

## Improvement of Cereal Product Safety by Enzymatic Way of Acrylamide Mitigation

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**Abstract:** Acrylamide as a probably carcinogenic compound is known to be formed in many high thermally treated products with a natural occurrence of amino acid asparagine and reducing sugars as well. Cereal products, especially gingerbreads are extensively affected by acrylamide formation up to 1000 µg/kg and more. This study compares pros and cons of enzyme treatment and a substitution of ammonium raising agent for sodium salt addition in manufactured gingerbreads with respect to their final sensory quality. More than 97% reduction of acrylamide content was achieved by the asparaginase application before baking with no observed detrimental effect on sensory quality of final products. On the other hand, sodium raising agents efficiently decreased acrylamide content, but with no acceptable impact on colour, texture, softness, delicacy and an expected appearance of gingerbread. For that reason, the application of L-asparaginase enzyme seems to be a perspective way to mitigate acrylamide.

**Keywords:** acrylamide; gingerbread; L-asparaginase; raising agent

### INTRODUCTION

After the revealing of acrylamide presence in foods a lot of studies have confirmed its presence in nearly all fried, baked and roasted foods. Acrylamide exposure varies depending upon the population's eating habits and the way the foods are processed and prepared. Generally, fried potato products, ready-to-eat breakfast cereals, baked goods and roasted coffee are the most important food categories that contribute most to acrylamide exposure. Among them, according to European-wide-database (IRMM 2006), gingerbreads belong to those with the highest content of acrylamide (number of samples: 1003; acrylamide content: minimum 5 µg/kg; median 303 µg/kg; maximum 7834 µg/kg). An average long-term exposure of acrylamide has been estimated of 0.3 to 0.8 µg/kg body weight/day (FAO/WHO 2002). The contribution of gingerbreads to the acrylamide exposure

for general population (1–97 year old) has been reckoned to be 6% (BONN *et al.* 2005). Although gingerbreads are ones of seasonal products especially made at the Christmas time, but not entirely, they are usually consumed by children during a year as a snack. Based on the reported data, the Committee JECFA in 2005 noted that children may have intakes of acrylamide around two or three times higher those of adult consumers when expressed on a body weight basis (JECFA 2007). It is expected that children and adolescents have consumption patterns different from adults. Most of the types of foods in which acrylamide was detected are popular among children and adolescents. Moreover, they have a lower average body weight and, consequently, a higher average food intake per kilogram of body weight than adults. For that, acrylamide intake by these individuals is considered a concern. A perspective way of efficient acrylamide mitigation seems to be an ap-

plication of L-asparaginase enzyme converting one of the crucial precursors amino acid asparagine to aspartic acid which does not enter the reaction of acrylamide formation. An unambiguous advantage of this approach is that no detrimental effect on sensory quality of final products is observed. The desirable efficiency of enzyme activity depends on the appropriate conditions during enzyme treatment.

The presented study is focused on the determination of enzyme and raising agent effects on acrylamide formation in manufactured gingerbreads and their sensorial evaluation.

## MATERIAL AND METHODS

**Baking procedure.** Dough for gingerbread making was treated with L-asparaginase enzyme addition in concentration of 100 U and 1000 U/kg of dough, respectively. Raising agents applied in dough were as follows: ammonium hydrogen carbonate (17.30 g of  $\text{NH}_4\text{HCO}_3$  per kg of dough) or a mixture of sodium hydrogen carbonate (24.22 g of  $\text{NaHCO}_3$ ) and sodium pyrophosphate (17.30 g of  $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$ ) per kg of dough for 30 min and 60 min, respectively, during kneading at 25°C. In the third case dough was staying for 48 h at ambient temperature. After shaping pastries were baked in an oven at the temperature of 250°C (upper heating) and 230°C (bottom heating) for 5 min. Final products were analysed to determine acrylamide and amino acid (asparagine, aspartic acid,

glutamine, glutamic acid) contents by LC/MS/MS (ČIESAROVÁ *et al.* 2009); as well as a dryness and pH value were measured and sensory properties were evaluated.

## RESULTS AND DISCUSSION

During a standard procedure of gingerbread making with ammonium hydrogen carbonate addition more than 1200 µg/kg of acrylamide was developed. This high level of acrylamide was successfully decreased by enzyme addition in concentration of 1000 U/kg of dough after 30 min lasting incubation (45% of acrylamide from the initial value) and 60 min long incubation (75% reduction of acrylamide). Long-term dough staying for 48 h at ambient temperature resulted in 97% of acrylamide reduction. A promising decrease of acrylamide (approximately 80%) was achieved also with a lower 100 U/kg of dough enzyme concentration, but only after 48 h incubation (Figure 1). A substitution of ammonium raising agent for sodium salts resulted in a substantial decrease of acrylamide content (70 µg/kg). Further acrylamide elimination up to the values between 20 and 30 µg/kg and even lower than LOQ was reached with 1000 U/kg enzyme application (Figure 2). However, conspicuous disadvantages of this raising agent replacement were inappropriate sensory properties of final products considering their colour, texture, softness, delicacy, and expected appearance as well. Thus, an enzymatic way of acrylamide re-

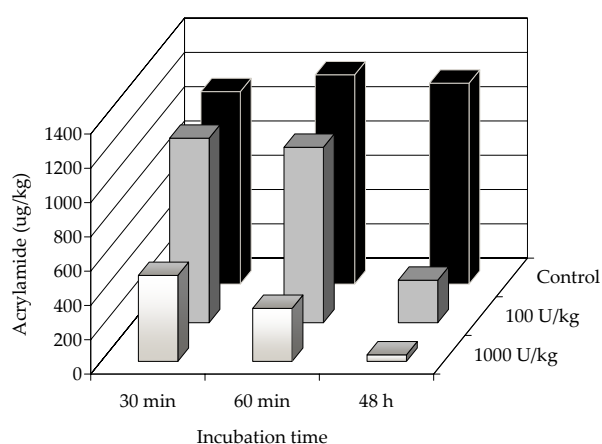


Figure 1. Acrylamide content in gingerbread with ammonium raising agent addition treated by L-asparaginase enzyme (0; 100; 1000 U/kg) for 30 min, 60 min and 48 h incubation at ambient temperature before baking

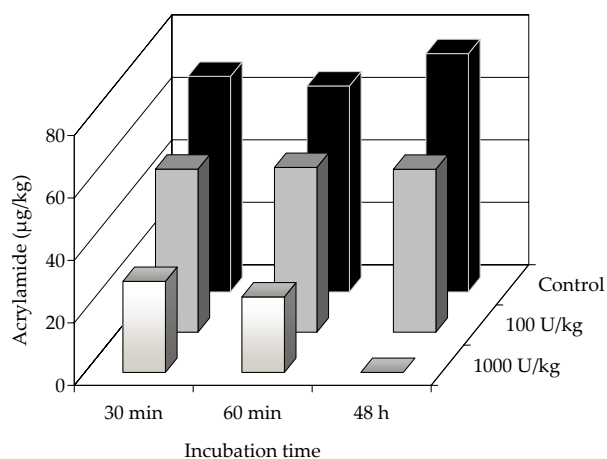


Figure 2. Acrylamide content in gingerbread with sodium salt raising agent addition treated by L-asparaginase enzyme (0; 100; 1000 U/kg) for 30 min, 60 min and 48 h incubation at ambient temperature before baking

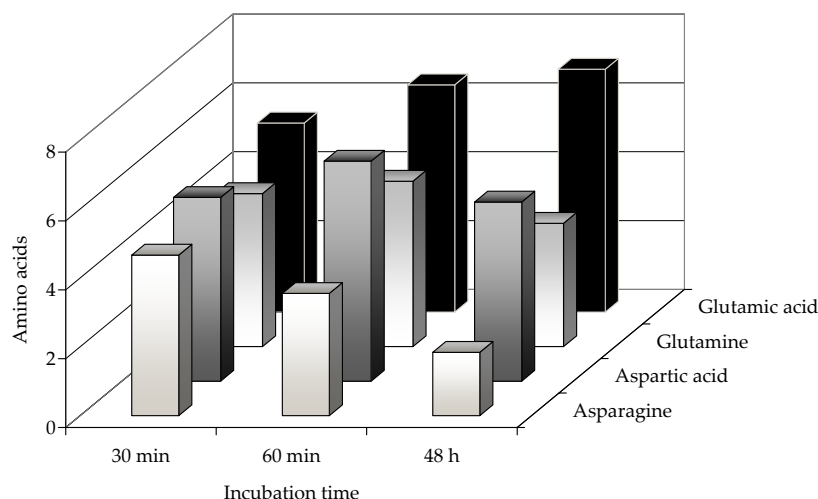


Figure 3. Amino acids (Asp – aspartic acid in  $\text{mg} \times 10^{-1}/\text{kg}$ ; Asn – asparagine in  $\text{mg}/\text{kg}$ ; Glu – glutamic acid in  $\text{mg}/\text{kg}$ ; Gln – glutamine in  $\text{mg}/\text{kg}$ ) in gingerbread with sodium agent addition treated by L-asparaginase enzyme (1000 U/kg) for different incubation time at ambient temperature before baking

duction seems to be more acceptable. Moreover, an interesting impact on amino acid profiles of enzymatically treated samples with different raising agents was observed. In both cases of raising agent alternatives, after enzyme addition an expected decrease of asparagine content related with an adequate aspartic acid increase in first 60 min was found out which was evident especially in higher 1000 U/kg enzyme concentration (Figure 3). Later the content of aspartic acid decreased probably due to entering further reactions. However, a noteworthy fact is that the content of glutamine weakly decreased and glutamic acid content was slightly growing especially in case of sodium agent addition (Figure 3). These observations could imply the presence of transamination and transamidation reactions between the mentioned amino acids as a consequence of amino acid metabolism as well as they open the question of the substrate specificity of L-asparaginase enzyme related to amino acid glutamine.

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