International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395 -0056
Volume: 03 Issue: 12 | Dec -2016 www.irjet.net p-ISSN: 2395-0072

Reduction of acrylamide formation in bread by lactic acid bacteria and *Nigella sativa* oil

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ABSTRACT

Acrylamide, a probable human carcinogen, is formed by a reaction between asparagine and reducing sugars via Maillard reaction and was detected in a wide range of cereal products.

The aim of the present study was to investigate the effects of lactic acid bacteria (LAB) and Nigella sativa oil on the acrylamide formation in bread. For the lactic acid fermentation, Lactobacillus plantrum was used as a starter at different levels (2 and 4%). Nigella sativa oil was applied on the surface of dough before baking at the level of 4% (v/w). The effects of the two factors on acrylamide formation were investigated in experimental design. Acrylamide determination was performed by high performance liquid chromatography coupled with UV detection. The results showed that both LAB and Nigella sativa oil could effectively reduce the formation of acrylamide. Lactic acid fermentation, as individual factor, has significant effect on acrylamide inhibition depending on the starter concentration in the bread models. Breads containing 2 and 4% of L.plantarum showed a decrease in acrylamide content of 16.6% and 27.4% respectively. Significant effect was also observed when Nigella sativa oil was applied. In fact, the amount of acrylamide decreased by 46.3% for bread models added by Nigella sativa oil. The maximum reduction rate (56%) was achieved when the addition level of LAB was 4% and the Nigella sativa oil was applied. In conclusion, both LAB and Nigella sativa oil showed their inhibitory effects on acrylamide formation in bread.

Key words: acrylamide, breads, *L.plantarum*, *Nigella sativa* oil, reduction

1. Introduction

Maillard reaction is a key operation for flavor and tastes formation during heat treatment of foods[1, 10]**.** However, some other compounds, called Neoformed Compound (NFC), could be synthetized during Maillard reaction and are currently known for their undesirable health effects [1]. The most NFC compounds studied are acrylamide and hydroxymethylfurfural. In April 2002, researchers reported the formation of acrylamide in heat treated foods [10].It is a low molecular weight compound known for its toxicity, formed by a reaction between asparagine and reducing sugars [2, 12]. It is classified as « probably human cancerogen», and neurotoxin [13]. Studies showed that acrylamide in foods is formed by a reaction between asparagine and reducing sugars [2, 12] and demonstrated that raw

materials and processing conditions such as time, temperature, water activity, pH and additives could influence acrylamide formation [11].

Several strategies to reduce acrylamide content in cereal products are recommended with a large range of reduction levels. [12] suggested that lactofermentation using lactic acid bacteria (NCIMB 40450) in sourdough preparation reduces acrylamide formation by 75% in crispbread. This effect is due to the reduced pH rather than to the consumption of asparagine by lactic acid bacteria. Despite of the important potential of this process strategy in acrylamide reduction, its application is not very occurred. Other studies recommended the addition of antioxidants and polyphenols in foods preparation to limit acrylamide formation [8, 14]. The mechanism reaction of these molecules is not completely clarified. Researchers supposed that antioxidants and polyphenols react with asparagine during Maillard reaction [7].

Nigella sativa L. is a spices plant belonging to the family *Ranunculaceae*. It has been used traditionally, especially in the Middle East and India, for the treatment of asthma, cough, bronchitis, headache, rheumatism, fever, influenza and eczema. It's known that *Nigella sativa* oil is a very rich polyphenols matrix [4]. *Nigella sativa* beans are, already, widely used in Tunisian bakery products.

The aim of the present study is to investigate the impact of lactic acid bacteria fermentation and *Nigella sativa* oil application on the acrylamide formation in bread. In order to explore the potential of LAB, *Nigella sativa* oil and their interaction on acrylamide reduction, a two-factor three levels experimental design was employed to achieve best combination of the variables.

A method for acrylamide extraction and detection using high performance liquid chromatography with UV detection was developed and optimized in our laboratory.

To our knowledge, this is the first report on the combined effect of lactic bacteria and *Nigella sativa* oil on acrylamide formation.

2. Materials and Methods

2. 1 Studies model effects of lactic acid bacteria and *Nigella sativa* oil on acrylamide formation

Effects of lactic acid bacteria fermentation and added *Nigella sativa* seeds oil were investigated through a design of experiments. Three levels for each factor were chosen (refer to Table 1) and all experiments were repeated three times. For the lactofermentation, *Lactobacillus plantarum* (obtained from Departement of Food Technology, ESIAT, Tunisia) was used as a starter. As for *Nigella sativa* oil application, two forms were tested: an ordinary oily form extracted from black cumin seeds and a dry form obtained by immobilization of *Nigella sativa* oil on maltodextrine support. The two forms of *Nigella sativa* oil were obtained from HERBIOTECH AROMA (Tunisia).

Table-1: Experimental design for effect evaluation of *L.plantarum* and *Nigella sativa* oil on acrylamide formation

Experiment	Lactic bacteria concentration (CFU/ml)	*Nigella sativa* oil form
1	10^4	-
2	-	-
3	10^8	Oil
4	-	Oil
5	-	Oil
6	10^4	Oil
7	10^4	Powder
8	10^4	-
9	10^8	Powder
10	-	Powder
11	10^8	Oil
12	10^8	-
13	-	-
14	10^8	Powder
15	10^4	Oil
16	10^4	Powder
17	10^8	-
18	-	Powder

2.2 Backing procedure

2.2.1 Preculture preparation

Lactobacillus plantarum was precultured on MRS broth at 30°C for 24h. The cells were collected by centrifugation at 4500 x g for 10 min, washed twice with sterile 0.15 M NaCl and resuspended in sterile 0.15 M NaCl in order to obtain a suspension of about 10^8 cfu/ml [4].

2.2.2 Backing procedure

The recipe of baking yeast-leavened wheat bread was adopted from [3]. A model system made up of wheat flour (61.7%), water (36.6%), salt (0.7%) and commercial dry yeast (1.7%) as leavening agent was used. In further experiments, lactic acid bacteria (three different concentrations: Control, 10^4 cfu/ml and 10^8 cfu/ml) was added to each basic formulation. The ingredients were mixed for 10 min at room temperature, the dough shaped manually in cylindrical baguettes of about 5 cm diameter and allowed to leave for 2 h at 30°C. In the end of fermentation time, *Nigella sativa* seeds oil was applied on the surface of rolls bread dough (5 ml for the oily form and 10 ml for the dry form) as described on the experimental design. Finally, all breads models were cooked at 260°C for 20 min.

2.3 Polyphenols analysis

The phenolic compounds of *Nigella sativa* seeds oil were determined by a colorimetric method using the Folin-Ciocalteau reagent as previously done by [5].

2.4 Acrylamide analysis in bread samples

2. 4.1 Acrylamide extraction from bread samples

Three grams of each sample were ground in a waring blender for 3 min and thoroughly homogenized with 30 ml of distillated water. After 30 min of agitation, the homogenates were filtred through Whatman filter paper. The filtrates were purified by adding 1 ml of Carrez I and Carrez II reagents. The precipitates were, then, removed by centrifugation at 12000 rpm for 20 min. One ml of hexane was added to the samples to extract remaining long chain fatty acids that could create problems in chromatographic analysis. The mixture was shaken vigorously and the upper hexane layer was removed. After filtration through 0.45 µm syringe filter, 20 µl of each solution was injected onto HPLC column for acrylamide analysis.

2.4.2. LC-UV analysis

LC-UV analysis were performed by Knauer HPLC system (Germany) consisting of a binary pump and a

temperature controlled column oven, coupled to UV-2500 detector. The analytical separation was performed on a Knauer C18 A column (150 x 3 mm) using the isocratic mixture acetonitrile - milliQ water (6:94 v/v) at a flow rate of 0.2 ml/min at 30 °C. Acrylamide was detected by its absorbance at 210 nm with UV-detector.

2.5. Statistics

The baking experiments were designed and evaluated using the software Minitab 14.0 (Minitab Inc., State College, PA, USA).

3. Results and discussions

Lactofermentation pretreatment and *Nigella sativa* oil addition have shown an important reduction effect on acrylamide formation in different bread models (Fig 1). The decrease of acrylamide content was found to range from 16.6 to 56% depending on the recipe.

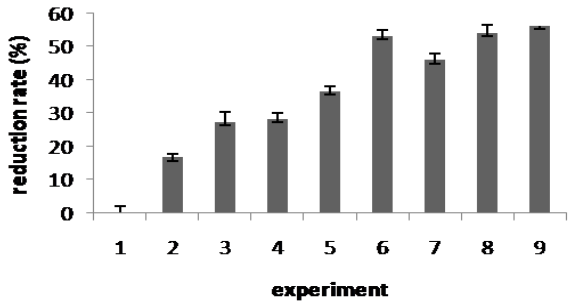

Fig .1 Reduction rate of acrylamide formation in different bread samples

Lactofermentation pretreatment, as individual factor, has significant effect (p<0.05) on acrylamide inhibition. The decline in acrylamide formation was affected by the lactic acid bacteria level in the system. Surface plots (fig 2) illustrate that the lowering of rates varied from 16.6% to 27.4% in breads containing 10^4 cfu/ ml and 10^8 cfu/ml respectively. LAB are reported to prevent acrylamide formation by their acidification properties. The pH influence the reactivity of both the sugar and the amino acid group. The suggested optimum pH for acrylamide formation is around 7-8. By lowering the pH, the free protonated amino-group of asparagine is converted to protonated amine (NH_3^+) thus blocking the Schiff's base formation, which is an essential step in the formation of acrylamide [11]. Besides to pH reduction, others studies suggested that lactofermentation increase asparagine consumption [12].

Nigella sativa oil has exhibited more important inhibiton of acrylamide than lactofermentation. The amount of acrylamide in bread decreased by 19.4% and 46.3% for the oily and dry form respectively. The higher effect shown by *Nigella sativa* oil is likely due to its high polyphenols content (201±5 méq gallic acid for le the oily form and 117,6±4 méq gallic acid for the dry form).

Polyphenols are recognized as powerful inhibitor of acrylamide formation. In this case, [8] reported that ferrulic acid decrease the acrylamide formation by 50%. [14] observed a significant reduction in acrylamide content when 0.1g/kg of tea extract was added to reducing sugars- asparagine model system.

Although the dry form containing on polyphenols is less than the oily form, its effect on acrylamide reduction is more important. Probably, the immobilization of *Nigella sativa* oil on maltodextrine support protect polyphenols from a direct exposure to hot temperature and then their active structure is not altered. The surface plot shows that the highest acrylamide reduction rate (56%) coincides with an increasing addition of *Lactobacillus plantarum* and the employment of *Nigella sativa* oil powder form. The two pretreatment have a synergic effect on acrylamide reduction.

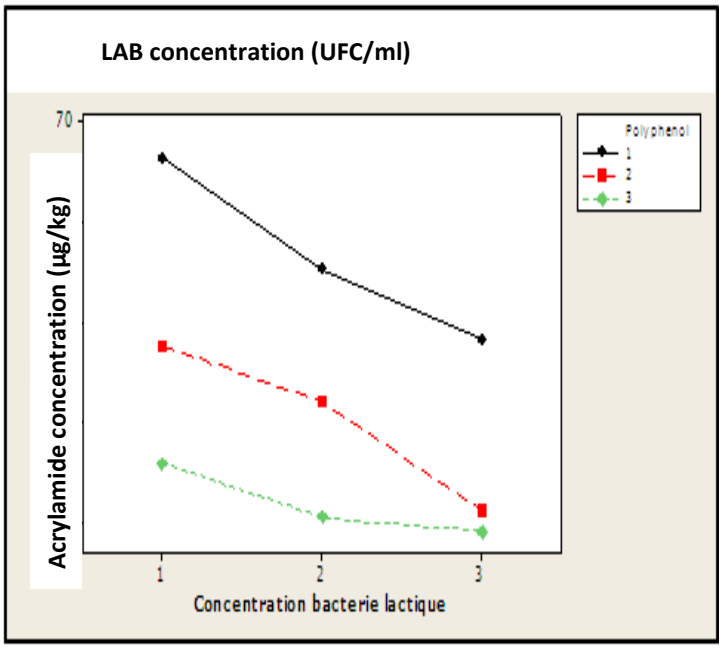

Fig 2. Effect of *L.plantarum* and *Nigella sativa oil* on acrylamide formation in different bread samples

Polyphenol levels: 1. Control; 2. Oil form; 3: dry form

LAB levels: 1. control; 2. 10^4 cfu/mL; 3. 108 cfu/mL

4. Conclusion

In this paper, the effect of lactic acid bacteria and *Nigella sativa* oil on the acrylamide formation in bread was studied. It was demonstrated that both strategies showed significant reducing effect on acrylamide formation. For lactic acid fermentation, acrylamide elimination increased linearly with increasing bacterial culture concentration. *Nigella sativa* oil addition seems to be, also, an important measure for the reduction of acrylamide content. Both strategies can be applied concurrently to reach the maximum reduction rate of acrylamide (56%).

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