Quantifying the formation of carcinogens during food processing: acrylamide

Wendie L. Claeys, Kristel De Vleeschouwer and Marc E. Hendrickx*

Department of Food and Microbial Technology, Faculty of Agricultural and Applied Biological Sciences, Katholieke Universiteit Leuven, Kasteelpark Arenberg, 22, B-3001 Heverlee, Belgium (Tel.: +32 16 32 15 85; fax: +32 16 32 19 60; e-mail: marc.hendrickx@agr.kuleuven.ac.be)

The presence of acrylamide has recently been demonstrated in diverse food products. A number of research studies followed concerning the effect of intrinsic and extrinsic factors on the yield of AA in processed food. However, reported data are mainly qualitative in nature, which does not allow the development of engineering models for design, evaluation and optimization of food processes. In this paper, the need for more quantitative research (kinetic and thermodynamic modeling) is emphasized by outlining a possible strategy based on a state of the art of research results available in literature.

During processing and preservation of food products heat treatments (cooking, baking, roasting, frying, sterilization) at temperatures between 90 and 220 °C are often applied. Such high temperatures may lead to the formation of toxic compounds reducing the nutritional value and safety of foods. Well-known carcinogenic/mutagenic compounds

* Corresponding author.

thus formed are e.g. heterocyclic amines (Knasmüller, Murkovic, Pfau, & Sontag, 2004), polycyclic aromatic hydrocarbons (Knize, Salmon, Pais, & Felton, 1999), and N-alkyl-N-nitrosamines (Tricker & Kubacki, 1992). Recently, also acrylamide (AA), produced commercially for many years for a variety of technical applications (e.g. as polymers for paper and textile industry, as soil conditioners, in waste water treatment), was added to this list. In April 2002, the 'Swedish National Food Administration' (SNFA) highlighted its presence in different foodstuffs (FAO/WHO, 2002). For some products, the measured amounts exceeded amply the drinking water limit of 0.5 µg AA/kg (WHO, 1996) and the existing European Union limits on chemical migration from plastic packaging of 10 µg AA/kg (E.C., 1992).

AA has been classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans (Group 2A) (IARC, 1994). The toxicology of AA (carcino- and mutagenicity, neuro- and reproductive toxicity) is discussed amongst others by Friedman (2003), Rudén (2004), Tareke (2003), and WHO (1996). AA is mainly reactive through its double bond (Fig. 1). Polymerization of AA e.g. occurs through radical reactions with the double bond. AA can also react as an electrophile by 1,4-addition to nucleophiles such as SH- or NH_2-groups in biomolecules. In the body, AA can be metabolized to glycidamide, a reactive compound formed through epoxidation and considered as a mutagenic and cancer inducing compound, or form an adduct to hemoglobin, which is used as a measure of AA exposure.

A number of theoretical mechanisms have been proposed for the formation of AA in heated food. Most probably, AA in food results largely from the Maillard reaction between amino acids (primarily asparagine) and a reactive carbonyl (e.g. glucose), proceeding through intermediates that include a Schiff's base (Mottram, Wedzicha, & Dodson, 2002; Stadler, Blank, Varga, Robert, & Riediker, 2002; Zyzak et al., 2003). In this reaction, acrolein could be one of the possible precursors of AA. Next to the Maillard reaction, acrolein (2-propanal) can also be formed by transformation of lipids or by degradation of amino acids and proteins, and of carbohydrates (Gertz & Klostermann, 2002; Lingnert et al., 2002). Oxidation of acrolein to acrylic acid and subsequent reaction of acrylic acid with ammonia generated from the pyrolysis of nitrogen-containing compounds present in food, results in the formation of AA
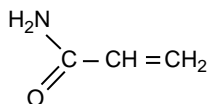
$$H_2N$$
$$C - CH = CH_2$$
$$O$$

Fig. 1. Structure of acrylamide.

(Becalski, Lau, Lewis, & Seaman, 2003). It is also suggested that alternatively, AA could be formed directly from rearrangement of nitrogen-containing compounds present in food without necessary involving acrolein (Becalski *et al.*, 2003; Lingnert *et al.*, 2002). Polyacrylamides from herbicides or used during soil preparation are no source of more AA in heated foods (Jongsung & Castle, 2003).

Analysis of acrylamide

Several analytical methods have been used to quantify AA in food. Gas chromatography (GC) with mass spectrometric detection (MS) with and without derivatization (bromination), and high-performance liquid chromatography (LC) with (tandem) MS appear to be the most commonly used methods (Gertz & Klostermann, 2002; Andrzejewski, Roach, Gay, & Musser, 2004; FDA, 2004; Jezussek & Schieberle, 2003; Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002; Wenzl, de la Calle, & Anklam, 2003). Differences between procedures are mainly situated at the level of sample preparation, namely AA extraction and clean-up. No uniform analytical method applicable to all food products currently exists (Wenzl *et al.*, 2003).

Most methods have shown limits of detection (LOD) of about 10–30 µg/kg (de la Calle, Ostermann, Wenzl, & Anklam, 2003). Results obtained using GC-MS, LC-MS or LC-MS/MS are generally comparable (de la Calle *et al.*, 2003; Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002; Jezussek & Schieberle, 2003).

Acrylamide in processed food and model systems

A range of AA levels has been reported in different food and food products (Becalski *et al.*, 2003; FDA, 2004; FSA, 2002; Konings *et al.*, 2003; Lingnert *et al.*, 2002; SNFA, 2002; Tareke *et al.*, 2002). So far, no AA has been detected in raw or boiled food. When heated above 100–120 °C, highest levels of AA were found in carbohydrate-rich food (100–4000 µg/kg) and lower levels in protein-rich food (< 100 µg/kg). AA levels showed significant variability within particular food categories and within batches of products processed under the same conditions. Generally, studies are mainly applied on fried and baked cut potato and to a lesser extent on baked cereal products. However, depending on consumer's consumption, products with a relatively low AA level can contribute significantly to the total dietary intake of AA and may not be disregarded. A good example is coffee, in which AA is formed during the roasting process (Andrzejewski *et al.*, 2004; Granby & Fagt, 2004).

Real food contains a number of variables that can influence AA formation. Not only intrinsic properties or compositional factors that in turn can be affected by e.g. storage and seasonal variation, but also extrinsic factors such as additives and heating medium, can affect AA formation significantly. As a consequence, results obtained in different tests or experiments may diverge. While the individual effect of variables on AA formation has to be known, the cocktail effect of such mixtures is most important. Only understanding at this level will allow determining accurately the specifications for foods and other systems with respect to AA formation. Therefore, it is interesting to start from simple and thus reproducible model systems under static conditions that in a second stage can be translated to a more complex in situ environment under dynamic conditions (real food system, factory setting).

AA is mainly formed through the Maillard reaction. Consequently, factors affecting the primary stage of the Maillard reaction such as pH and water activity (a_w) will most likely also influence the AA formation. Starting points of the Maillard reaction and AA formation are reducing sugars and amino acids, in particular asparagine. Table 1 summarizes values of sugars and asparagine in different food products reported in literature.

Next to reducing sugars and amino acids, it was suggested that also fat and oils could participate in the AA formation pathway. Another factor that needs to be considered is the general background of the food system (i.e. carbohydrate-rich versus protein-rich matrix).

Reducing sugars

Starch and sugars such as sucrose, lactose and maltose, can easily hydrolyze upon heating at temperatures above 100 °C at slightly acidic pH. Consequently, thermal processing often results in a continuous supply of monosaccharides formed from complex carbohydrates (Lingnert *et al.*, 2002). Zyzak *et al.* (2003) demonstrated that a variety of monosaccharides (e.g. glucose, deoxyglucose, ribose, glyceraldehyde, glyoxal) can generate AA from asparagine. The shorter the sugar chain, the more AA was formed, which is in accordance with the Maillard reaction. Generally, the shorter the carbon chain of the sugar, the greater the lysine loss in the Maillard reaction and thus the more reactive the sugar. As the sugar chain is shorter, the molecule is strained to form a cyclic hemiacetal structure and subsequently the carbonyl becomes more readily available for nucleophilic attack from the α-amine of asparagine.

The yield of AA is reported to be proportional to the glucose and fructose levels of the food (Biedermann-Brem *et al.*, 2003; Chuda *et al.*, 2003). For example, the AA content in heated potato increased linearly when glucose or fructose was added up to a concentration of about 3%. With higher additions the AA content diminished or its increase

Table 1. Concentration (mg/kg) of sugars and free amino acids in different food products

Product	Asparagine	Glucose	Fructose	Sucrose	Comments	References
Potato	2010–4250	97–2550	30–1537	430–1597	Different cultivars, FW[a]	Amrein et al., 2003
	6350–36540				Differing nutrient levels (Bintje), DW[b]	Eppendorfer, 1996
		170–270	60–250	1480–2080	Panda, Saturna and Tomensa variety, FW	Haase et al., 2003b
		97–1800	24–330		Fontane, Lady Claire and Naturella with different N-fertilization, FW	Biedermann, Noti, Biedermann-Brem, Mozzetti, & Grob, 2002a
	11500	370	180		DW	Biederman & Grob, 2003
Maize	< 10				Corn starch	Biederman & Grob, 2003
		64–139	155–156	73–366	Yellow corn from cob, FW	Li, Andrews, &, Pehrsson, 2002
	596–1073				Flour (maize Oh545o2 inbred)	Wang, Stumpf, & Larkins, 2001
Wheat	167				Flour	Mottram et al., 2002
	270				Dark flour	Biederman & Grob, 2003
Rye	173				High protein rye	Dembinski & Bany, 1991
		750–760	450–550	9070–12000	Flour	Haase et al., 2003a
Coffee	~ 22–30	5000–6500[c]		18000–95000	Beans; increasing from immature (black) to mature beans	Mazzafera, 1999
Spinach	505–1621				Differing nutrient levels	Eppendorfer, 1996
		2	51		FW	Li et al., 2002
Cocoa	309				Powder	Bonvehi & Coll, 2002
Meat	0.17–0.40				Bovine, different muscles	Feidt, Petit, Bruas-Reignier, & Brun-Bellut, 1996
	1.1–10.7				Pork, different quality classes	Flores, Moya, Aristoy, & Toldrá, 2000

[a] Fresh weight.
[b] Dry weight.
[c] Reducing sugars.

slowed down, presumably because of increased elimination (Biedermann & Grob, 2003).

As to the reactivity, Stadler et al. (2002) found fructose, galactose, lactose and sucrose to release AA with comparable yields. Becalski et al. (2003) on the other hand, observed a lower relative yield of AA from a dry asparagine-sugar system when sucrose (0.48) was used compared to glucose (1) or fructose (1.8). Also, addition of 40% sucrose to dried grated potato or to wheat flour did not result in increased AA levels (Biedermann et al., 2003). Comparing glucose and fructose, fructose appeared to result in a higher AA yield than glucose in an asparagine-sugar system or when added to dry potato or to wheat flour (Biedermann, et al., 2002a; Biedermann & Grob, 2003; Becalski et al., 2003; Leufvén & Lingnert, 2003).

Amino acids

By means of isotope substitution experiments, it was demonstrated that asparagine and not the reducing sugar directly provides the backbone of the AA molecule (Zyzak et al., 2003). The asparagine content represents 40% of the total amino acid content of potato (Martin & Ames, 2001)

and 14% and 18% of the total free amino acids of wheat flour and high-protein rye, respectively (Dembinski & Bany, 1991; Mottram et al., 2002). In terms of asparagine/glucose ratio, a 0.5 to an equimolar ratio seems to be favorable for AA formation (Becalski et al., 2003; Leufvén et al., 2003).

Even though amino acids in general and asparagine in particular play a primary role in the formation of AA in heat-treated food, the AA content seems to be little or not at all correlated with the total amino acid content or with the concentration of asparagine in potato tubers (Amrein et al., 2003; Chuda et al., 2003). In wheat and rye flour however, a high correlation between the AA yield and the level of asparagine was observed. Moreover, addition of fructose to wheat endosperm flour dough with low asparagine and fructose content did not seem to influence the AA content in the crust of yeast-leavened bread, whereas added asparagine significantly increased the AA content (Surdyk, Rosén, Andersson, & Aman, 2004). Asparagine seems thus to be as determining for AA formation in cereals as the reducing sugars in potato (Biedermann et al., 2003; Springer, Fischer,

Lehrack, & Freund, 2003; Surdyk *et al*., 2004). The content of free asparagine in potatoes is generally more abundant than sugars, whereas for wheat and rye the asparagine content is relatively low (Table 1).

The formation of AA from amino acids other than asparagine is not clear-cut. No or only trace amounts of AA have been reported to be formed in a model system consisting of glutamine, methionine, cysteine, aspartic acid, glutamic acid or an amino acid mixture of serine, threonine, alanine, proline and phenylalanine, and glucose (Mottram *et al*., 2002; Stadler *et al*., 2002; Leufvén *et al*., 2003). Nonetheless, impurities in stock amino acids almost certainly account for some AA. Becalski *et al*. (2003) e.g. reported asparagine (0.3%) and cysteine (0.8%) impurity in their aspartic acid and glutamine standard, respectively.

Fats and oils

A hypothetical mechanism that has been suggested for AA formation is through acrolein formed from glycerol or as a result of oxidation of polyunsaturated fatty acids and their degradation products. When oil is heated at temperatures above smoke point, glycerol is degraded to acrolein. Formation of acrolein is known to increase with an increased level of unsaturation in the oil and to lead to a lowering of the smoke point (Lingnert *et al*., 2002).

However, it seems that AA is not principally formed from precursors (especially acrolein) present in oil itself and that AA formation is non-oxidative in nature (Biedermann & Grob, 2002a; Becalski *et al*., 2003; Leufvén *et al*., 2003; Tareke, 2003; Vattem & Shetty, 2003). Oxidized fats could compete with other carbonyl compounds for only a trivial role in AA formation through the asparagine/carbonyl pathway. The type of oil, on the other hand, could influence the rate of AA formation (see "Heating Medium" section).

Carbohydrate-rich versus protein-rich matrix

Roasted or fried meat and fish products contain low AA values compared to potato or cereal products (Biedermann & Grob, 2003; Tareke, 2003; Vattem & Shetty, 2003) and more AA is formed when reaction mixtures of asparagine and glucose are added to a potato starch matrix compared to whole wheat flour or beef homogenate matrices (Leufvén *et al*., 2003). It is clear that the food matrix has an influence (promoting, neutral or retarding) on AA formation, but it is not yet totally elucidated to which factors this effect can be ascribed. Catalytic factors associated with the matrix as well as the water binding capacity of the matrix, the matrix favoring competitive reactions, and a capacity of the matrix to retain AA formed by further reaction or by bond-formation are all proposed theories. The influence of the matrix does not appear to be a consequence of shortage of precursors. When corn starch

and wheat flour were fortified with asparagine to the concentration in potato, the AA concentration formed was still five times lower than in potato (Biedermann & Grob, 2003). Also the degree of AA elimination was substantially lower in wheat flour or starch than in potato (Biedermann *et al*., 2002a).

Considering a protein-rich versus a starch-rich food product, Biedermann *et al*. (2002a) observed that the degradation of AA was more pronounced in a beef system than in other matrices, which supports that in this case the elimination mechanism may be of importance. The binding of AA to proteins in the beef matrix is a plausible explanation since AA is known to react with amino acids in proteins. The adduct formation to hemoglobin is an example. The hypothesis is further supported by the observation that addition of β-lactoglobulin to a glucose-asparagine model system resulted in an even lower AA concentration than when beef homogenate was added (Leufvén *et al*., 2003).

Addition of lysine to a glucose-asparagine model system or to homogenate potato samples also lowers the yield of AA significantly (Becalski *et al*., 2003; Leufvén *et al*., 2003; Rydberg *et al*., 2003). In this case, most probably the high activity of lysine in the Maillard reaction, attributed to the reactivity of its free amino group, is responsible for the diminished yield of AA. The presence of cysteine in a glucose-asparagine system decreased the formation of AA as well (Leufvén *et al*., 2003). Here both hypotheses apply, namely cysteine influences the reaction paths leading to AA and/or AA is reacting with cysteine. A reduced AA yield was also observed when glycine, alanine, glutamine or glutamic acid was added to homogenate potato samples (Rydberg *et al*., 2003).

Hardly any AA is formed in purified starch. Starch is not involved in AA formation and does not seem to influence the rate of AA elimination. In contrast to starch that appears to be a rather inert medium, protein-rich media enhance AA elimination, thus hampering high AA concentrations to build up (Biedermann *et al*., 2002a).

Regarding the size of the food matrix particles, i.e. coarse particles against fine powder, it does not seem to influence the AA yield in grated potato (Biedermann, Biedermann-Brem, Noti, & Grob, 2002b), but this may not be generalized. For cereals, e.g. the extent of milling and thus flour particle size influences the protein and starch contents of the cereal. A portion of the starch granules are mechanically damaged, of which the level varies with the intensity of grinding and the hardness of the cereal. The more damaged the starch granules, the higher the susceptibility for enzymatic hydrolysis (Belitz & Grosch, 1987). The extent of milling affects both the asparagine and the reducing sugar content. Accordingly, lower levels of AA can result from less milled flours

(Haase, Matthäus, & Vosmann, 2003a; Springer *et al.*, 2003).

pH

Formation of AA is like the Maillard reaction dependent on the pH of the system. The pH will influence the reactivity of both the sugar and the amino group. A higher pH favors the open chain form of the sugar and the unprotonated form of the amino group, considered to be the reactive forms. The suggested optimum pH for AA formation is around 7–8 (Brown, 2003; Rydberg *et al.*, 2003). The AA formation in a model system containing asparagine and glucose could be diminished with 99% by lowering the pH of the model phosphate buffer from 7 to 4 (Jung, Choi, & Ju, 2003). By lowering the pH the free non-protonated α-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 AA.

Jung *et al.* (2003) demonstrated that lowering the pH by soaking baked or fried corn chips and French fries in a citric acid solution of 0.1–0.2% or 1–2%, respectively, results in lower AA levels in the product. Similar observations were made by Pedreschi, Kaack, and Granby (2004) for 1 and 2% citric acid treated potato slices fried at 150 °C. There appeared to be no significant removal of asparagine or sugars from the in citric acid immersed samples, so that the reduction of AA seems to be principally a pH effect. In their frying experiments at 170 and 190 °C however, no significant effect of citric acid was observed on the AA content. A drawback of immersion of food products in a citric acid solution is possible souring or change in color and texture if a precise procedure is not followed. Furthermore, also frying formulations can be affected and become rancid.

Another factor altering the pH is heating itself. Heating can cause a decrease in pH because of increased water dissociation and water activity in the crust producing Maillard products (Gertz & Klostermann, 2002).

Humidity and a_w

AA formation seems to be mainly a surface phenomenon. Formation of AA is limited to cooking methods that result in drying with at least crust formation (FAO/WHO, 2002; Lingnert *et al.*, 2002). Formation of AA in 'wet' samples (e.g. fresh potato or dough) is negligible.

When humidity of potato samples was situated between 10–20%, the amount of AA was comparable to the amount observed in 'dry' samples. This seems to be in contrast with the Maillard reaction being most efficient at 12–18% humidity (Eichner & Karel, 1972). However, at a low humidity not only formation but also elimination is accelerated, holding the net AA content in the potato samples approximately constant (Biedermann *et al.*, 2002b).

The AA formation and elimination reaction take likely completely different routes, but no explanation is at hand for their similar acceleration at 10–20% humidity. In dough of white wheat flour, the performance seems to be different compared to potatoes. When the dough contained approximately 10% residual water, a tenfold lower AA content was measured than when the dough was dry (Biedermann *et al.*, 2002b).

In situ environment

Extrapolation of results observed in a model system to a real food system involves an increase in complexity with the AA content being the net result of inter-related intrinsic (e.g. cultivar) and extrinsic (e.g. storage, pre-treatment) variable factors. Additionally, when going from a closed to an open system, the relevant temperature and conditions for the formation of AA depend on the heat and mass transfer phenomena taking place.

Heating medium

Because food and heating medium (dry air, oil) are directly in contact with each other, dynamic heat and mass transfer phenomena have to be taken into account. Factors that affect heat and mass transfer and therefore AA formation, are the thermal and physicochemical properties of the food, the heating medium and the process equipment, the geometry of the food and the temperature of the heating medium.

The heating medium often reaches temperatures of e.g. 180–200 °C, but the food itself is cooled by moisture evaporation. Most foods stay humid inside, with temperature decreasing from a high value at the surface to approximately 100 °C below the crust. Temperatures above 100 °C are reached only when the food is almost completely dry. During frying in oil, mass transfer phenomena taking place mainly concern an exchange of water and oil or fat. Water vapor creates a kind of buffer around the food, restricting the heat transfer and keeping the temperature in the food below the temperature of the oil. The type of the frying medium and additives (e.g. anti-foaming agent) may influence the heat transfer by changing the surface tension of the oil. Change of surface tension affects the size of the vapor bubbles surrounding the food and their release, and as a consequence the heat transfer. For example, in French fries a higher content of AA was observed when palm oil was used or when silicone was added to the frying oil (Gertz & Klostermann, 2002). Palm oil contains 6–8% diglycerides that probably increase the heat transfer by emulsification of the components. A correlation between the degree of oil degradation expressed as the content of polar materials and AA level was not found. Tareke (2003) observed an enhanced AA formation in potatoes when different oils (sesame, corn and olive oil) were added, dependent on the

amount and type of oil used. When the oils were heated alone, no detectable AA levels were observed (see "Fats and Oils" section).

Due to water evaporation, results can differ when the amount of AA is expressed in terms of weight prior to heating or in terms of dry weight. For potatoes e.g. containing ∼80% water, the weight loss during heating in an oven is mainly due to evaporation of water and can be as high as 50% of the fresh weight (Rydberg *et al.*, 2003; Tareke *et al* 2002). This implies that AA values expressed on a fresh weight basis can be two times higher than when expressed on a dry weight basis. When heating in a frying pan instead of in an oven, the loss of water will be partially compensated by oil uptake and weight loss will therefore be less. Water loss and oil uptake phenomena become more intense at higher temperatures and smaller sample thickness (Krokida, Oreopoulou, & Maroulis, 2000).

Cultivar

Different cultivars of e.g. cereal or potato are characterized by different concentrations of precursors, which is reflected by the range of asparagine or sugar content observed in Table 1. Some comparative data of sugar and amino acid concentration for various potato cultivars together with their potential for AA formation are given by Amrein *et al.* (2003). Biedermann *et al.* (2002a) compared the AA formation in commercial potato samples of different cultivars. Results did not disprove an influence of the cultivar, but indicated that other factors seemed to be at least equally important. As the samples were from commercial sources, e.g. storage conditions could not be verified.

Also individual potato tubers sometimes differ strongly in their AA formation due to amongst others differences in their sugar content. Some potatoes, e.g. are slightly greenish (i.e. influence of light) and can contain 5.5 times more reducing sugar (Biedermann *et al.*, 2002a).

Storage

Cooling of potato tubers below 8–10 °C causes a strong increase in reducing sugars that are released from the starch, a process known as 'low temperature sweetening' (LTS). The liberated sugars protect the potato against frost. Unfortunately, a high reducing sugar concentration enhances the AA formation (Biedermann *et al.*, 2002b; Blenkinsop, Copp, Yada, & Marangoni, 2002; Noti *et al.*, 2003; & Olsson, Svensson, & Rosmund, 2004). Even short term cooling of potatoes (around 20 days) at 4 °C strongly increases the potential of AA formation. Noti *et al.* (2003) observed a kind of lag phase of ∼5 days before the reducing sugar content and the potential for AA formation started to rise. However, this increase seems not to be avoidable by storage at 4 °C for less than 5 days. After a storage period of 40 h at 4 °C, during which no increase was observed, the potential for AA formation showed a significant rise. It was

suggested that this could be due to a higher enzyme activity at ambient temperature. When potatoes stored at low temperature are reconditioned at higher temperatures (12–25 °C), the content of reducing sugars decreases again. Long-term LTS though, is considered to be irreversible (Biedermann *et al.*, 2002a; Blenkinsop *et al.*, 2002; Noti *et al.*, 2003).

With respect to the free amino acid content during storage, different observations were made probably related to the length of the storage period. Olsson *et al.* (2004) observed independently of storage temperature similar levels of asparagine and glutamine in potato tubers stored during 8 months at 3 and 10 °C. Brierley, Bonner, & Cobb (1997) observed after a storage period of 25 weeks an equal accumulation of asparagine and glutamine, but the accumulation was greater at 10 °C than at 5 °C and could be linked to an upturn in proteinase activity on the break of dormancy.

Next to temperature, also light seems to activate potatoes, initiating an increase in the concentration of reducing sugars (Biedermann *et al.*, 2002a). So, potatoes should be stored in the dark and above 8 °C. Storage at temperatures higher than 8 °C involves however, a practical problem for long term storage. To maintain a potato supply throughout the year, it is necessary for producers to store potatoes for periods of several months. This can only be achieved successfully if the storage conditions prevent the potatoes from spoiling or sprouting. Alternatively to cooling at lower temperatures, chemical sprout suppressing agents can be used. The use of such chemicals though, may not be permitted and is not always desired by the consumer. Sprouting itself has only a weak effect on AA formation. Stopping sprouting (breaking of the germs at an early stage) does not result in potatoes with a high potential for AA formation (Noti *et al.*, 2003).

According to Noti *et al.* (2003) freezing of potatoes should have no effect on sugars nor on AA formation, i.e. there is no objection against using frozen prefabricates e.g. for the preparation of French fries. Tareke *et al.* (2002) on the other hand observed that freezing of potatoes before heating enhanced AA formation. It was suggested that this could be due to rearrangement of starch and the breakage of cell walls leading to increased available concentrations of AA precursors. Cooling of cooked potatoes has no effect on AA formation because the enzymes that liberate the sugars are inactivated (Noti *et al.*, 2003).

Cereals can be stored without loss of quality for 2–3 years, provided that the kernel moisture content, which is 20–24% after threshing, is reduced to at least 14% (Belitz *et al.*, 1987). No information was found on the effect of storage conditions on AA precursors in baking flour. Concerning finished bakery products, Biedermann and Grob, (2003) observed a decrease of AA content in gingerbread from 850 to 500 µg/kg during storage at room temperature for a period of about 6 months (see also "Additives and Miscellaneous" section).
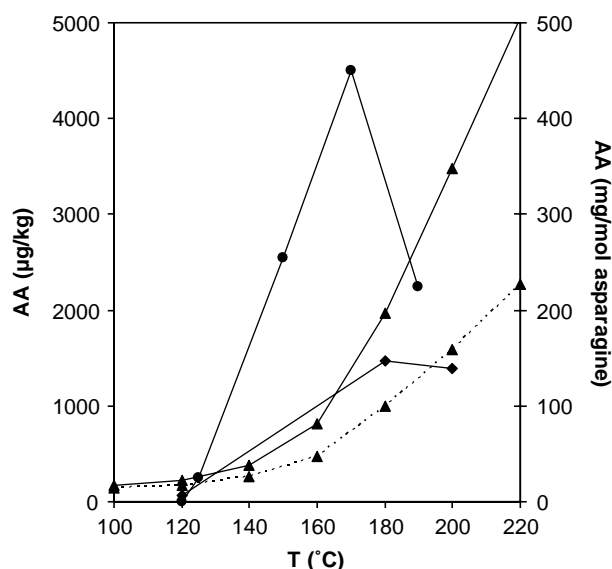
Fig. 2. Acrylamide levels (AA) measured in (i) a "wet" glucose:asparagine (1:1) model system heated in sealed glass tubes for 20 min (•, mg/mol asparagine, Mottram et al., 2002), in (ii) French fries heated in an oven for ~15 min (▲, µg/kg product, Tareke et al., 2002), and (iii) in wheat flour heated in an oven for 30 min (♦, µg/kg product, Biedermann & Grob, 2003). Values with and without compensation for weight loss are presented by a dotted and a full line, respectively.

Strategies for lowering AA

An obvious way to reduce the AA content in food is by adjusting the process parameters time and temperature or by lowering the amount of AA precursors. Other strategies for
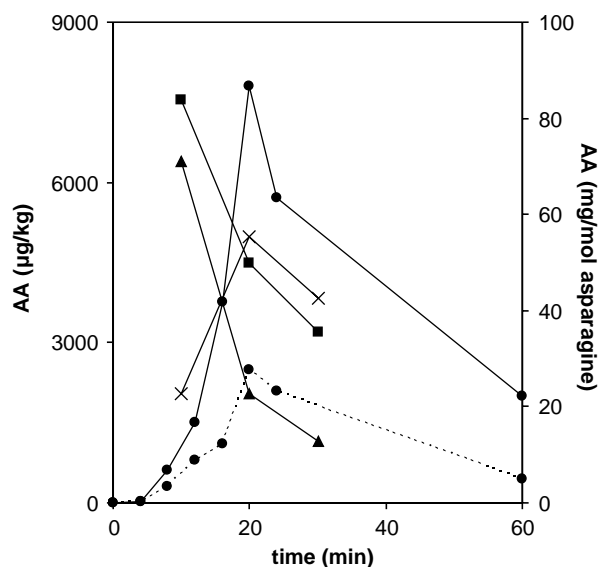

Fig. 3. Formation of acrylamide (AA) in (i) a 'dry' glucose:asparagine (1:1) model system heated in an oven at 155, 165 and 185 °C (x, ■, ▲, respectively, mg/mol, derived from Becalski et al., 2003) and in (ii) potato strips heated in an oven at 200 °C (•, µg/kg product, derived from Rydberg et al., 2003). Values with and without compensation for weight loss are presented by a dotted and a full line, respectively.

diminishing AA formation mainly involve pre-treatment or the use of product-related additives.

Process parameters

The level of AA increases with increasing temperature and time (Figs. 2 and 3). AA levels can thus easily be reduced by lowering temperature and time if possible. However, after reaching a maximum, the level of AA decreases again indicating that excess baking, frying, roasting can have a similar effect on AA levels (Becalski et al., 2003; Biedermann et al., 2002a; Mottram et al., 2002; Rydberg et al., 2003). This decrease in AA content is not correlated with the decrease of reducing sugars (in potato samples) and is thus not the result of an exhaustion of precursor sugars. The decrease in AA content appears neither to be due to drying (Biedermann et al., 2002a). Probably it can be attributed to AA elimination presumably by bonding to reactive food constituents, such as thiol functions (see "Carbohydrate-rich versus protein-rich" section), or by further reaction.

Reactants

Since the level of AA formed is positively correlated with the reducing sugar content, lowering the amount of reducing sugars will result in less AA. Reducing sugars are however, necessary for amongst others browning. For the production of, e.g. French fries and potato chips, a potato cultivar with low reducing sugar content is selected, because fries have to be crispy before browning starts. Potatoes containing less than 0.2 g/kg reducing sugar on the other hand are not suitable for such products for which some browning and roasting flavor is required. For roasted products of optimum culinary quality, potatoes have to contain 0.2–1.0 g/kg reducing sugars (Blenkinsop et al., 2002; Biedermann & Grob, 2003).

Instead of diminishing the amount of reducing sugars, sugars reactive in AA formation could in some cases be replaced by less reactive ones, taking their properties into account. In bakery ware, AA contents could thus be reduced by exchanging fructose by glucose (see "Reducing sugars" section).

On the level of amino acids, the extent of heat-induced AA formation in food can be reduced by selecting cultivars that contain low levels of asparagine and/or by removing (hydrolysis) or blocking asparagine prior to processing. In case of cereal products, grain varieties can be selected for lower asparagine levels (Table 1). The nutritional and qualitative effects of selecting alternative cultivars should be carefully considered. For example, a highly significant relationship was observed between food color and AA content. Addition of asparagine to wheat endosperm flour dough, however, did not increase the color of the wheat bread crust showing that mainly other amino compounds are involved in the browning reactions (Surdyk et al., 2004).

Hydrolyses of asparagine to aspartic acid and ammonia can be performed chemically (acid- or base-catalyzed) or enzymatically with asparaginase or other amidases. Zyzak et al. (2003) evaluated the effectiveness of asparaginase in reducing AA formation in a microwaved mashed potato product. Asparaginase pre-treatment of the snack produced an asparagine reduction of 88% and an AA reduction larger than 99% when compared to samples prepared by the exact same process but without asparaginase pre-treatment. A limitation of this method is that disruption of cell membranes is needed to liberate asparagine and allow it to come into contact with asparaginase. Asparaginase is already widely used in medicine in the treatment of childhood acute lymphoblastic leukemia (Sallan et al., 1983). Nevertheless, assessment of the safety of asparaginase-treated food and evaluation of available enzyme preparations for their ability to hydrolyze asparagine in food should be performed (Friedman, 2003).

Another approach for diminishing the available asparagine is blocking the primary amine group of asparagine thus preventing formation of N-glycoside intermediates that form AA. Similarly, asparagine occurring as a protein component does not have an accessible primary amine group for Schiff's base formation and would not be expected to participate in the production of AA. An effective analogue test was performed where asparagine was acetylated to N-acetylasparagine (Friedman, 2003).

Pre-treatment

Pre-treatment as a means of lowering the AA level in heated food is aimed at lowering the amount of AA precursors in the food. Blanching of potatoes for example, is applied to reduce the formation of brown spots during heating by extracting reducing sugars at the surface. In addition, blanching reduces the oil absorption by gelatinization of the surface starch. Blanching extracts asparagine as well and consequently, results in less AA formation. Soaking in cold water is less efficient than blanching, because blanching renders membranes permeable thus facilitating the extraction of AA precursors (Haase, Matthäus, & Vosmann, 2003b; Jung et al., 2003; Pedreschi, et al., 2004). Extraction by moving water or by movement of the potato in still water additionally washes the starch out of the open cells. Starch however, should be available at the surface to support crust formation. Similar to blanching or soaking, boiling of potatoes prior to frying attenuated the formation of AA (Tareke et al., 2002).

In case of grain, the extent of milling determines indirectly the amount of AA that potentially can be formed (see "Matrix" section). With regard to coffee, convection roasting has been indicated as a possible approach for decreasing the levels of AA formed, but whether this would be feasible is not clear yet (E.C., 2003).

Additives and miscellaneous

The use of antioxidants or other compounds, which react with AA or its precursors, may reduce the AA content in heated foods. It is difficult to draw firm conclusions about antioxidant effects on AA. The few reports published seem conflicting. Addition of the antioxidants butylated hydroxytoluene (BHT), sesamol and vitamin E to lean meat before heating resulted in a higher AA level compared to the control sample (Tareke, 2003). It was suggested that antioxidants may lead to increased AA levels probably by protecting the formed AA from radical initiated reactions. On the other hand, a decreased AA formation has been demonstrated with addition of a flavonoid-containing spice and rosemary during frying of potato slices (Becalski et al., 2003; Fernandéz, Kurppa, & Hyvönen, 2003). These different effects are not necessarily due to antioxidative properties of the additives (Leufvén & Lingnert 2003). In potato and other starch-containing plants, starch granules are usually complexed to the proteins and other biological polymers that are present in the tissue. Because the AA content rather increases than decreases when exogenous phenolics are present, Vattem and Shetty (2003) suggested that phenolics may interact with proteins altering their tertiary structure and as such be present in the starch complexes. This would lead to the dissociation of the stable starch complexes, which may make the starch more available for reactions with proteins and for thermal degradation when fried, thus resulting in a rather higher formation of Maillard reaction products and AA.

Another well-known antioxidant is ascorbic acid (vitamin C) naturally occurring in fruits and vegetables. In potato tubers e.g. ascorbic acid concentrations typically found, range from 8 to 30 mg per 100 g fresh weight, but diminish during storage. During pre-treatment and processing of potatoes, ascorbic acid is further degraded by enzymic and thermal degradation and by leaching in blanching and cooking water (Blenkinsop et al., 2002). Biedermann et al. (2002a) reported a weak decrease of AA formation by the addition of ascorbic acid (1%) to a potato model. Ascorbic acid seemed neither to inhibit AA formation, nor to influence AA elimination significantly. Rydberg et al. (2003) observed a significant reduction in AA content when 1.7% ascorbic acid was added to a potato sample and suggested that the effect could be ascribed to a combination of the lowering of pH and the binding of water.

As to fertilization or farming method, the ammonium concentration in strongly fertilized (nitrogenous fertilizer) potato increases, but a clear effect on AA formation was not reported. Other factors such as characteristics of the cultivar and storage conditions usually play a more important role (Amrein et al., 2003; Biedermann et al., 2002a).

In contrast to the effect of nitrogenous fertilization or farming method on the AA yield of potatoes, the use of the

raising agent ammonium bicarbonate in cereal products has been found to increase the potential of AA formation in industrial baking trials (E.C., 2003; Leufvén & Lingnert 2003). A similar effect was observed in model systems of wheat flour fortified to 1.15% asparagine and with various amounts of fructose, where between 54 and 75 times more AA was formed when 1% ammonium was added. Addition of ammonium and fructose to corn starch on the other hand, did not provide a detectable amount of AA, probably due to a small amount of asparagine (Biedermann & Grob, 2003). It was demonstrated that ammonium added does not directly form AA by reaction with a carbon source, but acts rather as a catalyst accelerating the reaction from asparagine to AA even at ambient temperature (Biedermann & Grob, 2003). Hence, storage of cereal products containing ammonium salts could lead to higher AA contents. In case of gingerbread, the decrease in AA content could be explained by an almost complete loss of ammonium during the baking process (see "Storage" section). Replacing ammonium salts by other raising agents, e.g. sodium or potassium carbonate, can lower the AA levels significantly, but for some products flavor implications need to be considered (Biedermann & Grob, 2003; E.C., 2003).

Attempts to remove AA in food products after formation include supercritical CO_2 extraction and UV light. Supercritical CO_2 extraction is reported to be very effective in removing AA, but will also remove other compounds thus destroying the product. UV light causes polymerization of AA. When product was exposed to wavelengths of light ranging from UV to red, no effect was observed at any wavelength. Potentially, the level of AA is too low in food products for this technique to be effective (Brown, 2003).

Modeling and quantitative analysis

A problem that arises with qualitative data is that, although indicative, measured AA contents are comparable and can be used for evaluation purposes only if the relevant experimental parameters are similar and known. Usually qualitative data are accompanied solely by the observation of a single parameter (e.g. temperature), which is too limited to describe heating processes and their effects on processed food in an adequate way. When modifying processes or foodstuffs to decrease the AA content, the influence on other food safety issues (e.g. micro-organisms, mutagens) and on food quality (e.g. color, odor, flavor, texture) should be considered as well. This only becomes feasible by combining AA formation kinetics with kinetic data of safety and quality aspects. For instance, the most straight forward way to reduce the AA level is by reducing baking, roasting or frying time and temperature. On the other hand, considering the decrease in AA level after prolonged heating, for some products (e.g. bakery products) excess baking can have the same effect. Whatever approach is used, a balance between product safety and product quality has to

be kept in mind. As such the moisture content of bakery products e.g. should be verified not only to maintain a desirable texture but also to avoid microbial and spoilage implications.
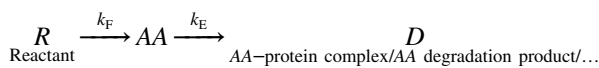
Kinetics of AA

In order to predict and to control the amount of AA formed, formation mechanisms and kinetics of AA in function of process- and product variables need to be known. Considering the various possible formation mechanisms of AA, it seems to be an almost impossible task to elucidate the kinetics of all the pathways involved. More than one reactant can be followed and modeled by means of Multiresponse Modeling. In this approach various reaction steps (reactants, intermediates and products) are followed to gain insight in the reaction mechanism (van Boekel, 1996). Nevertheless, a pragmatic approach that considers the overall formation kinetics rather than details of the complex chemistry could already be sufficient for obtaining information of direct applicability to food processing. As such, use of a simple reaction order for complex formation pathways can be equally useful for modeling chemical changes during processing, when knowledge of pure chemistry or mechanism of the reaction is of no importance.

The influence of temperature on the formation of AA has been repeatedly demonstrated (Becalski *et al.*, 2003; Mottram *et al.*, 2002; Tareke *et al.*, 2002; Biedermann & Grob, 2003; Leufvén & Lingnert, 2003; Rydberg *et al.*, 2003). Fig. 2 presents (a reproduction of) some of the values reported in literature. Note that heating times differ and therefore values are not merely comparable. From this figure it is clear that the onset of AA formation is around 120 °C. It is also demonstrated that qualitative as well as quantitative (kinetic) data depend on the food model studied. Not only the nature of the reactants, but also the molar ratio, the a_w, the matrix, the heating equipment, etc. will influence results as is outlined above. Besides, factors influencing the AA content and AA kinetics are interrelated. To demonstrate, Fig. 2 gives an example of how results can differ when the amount of AA is expressed in terms of weight prior to heating, thus compensating weight loss during heating, or in terms of dry weight. Since the weight loss is temperature (and time) dependent, also kinetics are affected. The effect of the food matrix on results is illustrated as well. In the simple model system without any matrix and in wheat flour, the AA content reaches its maximum around 175–180 °C, whereas in French fries the AA content is still augmenting, although the heating time is shorter.

The influence of heating time on AA formation at constant temperature is given in Fig. 3 for a model system and potato strips heated in an oven. The level of AA increases loglinearly in function of time at constant heating temperature, indicating first order kinetics. After prolonged

heating, a similar loglinear decrease of the AA content is observed (Becalski *et al*., 2003; Mottram *et al*., 2002; Rydberg *et al*., 2003). The decrease of AA content after a period of heating seems to be mainly attributed to elimination of AA becoming predominant over formation (Becalski *et al*., 2003; Biedermann *et al*., 2002a; Rydberg *et al*., 2003). Information on the elimination rate of AA can be provided by means of isotopic labeled AA, e.g. deuterated AA (D$_3$-AA). The observed elimination by means of added isotopic labeled AA however, will not directly correspond to the real elimination of AA. As to D$_3$-AA, it can undergo some deuterium-hydrogen exchange in wet samples (e.g. 19% humidity) (Biedermann & Grob, 2003; Biedermann *et al*., 2002a,b). Biedermann *et al*. (2002c), monitoring AA elimination by D$_3$-AA, proposed (pseudo-) 1st order kinetics. Next to isotopic labeled AA, elimination of AA can also be deduced from the kinetic model fitted on the net formation data of AA.

Based on results reported in literature, formation and elimination of AA can be described by first order kinetics. Simplified, the net content of AA in food can be assumed to result from two consecutive reactions, with k_F and k_E the first order formation and elimination rate constants at the temperature studied:

$$\underset{\text{Reactant}}{R} \xrightarrow{k_F} AA \xrightarrow{k_E} \underset{AA-\text{protein complex}/AA \text{ degradation product}/\ldots}{D}$$

The rate for each step can be expressed as (Smith, 1981):

$$\frac{dC_R}{dt} = -k_F C_R, \tag{1}$$

$$\frac{dC_{AA}}{dt} = k_F C_R - k_E C_{AA}, \tag{2}$$

$$\frac{dC_D}{dt} = k_E C_{AA}. \tag{3}$$

At time $t=0$, the concentrations of AA and D (C_{AA} and C_D, respectively) can be considered zero and the concentration of R (C_R) equal to C_{R0}. When kinetics are analysed under isothermal conditions (constant temperature), k can be assumed constant and Eq. (1) can be easily integrated:

$$\frac{C_R}{C_{R0}} = \exp(-k_F t). \tag{4}$$

Dividing Eq. (2) by Eq. (1) gives:

$$\frac{dC_{AA}}{dC_R} = -1 + \frac{k_E C_{AA}}{k_F C}, \tag{5}$$

which can be further integrated to:

$$\frac{C_{AA}}{C_{R0}} = \frac{k_F}{k_F - k_E} \left[\left(\frac{C_R}{C_{R0}} \right)^{k_E/k_F} - \frac{C_R}{C_{R0}} \right]. \tag{6}$$

This ratio or the yield of AA (*i.e.* the fraction of reactants converted to AA, e.g. the AA concentration over the asparagine concentration) can be expressed as a function of time by substituting the ratio of C_R over C_{R0} with Eq. (4) (Smith, 1981):

$$\frac{C_{AA}}{C_{R0}} = \frac{k_F}{k_F - k_E} (e^{-k_E t} - e^{-k_F t}). \tag{7}$$

Notice that this equation applies only for the ideal situation of isothermal conditions. In case of non-isothermal conditions (variable temperature), the assumption of a constant k-value does not longer apply and the integrated effect of temperature on the reaction rate constant has to be taken into account. Mostly, the effect of temperature can be expressed by the Arrhenius relation (Arrhenius, 1889), in which the temperature dependence of the rate constant k is quantified by the activation energy E_a (J/mol) according to:

$$k = k_{\text{ref}} \exp \left(\frac{E_a}{R} \left(\frac{1}{T_{\text{ref}}} - \frac{1}{T} \right) \right), \tag{8}$$

where R is the universal gas constant (8.314 J/mol,K), T the temperature concerned, and k_{ref} the reaction rate constant at reference temperature T_{ref}.

With respect to AA, so far no kinetic data are reported in literature. Activation energy data for the Maillard reaction are situated within a wide range of 10–160 kJ/mol, depending on the system studied (e.g. water activity, pH) and on what part of the reaction has been measured (e.g. lysine loss, browning) (Lingnert *et al*., 2002).

Mass and heat transfer models

Changes taking place during heating (frying, roasting, …) are difficult to model since there is a moving boundary that divides the crust and core regions and involves coupled heat and mass transfer. Moreover, there are a number of interrelated factors that have to be taken into account. For instance, the lower the initial moisture content, the higher the fat uptake by potatoes. Oil and moisture exchange are in turn related to structural changes taking place during processing such as the porosity of the product (Saguy & Pinthus, 1995).

Mathematical models of different complexity have been developed to describe transport phenomena occurring during heating, for which different assumptions were made such as constant physical properties, a simplified geometric shape, no volume or structure changes, *etc.* (Hubbard & Farkas, 2000; Krokida *et al*., 2000; Sablani, Marcotte, Baik, & Castaigne, 1998; Sahin, Sastry, & Bayindirli, 1999; Rovedo, Zorrilla, & Singh, 1999; Savoye, Trystam, Duquenoy, Brunet, & Marchin, 1992; Yamsaengsung & Moreira, 2002).

A distinction can be made between numerical and empirical approaches for modeling mass and heat transfer. In the numerical approach, heat transfer through solid foods

is usually modeled by Fourier's equation for heat conduction and moisture transfer is generally described by Fick's law of diffusion. Methods used for solving the model, include the finite difference (FD) method normally used for simple geometries, the finite element (FE) method for irregular geometries, and the finite volume (FV)/computational fluid dynamics (CFD) method, which is derived from the FD method. Standard software packages are available (Wang & Sun, 2003).

Krokida *et al.* (2000) developed an empirical model to describe moisture loss and oil uptake as a function of frying time. Based on the assumptions that the oil temperature is constant during frying, the initial moisture content in the food (potato strips) is uniform, and the two flows of moisture loss and oil uptake are independent of each other, both mass transfer phenomena could be described by a fractional conversion model. In this concept, which is widely used in chemical engineering (Levenspiel, 1972), the extent of the transfer or the fraction f is defined as:

$$f = \frac{X - X_0}{X_\infty - X_0}$$
$$= \frac{\text{moisture/oil content after a specified process time}}{\text{moisture/oil content after an infinite process time}},$$
(9)

with X_0 and X_∞, respectively, the initial and maximal moisture or oil content. The plot of the logarithm of $(1 - f)$ yields a straight line, resembling 1st order kinetics. Applying this on Eq. (9) gives:

$$\ln(1 - f) = kt = \ln\left(\frac{X_\infty - X}{X_\infty - X_0}\right)$$
(10)

or

$$X = X_\infty + (X_0 - X_\infty)\exp(kt).$$
(11)

For oil uptake, Eqs. (10) and (11) can be simplified by deleting X_0 since the oil content at the beginning of the frying process is zero. By means of empirical equations, the rate constant k and the equilibrium value X_∞ of moisture loss and oil uptake during frying could be related to the oil temperature and the sample thickness (Krokida *et al.*, 2000). A drawback of this approach is that it describes the average change of oil and water content in the system and assumes isotropic heating. Numerical methods on the other hand, give an estimation of the thermal behavior of the food under more realistic conditions by taking e.g. non-linear and non-isotropic thermal properties, irregular shaped bodies and time-dependent boundary conditions into account. As a consequence, only numerical heat and mass transfer models have the potential to incorporate with the formation kinetics of AA.

Conclusions

AA was found to be formed in food as a result of common cooking practices, such as frying, roasting, and baking. Therefore, finding ways to prevent AA formation and to lower AA levels in food is not straight-forward. Moreover, the level of AA in heated foodstuffs is the net result of complex reactions leading to formation and elimination of this compound.

Many reports in literature study the effect of different variables (heat load, pH, additives …) on AA, but mainly the qualitative or the singular effect of these variables on AA is discussed. To find optimal chemical and/or physical conditions to reduce the AA level and to retain at the same time the required safety standards and quality characteristics of the food, a multi-component approach is essential. Additionally, to be able to directly evaluate the safety and quality of a food product during processing, to optimize existing processes or to design new processes, more research on kinetic and/or dynamic modeling of the different actors is needed. Formation and elimination kinetics of AA together with heat and mass transfer models thus need to be combined with kinetic models describing microbial inactivation and degradation or enhancement of quality-related properties.

The range of products in which AA can be found, is extremely wide and initial findings for reducing AA apply in some cases only to limited product types. Any modifications performed on the raw material or process conditions needs to be viewed from a consumer perspective. Findings in laboratory studies thus need testing for feasibility in commercial practice.

A number of provisional recommendations for industry and consumers are given by the FAO/WHO (FAO/WHO, 2002), the European Commission (E.C., 2003), and the FDA (2004).

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