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REVIEW PAPER

Acrylamide in Baking Products: A Review Article

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Abstract Acrylamide or 2-propenamide is a chemical compound, with chemical formula CH_2=CH–CO–NH_2, that can be produced at high levels in high-carbohydrate heat-treated foods. The risks of acrylamide to health and its toxic properties (neurotoxicity, genotoxicity, carcinogenicity and reproductive toxicity) were demonstrated by the Scientific Committee on Toxicity, Ecotoxicity and the Environment in 2001. Potato and bakery products account for around 50% and 20% of human exposure to acrylamide, respectively. Factors affecting acrylamide formation and degradation in foods are acrylamide precursors such as free amino acids (mainly asparagine), reducing sugars and processing conditions (i.e. baking time and temperature, moisture content and matrix of product). The aim of this review was to present some results from recent investigations of the effects of different factors affecting acrylamide formation in bakery products. Finally, recommendations are proposed as guidelines for baking manufacturers to reduce the level of acrylamide in their products.

Keywords Acrylamide review · Baking · Acrylamide formation · Risk of acrylamide to health · Asparagine · Reducing sugars

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Introduction

Discovery and Importance of Acrylamide

Acrylamide (CH2=CH–CO–NH2; 2-propenamide) is a white crystalline solid with a molecular weight of 71.08 kDa. It has a melting point of 84.5±0.3 °C, low vapour pressure of 0.007 mmHg at 25 °C and a high boiling point (136 °C at 3.3 kPa/25 mmHg; Norris 1967: 2; Ashoor and Zent 1984; Eriksson 2005). Some toxicological studies in 1984 and 1991 suggested that acrylamide vapours irritate the eyes and skin and cause paralysis of the cerebrospinal system (Zhang et al. 2005; Johnson et al. 1986; Smith and Oehme 1991). The risk of acrylamide to health was also shown in 1997 when a large water leakage happened during the building of a tunnel in Sweden and large numbers of dead fish and paralyzed cattle were found near the construction site. The walls of the tunnel contained monomeric acrylamide and *N*-methylolacrylamide, and a large leak of these compounds into the environment appeared to be the cause of the health problem. Through the measurement of reaction products (adducts) with protein haemoglobin in blood, it was shown that several of the tunnel workers had developed peripheral nerve symptoms similar to those reported for acrylamide poisoning (Eriksson 2005; Hagmar et al. 2005).

In 1994, the International Agency for Research on Cancer (IARC 1994) classified acrylamide as "potentially carcinogenic to humans", and in 2001, the Scientific Committee on Toxicity, Ecotoxicity and the Environment demonstrated its inherent toxic properties (neurotoxicity, genotoxicity to both somatic and germ cells, carcinogenicity, and reproductive toxicity).

The importance of acrylamide in food was mentioned for the first time in 2000 by Tareke et al. who showed that feeding rats with fried feed led to a large increase in the level of the haemoglobin adduct, which was concluded to be *N*-(2-carbamoyl methyl) valine. Also, in April 2002, a group of Swedish researchers reported that some heat-treated starch-rich foods such as potato and cereal products and coffee contained high levels of acrylamide (Surdyk et al. 2004; Tareke et al. 2002; Svensson et al. 2003). In 2005, the Swedish National Food Administration announced that foods processed and cooked at high temperatures contain relatively high levels of acrylamide (Zhang et al. 2005).

Food Exposure and Risk of Acrylamide to Health

Direct consumer exposure to acrylamide may result from ingestion of high-carbohydrate foods such as potato crisps and chips, roasted cereals, and breads (Fig. 1). Indirect exposure may result from residual traces of the monomer in food packaging where polyacrylamide is used as a binding agent (Zhang et al. 2005; Samuelson 2003).

Assessment of the presence of acrylamide is a great concern in many countries. According to the results obtained so far, potato products account for around 50% and baking products and bread for around 20% of human exposure to acrylamide. Bakery goods, bread and rolls have relatively low values whereby the highest levels have been detected in the crust of the bread, whereas the crumb contains almost no acrylamide. One exception is crisp bread which contains considerable amounts of acrylamide. Several observations have led to the hypothesis that heating of food could be an important source of human exposure to acrylamide. Acrylamide is formed in foods, if the heating/frying is done in an oven, on a frying pan or by microwave heating, whilst no acrylamide has been detected in boiled food products (Eriksson 2005; Törqvist 2005).

Acrylamide is genotoxic (mutagenic), which increases the incidence of cancer in rats at doses of 1–2 mg/kg body weight per day. Even though the maximum acrylamide intake from foods (in Europe) has been reported to be 0.05 mg/kg body weight per day, there was an urgent need (in 2002) for action to minimize the level of acrylamide in foods. In November 2003, the Heatox project was started to investigate the risk of acrylamide to human health. The final report of the project was released in 2007, which identified the following risks: (1) Evidence for acrylamide posing a cancer risk for humans has been strengthened. (2) Since acrylamide levels in bread and potatoes have been reduced in laboratory experiments, human exposure can potentially be decreased. (3) Acrylamide is not the only genotoxic compound formed when heating food. Totally, around 50 compounds have been highlighted as potential carcinogens (HEATOX Project 2007).

Factors Affecting Acrylamide Formation in Baking Products

Precursors

It is now well established that free amino acids, mainly asparagine, and reducing sugars are important precursors to acrylamide in foods and that processing conditions, such as temperature, water activity and matrix, influence its formation and degradation. However, further research is needed to better understand how these factors will influence acrylamide content in baking products (Surdyk et al. 2004; Svensson et al. 2003).

Even though it has been shown that a temperature of 120 °C or higher is needed for the formation of acrylamide, there are reports confirming that this compound can be formed at temperatures below 100 °C, particularly in drying processes at 65–130 °C (Eriksson 2005; Biedermann and Grob 2003). Prolonged storage and heating time at high temperatures (higher than 120 °C) decrease the acrylamide content. Thus, acrylamide content of food is the net amount of acrylamide, i.e. the result of formation and disappearance. The type of reaction responsible for this disappearance is still unclear.

Acrylamide is formed predominantly from the amino group of asparagine and a carbonyl compound derived from reducing sugars (mainly glucose, fructose and maltose). Free asparagine in combination with reducing sugars generates significant amounts of acrylamide when pyrolysed at temperatures >120°C (Mottram et al. 2002; Stadler et al. 2002). It was confirmed that free asparagine is the limiting factor for the formation of acrylamide in yeast-leavened wheat bread and ginger bread (Surdyk et al. 2004; Amrein et al. 2004). It was shown that added asparagine to the flour dramatically increased acrylamide content in yeast-leavened breads whilst added fructose did not influence the content. These results clearly show that low acrylamide yeast-leavened breads should be baked with ingredients with a low level of free asparagine or a process that lowers the content of free asparagine before it is converted to acrylamide (Surdyk et al. 2004). Curtis et al. (2010) studied acrylamide precursors (free amino acid and sugar concentrations) in rye varieties and showed free asparagine concentration to be the main determinant of acrylamide formation in heated rye flour, as it is in wheat. Hamlet et al. (2008) showed that in cooked flours and doughs (mainly rye and wheat), asparagine was the key determinant of acrylamide generation. They found that in biscuit and rye flours, levels of asparagine were correlated with fructose and glucose, so selection based on low fructose and glucose contents, and hence low asparagine, could be beneficial in reducing acrylamide in products (e.g. crackers and crisp breads) that have no added sugars. The most common strategies to decrease acrylamide formation

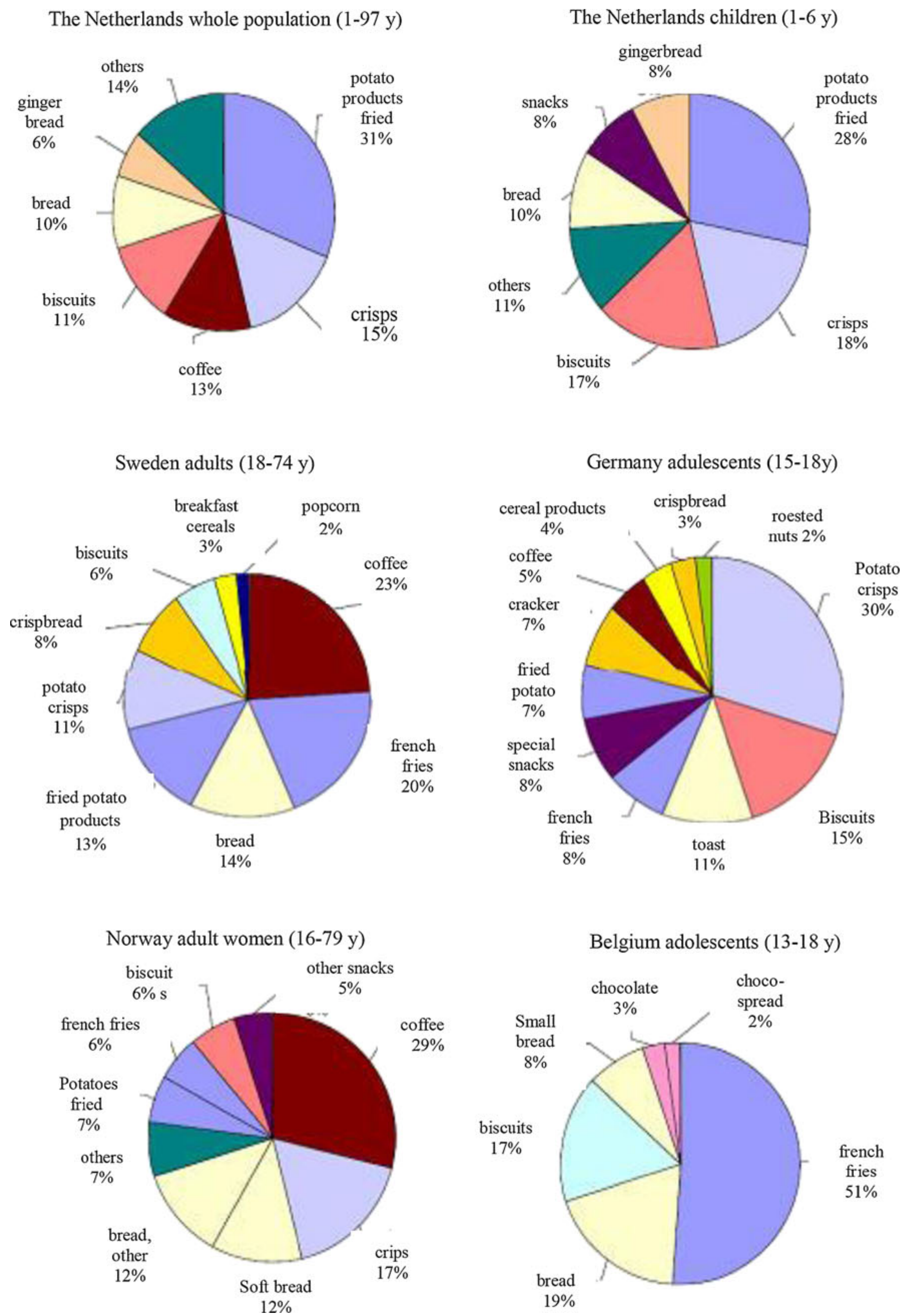

Fig. 1 Contribution of food groups to acrylamide exposure in different countries for different age groups (HEATOX Project 2007)

have focused on reducing or diluting precursors such as free asparagine: (1) consuming asparagine by either adding an enzyme or using yeast or another microorganism (Fredriksson et al. 2004; Hamlet et al. 2005); (2) adding other amino acids (Brathen et al. 2005; Claeys et al. 2005); (3) binding asparagine with a complexing agent, for example by adding divalent metal ions (Gökmen and Senyuva 2007; Lindsay and Jang 2005); and (4) removing accelerants such as ammonium salts (Amrein et al. 2004, 2006; Biedermann and Grob 2003).

It is assumed that the mechanism leading to the formation of acrylamide derives from Maillard reaction, i.e. the reaction between reducing sugars and proteins/amino acids (mainly asparagine; Fig. 2). However, other routes may also be responsible (Wenzl et al. 2003; Stadler et al. 2002; Becalski et al. 2003; Mottram et al. 2002). There are similar confusions about the effects of the amount of precursors on acrylamide formation. For instance, addition of 35 mmol/kg of glucose and fructose in a model system including reducing sugar and asparagine increased acrylamide formation, but the extreme addition of glucose (up to 140 mmol/kg) would decrease the acrylamide content to lower than that expected from 35 mmol/kg addition. Also, the addition of protein or amino acids could to some extent lead to a reduced formation of acrylamide.

pH and Moisture

The effects of pH and moisture content have also been investigated. Researchers showed that the addition of consumable acids is a very simple but efficient method of minimizing acrylamide in bakery products. When increasing amounts of citric acid were added to baked corn chips, acrylamide decreased almost linearly (Jung et al. 2003). Similar effects were reported when lactic, tartaric, citric and hydrochloric acids were added to semi-finished biscuits and cracker models (Graf et al. 2006; Levine and Smith 2005; Taeymans et al. 2004). Addition of acidity has been shown to result in a decreased acrylamide formation, but to an increased degradation of the acrylamide formed. Generation is significantly reduced due to hydrolysis of the carboxamide group leading to aspartic acid at lower pH. Furthermore, reduced pH values resulted in only moderate Maillard reactions, accompanied by lower acrylamide formation. There are some complexities about the effect of water activity. For example, acrylamide is not formed before water activity is reduced to below 0.8, and in low water activity foods, maximum acrylamide formation is observed when water activity is about 0.4; further reduction of water activity tends to decrease the amount of acrylamide (Eriksson 2005; Stadler et al. 2002; Mottram et al. 2002; Biedermann et al. 2002a, b; Weisshaar 2004; Hoenicke and Gatermann 2004, 2005; Delatour et al. 2004; Brathen and Knutsen 2005).

Lipid Oxidation

Lipid oxidation is one of the major chemical reactions occurring during food processing or storage and may have a strong impact on the final quality of foods. It is well known

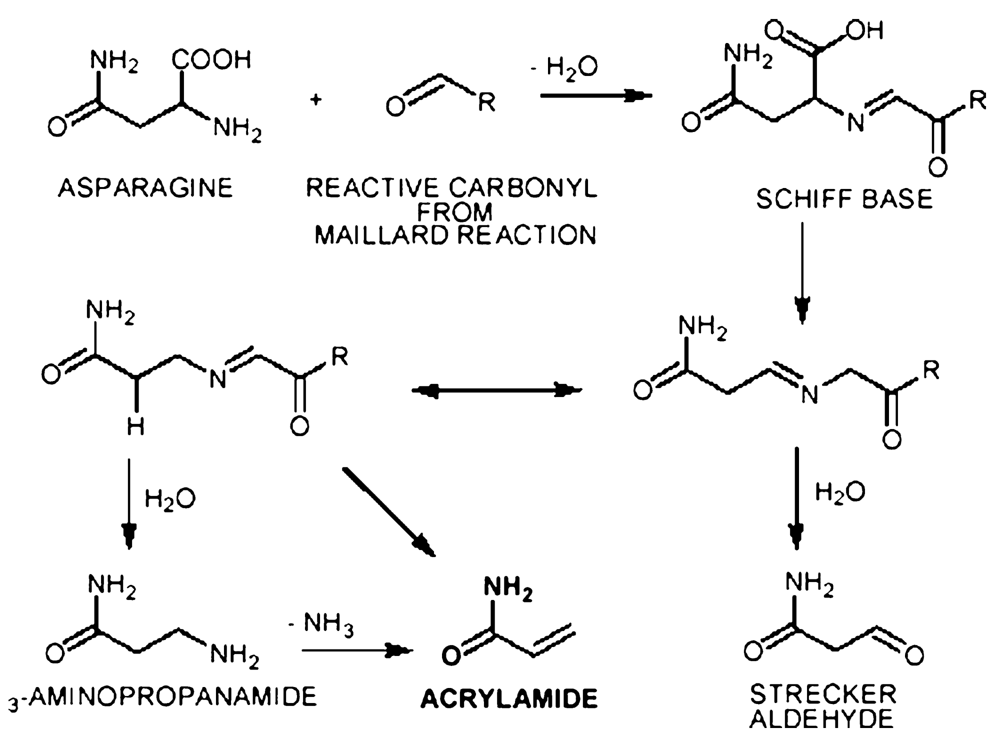

Fig. 2 Proposed mechanism for the formation of acrylamide in heat-treated foods (HEATOX Project 2007)

that lipids (triglycerides) produce a large amount of acrolein by heat treatment (Umano and Shibamoto 1987). Acrolein can further react via oxidation to generate acrylic acid or by formation of an intermediate acrylic radical. Both of the intermediates could then induce acrylamide formation in the presence of a nitrogen source under favourable reaction circumstance in lipid-rich foods upon heat treatment (Fig. 3). In a model system using asparagine, significant amounts of acrylamide (114 μg/g of asparagine) were formed, but under the same condition using glutamine, acrylamide was formed only at the level of 0.18 μg/g of glutamine. Considering that both amino acids contain an amide moiety, this may be due to differences in the amount of ammonia formation from asparagine and glutamine rather than due to their amide moiety. Also, asparagine may produce a three-carbon unit more readily than glutamine does (Yasuhara et al. 2003).

Researches showed that some secondary lipid oxidation products can convert amino acids into the corresponding vinylogous derivatives (Hidalgo and Zamora 2007; Zamora et al. 2007), and some lipid oxidation products can degrade asparagine to acrylamide (Zamora et al. 2007). They proposed α,β,γ,δ-diunsaturated carbonyl compounds as the most reactive, followed by hydroperoxides, likely because of their thermal decomposition upon heating. Arribas-Lorenzo et al. (2009) investigated the effect of oil oxidation level as well as the oil phenol profile on acrylamide formation in cookies and claimed that lipid oxidation products can be regarded as an important factor in acrylamide formation in fat-rich dry foods and that the amount and the type of antioxidant compounds of oil clearly affect acrylamide concentrations after baking. Capuano et al. (2010) showed that oil oxidation level positively influences the formation of acrylamide in different formulations with three oil samples at different oxidation levels, but catechin presence reduced acrylamide formation in systems containing unheated oil and partially oxidized oil whilst no significant lowering effect was observed when highly oxidized oil was used.

In sugar-containing model systems, lipid oxidation level moderately influenced acrylamide formation, whilst the effect became more pronounced in systems with low water content and with low carbohydrate concentration. The type of oil or fat used in the formulation also influences acrylamide levels (Fig. 4), with acrylamide formation higher in systems containing sunflower oil than in systems made with palm oil, which has a lower susceptibility (Capuano et al. 2010).

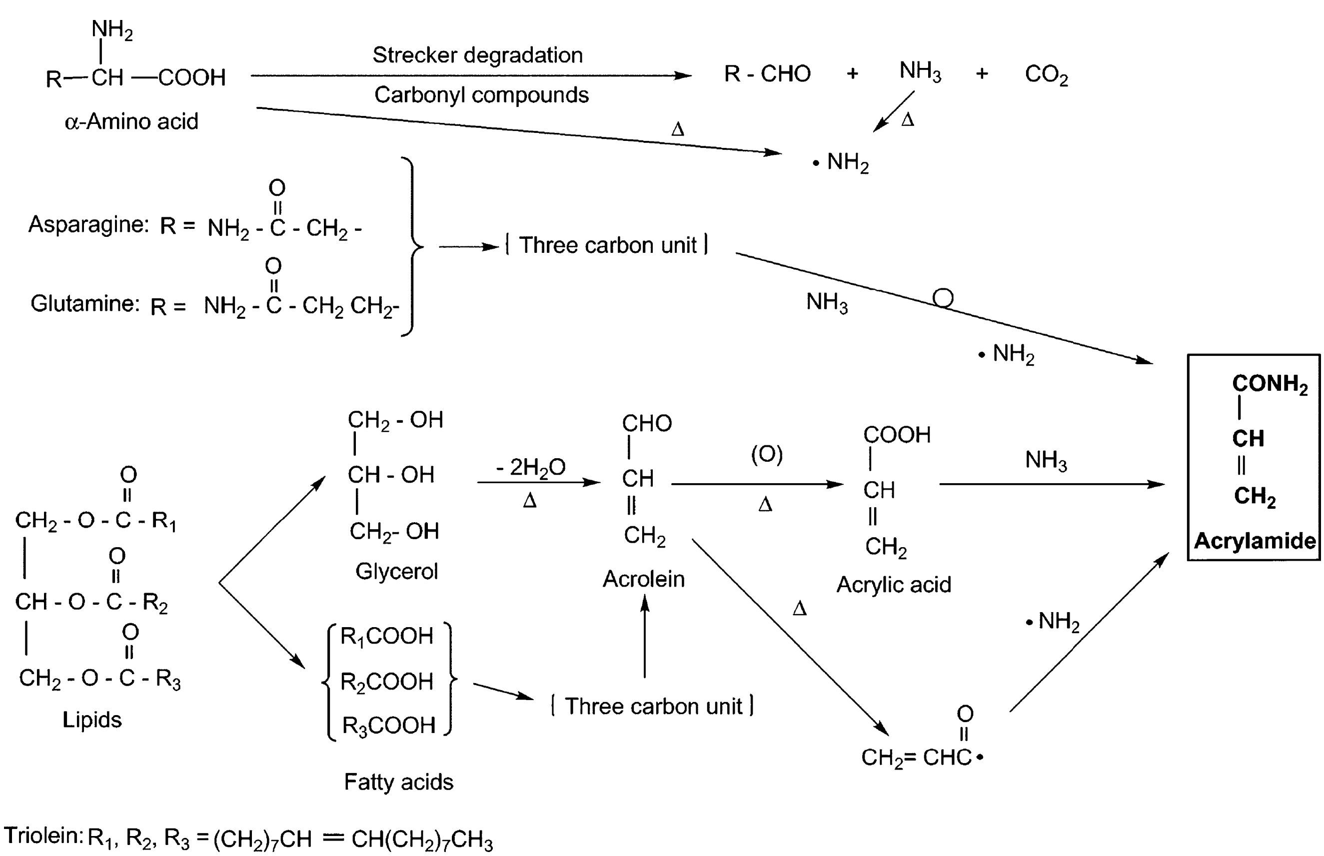

Fig. 3 Hypothesized formation mechanisms of acrylamide from an amino acid and a lipid (Yasuhara et al. 2003)

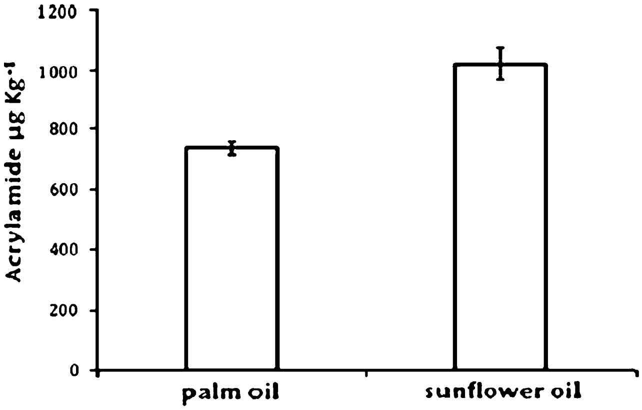

Fig. 4 Acrylamide concentration formed in sugar-free model systems containing palm oil versus sunflower oil. Results are expressed on a dry basis (Capuano et al. 2010)

Addition of Cations

Recently, there has been interest in studying the use of monovalent, divalent and trivalent metal cations, in particular Na^+ and Ca^{2+}, to prevent the formation of acrylamide in model systems with asparagine and sugar (Gökmen and Senyuva 2007; Kolek et al. 2006), potatoes (Lindsay and Jang 2005; Mestdagh et al. 2008) and wheat (Claus et al. 2008; Sadd et al. 2008). A patent application also showed that polyvalent cations are able to reduce acrylamide formation during heating (Tomoda et al. 2004). Metal cations have long been known to cause pH reduction (Vadlamani and Seib 1999). Therefore, the study of potential mitigating factors, such as Ca^{2+} and other cations, should properly control for pH if comparable data are desired. Researches showed that adding divalent cations such as Ca^{2+} or Mg^{2+} to the dough prior to baking caused a remarkable effect on the acrylamide contents of the products. Elder et al. (2004) reported that adding divalent cations (Ca^{2+}, Mg^{2+}) caused a 20% reduction in acrylamide content. Gökmen et al. (2007) showed that in a fructose–asparagine model system, added divalent cations, such as Ca^{2+}, prevent acrylamide formation completely. They found that pyrolyzing the equimolar mixture of asparagine and glucose with equimolar amounts of monovalent, divalent and trivalent cations such as K^+, Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{3+} led to a 97% or more reduction in the amounts of acrylamide formed during heating at 150 °C for 20 min. This was also true for Na^+ to a certain extent. Levine and Ryan (2009) studied the effect of calcium cations on acrylamide formation and showed that when 1% $CaCl_2$ was added to flour, salt and water bread dough or cracker dough, acrylamide formation during cooking was reduced about 35% or 60%, respectively. In sweet and savoury biscuits with 2% $CaCl_2$, the reduction was about 60%, and it was suggested that $CaCl_2$ can be applied more efficiently to baked products by adding it to a commercial tin-release agent. $CaCO_3$ is used as a preferred source of calcium for the fortification of cereal-based foods since, being water-insoluble, it minimally affects product quality and acrylamide formation. Generally, there was no reduction when $CaCO_3$ was added to simulate a calcium-enriched flour.

Antioxidant Effects

During these years, many correlative tests have been performed and positive or negative effects on acrylamide reduction have been demonstrated using different kinds of antioxidants. In fact, both reduction and enhancement results of acrylamide formation via the addition of different antioxidants were validated in different published researches, which suggested the dual effects of antioxidants on the generation of acrylamide. Tareke (2003) showed that addition of BHT, sesamol and vitamin E to meat prior to heating enhanced the formation of acrylamide probably by protection of acrylamide against free radical-initiated reactions. On

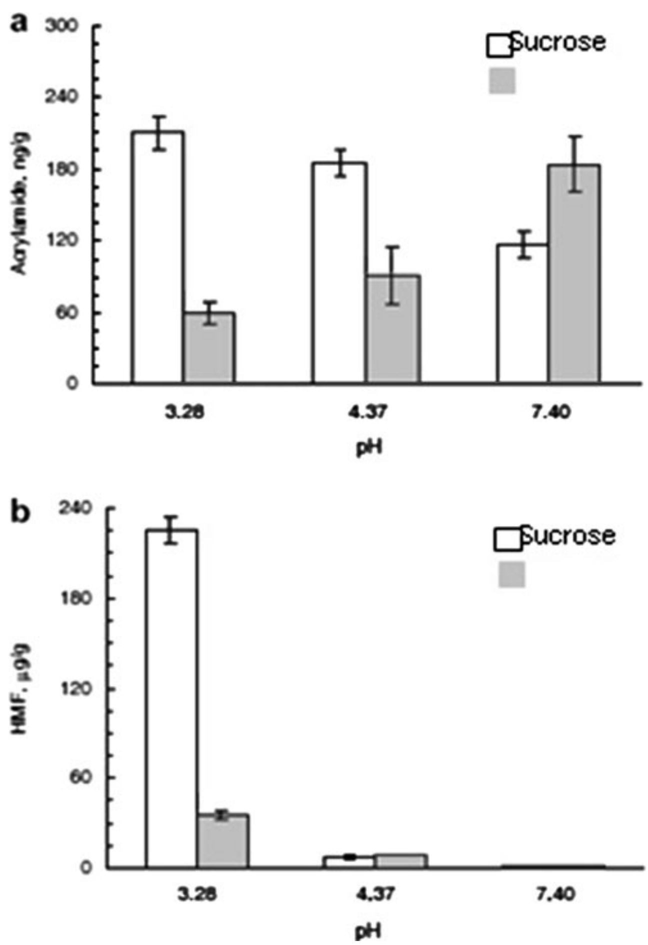

Fig. 5 Effect of initial dough pH on the formation of acrylamide (**a**) and HMF (**b**) in cookies comprising sucrose and glucose upon baking at 205 °C for 11 min (Gökmen et al. 2007)

the other hand, flavonoids are known to have antioxidative properties. The green tea flavonoids such as epicatechin and epigallocatechin gallate were recently found to hamper Maillard reactions due to the carbonyl-trapping capacity of these compounds. Zhang and Zhang (2007) demonstrated that addition of antioxidant of bamboo leaves (AOB) and extract of green tea (EGT) greatly reduced the acrylamide content in fried bread sticks, and results showed opposite concentration-dependent relationships in different ranges of AOB and EGT treatments that may relate to the inherent property of these two antioxidants and the antioxidant activity of food matrices, which is the so-called antioxidant paradox. On the other hand, addition of antioxidants could block the oxidation of acrolein to a certain extent and further mitigate the generation of acrylamide. Hedegaard et al. (2008) evaluated the effect of antioxidants on the content of acrylamide in wheat bread as a heated food product often spiced with herbs such as rosemary and dittany, which are known to have antioxidative properties and are used in many types of food. They found that rosemary decreases acrylamide formation when added to dough prior to baking, but the spice dittany showed less effect compared to rosemary and even increased acrylamide formation slightly. In a cracker model based on wheat flour and water, $NaHSO_3$ was demonstrated to enhance acrylamide elimination (Levine and Smith 2005), and Casado et al. (2010) suggested that $NaHSO_3$ (as a preservative and antioxidant compound) may inhibit the production of intermediates that induce the formation of acrylamide and then reduced acrylamide formation without a negative repercussion on the sensory quality of heated olive juice. Summa et al. (2006) studied the correlation of the acrylamide content and the antioxidant activity in model cookies. A direct correlation was found between the acrylamide level and the antioxidant activity.

This review presents the results obtained from recent investigations of the effects of different factors involved in food processing, particularly baking, on acrylamide formation.

Effects of Processing Conditions

Reduction of acrylamide formation in bread has been the subject of much research. A key factor in the sensory quality of bread is the colour of its surface (crust), but unfortunately, there is a strong correlation between intensity of crust colour and acrylamide formation, particularly when bread is baked at temperatures higher than 200 °C (Ahrné et al. 2007).

Factors of greatest importance in this regard are temperature and air humidity profile during baking. Compared with conventional baking conditions, optimized conditions may result in a 50% reduction in acrylamide formation. It is a common observation that the formation of crust starts when the surface temperature of the bread is over 100 °C. A dehydration process accompanies crust formation. Therefore, the exact temperature and moisture content of the crust are two important factors in acrylamide formation (Ahrné et al. 2007).Ahrné et al. (2007) studied the effects of crust temperature and water content on acrylamide formation and colour development in the crust. For this purpose, they used three baking systems and temperatures: traditional (baking in a deck oven without air circulation), steam and falling-temperature baking systems at 200, 230 and 260 °C. It is well known that there is a strong correlation between acrylamide formation and baking temperature and time (up to 15 min at 260 °C; Surdyk et al. 2004; Ahrné et al. 2007; Brathen et al. 2005). However, Ahrné et al. (2007) found that increasing baking time up to 20 min at 260 °C decreased acrylamide concentration particularly in the outer crust, whilst its concentration in the inner crust was 25–75% that of the outer crust for baking at 200–230 °C. Their results showed that the lowest crust temperature for which traces of acrylamide were detected was approximately 150 °C and that formation of acrylamide apparently started at approximately 120–130 °C. Consequently, no acrylamide was observed in the bread crumb. However, these results may be challenged on the grounds that the amount of acrylamide should be determined on the basis of dry matter. The same researchers observed strong correlations between colour and acrylamide concentration, on the one hand, and between crust temperature and colour, on the other. Therefore, acrylamide concentration and colour are well correlated, but only up to a total colour difference of ΔE = 65, above which acrylamide content starts to decline. However, such crust colours (total colour difference of above $\Delta E > 60$) would be considered as unacceptable by the consumer.

The authors also found steam injection to influence water content, crust colour and acrylamide formation, resulting in a lower acrylamide concentration by almost 50% in comparison with baking without steam. Lowering oven temperature after 5, 10 and 15 min of baking reduced acrylamide concentration by 67%, 36% and 35%, respectively, compared with that produced by baking at a constant temperature (the authors did not mention the exact temperatures). Such baking conditions resulted in a lighter crust colour. Therefore, it is possible to use steam for baking bread to approximately the same colour level as that produced by traditional baking, but with considerably lower acrylamide levels.

Brathen and Knutsen (2005) determined the effects of baking time and temperature on the formation of acrylamide in starch gels and cereal food products, including freeze-dried rye-based flat bread dough, flat bread and bread (Brathen et al. 2005; Cochran and Cox 1971). They reported that the highest and lowest amounts of acrylamide were observed in the starch system and bread crust, respectively. Also, more acrylamide was formed in freeze-dried flat breads than in flat bread. Furthermore, they found that the proportion of crust to whole bread (from 16.9% to 22.4%), its thickness (from thin to thick) and its colour (from light to dark) corresponded to the amount of acrylamide formed in the crust.

For the two dry systems (starch gels, freeze-dried flat bread and conventional-baked flat breads), the amount of acrylamide went through a maximum at around 190–210 °C. In the case of freeze-dried flat bread, the temperature which yielded the highest amount of acrylamide was highly dependent on baking time. In the starch system, the amount of acrylamide went through a maximum as the amount of asparagine increased, whilst no such effect was found for glucose. They observed a positive relationship between acrylamide and dry matter and also between time and temperature irrespective of the amounts of reactants (glucose and asparagine).

Becalski et al. (2003) experimented with dry systems and Mottram et al. (2002) experimented with aqueous solutions. Both studies reported reducing amounts of acrylamide with increasing temperature from 155 to 185 °C for more than 10 min. Although the presence of water has been reported not to hinder the formation of acrylamide, it is worth mentioning that the effective temperature is kept down by water evaporation as long as water is present. This explains the difference between dry systems and those containing residual water and also why the amount of acrylamide is lower in flat breads than in the starch system but increases only when moisture content falls to below 4%.

Becalski et al. (2003) also observed a positive relationship between acrylamide and dry matter. Prolonged heating tends to reduce acrylamide content in dry systems, but not in bread crust. In many other systems, the same dependence on time has been reported. This indicates that acrylamide reacts further and/or is eliminated through evaporation. However, at low temperatures, the decrease in acrylamide with increasing baking time does not occur even in dry systems. This strengthens the hypothesis that the differences between dry systems and those containing residual water are due to the evaporation of water and the reduction of effective temperature. Furthermore, the rate of acrylamide elimination depends on temperature (faster rates at higher temperatures).

Mustafa et al. (2005) studied the effect of time and temperature of baking and addition of fructose, asparagine and oat bran concentrate on the acrylamide content and colour of rye crisp bread. Acrylamide content increased with time and temperature of baking, with higher effects perceived at higher temperatures and longer times in an accelerating slope. They showed that baking rye crisp bread at different combinations of time and temperature favoured the browning reactions.

The same authors used proper conditions to show that little acrylamide (<20 μg/kg dry crust) was detected at low baking time and temperature (15 min at 150 °C) but that the highest (1,800 μg/kg of dry crust) was observed when bread was baked for 25 min at 290 °C. Furthermore, based on the regression analysis of the response surface model, they found significant effects for both temperature and time as well as their interaction (Tareke et al. 2002; Stadler et al. 2002; Grob et al. 2003; Rydberg et al. 2003; Yasuhara et al. 2003).

Based on the above results, HEATOX (European project) verified that both baking time and temperature increase acrylamide content in crusts and rye crisp bread and that a significant interaction exists between baking time and temperature.

The HEATOX report shows that conventional baking conditions yield the highest amount of acrylamide in the outer part of the crust but that it increases with increasing baking temperature (200–260 °C) and baking time (10–25 min). However, for the highest temperature–time combination (260 °C, 20 min), the acrylamide level decreases and is lower in the outer crust than in the inner crust. Using this knowledge base, scientists in that project worked along two lines: application of steam to traditional baking and alternative heating techniques such as infrared radiation and air jet impingement.

Steam Baking

The HEATOX project investigated the effects of using steam during the final part of baking. The reference baking parameters were 200 °C for 20 min. This was compared with baking when steam was introduced after 5, 10 or 15 min of baking. Both steam and "falling-temperature" (i.e. reducing of baking temperature at the last stage of baking) baking processes substantially lowered acrylamide levels in the final bread crust. However, crust colour was influenced more in the falling-temperature breads than in the steam-baked ones. With steam baking, it was possible to find conditions that gave bread of the same colour as that achieved by traditional baking, but with considerably lower acrylamide levels. With falling-temperature baking, however, though acrylamide levels were lowered, bread colour was lighter. Using sensory analysis, it was shown that it was possible to bake bread (using steam during the final 5 min) that was not significantly different from the control in terms of odour, appearance, texture and flavour, but with 40% lower acrylamide level (HEATOX Project 2007).

Alternative Baking Techniques

The effects of new baking techniques, such as air jet impingement and infrared radiation baking, on bread crust formation and its characteristics were studied. Using information obtained from such experiments, baking conditions were defined to achieve similar crust colour with various baking techniques. It was obvious that the acrylamide content in bread crust could be reduced using alternative baking technologies. Reduced levels were obtained with both infrared radiation and air jet impingement baking.

Preliminary experiments were also performed to study the flavour of breads baked by these new technologies. A close relationship was found to hold between the presence of a large number of volatiles, especially those volatile and odorous compounds that are known to be formed via browning reactions in the bread crust like Strecker aldehydes and alkyl pyrazine, and the formation of acrylamide for the baking methods studied. Obviously, the baking method that produced low amounts of acrylamide in these experiments also tended to result in low contents of important flavour substances.

IR baking has been further focussed on to evaluate how it can be optimized with regard to acrylamide minimization and sensory product quality. Crust colour, acrylamide, flavour compounds and sensory characteristics were investigated as a function of baking time under various baking conditions. One important finding from these experiments was that, with IR baking, it is possible to obtain a sensory profile almost identical to that of conventionally baked bread, but with considerably lower acrylamide content. A reduction of acrylamide content by 60% could be shown (HEATOX Project 2007).

Effects of Precursors and Reaction Conditions

The influence of the composition of food and thermal conditions of the process are the main factors that interact with acrylamide formation (Berg and Van Boekel 1994; Morales et al. 1997; Gökmen et al. 2007; Gökmen and Acar 1999; Gökmen and Şenyuva 2006). Since reduction of acrylamide content is a major concern of baking producers, Gökmen et al. (2007) studied the effects of dough formula and baking conditions on acrylamide formation in cereal products (cookies) (Fig. 5). They investigated the type and concentrations of sugars and pH levels along with the effects of six different formulae and measured hydroxymethylfurfural (HMF) as a chemical indicator and as an acrylamide precursor to assess the quality of thermally processed food products. They reported that the amount of sucrose in the recipe was less effective on the yield of acrylamide than glucose because increasing amounts of sucrose from 10 to 35 g in the recipe almost doubled the amount of acrylamide formed during baking. This is while replacing sucrose with glucose in the recipe resulted in a drastic increase in the amount of acrylamide formation upon baking at 205 °C for 11 min (74.1 ± 5.60 ng/g of acrylamide in cookies). The reason for this, they claimed, is that the hydrolysis of sucrose might be very limited under these baking conditions (baking for 11 min at 205 °C). Reduction of acrylamide formation by a factor of 50% or more using sucrose instead of reducing sugars (e.g. glucose) confirms earlier findings by other researchers (Amrein et al. 2004; Graf et al. 2006; Vass et al. 2004). Some authors have also reported that a reducing sugar is needed to form acrylamide from asparagine (Mottram et al. 2002; Stadler et al. 2002; Yaylayan et al. 2003). Sugar seems to be the most important ingredient in the dough formula for acrylamide formation because the free asparagine is relatively low in wheat flour (0.15–0.40 g/kg; Surdyk et al. 2004; Noti et al. 2003). Rather than sugar, organic acids added to baking powders as an acidic salt to reduce pH decrease the amount of acrylamide formed during baking (Surdyk et al. 2004; Rydberg et al. 2003; Jung et al. 2003; Kita et al. 2005). However, when reducing sugars are used in place of sucrose, the reduced pH increases the amount of acrylamide by 1.8 times in cookies under the same baking conditions. This is probably due to the excessive hydrolysis of sucrose (Gökmen et al. 2007).

Finally, they showed that the kinetics of acrylamide formation significantly differed between the recipes comprising sucrose (with a lower initial rate of formation) and those with glucose (with a rapidly increasing rate of formation). These results are comparable with those reported by Summa et al. (2006) who confirmed a linear increase in acrylamide concentration of cookies for the recipe comprising glucose, but a lower initial rate of acrylamide formation within a baking time of 15 min with the recipe using sucrose instead of glucose.

Even though acrylamide concentration increases during frying and baking, addition of different additives, such as rosemary, amino acids or protein, reduces the level of acrylamide. Also, reduced pH dramatically reduces acrylamide content during frying and baking (Tareke et al. 2002; Becalski et al. 2003; Brathen et al. 2005; Grob et al. 2003; Rydberg et al. 2003; Jung et al. 2003).

It has been found that in baking products, addition of asparagine dramatically increases the amount of acrylamide, but no effect of glucose addition is observed. However, the increase in acrylamide content occurs only with lower concentrations of asparagine, whilst at higher concentrations, a decrease is observed in acrylamide content. This indicates that one acrylamide elimination mechanism might be its reaction with excess amounts of amino acids. Rydberg et al. (2003) reported that addition of amino acids or protein-rich ingredients reduced the amount of acrylamide. No similar decrease at high concentrations was found for glucose, indicating that reaction of acrylamide with carbohydrates is insignificant in the removal of acrylamide (Brathen et al. 2005).

Surdyk et al. (2004) established a model to study the internal and external factors affecting acrylamide content in yeast-leavened wheat bread and to investigate the effects of asparagine and fructose additions on acrylamide level in the bread along with the impacts of baking temperature and time.

In wheat flour with low levels of asparagine and reducing sugars and when no precursors were added to the dough, about 80 μg of acrylamide per kilogram of dry crust was detected. In order to determine the effects of the precursors on acrylamide formation in bread, asparagine and reducing sugars were added to the dough at amounts higher than their natural levels found in wheat (Fredriksson et al. 2004; Tkachuk 1979; Aman 1988). At the highest level of asparagine added (0.70 g/100 g flour), up to 6,000 μg of acrylamide per kilogram of dry crust was reported, but fructose did not show any influence on the content of acrylamide in bread. They concluded that because of the presence of free sugars which are formed during baking, the amount of reducing sugars is not a limiting factor. They also reported that addition of asparagine increased the amount of acrylamide formed in the isolated crumb of yeast-leavened wheat bread, but to a much lower level (about 30 μg of acrylamide per kilogram of dry crumb). They confirmed that more than 99% of the acrylamide in bread formed in the crust, which is in accordance with the results reported from in vitro studies indicating that acrylamide does not form from asparagine and reducing sugars at temperatures below 100 °C. Therefore, it is very likely that the detected acrylamide in the crumb originated from crust parts remaining in the crumb due to incomplete separation of the crust.

The authors also reported that within the range of asparagine added, the response surface model revealed an almost a linear increase from 200 to about 1,200 μg per kilogram fresh bread.

Even though they found a highly significant correlation ($p<0.001$) between colour development and acrylamide content in the crusts with the same recipe, baking (15 min at 270 °C) with higher levels of added fructose and asparagines did not significantly change the colour of the crust, whilst the acrylamide content increased dramatically. Therefore, they concluded that amino compounds other than asparagine and/or reducing sugars should be mainly involved in the browning reactions of the crust (Surdyk et al. 2004).

As the HEATOX report shows, added asparagine dramatically increased the content of acrylamide in crust dry matter whilst added glycine decreased its content. The more asparagine in the dough, the stronger was the reducing effect of glycine. Furthermore, when glycine was applied on the surface of the fermented dough, the acrylamide content of the bread exhibited a significant reduction. Since glycine is more reactive than asparagine, with respect to Maillard reactions, its addition caused an increased browning reaction during baking. No such effect was observed, however, on acrylamide formation. Reducing sugars such as fructose are not a limiting factor for acrylamide formation in bread; therefore, their addition does not influence acrylamide content. On the other hand, baking bread under various (baking time–temperature) conditions with the same ingredients showed a strong correlation between colour and acrylamide content, but this correlation was not found in the experiment with added precursors. Also, oat bran concentrate with a high content of mixed-linkage β-glucan did not influence the acrylamide content in breads.

Effects of Milling Process and Fermentation

Reduced values of such factors as flour milling intensity and baking temperature decrease the amount of bread acrylamide content during the baking process (Ahrné et al. 2007; Haase et al. 2003). However, using a lower amount of ash flour and extensive yeast fermentation may reduce the acrylamide formed without affecting the crust colour (Surdyk et al. 2004; Fredriksson et al. 2004). Also, addition of glycine to dough can significantly reduce the acrylamide content of both flat bread and bread crust (Brathen et al. 2005; Fink et al. 2006).

According to the HEATOX report, asparagine which is mainly concentrated in cereal bran is the limiting factor for the formation of acrylamide in bread. Thus, high-fibre flour compared with sifted flour yields breads with a higher acrylamide content. However, fermentation of dough made with different milling fractions showed that most of the asparagine was used up after 2 h of fermentation with baker yeast. Therefore, new fermentation techniques could help the breakdown of asparagine to reduce acrylamide, as a consequence. Sourdough, on the other hand, did not reduce the content of free asparagine as efficiently, but had a strong negative impact on asparagine utilization by the yeast. This indicates that this type of fermentation may result in a bread with a higher acrylamide content than breads fermented with yeast only. Short fermentation time compared with longer fermentation reduced the acrylamide content by 87% in the bread made with whole grain wheat and by 77% in bread made with rye bran.

Chemical Indicators of Acrylamide Formation in Baking Products

Measuring the compounds which form during the early stages of Maillard reaction and known as acrylamide precursors might be a good index for evaluating process conditions involved in acrylamide formation during the manufacture of cereal products. For instance, determination of furosine (∊-*N*-(furoyl-methyl)-L-lysine) and HMF has been used as an indicator to evaluate the heat effects induced during the manufacture of cereal products such as sliced bread toasting (Ramírez-Jiménez et al. 2000; Henle et al. 1995; Ramirez-Jimenez 1998).

Furosine amino acid is formed during the hydrolysis of Amadori compounds, including fructosyl-lysine, lactulosyl-lysine and maltulosyl-lysine, produced by the reaction of ∊-amino groups of lysine with glucose, lactose and maltose, respectively (Erbersdobler and Hupe 1991). HMF is an intermediate product in Maillard reaction and is also formed from the degradation of sugars at high temperatures (Ramírez-Jiménez et al. 2000; Kroh 1994; Berg and Van Boekel 1994; Morales et al. 1997).

Ramírez-Jiménez et al. (2000) used furosine and HMF determinations as indicators of browning reactions in bread to evaluate their usefulness in process control. They reported a linear correlation between HMF and colour index in different commercial breads, but no linear correlation was found between furosine/HMF and furosine/baking temperature. They found that when the water content of the bread samples was lower, a smaller extension of the browning reaction happened and a lower colour was observed, as can be verified by the higher furosine content (early stage indicator) and the lower HMF value. In the crust, higher baking times produced a greater extension of the Maillard reaction and, therefore, a degradation of furosine. The study of toasted sliced bread showed that furosine levels began to descend after the first 10 min of the toasting process (Ramírez-Jiménez et al. 2000). From the above results, it may be concluded that negative correlations exist between furosine and colour index and between furosine and acrylamide formation. Therefore, when furosine content begins to decrease in bread crust, acrylamide can be expected to start forming. This might be a useful indicator for evaluating the effects of baking conditions on acrylamide formation.

As regards HMF, it is shown that there should be a linear correlation between HMF and acrylamide content in bread so that HMF determination can be recommended as a quality control measure instead of acrylamide determination, which is much more difficult and expensive.

Ruiz et al. (2004) identified the three browning indicators, namely furosine, HMF and glucosylisomaltol, to be used as a complex indicator of utility for monitoring the processing of pre-baked bread. They reported no detectable furosine in raw dough. However, they observed that the early stages of Maillard reaction were favoured during the pre-baking process (14–15 min at 175 °C) so that furosine increased in content throughout the process whilst colour did not change when compared with the original dough and no HMF could be detected in the pre-baked bread. Therefore, no acrylamide formation could be expected. They also reported that furosine continued to increase after baking for 14 min at 220 °C, but declined after 30 min. This is while the HMF content increased for all baking times. Their results are consistent with those by other authors (Ramírez-Jiménez et al. 2000; Ramirez-Jimenez 2001).

It can be concluded that furosine and HMF might be the useful indicators for the estimation of acrylamide formation during the bread baking process.

Conclusion

Acrylamide is a non-volatile compound which is classified as "potentially carcinogenic to humans". Man can be directly exposed to it by consuming heat-treated foods rich in carbohydrates, such as baked foods, or indirectly through certain packaging materials. The recent risk characterization of acrylamide concludes that the evidence of acrylamide posing a cancer risk for humans has been strengthened. Therefore, reducing the acrylamide formed in baked foods is still a major concern.

This paper provides an overview of acrylamide formation during the bread making process. Even though baking is by evidence an important step during which acrylamide is formed, several other parameters such as formulation, flour quality, fermentation conditions among others play their roles in its formation. The following generalizations may be drawn from the literature cited in this review:

There is a strong positive correlation between baking temperature and time and acrylamide formation, whilst replacement of reducing sugars with sucrose and the use of flours with a lower asparagine content (in recipe) may decrease the acrylamide content of baked foods. On the other hand, reduced pH in some recipes such as cookies has an opposite effect.

Chemical indicators such as furosine and HMF may be used for controlling the acrylamide content in pre-baked and fully baked products, respectively.

Finally, it is possible to reduce the acrylamide content whilst retaining the sensory quality by either introducing steam in traditional baking or by using new alternative baking techniques. The experimental works showed that it is possible to reduce acrylamide content by 40% in white bread by applying steam during the final 5 min of baking with no significant changes in the sensory quality. Also, using infrared radiation heating makes it possible to reduce the acrylamide level in flat bread cakes by 60% with retained sensory properties.

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