

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

F. Dastmalchi<sup>1</sup>, S. H. Razavi<sup>2\*</sup>, M. Labbafi<sup>2</sup>, and M. Faraji<sup>1</sup>

### 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  $\mu\text{g kg}^{-1}$ ). Fermentation by LAB and yeast reduced acrylamide formation. Fermented bread with *Lactobacillus paracasei* showed the lowest amount of acrylamide (131.06  $\mu\text{g kg}^{-1}$ ) due to its lower pH of sourdough (3.51) and glucose content (5.44  $\text{mg g}^{-1}$ ). 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

<sup>1</sup> Department of Food Science and Technology, Faculty of Food Industry and Agriculture, Standard Research Institute (SRI), Karaj, Islamic Republic of Iran.

<sup>2</sup> Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, University of Tehran, Postal code: 31585-11167, P. O. Box 4111, Karaj, Islamic Republic of Iran.

\* Corresponding author email: srazavi@ut.ac.ir



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; Granby *et al.*, 2008; Morales *et al.*, 2009).

Among the food mentioned above, bread is one of the most consumed product 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<sup>-1</sup>, 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<sup>-1</sup>.

### 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 10<sup>9</sup> CFU g<sup>-1</sup> 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

Moisture, wet gluten and ash contents were determined using International Organization for Standardization (ISO) Methods No.712, 2171, 21415-2 respectively (ISO, 2009; ISO, 2007; ISO, 2006).

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. <sup>a</sup>

Specifications	Moisture (%)	Ash (%)	Protein (%)	Gluten (%)	Gluten Index	pH
wheat flour	11.2±0.0	0.58±0.0	11.3±0.0	29.6±0.	72±0.	5.6±0.0
	1	1	3	1	3	2

<sup>a</sup> 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.

	Control bread	Bread with different-sourdough
Wheat flour (g)	100	100
Sourdough (g)	0	13
Instant dry yeast (g)	1.7	1.7
Bread improver (g)	16	16
Vegetable oil (g)	5	5



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 4,000×g 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-d<sub>3</sub> (1.0 mg L<sup>-1</sup> 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 NaHCO<sub>3</sub>/K<sub>2</sub>CO<sub>3</sub> (3:1, w/w). Then DLLME

procedure was applied to extraction of xanthy-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 xanthy-acrylamide solution. The mixture was gently shaken and centrifuged at 4,000×g 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-PhtaldiAldehyde (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  $\mu\text{g L}^{-1}$  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  $\mu\text{g L}^{-1}$ . 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  $\mu\text{g L}^{-1}$ ).

### Accuracy and Enrichment factor

Accuracy of the method was tested by spike bread samples at known level of 50  $\mu\text{g L}^{-1}$ , 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  $\mu\text{g L}^{-1}$  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  $\mu\text{g L}^{-1}$  levels, respectively. Results showed that the intra-day and inter-day *RSD%* are less than 10% for both of 10 and 100  $\mu\text{g L}^{-1}$  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*, *Pediococcus acidilactici* and *Pediococcus pentosaceus* 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 3. Acrylamide and some physicochemical specifications of bread.<sup>a</sup>

Samples	LAB strain (in sourdough)	Acrylamide ( $\mu\text{g kg}^{-1}$ )	Moisture (%)	Acidity (%)	pH of bread	pH of sourdough	Scores of sensory evaluation
Bread	<i>L. plantarum</i>	165.62 ± 9.10 c	23.20±0.00 a	2.24±0.01 a	5.02±0.02 c	3.60 ±0.10 a	4.60 ± 0.0 b
Bread	<i>L. paracasei</i>	131.06 ± 8.29 d	21.52±0.02 e	2.25±0.02 a	4.95±0.02 d	3.51±0.12 b	4.70 ± 0.5 a
Bread	<i>L. casei – casei</i> ,	219.11 ± 5.31 b	21.90±0.05 c	2.01±0.03 b	5.02±0.01 c	3.60±0.12 a	4.60 ± 0.0 b
Bread	<i>L. sanfranciscensis</i>	229.34 ± 8.76 ab	22.61±0.02 b	2.00±0.03 b	5.12±0.01 b	3.53±0.10 b	4.60 ± 0.3 b
Control Bread	-	239.12 ± 1.87 a	21.81±0.01 d	2.00±0.02b	5.19±0.01 a	-	4.00±0.0 c

<sup>a</sup> 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.

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<sup>-1</sup> dry weight (dw)] compared to whole wheat flour [0.5 g kg<sup>-1</sup> dw]. In the present study, because of the low amount of free asparagine in the wheat flour (0.08±0.005 g kg<sup>-1</sup>), 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<sup>-1</sup>, respectively) 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  $\alpha$ -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. sanfranciscensis* 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<sup>-1</sup> dw basis) in flour, control bread and treated bread. <sup>a</sup>

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

<sup>a</sup> 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 ( $0.14 \text{ g kg}^{-1}$ ) 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).

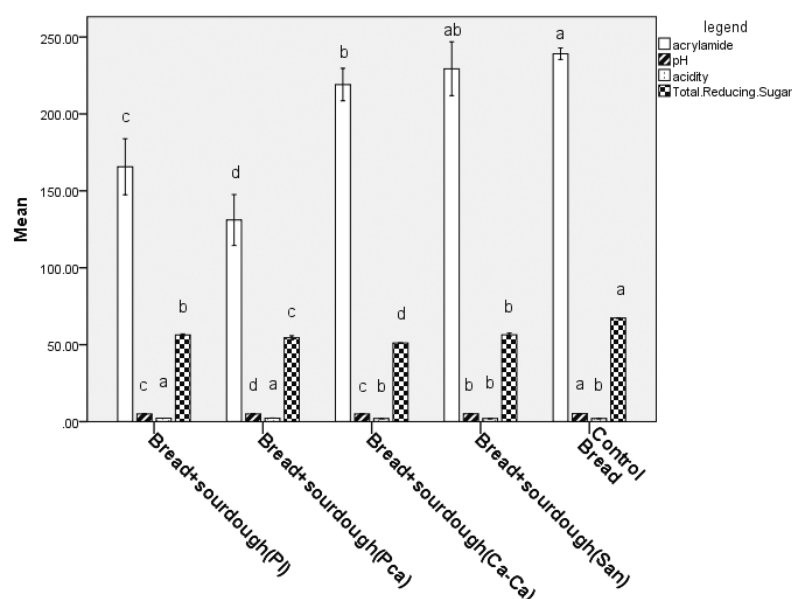
Pearson correlation was used to analyse the relationship between the dependent variables. Glucose, fructose and total reducing saccharides of bread influenced the pH value in the bread ( $r= 0.807$ ,  $r= 0.699$ ,  $r= 0.788$ ), also acrylamide contents of bread, correlated with pH and acidity ( $r= 0.842$ ,  $r= -0.953$  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; Granvogel *et al.*, 2004).

#### **Effect of Sourdough on Bread Properties**

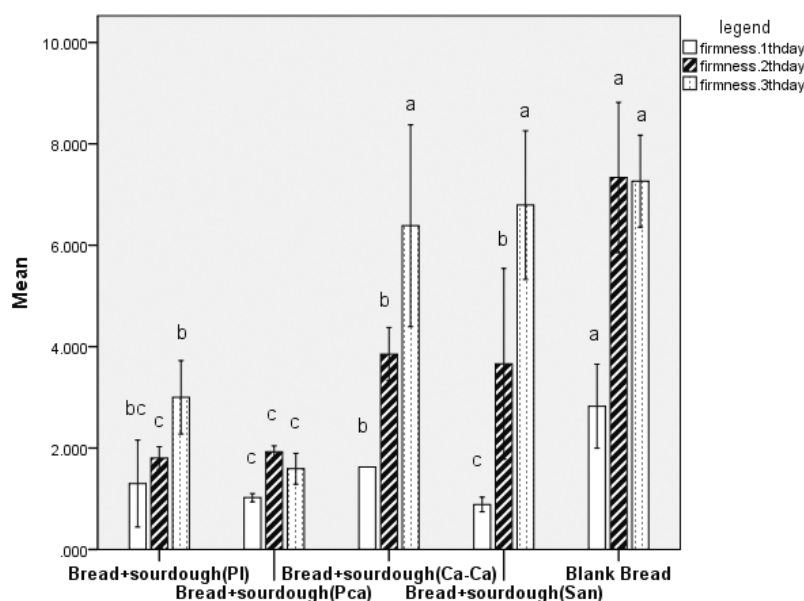
The overall sensory evaluations and firmness of the bread are shown in Table 3 and Figure 2.

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 $\pm$ 2 SD (error bars) of three determinations.



**Figure 2.** Compare means of bread firmness on first, 2<sup>nd</sup> and 3<sup>rd</sup> 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 $\pm$ 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



(Gamel *et al.*, 2015; Katina, 2005; Martinez-Anaya, 2003). 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  $\mu\text{g kg}^{-1}$ ) 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 *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.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support from the University of Tehran, Standard Research Institute, Iranian Center of Excellence for Application of Modern Technologies for producing functional foods and drink and Persian Enzyme Iranian Co.

## REFERENCES

1. AACC. 2000. *Approved Method of the American Association of Cereal Chemists*. 10<sup>th</sup> Edition, American Association of Cereal Chemists, St. Paul, MN, USA.
2. Amrein, T. M., Scho'nba'chler, B., Escher, F. and Amado, R. 2004. Acrylamide in Gingerbread: Critical Factors for Formation and Possible Ways for Reduction. *J. Agric. Food Chem.*, **52**: 4282–4288.
3. Amrein, T. M., Limacher, A., Conde-Petit, B., Amadò, R. and Escher, F. 2006. Influence of Thermal Processing Conditions on Acrylamide Generation and Browning in a Potato Model System. *J. Agric. Food Chem.*, **54**: 5910–5916.
4. AOAC. 1995. *Official Method of Analysis of AOAC Intl.* 16<sup>th</sup> Edition, Association of Official Analytical Chemists, Arlington, VA, USA.
5. Azizi, M. H., Sayeddin, S. M. and Payghambardoost, S. H. 2006. Effect of Flour Extraction Rate on Flour Composition, Dough Rheological Characteristics and Quality of Flat Bread. *J. Agri. Sci. Tech.*, **8**: 323-330.
6. Bartkiene, E., Jakobsone, I., Juodeikiene, G., Vidmantiene, D., Pugajeva, I. and Bartkevics, V. 2013. Study on the Reduction of Acrylamide in Mixed Rye Bread by Fermentation with Bacteriocin-like Inhibitory Substances Producing Lactic Acid Bacteria in Combination with *Aspergillus niger* Glucoamylase. *Food Control.*, **30**: 35-40.
7. Bemiller, J. N. and Huber, K.C. 2008. Carbohydrates. In: "*Fennemas Food Chemistry*", (Eds.): Damodaran, S., Parkin, K. L. and Fennema, O. R. CRC Press, Taylor and Francis Group, Boca Raton, PP.101-105.
8. Brummer, J. M. and Lorenz, K. 1991. European Developments in Wheat Sourdoughs. *Cereal Food. World*, **36**: 310-314.
9. Bütikofer, U. and Ardö, Y. 1999. Quantitative Determination of Free Amino Acids in Cheese. *International Dairy Federation, Belgium, Bulletin-FIL-IDF*.
10. Capuano, E., Garofalo, G., Napolitano, A., Zielinski, H. and Fogliano V. 2010. Rye Flour Extraction Rate Affects Maillard Reaction Development, Antioxidant

- Activity, and Acrylamide Formation in Bread Crisps. *Cereal Chem.*, **87**: 131-136.
11. Capuano, E. and Fogliano, V. 2011. Acrylamide and 5-HydroxyMethylFurfural (HMF): A Review on Metabolism, Toxicity, Occurrence in Food and Mitigation Strategies. *LWT-Food Sci. Technol.*, **44**: 793-810.
  12. Charoenpanich, J. 2013. Removal of Acrylamide by Microorganisms. In: "Applied Bioremediation: Active and Passive Approaches", (Eds): Patil, Y. B., Rao, P. <http://dx.doi.org/10.5772/56150>
  13. Curtis, T. Y., Muttucumar, N., Shewry, P. R., Parry, M. A., Powers, S. J. and Elmore, J. S. 2009. Effects of Genotype and Environment on Free Amino Acid Levels in Wheat Grain: Implications for Acrylamide Formation during Processing. *J. Agric. Food Chem.*, **57**: 1013-1021.
  14. De Vuyst, L. and Neysens, P. 2005. The Sourdough Microflora: Biodiversity and Metabolic Interactions. *Trend. Food Sci.*, **16**: 43-56.
  15. Elder, V. A., Fulcher, J. G., Leung, H. K. H. and Topor, M. G. 2007. *Method for Reducing Acrylamide Formation in Thermally Processed Foods*. Patent US 2007/0292589 A1.
  16. Fink, M., Andersson, R., Rosen, J. and Aman, P. 2006. Effect of Added Asparagine and Glycine on Acrylamide Content in Yeast-leavened Bread. *Cereal Chem.*, **83**: 218-222.
  17. Friedman, M. 2003. Chemistry, Biochemistry, and Safety of Acrylamide. A Review. *J. Agric. Food Chem.*, **51**: 4504-4526.
  18. Fredriksson, H., Tallving, J., Rosen, J. and Aman, P. 2004. Fermentation Reduces Free Asparagine in Dough and Acrylamide Content in Bread. *Cereal Chem.*, **81**: 650-653.
  19. Gamel, T. H., Abdel-Aal, E. S. M. and Tosh, S. M. 2015. Effect of Yeast-fermented and Sourdough Making Processes on Physicochemical Characteristics of  $\beta$ -Glucan in Whole Wheat/Oat Bread. *LWT-Food Sci. Technol.*, **60**: 78-85.
  20. Ganzle, M. G., Vermeulen, N. and Vogel, R. F. 2007. Carbohydrate, Peptide and Lipid Metabolism of Lactic Acid Bacteria in Sourdough. *Food Microbiol.*, **24**: 128-138.
  21. Ghasemzadeh-Mohammadi, V., Mohammadi, A., Hashemi, M., Khaksar, R. and Haratian, P. 2012. Microwave-assisted Extraction and Dispersive Liquid-liquid Microextraction Followed by Gas Chromatography-mass Spectrometry for Isolation and Determination of Polycyclic Aromatic Hydrocarbons in Smoked Fish. *J. Chromatography A.*, **1237**: 30-36.
  22. Granvogl, M., Jezussek, M., Koehler, P. and Schieberle, P. 2004. Quantitation of 3-Amino -propionamide in Potatoes: A Minor but Potent Precursor in Acrylamide Formation. *J. Agr. Food Chem.*, **52**: 4751-4757.
  23. Granby, K., Nielsen, N.J., Hedegaard, R.V., Christensen, T., Kann, M. and Skibsted, L. H. 2008, Acrylamide-asparagine Relationship in Baked/Toasted Wheat and Rye Breads. *Food Addit. Contam. A.*, **25(8)**: 921-929.
  24. Hamlet, C. G., Baxter, D. E., Sadd, P. A., Slaiding, I., Liang, L., Muller, R. Jayaratne, S. M. ; Booer, C. 2005. *Exploiting Process Factors to Reduce Acrylamide in Cereal-based Foods, C03032 and C03026*. Report Prepared on Behalf of the UK Food Standards Agency, RHM Technology, High Wycombe, UK.
  25. IARC (International Agency for Research on Cancer). 1994. Acrylamide. In *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, **60**: 389-433.
  26. ISO. 2009. *Cereal and Cereal Products: Determination of Moisture Content Reference Method*. Approved Method of the ISO No. 712, International Organization for Standardization.
  27. ISO. 2007. *Cereals, Pulses and by Products: Determination of Ash Yield by Incineration*. Approved Method of the ISO No. 2171, International Organization for Standardization.
  28. ISO. 2006. *Wheat and Wheat Flour: Gluten Content. Part 2. Determination of Wet Gluten by Mechanical Means*. Approved Method of the ISO No. 21415-2, International Organization for Standardization.
  29. Katina, K. 2005. Sourdough: A Tool for the Improved Flavour, Texture and Shelf-life of Wheat Bread: Academic Dissertation. Espoo. VTT Publications 569, Finland, PP. 17-25.
  30. Keramat, J., LeBail, A., Prost, C. and Jafari, M. 2011. Acrylamide in Baking Products: A



- Review Article. *Food Bioprocess Tech.*, **4**: 530-543.
31. Lim, H. and Shin, H. 2013. Ultra Trace Level Determinations of Acrylamide in Surface and Drinking Water by GC-MS after Derivatization with Xanthidrol. *J. Sep. Sci.*, **36(18)**: 3059-3066.
  32. Lopachin, R. M. and Lehning, E. J. 1994. Acrylamide Induced Distal Axon Degeneration: A Proposed Mechanism of Action. *Neurotox Res.*, **15**: 247-260.
  33. Martinez-Anaya, M. A. 2003. In: "Handbook of Dough Fermentations", (Eds.): Kulp, K. and Lorenz, K. (Eds.) Marcel Dekker, New York, pp 63-95.
  34. Morales, F. J., Martin, S., Acar, O. C., Arribas-Lorenzo, G. and Go'kmen, V. 2009. Antioxidant Activity of Cookies and Its Relationship with Heat-processing Contaminants: A Risk/Benefit Approach. *Eur. Food Res. Technol.*, **228**: 345-354.
  35. Mottram, D. S., Wedzicha, B. I. and Dodson, A. T. 2002. Acrylamide Is Formed in the Maillard Reaction. *Nature*, **419**: 448-449.
  36. Muttucumar, N., Elmore, J. S., Curtis, T., Mottram, D. S., Parry, M. A. and Halford, N. G. 2008. Reducing Acrylamide Precursors in Raw Materials Derived from Wheat and Potato. *J. Agric. Food Chem.*, **56**: 6167-6172.
  37. Rahangadale, S., Jangir, B. L., Patil, M., Verma, T., Bhandarkar, A. and Sonkusale, P. 2012. Evaluation of Protective Effect of Vitamin E on Acrylamide Induced Testicular Toxicity in Wister Rats. *Toxicol. Int.*, **19(2)**: 158-61.
  38. Sadat Mousavian, D., Niazmand, R. and Sharayei, P. 2015. Decreasing Acrylamide Formation in Fried Potato Slices Using Hydrocolloid Coatings and Bene Kernel Oil. *J. Agr. Sci. Tech.*, **17**: 1725-1734.
  39. Swedish National Food Administration . 2002. *Information about Acrylamide in Food*. <http://www.slv.se>.
  40. Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J. and Guy, P. A. 2002. Acrylamide from Maillard Reaction Products. *Nature*, **419**: 445-450.
  41. Surdyk, N., Rosén, J., Andersson, R. and Åman, P. 2004. Effects of Asparagine, Fructose and Baking Conditions on Acrylamide Content in Yeast-leavened Wheat Bread. *J. Agric. Food Chem.*, **52**: 2047-2051.
  42. Viklund, G. Å. I., Olsson, K. M., Sjöholm, I. M. and Skog, K. I. 2008. Impact of Harvest Year on Amino Acids and Sugars in Potatoes and Effect on Acrylamide Formation during Frying. *J. Agric. Food Chem.*, **56**: 6180-6184.

### بررسی تاثیر لاکتوباسیلوس پلانتاروم، پاراکازئی، کازئی - کازئی و سانفرانسی سنسیس بر کاهش میزان آکریلامید در نان گندم

ف. دستمالچی، س.ه. رضوی، م. لبافی، و م. فرجی

#### چکیده

آکریلامید یک ترکیب سمی است که در لیست ترکیبات احتمالی سرطان زا برای انسان، قرار دارد و در مواد غذایی غنی از کربوهیدرات مانند نان تولید می شود. در این تحقیق، تاثیر فرایند تخمیر بوسیله ۴ باکتری اسید لاکتیک و مخمر ساکارومایسس سروزیه، در کاهش آکریلامید نان در معیار صنعتی بررسی شد. نتایج نشان داد که ویژگی های آرد و نوع میکروارگانیسم های شرکت کننده در فرآیند تخمیر، مهمترین فاکتورها در فرآیند تشکیل آکریلامید در نان هستند. نان شاهد که بوسیله مخمر

ساکارومایسس سروزیه تخمیر شد، دارای بالاترین میزان آکریلامید ( $239/12 \mu\text{g/kg}$ ) و قندهای احیاکننده است. تخمیر بوسیله باکتری های اسید لاکتیک به همراه مخمر موجب کاهش آکریلامید و بهبود کیفیت نان شد. نان تخمیر شده با لاکتوباسیلوس پاراکازئی دارای کمترین میزان آکریلامید ( $131/06 \mu\text{g/kg}$ ) بود. این کاهش در نتیجه افزایش تولید اسیدهای آلی در خمیر ترش تولید شده با این باکتری و کاهش میزان گلوکز در نان تولید شده با این خمیر ترش می باشد. افزودن آغازگرهای لاکتیکی با میانگین  $\text{pH} = 3/56$ ، موجب کاهش  $\text{pH}$  خمیر و در نتیجه بهبود بافت و ویژگی های حسی همراه با کاهش آلاینده ها مانند آکریلامید گردید. نان تخمیر شده با آغازگرهای باکتری های اسید لاکتیک، دارای بافتی نرمتر نسبت به نان کنترل بودند، بویژه نان تخمیر شده با لاکتوباسیلوس پاراکازئی که دارای کمترین میزان آکریلامید و نرمترین بافت در طول سه روز نگهداری بود.