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Reducing acrylamide in fried potato pancake using baker's yeast, lactobacilli and microalgae

G. Mousavinejad[1], K. Rezaei[1,2*] and F. Khodaiyan[1]

[1]Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, University of Tehran, Chamran street, 31587-77871 Karaj, Iran; [2]Center of Excellence for Application of Modern Technologies for Producing Functional Foods and Drinks, University of Tehran, Chamran street, 31587-77871 Karaj, Iran; krezaee@ut.ac.ir

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RESEARCH ARTICLE

Abstract

In preparation of fried potato pancake, dough was fermented with six strains of lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus delbrueckii*, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Lactobacillus reuteri* and *Lactobacillus casei*; 10^8 cfu/g dough), baker's yeast (2.6×10^7-7.8×10^7 cfu/g dough), and also treated with inactivated microalgae powder (*Dunaliella salina*; 0.01 g/g dough) as an additive, in combination or not. After treatment, texture and colour attributes of fried potato pancakes were determined. After fermentation for 2 h, baker's yeast reduced 85% of acrylamide (AA). *L. acidophilus* was the most efficient bacterial strain under study and reduced 60% AA, and similar results were obtained with microalgae. Compared to the control, AA was eliminated when microalgae were combined with either yeast or lactic acid bacteria, and the resulting fried potato pancake was lighter in colour and had a softer texture when fermented with lactic acid bacteria or yeast.

Keywords: acrylamide, commercial baker's yeast, commercial microalgae, *Lactobacillus*, potato pancakes

1. Introduction

Nowadays, high level of acrylamide (AA) in many processed food products has become a matter of concern. In April 2002, the National Food Administration of Sweden reported the presence of high amounts of AA in heat-treated foods rich in carbohydrates such as grains and potato-based foods. Many studies demonstrated that higher levels of AA (150-4,000 mg/kg) have been found in carbohydrate-rich foods that are fried and also in certain heated commercial potato products and crisp bread (Claus *et al.*, 2008; Dybing and Sanner, 2003; Surdyk *et al.*, 2004). Potato is one of the basic food commodities in the world. It is a suitable source for energy and protein that is consumed at high levels and different forms and shapes throughout the world. Unfortunately, in the fried forms such as French fries, crisps and potato pancakes, AA is produced at high levels (Michalak *et al.*, 2011; Pedreschi *et al.*, 2005; Wicklund *et al.*, 2006). AA is formed in foods via the Maillard reaction between free asparagine (ASP) and reducing sugars as the precursors. Different methods have been reported for reducing AA in different food products. Meanwhile, some microbial treatments have been proposed for reducing AA in bakery products (Fredriksson *et al.*, 2004; Huang *et al.*, 2008). It was demonstrated that AA content could be reduced up to 90% with 2 h fermentation processes of dough in bakery products (Fink *et al.*, 2006; Fredriksson *et al.*, 2004), due to the consumption of limiting precursor (ASP) by the yeast. Fermentation on the free ASP and reducing sugars in *you-tiao*, a traditional Chinese fried food, revealed that the addition of 0.8% (w/w) yeast to dough and fermentation for 1 h could reduce the amount of AA to 66.7% (Huang *et al.*, 2008). Other studies also introduced fermentation as one of the most important process that reduces AA levels, which in turn, provides quality assurance and safety of the bakery products (Matthäus and Haase, 2014).

However, only a few studies have been reported concerning the effect of fermentation on the AA level in potato-based foods. Baardseth *et al.* (2006) and Blom *et al.* (2009) showed that lactic acid fermentation of potato rods using

Lactobacillus plantarum strain NC8 can considerably reduce up to 90% Maillard reaction components and AA in French fries, which was accompanied by a loss in the development of both colour and taste of French fries (Baardseth *et al.*, 2006; Blom *et al.*, 2009).

Addition of plant extracts in the various products is a possible way to reduce AA. Markova *et al.* (2012) reported a 23% reduction in AA level after adding some spices to buckwheat ginger cakes. Similarly, a 76% reduction in the AA level was achieved when antioxidant solution of bamboo leaves was added before the processing of French fries and potato crisps (Zhang *et al.*, 2007). In a similar study, an 83% reduction in AA level was reported for fried bread sticks by adding green tea extract (Zhang and Zhang, 2007).

Pan-fried potato pancake (PFPP) is an easily prepared product and there are varieties of formulations for the production of PFPP. Since in all formulations potato is a basic ingredient and the processing is mainly frying at high temperature, high levels of AA are formed in this product (531-692 μg/kg) (Michalak *et al.*, 2011). Potato pancake is a prevalent and inexpensive food product that is popular among most consumers and it has a high potential to improve dough formulation. No specific report has so far been reported on the reduction of AA content in potato pancake and no previous study has addressed the microalgae application as an effective additive to inhibit AA formation in the food products. Thus, the purpose of this study was to investigate the possibility of reducing AA in potato pancake by different microbial treatments including lactic acid bacteria, yeast and inactive microalgae and to compare the effects of different fermentation treatments on AA formation. Texture and colour of the fried potato pancakes were also investigated as a function of the fermentation process.

2. Materials and methods

Materials

Potato (variety 'Marphona') was purchased from the Seed and Plant Improvement Institute (Karaj, Iran) in June 2012 and stored at 10 °C to prevent the accumulation of reducing sugars in potato during the experiments (Meulenaer *et al.*, 2008). Textured soy protein (defatted soybean product with 50% protein) was purchased from Max Soy Co. (Tehran, Iran). Other raw materials (eggs and salt) were purchased from a local supermarket in Tehran (Iran).

Chemicals

Carrez-I and Carrez-II solutions for the clarification of AA extract were prepared by dissolving 37.5 g potassium ferric cyanide and 75 g of zinc sulphate in 250 ml deionised water, respectively. Ethyl acetate, *n*-hexane and all of other solvents and chemicals used for the AA determination were of analytical grade and supplied by Merck Chemical Co. (Darmstadt, Germany). AA as external standard (with >99.5% purity level) and *di-n*-butyl phthalate as the internal standard were provided from Sigma-Aldrich Chemical Co. (Schnelldorf, Germany) for use in the GC analysis.

Microorganisms and culture

Lactobacillus delbrueckii (DSM 15996), *Lactobacillus helveticus* (DSM 20075), *L. plantarum* (DSM 200179), *Lactobacillus acidophilus* (DSM 20079), *Lactobacillus reuteri* (DSM 20016) and *Lactobacillus casei* (DSM 20011) were purchased from DSMZ (German Collection of Microorganisms and Cell Culture, Braunschweig, Germany). Lactic acid bacteria (LAB) were cultured and handled according to the method applied by Baardseth *et al.* (2006). They were cultured in 10 ml De Man, Rogosa and Sharpe broth overnight at 37 °C (Merck Chemical Co.) and harvested at a maximum density of 10^9 colony-forming units (cfu/ml) by centrifugation (Universal 320; Hettich, Tuttlingen, Germany) at 2,240×*g* for 3 min and the pellet was dissolved in 1 ml water at 37 °C before addition to pancake doughs. *Saccharomyces cerevisiae* was supplied by S.I. Lesaffre (Marcq-en-Barœul, France; 1.3×10^{10} cfu/g compressed yeast). It was activated by dissolving 10 g yeast in 50 ml water and incubated for 10 min at 38 °C according to the company's instruction. Proper volume for each concentration of yeast was taken before the addition to pancake dough. Microalgae whole cell powder (MWCP) of *Dunaliella salina* with high amount of total phenolic content (averagely 2.7 mg gallic acid equivalent/g dried biomass) and antioxidant activity (averagely 28.3 mg diphenyl picrylhydrazyl/g dried biomass) was supplied by Qingdao Sinostar Co., Ltd. (Qingdao, China). Microalgae were not alive and 0.01 g MWCP/g dough (1%, w/w) of this powder was used as an additive in all formulations of PFPP in the current study.

Preparation of pan-fried potato pancakes

The ingredients for preparation of examined PFPP included 400 g of blanched (parboiled) potato slices, 100 g of hydrated textured soy protein, 2 whole eggs and 7.5 g salt. For blanching purposes, potato slices were soaked in boiling water for 15 min. For hydrating textured soy protein, the product was soaked in water for 30 min. Potato and textured soy protein were mashed and mixed using a mill system (model 32002; Moulinex, Paris, France). The eggs were mixed completely with the salt and added to the mashed potato and mixed again for 3 min.

After the preparation of dough, it was divided into 4 portions and each portion was treated with a specific microorganism

according to the experimental plan of the study. For lactic acid fermentation, the harvested LAB of each species was dissolved in 1 ml deionised water, added to 100 g of potato pancake dough (final concentration of 10^8 cfu/g dough) and mixed for 3 min. The inoculated doughs were then incubated at 37 °C and 70% relative humidity (RH) for 90 min. For experiments with baker's yeast, the effects of yeast concentration and fermentation time on the AA level were also investigated. In one set of the experiments, the yeast concentration was varied within 0.2-0.6% (w/w) (final concentration of 2.6×10^7-7.8×10^7 cfu/g dough), for 90 min. In a different set of experiments, the fermentation time was changed from 30 to 180 min at a yeast concentration of 0.2% (w/w) (final concentration of 2.6×10^7 cfu/g dough). The inoculated doughs were then incubated at 37 °C and 80% RH. For the interaction experiments, crude potato dough was divided into four equal portions and each portion was treated with a specific mixture of microorganisms. For investigating the Y-B (yeast-bacteria) interaction, yeast at 0.2% level (w/w) and the harvested *Lactobacillu*s were dissolved in 1.0 ml deionised water and added to 100 g of the dough. MWCP was prepared at 1.0% (w/w) in 100 g dough and added with *Lactobacillus* and yeast (0.2%, w/w) to study B-M (bacteria-microalgae) and Y-M (yeast-microalgae) interactions, respectively. Triple interaction effect of Y-B-M (yeast-bacteria-microalgae) was also studied by mixing 1.0% of MWCP in 100 g dough inoculated with *Lactobacillus* and yeast (0.2%, w/w). All the inoculated dough samples were then mixed for 3 min and incubated for 2 h at 37 °C and 80% RH.

At the end of the fermentation period, 60 g of dough were shaped into 0.5-cm (in diameter) pancakes before the frying process. The pancake samples were then fried with vegetable oil (a combination of palm, sunflower and corn oil) at 180 °C for 2 min on one side, and then turned over and fried for an extra 2 min as well. Frying procedure was obtained according to the method of Michalak *et al.* (2011) with minor changes and also our preliminary experiments in the laboratory.

Analytical measurements

Colour determination

Changes in the colour of PFPPs were investigated using a colour meter (CR-400; Konica Minolta Co., Osaka, Japan). Lightness (L*), redness (a*), and yellowness (b*) colour components were the parameters used for this part of the study.

Texture evaluation

A universal testing machine (M350-10CT; Testometric Co. Ltd., Rochdale, UK) equipped with a 12.7-mm in diameter stainless steel punch probe was used to evaluate the pancake hardness. A stack of three pancakes were placed fried-side up on the texture analyser platform. The pancake stack was compressed to 50% of its original height (5 mm) at a constant rate of 1 mm/s. Maximum peak force recorded during the penetrometer analysis was considered as hardness. These assays were performed to compare the texture of fermented pancakes with that of the control.

Acrylamide extraction

AA extraction from PFPP was carried out according to method 8032A from EPA (1996) with minor modifications. The pulverised PFPP (5 g) was defatted twice using 30 ml *n*-hexane in a 50 ml tube with shaking for 10 min. After the second hexane extraction, residual solvent was evaporated and the sample was extracted with 100 ml water with stirring for 60 min on a magnetic stirrer (700 rpm) and centrifuged at 2,240×*g* for 15 min. The supernatant was removed and after use of Carrez-I and Carrez-II and stirring at 500 rpm, centrifuged at 2,240×*g* for 15 min. The supernatant was then brominated using bromine for the derivatisation of AA. The product (2,3-dibromopropenamide) was then separated from the reaction mixture by liquid-liquid extraction using ethyl acetate as the organic phase. The collected organic phase was then evaporated by means of a rotary evaporator (LABOROTA 4003-control; Heidolph, Schwabach, Germany) under vacuum at 30 °C until about 10 ml of the extract was obtained.

Acrylamide determination by gas chromatography-electron capture detector

The final extract from the previous stage was analysed using a gas chromatography system (7890A; Agilent Technologies, Folsom, CA, USA) equipped with an electron capture detector and an injector (both set at 300 °C) and an HP-5 column (60 m × 0.320 mm i.d. × 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). Sample volume for injection was 1.2 µl. Oven temperature was held at 120 °C for 1 min, ramped at 10 °C/min to 210 °C and ramped again at 25 °C/min to 285 °C. Identification of AA was based on its retention time (6.4 min) using the external standard and its quantification was based on its relative response factor (compared to di-*n*-butyl phthalate as internal standard, retention time: 11.6 min). A calibration curve was obtained for AA standards at 5-20 mg/kg concentration levels.

Statistical analysis

Each experiment comprised three replicates. Statistical analysis was performed using SAS 9.1 software package (SAS Institute Inc., Cary, NC, USA) by applying Duncan's multiple range tests at 95% confidence level. Correlation analysis was also carried out employing Pearson's test using SPSS 13 (SPSS Inc., Chicago, IL, USA) software.

3. Results and discussion

Acrylamide content in the control sample

It was not surprising to note that the control sample (pancake produced from blanched potato with no fermentation) had high level of AA (567 µg/kg; Figure 1). Michalak *et al.* (2011) reported the mean values of 437, 422, 564 and 694 µg/kg AA in potato pancakes prepared by pan-frying, deep-frying, roasting and microwave processes, respectively. It should be mentioned that although blanching might have some effects on AA formation in the final product, such effects were not considered in the current study. Grob *et al.* (2003) reported about 50% reduction in AA level of French fries after blanching of the potato rods before deep-frying. Matthäus and Haase (2014) reported that a reduction of the sugar content by blanching could result in about 60% reduction in the AA level, depending on the raw material and the production process. The longer blanching time lead to lower glucose and ASP contents in the potato strips and, as a consequence, it lowered AA formation during the frying process (Wicklund *et al.*, 2006).

Effect of lactic acid fermentation on acrylamide level

Fermentation of potato dough with different species of *Lactobacillus* significantly reduced AA formation during the production of potato pancakes (Figure 1). A 90-min fermentation with LAB in the current study resulted in 39-60% reduction in the AA levels of final products. *L. acidophilus* (60%), *L. reuteri* (52%) and *L. delbrueckii* (51%) had the highest AA reduction. The effect of the strain of *Lactobacillus* on the reduction of AA level can be associated with the consumption/elimination of reducing sugars as limiting factors in AA formation (Fredriksson *et al.*, 2004; Jung *et al.*, 2003). Baardseth *et al.* (2006) also showed that lactic acid fermentation of potato products reduced glucose, fructose and sucrose content after 3 h fermentation, while ASP content remained largely unaffected between 0 h and 4 h and increased slightly after 5 h fermentation. They confirmed that the reduction of AA content is due to the reduced levels of reducing sugars rather than the reduction of available ASP (Baardseth *et al.*, 2006).

L. plantarum is the only species that has been reported by other studies for reducing AA in French fries (Baardseth *et al.*, 2006; Blom *et al.*, 2009). Baardseth *et al.* (2006) showed

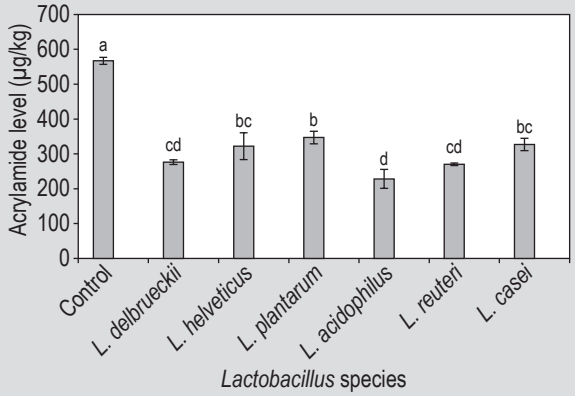

Figure 1. Acrylamide levels in fried potato pancakes prepared with dough fermented with lactic acid bacteria (10^8 cfu/g dough and 90 min fermentation time). Bars with different letters are significantly different ($P<0.01$).

that lactic acid fermentation (for 45 and 120 min) of non-blanched potato rods reduced AA levels in French fries to 48 and 71%, respectively. Blom *et al.* (2009) also showed that the application of the same method to French fries shortly prior to the pre-frying step reduced AA formation as much as 90% and browning reactions, colour and burnt smell were consequently reduced as well. In the current study, the obtained results surprisingly demonstrated that among the selected types of *Lactobacillus*, *L. plantarum* had the lowest effect on the AA reduction (only 39% reduction). *L. plantarum* and *L. acidophilus* have been applied for reducing phytic acid in traditional Iranian bread (*sangak*) (Najafi *et al.*, 2012). Bartkiene *et al.* (2013) used proteolytic *L. sakei* for AA reduction in bread supplemented with lupine. They showed that the addition of lupine could result in a higher AA level (43.3%) when compared to wheat bread as control (19.4 µg/kg dry weight). Using the mentioned bacterium to ferment the lupine reduced AA in this product (15.6 µg/kg dry weight) (Bartkiene *et al.*, 2013).

Effect of yeast fermentation on acrylamide level

Fermentation with yeast has been applied for reducing AA in the dough of cereal-based products (Fink *et al.*, 2006; Fredriksson *et al.*, 2004; Matthäus and Haase, 2014; Huang *et al.*, 2008). Since the physical properties and matrix of potato dough are similar to cereal dough, the effect of yeast fermentation on the AA level in potato dough was considered in this study (Figure 2). In the samples fermented for 90 min, addition of the yeast at 0.2 and 0.4% (w/w) considerably decreased the AA levels (up to 76 and 74%, respectively) when compared to that in yeast-free (control) sample. AA level was further reduced (84%) as the yeast was added at 0.6% (w/w) (Figure 2A). The yeast metabolises free amino acids during the fermentation and thus provides

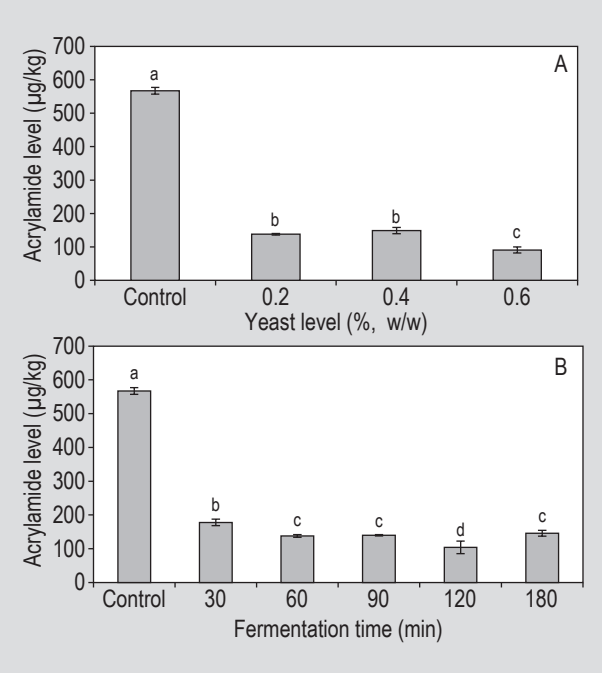

Figure 2. Acrylamide levels in fried potato pancakes prepared with dough fermented with baker's yeast. (A) Effect of yeast concentration (2.6×10^7-7.8×10^7 cfu/g dough) with 90 min fermentation. (B) Effect of fermentation time (with 2.6×10^7 cfu/g dough yeast concentration). Bars with different letters are significantly different ($P<0.01$).

a potential control point for ASP content and subsequent formation of AA (Surdyk *et al.*, 2004).

The effect of fermentation time on the AA content is shown in Figure 2B. As the fermentation time was increased (from 0 to 60 min) the AA content was decreased (from 567 to 138 µg/kg or 76% reduction). These results are in agreement with those reported by Fredriksson *et al.* (2004), who determined the contents of free ASP in 11 milling fractions from wheat and rye during the dough fermentation and pointed out that the ASP as an important precursor for AA formation was mostly utilised after 2 h of fermentation with yeast. Mustafa *et al.* (2009) also showed that increasing fermentation time had a significant effect on AA reduction in yeast-leavened wheat bread and the effects of fermentation were more pronounced in the samples with low ASP levels. Huang *et al.* (2008) showed that both free ASP and AA were dramatically reduced in a traditional Chinese fried-bread (*you-tiao*) after fermentation for up to 80 min. They also showed that during the yeast fermentation reducing sugars achieved a maximum level (1,850 mg/100 g) at about 80 min and decreased rapidly as fermentation proceeded further. So, they proposed that the simultaneous decrease in ASP is limiting the AA formation and the liberating sugar from starch in the initial fermentation stage was likely due to the amylase activity of yeast (Huang *et al.*, 2008). Other studies also demonstrated that change in the ASP content in carbohydrate-rich products is directly connected with AA content in the final product (Hendriksen *et al.*, 2005; Pedreschi *et al.*, 2007).

Interaction effects of yeast, lactobacillus and microalgae on the acrylamide level

In the current study, *D. salina* was selected as a new microbial additive to reduce AA, mainly due to its high antioxidant potential and phenolic compounds (Ye *et al.*, 2008). Zamani and Moradshahi (2013) reported that total phenolic content and antioxidant activity of *D. salina* cells were 3.68 pg gallic acid equivalent/cell and 11.04 pg trolox equivalent/cell, respectively. Although other edible microalgae that are commonly used as food additives (*Spirulina* spp. and *Chlorella* spp.) are in green colour, *D. salina* has a proper colour (pale red/brown) near to the original colour of potato pancakes. Addition of MWCP (*D. salina* species) individually to the pancake dough before pan-frying resulted in similar effects on AA reduction in comparison to when lactic acid bacteria was used in the current study (Figure 3).

PFPP samples fermented with Y-B showed a 26% inhibition of AA formation, while a 100% inhibition was achieved in PFPP samples treated with Y-M, B-M and Y-B-M. A lower reduction in AA level was observed using the combination of yeast and bacteria in comparison to the application of the yeast or bacteria alone. Forstova *et al.* (2014) also studied the influence of yeast and commercial starter (*Lactobacillus* spp.) on AA content of Czech leavened wheat-rye breads and demonstrated that the content of AA was rather higher

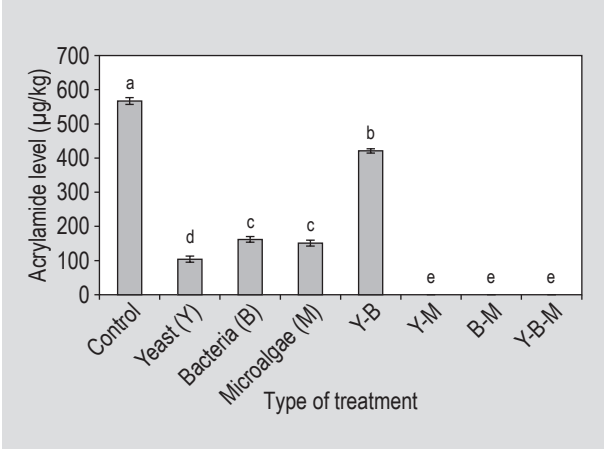

Figure 3. Acrylamide levels in the fried potato pancakes prepared from dough treated with *Lactobacillus acidophilus* (10^8 cfu/g dough), baker's yeast (2.6×10^7 cfu/g dough) and *Dunaliella salina* (0.01 g/g dough) (fermentation time: 2 h). Bars with different letters are significantly different ($P<0.01$). Y-B, Y-M, B-M and Y-B-M represent yeast-bacteria, yeast-microalgae, bacteria-microalgae and yeast-bacteria-microalgae, respectively.

in breads prepared using lactic acid bacteria and yeasts than in bread prepared using natural rye sourdough only. It seems that in the mixed starters, lactic acid bacteria have a strong negative effect on the yeast activity. It has also been shown that the use of 50% sourdough (fermented with LAB) in dough formulation can reduce only 17% of free ASP (Fredriksson *et al.*, 2004) but using less sourdough (25%) resulted in more ASP utilisation by the yeast (about 50%). They demonstrated that breads obtained with this type of fermented dough (yeast and LAB) may be contained more AA than the similar breads fermented with only yeast. The negative impact of lactic acid bacteria might be due to the fact that it reduces yeast activity which, in turn, results in low pH and increased proteolytic activity of LAB and plant proteases (Di Cagno *et al.*, 2002; Loponen *et al.*, 2004), followed by the production of amino acids and low-weight peptides. Although yeast is able to consume all types of these nitrogen sources (except lysine, cystein and histidine), most of the initial ASP of the dough will remain in the product and lead to AA production (Thiele *et al.*, 2002). A 100% inhibition in AA formation was observed when starters included microalgae in combination with either lactic acid bacteria or baker's yeast. These results might be related to the antioxidant activity and phenolic compounds in microalgae (Zhu *et al.*, 2009, 2011). Several mechanisms have been suggested for the inhibitory effect of antioxidants on the AA formation. Ciesarova *et al.* (2008) studied the effect of different spice extracts on the AA reduction in a model mixture simulating a fresh potato matrix. They suggested an interactive reaction of saccharide fragments from potato with the conjugated system of polyphenols to be responsible for the inhibition of AA formation. Cheng *et al.* (2008) and Arribas-Lorenzo *et al.* (2009) reported similar justification for the direct trapping of Maillard intermediates in AA formation mechanism. Similar results also demonstrated AA reduction in the different ASP/glucose model systems (Kotsiou *et al.*, 2011; Zhu *et al.*, 2009) and also in potato starch-based model systems (Zhu *et al.*, 2011).

Differences in AA levels among different microbial groups (bacteria, yeast or microalgae) could be attributed to the different concentrations of starters and various produced metabolites by each microorganism used in this study. Such effects can in turn influence the colour and texture of the final product that will be discussed in the following sections.

Effect of fermentation on pancake texture

The textural characteristics of samples fermented with the bacteria and yeast are shown in Table 1 and 2. There were significant differences in hardness values between the lactic fermented samples and the control (P<0.01) and a softening effect on potato pancakes fermented by LAB was found. An increase in the yeast level and fermentation time led to a significant decrease in the PFPP hardness in comparison to the control (P<0.01). The hardness values of samples fermented with the combination of yeast and other microorganisms were also decreased significantly. This can be due to the production of CO_2 through the alcoholic fermentation by yeast (Boekhout and Robert, 2003). On the contrary, addition of microalgae (with or

Table 1. Effect of lactic acid fermentation on the texture and colour of potato pancakes.[1]

Lactic acid bacteria type	Hardness (N)	Colour attributes[2]		
		L*	a*	b*
Lactobacillus delbrueckii	0.86^{c}	31^{d}	13^{b}	12^{d}
Lactobacillus helveticus	0.99^{b}	42^{b}	14^{ab}	21^{b}
Lactobacillus plantarum	0.77^{d}	37^{c}	15^{ab}	18^{c}
Lactobacillus acidophilus	0.75^{d}	50^{a}	10^{c}	22^{a}
Lactobacillus reuteri	0.67^{e}	43^{b}	13^{b}	20^{b}
Lactobacillus casei	0.73^{d}	36^{c}	17^{a}	18^{c}
Control	1.07^{a}	28^{d}	10^{c}	10^{e}

[1] Values in the same columns followed by different superscript letters (a-e) are significantly different (P<0.01).
[2] L* = lightness; a* = redness; b* = yellowness.

Table 2. Effect of yeast fermentation on the texture and colour of potato pancakes.[1]

Parameter	Hardness (N)	Colour attributes[2]		
		L*	a*	b*
Yeast level (%, w/w)[3]				
0.2	0.64^{b}	53^{a}	7^{b}	22^{a}
0.4	0.52^{c}	48^{b}	11^{a}	22^{a}
0.6	0.46^{d}	47^{b}	11^{a}	21^{b}
Control	1.07^{a}	28^{c}	10^{a}	10^{c}
Fermentation time (min)[3]				
30	1.18^{a}	30^{e}	11^{a}	11^{d}
60	0.59^{b}	39^{d}	11^{a}	17^{c}
90	0.44^{b}	45^{c}	10^{a}	20^{b}
120	0.50^{b}	53^{b}	6^{b}	23^{a}
180	0.53^{b}	56^{a}	7^{b}	23^{a}
Control	1.07^{a}	28^{e}	10^{a}	10^{d}

[1] Values in the same columns followed by different superscript letters (a-e) are significantly different (P<0.01). Statistical analysis was performed independently within each series of experiments: yeast level or fermentation time.
[2] L* = lightness; a* = redness; b* = yellowness.
[3] Fermentation time was 90 min for yeast level experiments and yeast level was 0.2% (w/w) in fermentation time experiments.

without lactic acid bacteria) had no effect on the texture of potato pancakes (Table 3). Among the studied interaction experiments, samples treated by B-M had the highest hardness (1.0 N). Sensory analysis also revealed that the texture and taste scores of PFPPs treated with LAB, yeast and microalgae had no significant differences compared to the control when the yeast level and fermentation time were no more than 0.4% (w/w) and 90 min, respectively. However, the samples fermented by high concentration of yeast (0.6%, w/w) and longer fermentation times than 90 min resulted in low sensory scores by the panellists (data are not shown). This could possibly be due to the partial disintegration of the samples.

Effect of fermentation on pancake colour

AA level in the fried potatoes is also correlated with the colour of potato products (Mottram *et al.*, 2002; Pedreschi *et al.*, 2005; Rosen and Hellenäs, 2002; Stadler *et al.*, 2002). The colours of fried potato products arise from the non-enzymatic Maillard browning reaction involving sugars and amino acids on the potato surface (Pedreschi *et al.*, 2006). The LAB fermentation process showed to have a significant effect on the colour of the potato pancakes as the colour turned lighter and more yellowish (higher L* and b* values) and changed to lower brown and red levels (lower a* values) when the potato dough was fermented before frying. Use of *L. acidophilus* resulted in the highest L* value and the lowest a* value (Table 1).

Studies published on deep-fried potato chips (Kaaber *et al.*, 1995) and potato rods (Baardseth *et al.*, 2006; Blom *et al.*, 2009) also indicated that lactic acid fermentation resulted in an increase in the lightness (L* value). In a similar way, addition of 0.2% (w/w) yeast resulted in the lightest sample (the highest L* value) with the lowest red-brown colour in yeast experiments. The results of this study showed that the yellowness and lightness were increased in the samples fermented for more than 60 min, while the redness (a* value) did not change significantly when compared to the control sample after 1.5 h of yeast fermentation (Table 2).

Table 3. Effect of different microbial treatments on the texture and colour of potato pancakes.[1]

Treatment	Hardness (N)	Colour attributes[2]		
		L*	a*	b*
Yeast	0.51^{c}	53^{a}	6^{cd}	23^{a}
Bacteria	0.75^{b}	50^{a}	10^{b}	22^{ab}
Microalgae	1.00^{a}	23^{e}	5^{d}	7^{f}
Yeast-bacteria	0.78^{b}	38^{b}	14^{a}	18^{c}
Yeast-microalgae	0.57^{c}	27^{d}	7^{c}	11^{d}
Bacteria-microalgae	1.00^{a}	34^{c}	6^{cd}	17^{d}
Yeast-bacteria-microalgae	0.56^{c}	39^{b}	6^{cd}	21^{b}
Control	1.07^{a}	28^{d}	10^{b}	10^{e}

[1] Values in the same columns followed by different superscript letters (a-f) are significantly different (P<0.01).
[2] L* = lightness; a* = redness; b* = yellowness.

The a* value (redness) decreased at higher levels in all the samples using microalgae alone or in combination with other microorganisms (Table 3). This drop in a* value is partially due to high levels of chlorophilic-green pigments in the MWCP and also due to the reduction of Maillard browning reaction (Pedreschi *et al.*, 2006). In the current study, all the interaction groups indicated a significant difference with the control sample (P<0.01) except the Y-M treatment which had a similar L* to that of the control. Treatments of Y-M, B-M and Y-B-M showed a lower red colour as indicated by a lower a* value because of simultaneous application of microalgae and fermentation process which reduces AA formation in the final product. The Y-B-M treatment had a higher b* and Y-B treatment showed the highest level red/brown colour (higher a* value) among the interaction groups. A highly positive correlation was also found between AA contents and a* colour values for the pancakes fermented by LAB (r=0.868, P<0.01) and yeast (r=0.641, P<0.01). Moreover, the AA values were negatively correlated with L* colour values of the products fermented with LAB (r=-0.700, P<0.01) and yeast (r=-0.943, P<0.01). However, no significant correlation was found between AA formation and different colour attributes in the pancakes containing *D. salina* microalgae. Although microbial treatments in some cases led to changes in colour of the final product (slightly red/brown colour in the samples prepared by microalgae and slightly pale colour in fermented samples), sensory evaluation showed that colours of PFPPs treated with LAB, yeast and microalgae had no significant differences ($P<0.01$) with that of the control sample (data are not shown). Taubert *et al.* (2004) reported that browning level was not a reliable measure of AA content in large-surface fried potato products, but the results of colour analysis in the current study showed that the colour changes in potato pancake are well associated with the changes in the AA content of the samples. Pedreschi *et al.* (2005) reported a linear correlation between AA content of the potato chips and their colour represented by the redness component a* at a temperature range between 120 to 180 °C.

4. Conclusions

The current study demonstrated that AA formation during the production of PFPP can be efficiently decreased by LAB, yeast and inactive microalgae. Fermentation of the potato dough using commercial yeast before pan-frying was more efficient than LAB in AA reduction. Among all studied LAB, *L. acidophilus* was more effective in the reducing

AA level in PFPP. Although the dough fermentation with *S. cerevisiae* yeast for 30 min was enough to reduce AA production, the pancake does not develop red/brown colour during the frying, which may lead to overcooking. Using the different combination treatments, the Y-M, B-M and Y-B-M interactions were completely effective in preventing the AA formation in PFPPs. The fermentation process generally caused lower red colour in the samples and microalgae addition resulted in more brown/green colour. Moreover, the use of the yeast and bacteria resulted in a softer product. B-M treatment of PFPP resulted in the highest hardness among the interaction experiments.

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Conflict of interest

The authors declare that no conflict of interest exists.

References

Arribas-Lorenzo, G., Fogliano, V. and Morales, F.J., 2009. Acrylamide formation in a cookie system as influenced by the oil phenol profile and degree of oxidation. European Food Research and Technology 229: 63-72.

Baardseth, P., Blom, H., Skrede, G., Mydland, L.T., Skrede, A. and Slinde, E., 2006. Lactic acid fermentation reduces acrylamide formation and other Maillard reactions in French fries. Journal of Food Science 71: C28-C33.

Bartkiene, E., Jakobsone, I., Juodeikiene, G., Vidmantiene, D., Pugajeva, I. and Bartkevics, V., 2013. Effect of lactic acid fermentation of lupine whole meal on acrylamide content and quality characteristics of wheat-lupine bread. International Journal of Food Science and Nutrition 64: 890-896.

Blom, H., Baardseth, P.,Sundt, T.W. and Slinde, E., 2009. Lactic acid fermentation reduces acrylamide formed during production of fried potato products. Aspects of Applied Biology 97: 67-93.

Boekhout, T. and Robert, V., 2003. Yeasts in foods. Beneficial and detrimental aspects. Behr's Verlag, Hamburg, Germany, 488 pp.

Cheng, K.W., Wong, C.C., Cho, C.K., Chu, I.K., Sze, K.H., Lo, C., Chen, F. and Wang, M., 2008. Trapping of phenylacetaldehyde as a key mechanism responsible for naringenin's inhibitory activity in mutagenic 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine formation. Chemical Research in Toxicology 21: 2026-2034.

Ciesarova, Z., Suhaj, M. and Horvathova, J., 2008. Correlation between acrylamide contents and antioxidant capacities of spice extracts in a model potato matrix. Journal of Food and Nutrition Research 47: 1-5.

Claus, A., Carle, R. and Schieber, A., 2008. Acrylamide in cereal products: a review. Journal of Cereal Science 47: 118-133.

Di Cagno, R., De Angelis, M., Lavermicocca, P., De Vincenzi, M., Giovannini, C., Faccia M. and Gobetti M., 2002. Proteolysis by sourdough lactic acid bacteria: effects on wheat flour protein fractions and gliadin peptides involved in human cereal intolerance. Applied and Environmental Microbiology 68: 623-633.

Dybing, E. and Sanner, T., 2003. Risk assessment of acrylamide in foods. Toxicological Sciences 75: 7-15.

Environmental Protection Agency of United States (EPA), 1996. Acrylamide by gas chromatography, method 8032A. Revision 1. EPA, Washington, DC, USA. Available at: http://tinyurl.com/ml9vty5.

Fink, M., Andersson, R., Rosen, J. and Aman, P., 2006. Effect of added asparagine and glycine on acrylamide content in yeast-leavened bread. Cereal Chemistry 83: 218-222.

Forstova, V., Belkova, B., Riddellova, K., Vaclavik, L., Prihoda, J. and Hajslova J., 2014. Acrylamide formation in traditional Czech leavened wheat-rye breads and wheat rolls. Food Control 38: 221-226.

Fredriksson, H., Tallving, J., Rosen, J. and Aman, P., 2004. Fermentation reduces free asparagine in dough and acrylamide content in bread. Cereal Chemistry 81: 650-653.

Grob, K., Biedermann, M., Biedermann-Brem, S., Noti, A., Imhof, D., Amrein, T.M., Pfefferle, A. and Bazzocco, D., 2003. French fries with less hydrogen carbonate on the formation of acrylamide in model systems. Journal of Agricultural and Food Chemistry 54: 10523-10561.

Hendriksen, H., Kornbrust, B., Ernst, S., Stringer, M. and Heldt-Hansen, H., 2005. Asparaginase mediate reduction of acrylamide formation in baked, fried and roasted products. Journal of Biotechnology 118: S1-S135.

Huang, W., Yu, S., Zou, Q. and Tilley M., 2008. Effects of frying conditions and yeast fermentation on the acrylamide content in you-tiao, a traditional Chinese, fried, twisted dough-roll. Food Research International 41: 918-923.

Jung, M.Y., Choi, D.S. and Ju, J.W., 2003. A novel technique for limitation of acrylamide formation in fried and baked corn chips and French fries. Journal of Food Science 68: 1287-1290.

Kaaber, L., Sundt T.W. and Slinde E., 1995. Production of chips from potato cultivars (*Solanum tuberosum L.*) with high sugar content using lactic acid fermentation. Potato Research 38: 39-45.

Kotsiou, K., Tasioula-Margari, M., Capuano, E. and Fogliano, V., 2011. Effect of standard phenolic compounds and olive oil phenolic extracts on acrylamide formation in an emulsion system. Food Chemistry 124: 242-247.

Loponen, J., Mikola, M., Katina, K., Sontag-Strohm, T. and Salovaara, H., 2004. Degradation of HMW glutenins during wheat sourdough fermentations. Cereal Chemistry 81: 87-93.

Markova, L., Ciesarova, Z., Kukurova, K., Zielinski, H., Przygodzka, M., Bednarikova, A. and Simko, P., 2012. Influence of various spiceson acrylamide content in buckwheat ginger cakes. Chemical Papers 66: 949-954.

Matthäus, B. and Haase, N.U., 2014. Acrylamide-still a matter of concern for fried potato food? European Journal of Lipid Science and Technology 116: 675-687.

Meulenaer, B.D., Wilde, T.D., Mestdagh, F., Govaert, Y., Ooghe, W., Farselle, S., Demeulemeester, K., Peteghem, C.V., Calus, A., Degroodt, J.M. and Verhe, R., 2008. Comparison of potato varieties between seasons and their potential for acrylamide formation. Journal of the Science of Food and Agriculture 88: 313-318.

Michalak, J., Gujska, E. and Klepacka, J., 2011. The effect of domestic preparation of some potato products on acrylamide content. Plant Foods for Human Nutrition 66: 307-312.

Mottram, D.S., Wedzicha, B.L. and Dodson, A.T., 2002. Acrylamide is formed in the Maillard reaction. Nature 419: 448-449.

Mustafa, A., Fink, M., Kamal-Eldin, A., Rosen, J., Andersson, R. and Aman, P., 2009. Interaction effects of fermentation time and added asparagine and glycine on acrylamide content in yeast-leavened bread. Food Chemistry 112: 767-774.

Najafi, M.A., Rezaei, K., Safari, M. and Razavi, S.H., 2012. Use of sourdough to reduce phytic acid and improve zinc bioavailability of a traditional flat bread (Sangak) from Iran. Food Science and Biotechnology 21: 51-57.

Pedreschi, F., Kaack, K. and Granby, K., 2006. Acrylamide content and colour development in fried potato strips. Food Research International 39: 40-46.

Pedreschi, F., Kaack, K., Granby, K. and Troncoso, E., 2007. Acrylamide reduction under different pre-treatments in French fries. Journal of Food Engineering 79: 1287-1294.

Pedreschi, F., Moyano, P., Kaack, K. and Granby, K., 2005. Colour changes and acrylamide formation in fried potato slices. Food Research International 38: 1-9.

Rosen, J. and Hellenäs, K.E., 2002. Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. Analyst 127: 880-882.

Stadler, R.H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P.A., Robert, M.C. and Riedker, S., 2002. Acrylamide from Maillard reaction products. Nature 419: 449-450.

Surdyk, N., Rosén, J., Andersson, R. and Aman, P., 2004. Effects of asparagine, fructose and baking conditions on acrylamide content in yeast-leavened wheat bread. Journal of Agricultural and Food Chemistry 52: 2047-2051.

Taubert, D., Harlfinger, S., Henkes, L., Berkels, R. and Schoming, E., 2004. Influence of processing parameters on acrylamide formation during frying of potatoes. Journal of Agricultural and Food Chemistry 52: 2735-2739.

Thiele, C., Gänzle, M.G. and Vogel, R.F., 2002. Contribution of sourdough lactobacilli, yeast, and cereal enzymes to the generation of amino acids in dough relevant for bread flavor. Cereal Chemistry 79: 45-51.

Wicklund, T., Ostlie, H., Lothe, O., Knutsen, S.H., Brathen, E. and Kita, A., 2006. Acrylamide in potato crisps. The effect of raw material and processing. Food Science and Technology 39: 571-575.

Ye, Z., Jiang, J. and Wu, G., 2008. Biosynthesis and regulation of carotenoids in *Dunaliella*: progresses and prospects. Biotechnology Advances 26: 352-360.

Zamani, H. and Moradshahi, A., 2013. Synthesis and coating of nanosilver by vanillic acid and its effects on *Dunaliella salina* Teod. Molecular Biology Research Communications 2: 47-55.

Zhang, Y. and Zhang, Y., 2007. Study on reduction of acrylamide in fried bread sticks by addition of antioxidant of bamboo leaves and extract of green tea. Asia Pacific Journal of Clinical Nutrition 16:131-136.

Zhang, Y., Chen, J., Zhang, X., Wu, X. and Zhang, Y., 2007. Addition of antioxidant of bamboo leaves (AOB) effectively reduces acrylamide formation in potato crisps and French fries. Journal of Agriculture and Food Chemistry 55: 523-528.

Zhu, F., Cai, Y.Z., Ke, J. and Corke, H., 2009. Evaluation of the effect of plant extracts and phenolic compounds on reduction of acrylamide in an asparagine/glucose model system by RP-HPLC-DAD. Journal of the Science of Food and Agriculture 89:1674-1681.

Zhu, F., Cai, Y.Z., Ke, J. and Corke, H., 2011. Dietary plant materials reduce acrylamide formation in cookie and starch-based model systems. Journal of the Science of Food and Agriculture 91: 2477-2483.