REDUCTION OF ACRYL AMIDE IN POTATO CHIPS USING LOCAL ISOLATE OF LACTOBACILLUS FERMENTUM NH91 MT 997784

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REDUCTION OF ACRYL AMIDE IN POTATO CHIPS USING LOCAL ISOLATE OF *LACTOBACILLUS FERMENTUM* NH91 MT 997784

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ABSTRACT : A local isolate of *Lactobacillus fermentum* NH91 MT997784 was used in fermentation of blanched and un blanched potato slices to reduce the acrylamide content of potato chips. The results revealed that addition of 1×10^9 CFU/ml of this isolate to potato slices for 60, 90 and 120 min of fermentation at 37°C were led to reduce pH from 6.57 to 4.72 in un blanched potato and from 6.5 to 4.62 in blanched potato, while the acryl amide were reduced to 88.18% and 90.68% in un blanched and blanched potato chips respectively after 120 min of fermentation. The reduction of glucose, fructose and sucrose in un blanched and blanched potato were increased from 55% to 82%, 45% to 77% and from 30% to 87%, respectively during the fermentation periods from 60 to 120 minutes, respectively for unblanched potato and for blanched potato the reduction were increased from 73% to 85%, 59 to 79% and from 37 to 71%, respectively, during the fermentation periods from 60 to 120 minutes. The results revealed that there was an increase in the level of amino acids in blanched and un blanched potato chips with about 6-12% in each of the asparagine and glutamine and more than that in both of aspartic and the glutamic acid, after one hour of fermentation with *Lactobacillus fermentum* NH91 MT997784. Significant changes in the level of amino acids were also observed in blanched and un blanched potato chips after 90-120 minutes of fermentation compared to beginning of the fermentation. All amino acids were decreased during the last period of fermentation. The level of amino acids in the blanched potato chips was lower compared to its level in un blanched potatoes.

Key words : *Lactobacillus fermentum*, potato chips, acrylamide, blanching potato.

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INTRODUCTION

Acrylamide (2-propenamide) is a chemical compound that has a structure of (CH_2 = CH $CONH_2$). It has been known since the 1950s. This polymer is used in many industrial applications such as Sewage treatment, mining, cosmetics and Paper Industry, as well as used in analytical laboratories in electrophoresis (Zhang and Zhang, 2007; Xu *et al*, 2014). Acrylamide is one of the compounds that could be carcinogenic if ingested in large quantities by the International Agency for Research on Cancer (IARC, 1994). The Swedish National Food Administration and Stockholm University have published data and comments about the presence of high levels of acrylamide in some foods (Tareke *et al*, 2002). Other studies revealed the presence of acrylamide in food, which are rich in carbohydrates and manufactured at high temperatures (above 120°C) as a result of Maillard reactions (Stadler *et al*, 2002; Tareke *et al*, 2002). These foods include chips potatoes, baked and fried potatoes, breakfast cereals, etc. (WHO, 2002).

The European Union (2002) and the World Health Organization (2002) listed acrylamide as a category II mutagen, and this result led to an increase in interest and research in this area. Studies have also shown that acrylamide is a by-product that is formed when carbohydrate-rich foods are fried, baked, or roasted at temperatures higher than 120°C (Pedreschi, 2007). At 180°C (Mottram *et al*, 2002; Stadler *et al*, 2002, 2004) also reported that levels of acrylamide were formed from reaction of reduced sugar with asparagine at a temperature of 180°C. Potato is characterized by its high content of free amino acid asparagine 93.6 mg/100 gm (Martin, 2001), as well as its high sugar content (Amrein *et al*, 2003). Therefore, large amounts of acrylamide are formed when exposed to heat. The precursors of *Lactobacillus* bacteria are widely used in several fermentation processes that are increasing in the world (Vidhyasagar *et al*, 2013). *Lactobacillus* quickly metabolizes simple sugars and produces lactic acid, which lowers the pH and reduces the occurrence of Maillard reactions that begin with high temperature. This method is used to reduce acrylamide in fried potato products (Blom *et al*, 2009). Many researcher confirmed that ability of lactic acid bacteria to convert sugars in vegetables to lactic acid, then the level of sugars decreases and the chance of Maillard reactions decreases. So acrylamide formation will decrease (Baardseth *et al*, 2006). Species of *Lactobacillus* are used for a wide variety of applications, including fermentation of a number of food and forage materials. It is defined as a probiotic as an environmentally friendly bacterium (Reque *et al*, 2000; Melo *et al*, 2017).

Lactobacillus fermentum is also a microorganism that is naturally found in the human intestine (Klein, 2011). Albuquerque *et al* (2018) indicated that among a number of strains *Lactobacillus fermentum* 296 isolates showed good performance in a series of tests for safety and physiological functions, such as adhesion, self-assembly, and antibody to pathogens. This indicates that bacteria are safe as well as GRAS.

MATERIALS AND METHODS

Lactic acid fermentation of blanched and un blanched potatoe

Unblanched potato were prepared by choosing of healthy and un infected potatoes, removing of dust, washing with tap water, then peeling and cutting as disc slices with about 5 cm diameter and 1.5-2 mm thickness, while blanched potato were prepared as same method in addition to blanching according to traditional method by heating at 80°C for 3min (Baardseth *et al*, 2006). Fermentation was carried out by adding 1×10^9CFU/ml of selected local isolate (calculated depending on Macfarland standard curve (Colle, 1996) for 60, 90 and 120 min in plastic container with 5 liter capacity contain one liter of tap water at 37°C. After fermentation all treatments were subjected to deep frying at 180°C for 3 min using liquid oil trade mark Alfia. Eight treatments were obtained, including blanched and unblanched potatoes as control without addition of inoculum and there are three periods of fermentation. Samples were stored in nylon bag in refrigerator at 4°C.

Chemical analysis

pH measurement : pH of fermented suspension of potato chips were determined before and after all periods of fermentation. Change in the pH, will indicate occurrence of the lactic fermentation of potato samples.

Determination of acrylamide in blanched and un blanched potato chips by HPLC : An extraction process of acrylamide was performed in all treatments according to Tateo and Bononi (2003). Grinding of potato samples separately, using electric mill with a speed of 3000 rpm to obtain powder form. Weight 10 g of powder, then putting in a Cellulose Extraction Thimble of Soxhlet apparatus. Extraction was achieved by hexan at 80°C for 5 hours to eliminate samples from lipids. Then samples were left in the air for an hour to allow the solvent to volatilize and drying 50 mL of methanol at a concentration of 99.9% was added to defatted sample in sealed flask with mixing for 20 minutes using a magnetic stirrer, then centrifugation process was carried out for 10 min at 5000 rpm. The precipitate was discarded and the suspension was filtered with filter paper Whatman No. 42. The filtrate (about 35 ml) was concentrated using vacuum rotary evaporator at 45°C until 2 ml of sample volume were obtained, then it will be ready to injected in HPLC apparatus. The column of HPLC was ODS-3.5µm, 250 × 4.6 mm, mobile phase was 70% distilled water and 30% acetonitrile, flow rate 1.2 ml/min. Detection was done by UV detector at 252 nm and 50 µl of sample was injected manually.

Determination of sugars in blanched and un blanched potato chips by HPLC : Sugars were extracted from blanched and unblanched potato chips according to Ohara-Takada *et al* (2005). Add 40 ml of 80% ethanol to 10 grams of mashed sample using airtight flask. Mix for 4 min by magnetic stirrer. Extraction was done by heating in a water bath at 80°C for an hour, then subjected to a centrifugation at 10,000 rpm for 10 minutes. Discard the precipitant and run the filtrate over the filter paper Whatman No. 42 in order to get rid of the sediment residue, then the filtrate was dried with vacuum rotary evaporator at a temperature of 45°C. Dried sample was dissolved with distilled water, then filtration was carried out using Microfilter with a diameter of 0.22 mm. 20 µl of sample was injected in HPLC using ODS C18 column with diameter of (4.6 × 250) mm, mobile phase contain Sodium phosphate 80% and Acetonitril 20% , flow rate was 0.8ml/min, at 40°C. Standard solutions of sugars include glucose, fructose and sucrose were prepared and injected in HPLC under same conditions to estimate retention time of each one.

Determination of amino acids in the blanched and un blanched potato chips by HPLC : Extraction of amino acids in blunched and unblanched potatoes were carried out, according to Ohara-Takada *et al* (2005).

Crushed with mortar, then added 40 ml of 80% ethanol. Homogenization was done by magnetic stirrer at room temperature for an hour. The extract was filtered with Whatman No.42 to obtain a clear solution, and the sediment was disposed. Ethanol was removed and the sample dried with a vacuum rotary evaporator. Then 10 ml of sodium citrate buffer (pH 2.2) was added and filterate using micro-filter with a diameter of 0.22 mm. Sample was ready for injection in HPLC, using ODS C18 column with (250 × 4.6 Id)mm, mobile phase contain 80% Metanol, 2.5% Tetra hydrofuran (THF) and 17.5% Sodium acetate and flow rate 1 ml/min at room temperature. Derivatives of amino acid were prepared by adding 25 µl of Orthophthal aldehyde to each sample.

RESULTS AND DISCUSSION

Factors effecting on acrylamide reduction in potato chips

Effect of blanching and lactic acid fermentation : Blanching of potato chips was carried out to remove as much of the reducing sugars and the amino acid asparagine present on the surface of the potatoes and then reducing the chance of acrylamide formation in the final product (Viklund *et al*, 2010; Gaikwad *et al*, 2016). Results showed that blanching and fermentation by local isolate of *Lactobacillus fermentum* NH91 MT997784 before frying were greatly contributed to reducing of acrylamide content of potato chips. At the time, fermentation of un blanched potatoes (control) reduced acrylamide content from 232 µg/kg to 74.6, 51.3 and 27.4 µg/kg in potato chips after 60, 90 and 120 minutes of fermentation respectively and with a maximum reduction of acryl amide was 88.18% (Table 1). While fermentation of blanched potatoes (control), led to reducing of acrylamide from 179 g/kg to 60.3, 38.9, 16.7 µg/kg after 60, 90 and 120 minutes of fermentation respectively, with a maximum reduction of 90.68% (Table 1). These results were indicate that the combination of lactic fermentation in addition to the blanching before frying has significantly contributed to reducing acrylamide in the potato chips. Reduction of acrylamide in blanched potato chips is due to that blanching helps to wash and remove most of sugars and amino acid from surface of potato chips and then it contributes to reduce formation of acrylamide (Kita *et al*, 2004). These results were agree with Grob *et al* (2003), Pedreschi *et al* (2004), who indicated that blanching before frying leads to reduce of acrylamide formation in potato chips by up to 50% compared to un blanched. More of studies noticed that blanching before frying has an important role in reducing the formation of acrylamide mainly by removing raw materials such as sugars and the amino acid asparagine, which is one of the substances that effect on formation of acrylamide (Viklund *et al,* 2010; Ismial *et al*, 2013). The study of Schettgen *et al* (2002) indicate that the average daily intake of acrylamide range between 0.3 - 0.85 µg/kg of body weight, while another study from European Food Safety Authority (EFSA, 2015) indicate that average daily intake of acrylamide for children with 3-10 old age should not exceed 0.7-2.05 µg/kg of body weight per day and children with 1-3 old age should not exceed 1.2-2.4 µg/kg of body weight per day. So the results of the current study indicate that the lactic fermentation of potato chips using *Lactobacillus fermentum* NH 91 in addition to the blanching process had a positive role in reducing the levels of acrylamide formed in potato chips to the allowed limits, where the amount of acrylamide remaining in un blanched and blanched potato chips after 120 minutes of fermentation attained to 27.4 µg/kg and16.7 µg/kg respectively (Table 1).

Effect of pH : Fresh potatoes has pH value about 6 (Nourian *et al*, 2003). After mixing pure water (pH 7) with potato chips, pH value of unblanched potatoes was became 6.57, while blanched potatoes was 6.5 (Fig. 1). The results generally showed a decrease in pH values during fermentation period by using local isolate of *Lactobacillus fermentum* NH91 MT997784 after 60, 90 and 120 minutes of fermentation. The results revealed that pH values were decreased from 6.57 to 4.72 and from 6.5-4.62 in unblanched and in blanched potato chips, respectively (Fig. 1). All these results were indicate the occurrence of the lactic fermentation process as a result of the metabolism of simple sugars leading to production

Table 1 : The effect of different periods of lactic fermentation of *Lactobacillus fermentum* NH91 MT997784 on reducing the percentage of acrylamide (%) in the blanched and un blanched potato chips HPLC estimation.

Fermentation period (min)	Unblanched potatoes		Blanched potatoes	
	Acrylamide concentration(µg/kg)	Reduction ratio (%)	Acrylamide concentration (µg/kg)	Reduction ratio (%)
Control	232	-	179	-
60	74.6	67.84	60.3	66.31
90	51.3	77.88	38.9	78.26
120	27.4	88.18	16.7	90.68

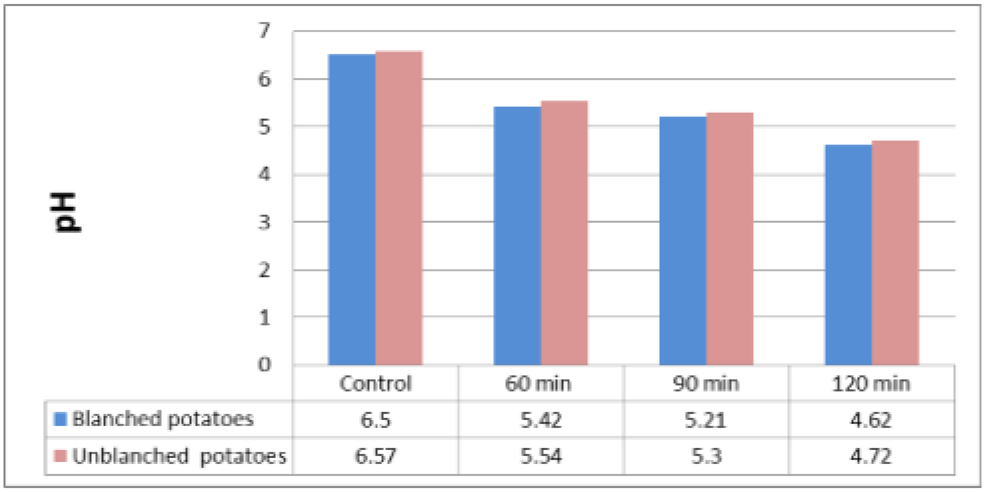

Fig. 1 : Effect of lactic fermentation of *Lactobacillus fermentum* NH91 MT997784 on the pH values of blanched and un blanched potatoes for different fermentation periods.

of lactic acid , which has a major role in reducing the pH values and then reducing the chance of Maillard reactions under high temperature conditions and then reduce the formation of acrylamide (Blom *et al*, 2009; Pyar and Peh, 2014). The results of the current study also indicated that decreasing of pH values were proportional with increasing of fermentation period.

Decreasing of pH value less than 5 may be led to prevent schiff base formation which consider intermediate compound in acrylamide path way formation (Zyzak *et al*, 2003; Yaylayan *et al*, 2005). It should be noted that optimal pH for acrylamide formation in food was pH 7. So that any decrease in pH value less than 7 leads to a decrease in the occurrence of Maillard reactions and thus lowering the level of acrylamide formation (Grob, 2007). These results were consistent with Bartkiene *et al* (2013) noticed that decrease in pH values in bread samples when using lactic acid bacteria for several species belonging to the genus *Lactobacillus* due to consumption of sugars and production of lactic acid. Also results present study were agree with Chauhan (2017) noticed that decreasing of pH values with 1.2 and 1.5 have significant role in reducing the formation of acrylamide during Maillard reactions.

Potatoes was consider one of richest field crop that contain sugars and asparagine. So this be a cause to form acryl amide in potato chips (Paul *et al*, 2016). It is also well known that lactic acid bacteria have the ability to metabolize monosaccharides and disaccharides, especially glucose, fructose and sucrose (Paucean *et al*, 2013). In general results showed a decrease in the sugars contents of un blanching and blanching potatoes during the fermentation periods. Percentage of decreasing in glucose, fructose and sucrose in un blanched potatoes ranged from 55% to 82%, 45% to 77% and from 30%. to 51%, respectively, in different fermentation periods of 60, 90 and 120 minutes respectively using local isolate *Lactobacillus fermentum* NH 91 (Fig. 2). These results were indicate that reducing of sugars content of potato chips corresponds to an increasing in fermentation period due to increasing of sugars consumption by bacteria as a carbon and energy source which reflected positively on reducing the pH and then reducing the percentage of acrylamide formation in potato chips. These results were agree with Baardeseth *et al* (2006), who indicated that reducing of sugars levels were a key in reducing the rate of acrylamide formation in potato chips. Results of current study also indicated that contents of blanched potato chips of glucose, fructose, and sucrose are also reduced to (73 - 85%), (59-79%) and (37 - 71%), respectively, in different fermentation periods (60, 90 and 120 minutes) respectively (Fig. 2). These results were indicate that the sugar contents of blanched potato chips was lower than un blanched potatoes during different fermentation periods. The reason may be led to that blanching was a cause to remove monosaccharides and amino acid (asparagin) during washing and blanching especially from the surface layer. These results are consistent with the findings of this study in explaining the effect of the fermentation process using lactic acid bacteria on reducing the acrylamide content of blanched potato chips, which was referred to in the previous paragraph. These results were agree with Blom *et al* (2009) and Ismial *et al* (2013), which indicated that the blanching process contributes significantly to reducing the level of sugars in potatoes and they emphasized that the fermentation process is a unique tool for reducing the concentration of acrylamide formed in potato chips. This process depends mainly on the density of the cultures, the fermentation period and the sugars content of the potato surface.

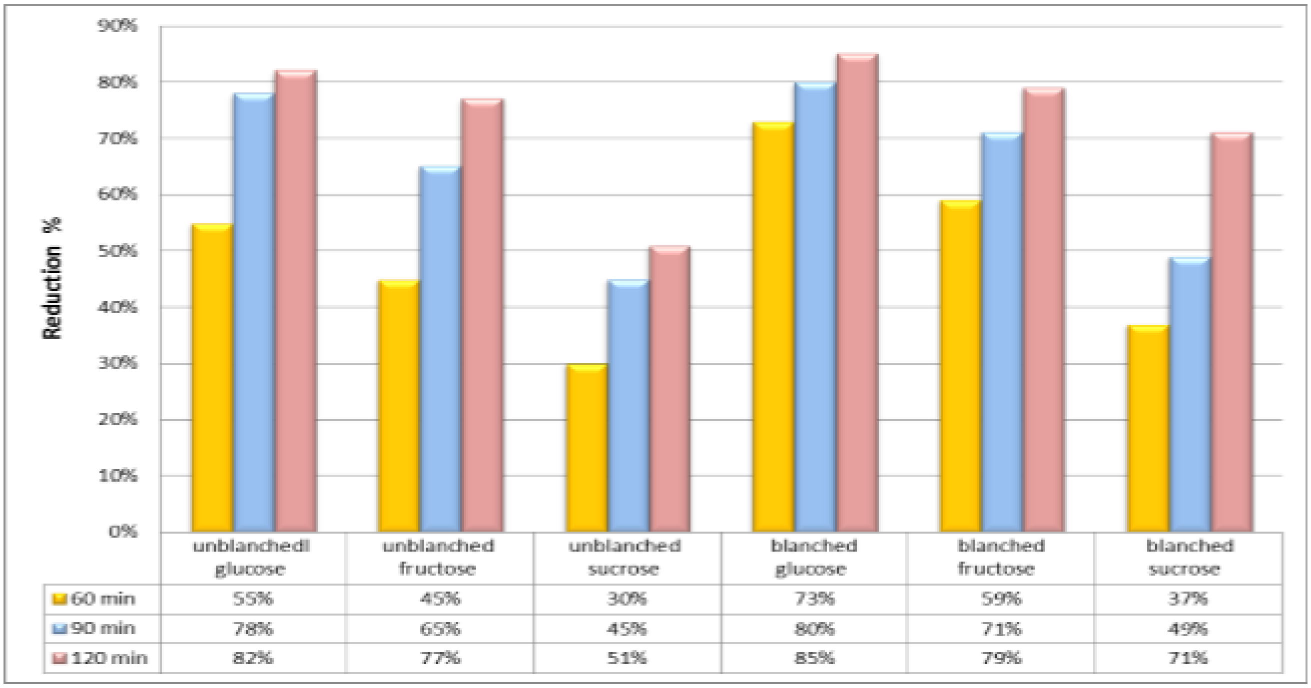

Fig. 2 : Effect of lactic fermentation of *Lactobacillus fermentum* NH91 MT 997784 on sugar concentrations calculated as % of reduction in blanched and un blanched potato chips.

Table 2 : The effect of lactic fermentation of Lactobacillus fermentum NH91 on amino acid concentration (μmol / 100ml) in potato chips, boiled and not boiled with HPLC.

Fermentation period (min)	**Unblanched potatoes**			
	Amino acids (μmol/100ml)			
	Asparatic acid	**Asparagine**	**Glutamic acid**	**Glutamine**
Control	613	33	711	68
60	657	153	811	93
90	476	124	628	75
120	379	73	448	54
	Blanched potatoes			
Control	421	23	612	51
60	489	142	691	56
90	379	96	490	49
120	312	50	412	32

Barkiene *et al* (2013) also used lactic acid bacteria with high activity to analyze the sugars in rye bread dough, and then it led to a decrease in the bread's content of reducing sugars and thus the level of acrylamide in the bread were reduced.

Effect of the amino acids content : Four amino acids in potato chips, namely asparagine, aspartic acid, glutamine and glutamic acid have been identified using HPLC (Table 2) before and after the fermentation which represent a highest levels of amino acids (25-30%) of the total free amino acids in potatoes (Amrein *et al*, 2004; Olsson *et al*, 2004). Amount of change in concentrations of these amino acids were observed and that asparagine is mainly involved in formation of acrylamide. Results were revealed increasing of 6-12% from each asparagine and glutamine, and more in both aspartic and glutamic acid, after one hour of fermentation using *Lactobacillus fermentum* NH 91 MT997784. This may be due to internal proteolysis that occurs in potato chips, as well as decomposition In bacterial cells added at the beginning of the fermentation (Baardseth *et al*, 2006). Clear changes were also observed in the level of amino acids in the blanched and un blanched potato chips after 90-120 minutes of fermentation compared to beginning of fermentation .As all amino acids were witnessed a clear decreasing during fermentation. These results were consistent with Kask *et al* (1999), who found a decrease in level of amino acids during the fermentation of potato chips due to using of amino acids by microorganisms for cell protein building. Decreasing of amino acids level, especially asparagine during fermentation gives an indicator of decreasing of acrylamide formation, because the presence of this acid in high concentrations greatly helps the formation of acrylamide in potato chips during fermentation and this fact was is supported by Zyzaki *et al* (2003). It was also observed from the results that the level of amino acids in blanched potato chips was lower compared to the levels of un blanched potatoes. The reason may be that the blanching process led to remove a percentage of amino acids during the washing and blanching, these results were consistent with Grob *et al* (2003) who indicated that the blanching of potato chips before the frying was helped to remove large quantities of amino acid asparagine and thus will contributed to reducing the percentage of acrylamide formed by 5-10 times compared to the unblanched fried potato chips.

CONCLUSION

This study was indicate that there is be able to use local isolate of *Lactcobacillus fermentum* NH 91 MT997784 in potato fermentation to reduce acryl amide formation in potato chips to be with allowable limits.

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