Food Chemistry: a Kazakhstan Perspective on the Maillard Reaction and Acrylamide Formation in Common Foods

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Abstract

The Maillard reaction is largely responsible for the colour, flavour, aroma and texture of fried, baked and roasted foods, including bread, biscuits, breakfast cereals and other foods made from wheat grain, French fries and crisps made from potato and a wide range of other popular foods. However, it also results in the formation of undesirable products, including the neurotoxin and probable carcinogen, acrylamide, and furans. Kazakhstan is a major wheat producer and exports wheat grain to many countries, including countries within the European Union. The European Commission has already issued "indicator levels" for the presence of acrylamide in food products. Although these are not regulatory limits, food producers strive to keep the levels of acrylamide in their products beneath the indicator levels in order to avoid intervention from food safety authorities and the associated bad publicity. Sourcing raw material with low acrylamide forming potential would enable food producers to achieve this without expensive changes to processesand this is likely to be an increasingly important issue for suppliers. This review describes the Maillard reaction, the evolving regulatory scenarios in Europe and the USA and the implications for Kazakhstan as a grain exporter.

Introduction

The Maillard Reaction

The Maillard reaction comprises a series of non-enzymic reactions between reducing sugars and amino groups, principally those of amino acids, which occurs during cooking and food processing. Temperatures in excess of 120 ºC are required, such as occur during frying, baking and roasting (but not boiling). The reaction takes its name from French chemist, Louis Camille Maillard, who first described it in 1912 [1], although the steps in the reaction as they are understood today were first proposed by an American chemist, John Hodge, in 1953 [2].

The products of the Maillard reaction include heterocyclic compounds such as pyrazines, pyrroles, furans, oxazoles, thiazolesand thiophenes [3-5]. The exact compounds that are formed depend on the food’s amino acid and sugar composition and the cooking or processing conditions. This defines the characteristic flavours, aromas and to some extent textures of particular food types and individual brands. The most important flavour and aroma volatiles of French fries and baked potatoes, for example, include pyrazines, pyrroles, pyridines, oxazoles, alkyloxazoles, thiazoles, furfural and hydroxymethyl furfural, while the most important flavour compound of bread is 6-acetyl-1,2,3,4 tetrahydroxypyridine, and 2-acetyl-1-proline provides the main flavour in wheat and rice crackers; all of these compounds are Maillard reaction products.

The reaction has been described in detail recently by Nursten [6] and Mottram [4],and we will not go into the same depth here. It requires a reducing sugar such as glucose, fructose or maltose, although sucrose can participate if it is first hydrolysed through enzymatic, thermal or acid-catalysed reaction [7]. The carbonyl (C = O) group of the reducing sugar condenses with an amino group to produce a Schiff base (Fig. 1). If the sugar is an aldose, such as glucose or maltose, the Schiff base cyclises to give an N-substituted aldosylamine, such as glucosylamine, which then rearranges to

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give a 1, 2-enaminol. This exists in equilibrium with its ketotautomer, an N–substituted 1-amino-2-deoxyketose, and together these are known as Amadori rearrangement products. Ketoses, such as fructose, give related Heyns rearrangement products through a similar series of reactions.

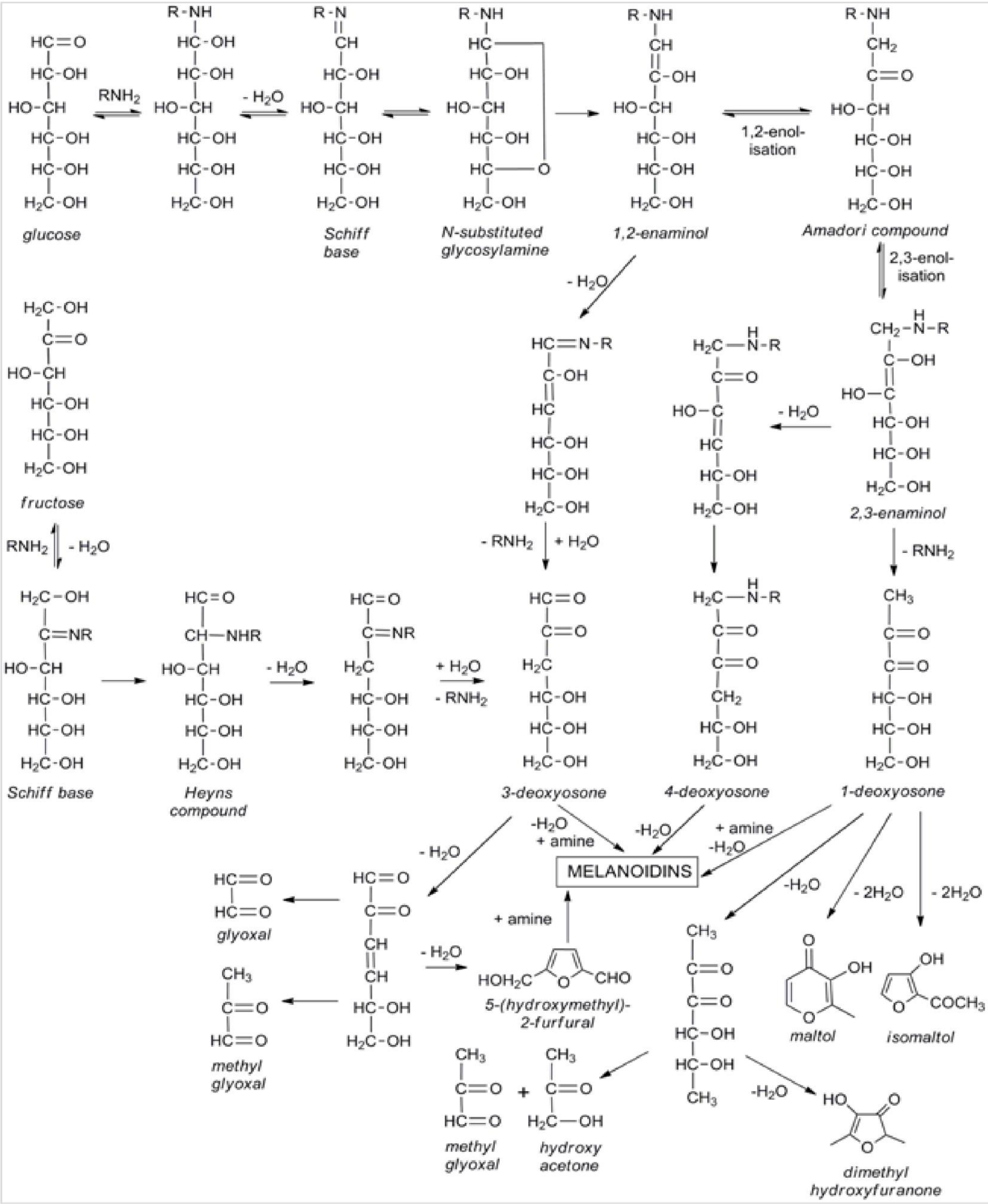

Fig. 1. The Maillard Reaction showing the formation of Amadori and Heynes intermediates and their breakdown to carbonyl compounds. Reproduced with permission from Halford et al. [5].

The Amadori and Heyns rearrangement products undergo enolisation, deamination, dehydration and fragmentation, leading to the formation of sugar dehydration and fragmentation products containing one or more carbonyl groups, including heterocyclic furfurals, furanones and pyranones (Fig. 1). These compounds contribute to the flavour characteristics of foods in their own right, but they also undergo further condensation reactions with amino groups and other components, resulting in the formation of many different flavour compounds (Fig. 2). This is important because it means that amino acids participate in the Maillard reaction at two different stages and this has implications for the relationship between precursor concentration and product formation [8]. One of the reactions involving carbonyl compounds and free amino acids is Strecker degradation, whereby an amino acid is de aminated and decarboxylated to give an aldehyde. In the case of cysteine, this provides a route for the incorporation of nitrogen and sulphur into heterocyclic flavour compounds, such as pyrazines, oxazoles and thiazoles [4] (Fig. 2). However, a Strecker-like degradation of asparagine gives rise to acrylamide (Fig. 2).

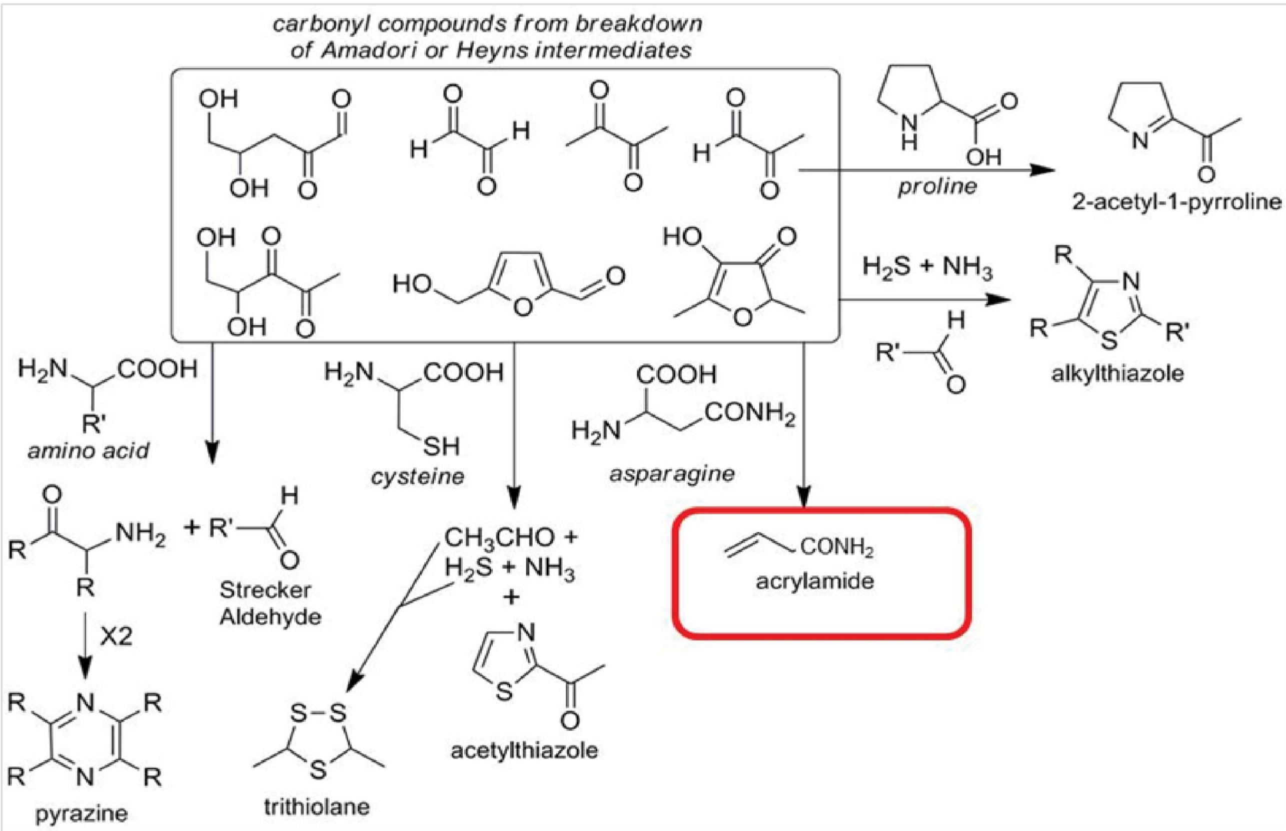

Fig. 2. Scheme for the formation of acrylamide and some classes of aroma compounds in the later stages of the Maillard reaction. Reproduced with permission from Halford et al. [5].

Amine-catalysed polymerisations of reactive intermediates from the breakdown of Amadori and Heynes rearrangement products, such as deoxysones, glyoxal, methylglyoxal, hydroxy-2-propanone, 3-hydroxy-2-butanoneglycoaldehyde and enaminols, also result in the production of melanoidins, which are brown, nitrogenous polymers (Fig. 1) [9]. The browning process is therefore often used to assess the extent of the Maillard reaction and there is usually a link between colour formation and the concentrations of other Maillard reaction products.

It is the Maillard reaction that makes a roast potato different from a boiled potato and gives French fries, potato crisps, bread crust, biscuits, rye crispbreads and a wide variety of other popular foods their characteristic flavour, aroma and texture. As well as the obvious benefits for palatability, colour and aroma, the Maillard reaction produces antioxidants, antibiotics and antimutagens. However, some of its products are considered as undesirable process contaminants, including heterocyclic aromatic amines found in grilled meat [10], furans, which have been shown to be carcinogenic in rodent models [11], and acrylamide (Fig. 2). Of these, it is acrylamide that has received most attention so far.

Acrylamide

The formation of acrylamide during high-temperature cooking and processing of mainly plant-derived raw materials was only reported as recently as 2002 [12]. Since then, the presence of acrylamide in foods has become one of the most difficult issues for the agrifood industry to deal with. Acrylamide is classified by the World Health Organization and the International Agency for Research on Cancer (IARC)as ‘probably carcinogenic to humans’, based on its carcinogenic action in rodents; it also has neurological and reproductive effects [13]. Its formation when asparagine participates in the final stages of the Maillard reaction was demonstrated in 2002 [14, 15], although additional routes from 3-aminopropionamide [16] or gluten [17] have been suggested.

In Europe, major contributors to dietary exposure to acrylamide for adults are fried potato products, coffee and soft bread, whereas for adolescents and children they are fried potatoes, soft bread, potato crisps (called chips in American English) and biscuits (http://www.efsa.europa.eu/en/press/news/datex110420.htm). Exposure is a function of concentration (Table 1) and intake, so bread is a major contributor (approximately 20 %) despite containing relatively low concentrations of acrylamide, typically below 100 parts per billion (ppb), because of the amount of bread that is consumed.

Table 1
Results of European Food Safety Authority Survey of Acrylamide Levels in Foods, 2009

Foodstuff	Mean (µ per kg (ppb))	Maximum (µg per kg (ppb))
Soft bread	32	364
Breakfast cereals	137	1435
Crisp bread	221	860
Wafers	245	725
Crackers	202	1320
Uncategorised biscuits	134	2640
French fries	327	3380
Potato crisps (US chips)	691	4804
Roast coffee	228	2223
Instant (soluble) coffee	593	1470

The mean exposure for adults in different member states of the European Union ranges from 0.43 to 1.36 µg per kg body weight per day and exposure is higher for children and adolescents. At present there is insufficient data available to enable a firm conclusion to be drawn on the risk, if any, that this level of intake represents. The toxicology studies that showed acrylamide to be carcinogenic in rodents used levels of acrylamide several orders of magnitude higher, as is usual for toxicology studies in order for effects to emerge within the time-course of the experiment and the relatively short lifespan of the animal, and to establish statistical significance. Epidemiological studies are difficult because almost everyone eats some of the affected foods. Two studies have shown a weak link between dietary acrylamide intake and endometrial cancer (cancer of the inner membrane of the uterus) [18, 19], but others have had inconclusive results [20].

Despite the lack of hard evidence on the risk that dietary acrylamide represents, the Food and Agriculture Organisation (FAO) of the United Nations/World Health Organisation (WHO) Expert Committee on Food Additives recommended that dietary exposure to acrylamide should be reduced. The food industry in Europe reacted rapidly and many strategies have been proposed for reducing acrylamide formation by modifying food processing. These are described in a ‘Toolbox’ produced by Food Drink Europe, the organization that represents European Food and Drink manufacturers (http://www.fooddrinkeurope.eu/uploads/publications_documents/Toolboxfinal260911.pdf). The methods include modification of time/temperature conditions during processing, lowering pH by addition of citric acid, pre-soaking in water, addition of antioxidants, and addition of divalent cations, such as calcium chloride. Use of these methods has resulted in significant reductions being made in some food types [21, 22]. Reducing levels of asparagine before cooking by adding the enzyme asparaginase, which converts asparagine to aspartate and ammonia, has been used successfully in some food products, such as rye crisp-breads, but it cannot be applied to all foods and has attracted adverse reaction from some quarters because it is also used as a drug in the treatment of acute lymphoblastic leukaemia and mast cell tumours. All of these methods have some limits to their application, being too difficult or expensive to apply to some foods, being ineffective in others, or having an unacceptably adverse effect on product quality. It is therefore important that the efforts being made by the food industry are augmented by a long-term programme of reduction in the acrylamide-forming potential of cereals, potatoes and other crops.

The Regulatory Situation

German regulators reacted to the news that acrylamide was present in some popular foods by setting a de facto maximum of 1000 parts per billion (ppb). Although this was not officially a regulatory limit, companies whose products exceeded this level were pressured into taking action. In some cases this resulted in adverse publicity and the shunning of products by retailers. The European Commission adopted a position that while reductions in dietary intake of acrylamide would be desirable if they could be achieved, guidelines or limits were not necessary; it was left to the food industry to take action to reduce acrylamide to levels "as low as reasonably achievable" (ALARA). However, the Commission did not object to Germany's approach and in 2011 it changed its stance and adopted a position similar to that which Germany had taken by issuing so-called 'indicative' levels (Table 2). These levels were generally significantly lower than the 1000 ppb that had been used by Germany and different levels were applied to different foods, based on the Commission's opinion of what should be achievable. The indicative levels are not regulatory limits, nor should they be regarded as a safety standard, although they have already been misused as such by the popular press. Nevertheless, the food industry is under pressure to ensure that concentrations of acrylamide in its products are below the indicative levels.

Table 2

Indicative levels for acrylamide content of cereal- and potato-based foods. Source: European Food Safety Authority (http://ec.europa.eu/food/food/chemicalsafety/contaminants/recommendation_10012011_acrylamide_food_en.pdf)

Foodstuff	Indicative level (μg per kg (ppb))
Soft bread	150
Breakfast cereals (other than muesli and porridge)	400
Biscuits, crackers, wafers, crisp bread	500
Biscuits and rusks for infants and children	250
Processed cereal-based foods for children	100
French fries	600
Potato crisps (US chips)	1000
Roast coffee	450
Instant (soluble) coffee	900
Baby foods, other than processed cereal based foods	80

There has been no national response to the acrylamide issue in the United States of America. However, the State of California took a number of food companies to court for failing to put warning labels on their products. Several companies contested the case at great expense but eventually conceded defeat in 2008 and agreed to label their products and to meet quite challenging targets for acrylamide reduction.

Factors Affecting Acrylamide-Forming Potential

The major determinant of acrylamide-forming potential in wheat and rye flour is the concentration of free asparagine [23-26]. Asparagine can accumulate in plants to very high concentrations when protein synthesis is low there is a plentiful supply of nitrogen [27], suggesting that plants use free asparagine, with its relatively high ratio of nitrogen to carbon and low reactivity,as a nitrogen store when they are unable to store nitrogen in the form of protein. Free asparagine may accumulate in response to exposure to toxic metals, pathogen attack, drought or salt stress [27]. It may also accumulate in response to a lack of nutrients other than nitrogen, such as potassium, sulphur, phosphorous and magnesium. Sulphur deficiency has a particularly dramatic effect on the concentration of free asparagine in wheat grain, causing increases of up to 30-fold, at which point free asparagine may make up more than 50 % of the free amino acid pool [23, 24, 26]. Even very small amounts of severely sulphur-deprived wheat grain should not be used as a raw material for foods in which acrylamide might form, making the even application of sulphur fertiliser over an entire wheat field very important. Furthermore, sulphur fertiliser application even at 10 kg sulphur per hectare (not untypical in the United Kingdom, for example) may not be sufficient to prevent a significant increase in free asparagine accumulation, depending on the intrinsic sulphur content of the soil [23].

Sulphur deficiency also affects the relative amounts of asparagine in different milling fractions [28], with asparagine accumulating mainly in the bran fractions under normal conditions but spreading to the endosperm (white flour fraction) when sulphur is deficient, thereby affecting more products. The recommended level of sulphur for wheat cultivation in the United Kingdom is currently 20 kg per hectare (Fangjie Zhao, Rothamsted Research, personal communication). Soil sulphur deficiency is getting worse in the United Kingdom and elsewhere because of the switch to nitric acid-based fertilisers in place of sulphur-containing fertilisers such as ammonium sulphate or superphosphate (a mixture of calcium sulphate dihydrate (gypsum) and calcium dihydrogen phosphate dihydrate) and, ironically, the reduction in atmospheric deposition of sulphur [29]. No data is available on the sulphur content of Kazakhstan soils.

Work is ongoing to screen United Kingdom and European wheat varieties and genotypes for free asparagine concentration. Data already available indicates that varietal selection could be a powerful tool in addressing the acrylamide issue. For example, an analysis of six wheat varieties grown at different locations over two harvest years in the United Kingdom [24] showed clear differences between varieties (genetic effect, G), with the average grain asparagine concentration in the best performer being 1.89 mmol per kg, while in the worst it was 2.59 mmol per kg, a difference of 37%. The study also showed significant effects of environment (E) and G×E interactions. Similarly, Taeymans et al. [30] reported that the asparagine content of different European wheat varieties harvested in 2002 ranged from 1.23 to 5.03 mmol per kg, while Claus et al.[21] reported flour asparagine content for different varieties from 0.37 mmol per kg to 1.89 mmol per kg. In all of these studies, free asparagine concentration in the grain correlated closely with acrylamide content in heated flour.

The relationship between asparagine and sugar concentrations in potatoes and acrylamide formation during processing is more complicated. Some studies have shown sugar concentrations to be the limiting factor for acrylamide-forming potential [31, 32], while another [33] found that asparagine and sugar concentrations contributed approximately equally to variation in acrylamide-forming potential within a segregating potato breeding population, while yet another [34] found that asparagine as a proportion of the total free amino acid pool was the determining factor in a study of three different potato varieties, suggesting that there was competition between different free amino acids for participation in the final stages of the Maillard reaction. This complex relationship between precursor concentration and acrylamide formation in potato requires further investigation. Currently, the best advice for potato is that the concentrations of reducing sugars, free asparagine and other free amino acids must all be considered in variety selection [8].

Implications for Kazakhstan Agriculture and Food Production

Awareness of the acrylamide issue amongst Kazakhstan's farmers, food processors, retailers, consumers and political leaders appears to be very low. While that is the case, the home market for foods derived from potatoes and cereal grains, as well as imported foods that are involved, such as coffee, is likely to be unaffected. However, the situation may change, and the food and agricultural industries should be prepared if consumer attitudes or the regulatory situation change. Of more immediate concern is the export market. Kazakhstan is the one of the leading grain producers in the world. The country produced 22 million tonnes (mt) of wheat grain in 2011, the biggest harvest since the Soviet era; approximately 2.5 mt was used for domestic food production, 5.5 mt for animal feed and the rest wasexported either as grain or flour (although exports were delayed by transport problems). Strong breadmaking and durum varieties are preferred, with a high content of gluten (up to 30% and more). These are the grades which enjoy high demand in the world market. The countries that imported Kazakhstan's wheat were Iran, Turkey, Greece, Albania, Cyprus, Italy, Georgia and Azerbaijan, so they included three countries within the European Union. If it is to protect this market, Kazakhstan will have to make sure that the acrylamide forming potential of its wheat grain and flour is low enough to enable European food processors to comply with European indicator values, bearing in mind that there is the potential for indicator values to be progressively reduced over time and to become regulatory limits.

Grain production in Kazakhstan is a leading sector of the economy. The major wheat growing areas are in the North Kazakhstan, Kostanai and Akmola oblasts in the north of the country. The soil in these regions is regarded as inherently fertile, but the yield is extremely low at 1-1.5 tonnes per hectare (for comparison, average wheat yield in the UK is over 8 tonnes per hectare); this suggests that most of the production is low intensity, with minimal inputs, despite the fact that fertiliser is heavily subsidised by the state. Most (95 %) of the wheat that is grown is spring wheat, and in 2012 production has been affected by a late frost. However, the main environmental factor affecting yield in most years is drought (there is very little irrigation), and some areas are also affected by high salt concentrations. The extremely high temperatures of the Kazakh summer are also likely to impact yield. On the other hand, the hot, dry conditions and the fertility of the soil combine to make Kazakhstan wheat mostly of very high quality.

It is difficult to say what impact these factors have on the acrylamide forming potential of Kazakhstan's wheat grain. Work is ongoing internationally to investigate the effects of environmental factors, including drought, heat and salt stress, on the free asparagine content of wheat grain but has not yet been completed. Traditionally, plant physiologists have focussed on the effects of environment on the vegetative tissues of plants because it was believed that responses in the vegetative tissues were more important for plant stress tolerance than responses in the grain. Only now is the link between plant stress responses, plant metabolism and crop quality being realised and investigated [35]. A worthwhile assessment of the potential impact of the acrylamide issue on Kazakhstan's wheat production and processing industries requires data on the typical free asparagine content of Kazakhstan wheat grain and the effect of Kazakhstan's sometimes extreme growing conditions.

Conclusions

The Maillard reaction produces the flavours, aromas and colours that make fried, baked and roasted foods so popular with consumers, as well as compounds believed to be beneficial to health, but it also produces compounds that are potentially harmful. Foods are complex mixtures of compounds and as the techniques for identifying the different components that are present improve it seems likely that other undesirable compounds will be discovered. This should be looked on positively because it will eventually lead to safer food. However, the issue of contaminants such as acrylamide looks likely to be a challenging one for the agrifood industry, as it seeks to operate within an increasingly complex regulatory environment while retaining the characteristics of products that consumers demand. The industry also faces the problem of communicating risk and benefit to the media and consumers, something that 'food scares' of the last thirty years, such as that associated with the Bovine Spongiform Encephalopathy (BSE) outbreak in the United Kingdom in the 1980s, and the ongoing debate on genetically modified crops in Europe and elsewhere, including Kazakhstan, have shown to be extremely difficult.

The as yet unknown risk, if any, of eating foods that contain acrylamide, must be set against the health benefits of eating some of the foods that are involved, and the contribution that cereal and potato products make to meeting our nutritional needs. Cereal and potato products are a valuable source of energy in the form of complex carbohydrate and an intrinsic part of the everyday diet of people throughout most of the world. Wheat is a valuable source of fibre, protein, B vitamins, iron, calcium, phosphoric acid, zinc, potassium and magnesium [36] and, uniquely, is used to make an array of products, including bread, noodles, cakes, biscuits/cookies, steamed bread, doughnuts, croissants, bagels, pizza, flat breads and chapattis, as well as the Kazakh specialities of bread, bauyrsaqs, shelpeks (chapattis fried in oil or routees), chak-chaks and fried dumplings. Nevertheless, raising awareness of the dietary acrylamide issue in Kazakhstan will enable the agrifood industry to be better prepared to protect consumers if more evidence emerges on the risk that dietary acrylamide poses, and to comply with the evolving regulatory situations in Kazakhstan's export markets. This will help to ensure the sustainability of Kazakhstan's cereal and potato production and processing industries in the coming years.

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