days. The results showed that firmness of bread on the first day correlated (r= 0.853) with firmness on the second day. In addition, firmness of bread on the second day correlated with (r= 0.800) the firmness on the third day, these correlations were significant at the 0.01 level (Figure 2). Therefore in this study, gradual firmness and staling of bread is logical and is confirmed by the other researchers.
The influence of sourdough on bread properties depends on acidity level, proteolytic activity, level of free amino acids and important flavour compounds in sourdough (Katina, 2005).

CONCLUSIONS

Food safety and quality is a matter of public concern and the Maillard reaction has a close relationship with this subject. The Maillard reaction produces different compounds such as pleasant color of the crust of bread, odor and aroma, also contaminants like acrylamide. The results of this study are in agreement with other authors that low pH values of dough by microorganism sources will be one of the solutions to prevent the production of acrylamide. The results showed that the amount of acrylamide in bread made with LAB starters (131.06 until 229.34 µg kg\(^{-1}\)) was lower than in the control sample up to 45%. Furthermore, in relation to the overall acceptability of bread, the associated acidic flavour of bakery products is just accepted in the case of sourdough, which might attribute to improvement of bread quality and prevention of acrylamide formation which is for other researchers to confirm. The bread fermented by \textit{L.parcasei} had the lowest amount of acrylamide and longer shelf life until three days. Control of staling and keeping the quality of bread for longer periods can lead to important economic benefits.

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مسکارومایس سروزیه تخمیر شده، دارای بالاترین میزان آکریلامید (µg/kg) 239/12 و فندهای احیاكنده است. تخمیر بوسله با کثرت های اسید لاکتیک به همراه مخمر موجب کاهش آکریلامید و بهبود کیفیت نان شده. نان تخمیر شده با لاکتوباسیلوس پاراکازیمی دارای کمترین میزان آکریلامید(µg/kg) 131/06 بود. این کاهش در نتیجه افزایش تولید اسیدهای آلی در تخمیر ترش تولید شده با این خمیرترش می‌باشد. افزودن آغازگرهای لاکتیکی با میانگین pH=3/56 موجب کاهش pH و در نتیجه بهبود بخشه و وزن‌گی های عصبی همراه با کاهش آلانین ها مانند آکریلامید گردید. نان تخمیر شده با آغاز گرهای باکتری های اسید لاکتیک، دارای داشت اثر نمی‌باشد ب به نان کنترل بوده، بیوزه نان تخمیر شده با لاکتوباسیلوس پاراکازیمی که دارای کمترین میزان آکریلامید و نرم‌ترین بخشه در طول سه روز نگهداری بود.