MIROIL

Addendum B

Toxic Breakdown Substances

Addendum B contains:

1. A Schematic of the Physical and Chemical Experiences During Frying
2. Abstract from Paper No. 17,961; by Paul B. Addi and Gregory J. Warner; for the Minnesota Agricultural Experiment
3. Potential Effect of Frypowder on Polycyclic Aromatic Compounds During Deep Fat Frying (December, 1999) - A report prepared for MirOil by Roman Bielska.
   MirOil markets *Frypowder®* and *fryliquid™*. Both are effective antioxidants. The comments relating to *Frypowder®* are relevant to *fryliquid™*

With traditional fryer operation you create toxic material when you turn ON the fryer. Time and temperature deplete antioxidants and drive breakdown reactions Many assert that filtering slows or alters this process. The breakdown substances are liquids that you can't remove by filtering. These breakdown substances are almost all colorless liquids that can't be "seen". Often paper filters can even make this worse by leaching other chemicals into hot oil[1].

It is true with chemical reactions in general and frying that as you heat the oil, the chemical reactions occur more rapidly. The progression of breakdown is not linear. It happens at an ever increasing rate with increase in temperature. This is why oil temperature is so important.

Fresh oil contains from 2% to 8% polar materials that are "good". All polar materials are not bad. We have proven free fatty acids (FFA) formed from thermal hydrolysis among "good" polar material. Oxidized fatty acids (OFA) from oxidation reactions join the "bad" polar material in these following classes:

- Mutagens
- Neurotoxins
- Carcinogens
- Others

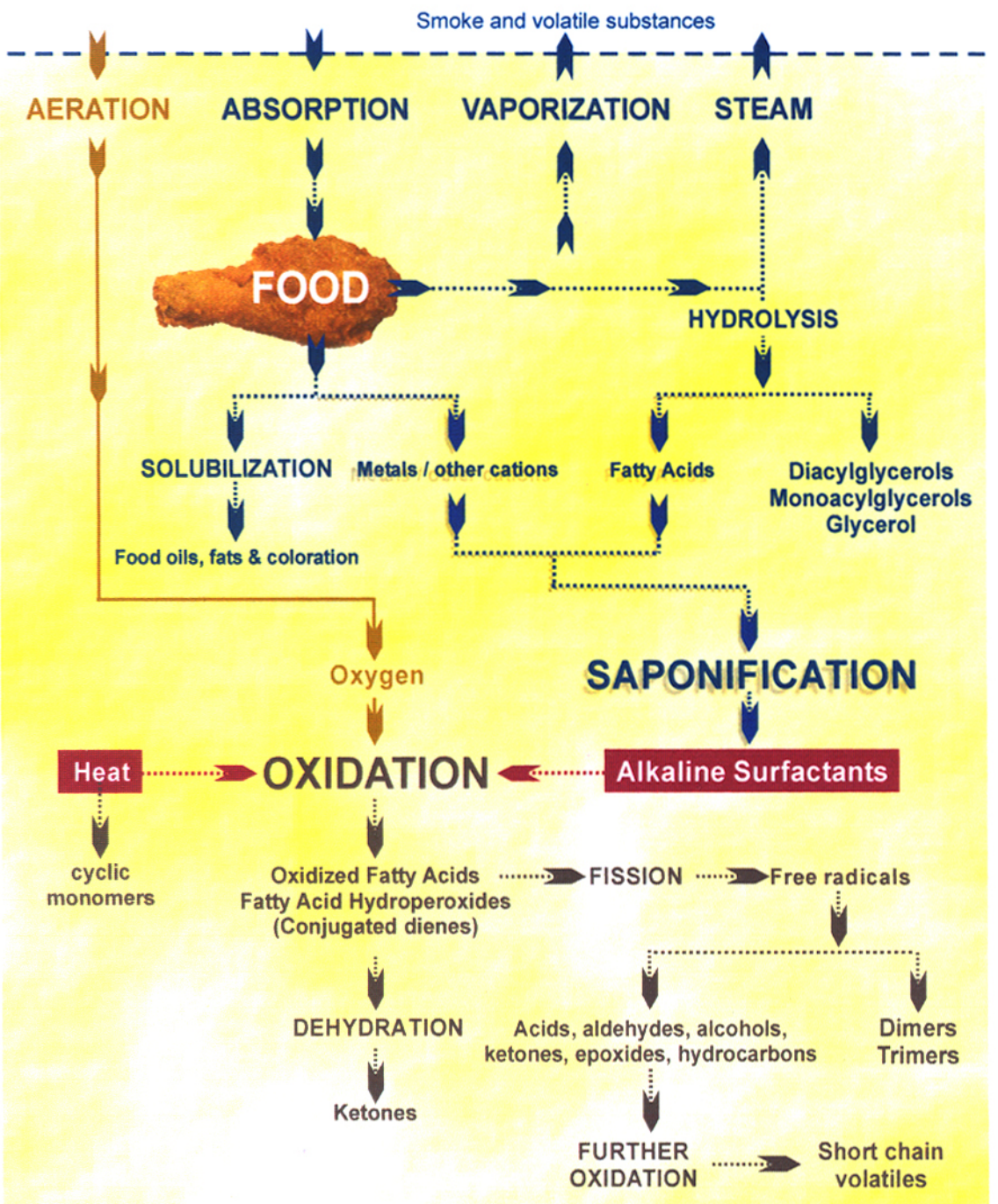
Fortunately, as these materials accumulate in the oil, they bring on diminished quality of food and motivate replacement of the oil.

Can the oil be always good without the presence of "bad" polar substances ? ? Yes! This happens with the application of MirOil antioxidant as part of oil management for Optimum Frying!

[1]Bleached paper has been shown to leach chemicals such as Polycyclic Aromatic Hydrocarbons into hot liquids. Regulating authorities have banned chlorine bleached paper filters from use in some countries.

THE IMPACT OF PROACTIVE ANTIOXIDANTS ON THE CHEMISTRY OF OIL DEGRADATION

This presentation is suggested by Handel[1] as an update of Fritsch[2]. -"Schematic of the Physical, Thermal and Chemical Experiences During Frying". Saponification is a reaction which was not shown by Fritsch[2]



MirOil antioxidant *fryliquid*™ ar *Frypowder*® are applied as part of c management for Optimum Frying maintain the cooking performanc and the nutritional profile of the oil at preferred target level. O management for Optimum Fryir provides 3 *interactive* effects th stabilize the amount and species polar substances at a level that comparable to much fresher oil use These are:

1. Antioxidants are restored to preve formation of unhealthy oxydize substances including fatty acid polymer and polar surfactants.
2. Food cooks just as fast with low temperatures that are much les damaging to the oil.
3. Fresh oil dilution maintains the lev of "good" polar substances at a targ low level and prevents formation "bad" and unhealthy polar substances

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2. C.W. Fritsch, J. Am. Oil Chem Soc 58:272 (1981)

The physical and chemical reactions shown by these colored arrows are diminished or arrested by MirOil antioxidants.

The application of MirOil antioxidants provide unexpected benefits:

- **More yield** - More fluids stay in the food.
- **Less oil absorption** (food-oil infiltration).
- **Food centers cook hotter**
- **Cook with lower oil temperatures**
- **Less polar substances** are formed in the oil.
- **The formation of OFA (oxidized fatty acids)is abated.**
- **No carbon and polymer deposits**

Chapter 5

The potential health aspects of lipid oxidation products in food

Paul B. Addis and Gregory J. Warner

Introduction

The idea that some of the products of *in vivo* lipid oxidation are deleterious to human health is not new. However, the concept that *dietary* lipid oxidation products and thermally altered lipids are injurious has attracted much interest recently and is the subject of this review. Regulatory activity by government agencies in the EEC has already occurred with rather tight controls placed on the degree of oil and shortening deterioration permitted during deep-frying. The US Food and Drug Administration (FDA) has been researching 'process-induced' toxic products such as the cholesterol oxidation products and also has an interest in determining any potentially deleterious effects on consumers of consumption of French-fried foods, especially if fried in heavily used, deteriorated oils, shortenings and tallows.

On balance, and in spite of the interest of regulatory agencies, the possible health aspects of lipid oxidation products remains highly controversial. Two excellent examples of the controversial nature of dietary lipid oxides are the areas of research dealing with possible toxic effects of heated fats and, a related area, the possible role of dietary cholesterol oxides and fatty acid oxidation products in coronary heart disease (CHD). On the heated-fat issue, numerous publications, in some cases published as early as the 1930s, reported toxic effects in rats consuming heated fats. Other studies showed no effects or no serious effects if reduced levels of heated fats were fed. Although it is difficult to know whether a consensus may have been reached by researchers on the heated-fat issue, a number of authors have opined that, where deleterious effects were seen, the studies did not reflect the practical situation of a restaurant for two reasons:

(1) levels of heated fats fed were unrealistically high; and
(2) fats were heated far more severely than would be the case in a restaurant.

However, recent studies will be cited to challenge the conclusion concerning the benign nature of abused oils on two accounts.

In the first place, it must be recognized that in the earlier studies the pathological end-points used were crude. In no work that the authors are aware of was arterial injury, atherosclerosis, or other phenomena related to CHD included. Yet, in recent years much evidence has accumulated on a lipid oxidation product—CHD connection. Secondly, research has also demonstrated that the levels of most atherogenic chemicals found in heated fats may in fact be reduced (when extremely abusive heat treatments are employed), by being replaced by dimers, trimers and polymers of fatty acids and triglycerides. The possible atherogenicity of the latter group of lipids has not been assessed. Clearly, it is time to reexamine the abused- or heated-fat issue using more precise and realistic heat treatments, relevant levels of intake, and appropriate pathological end-points.

A second but closely related area of intense debate and controversy deals with the relative atherogenicity of lipids vs. oxidation products of lipids, *viz.* cholesterol vs. cholesterol oxides and fatty acids vs. fatty acid hydroperoxides and/or secondary oxidation products. Of course, superimposed upon the foregoing questions are the sometimes acrimonious debates about the degree to which diet influences serum cholesterol and the degree to which serum cholesterol is related to CHD. The viewpoint that hypercholesterolaemia is an epidemic in most Western countries and is the primary cause of CHD (the lipid hypothesis) is promoted by the National Heart, Lung and Blood Institute (NHLBI) and the American Heart Association (AHA). In the face of severe criticism the NHLBI and AHA have steadfastly supported the 'lipid hypothesis' and have, in terms of funding, studiously ignored other promising areas of research such as dietary lipid oxidation products.

A very large number of research publications support the idea that oxidized lipids are far more deleterious to arterial health than the native lipids themselves. Therefore, cholesterol is viewed as harmless unless it is converted to one or more of a number of autoxidation products. However, here again unanimity is not to be found and one of our objectives is to explore both sides of this important issue.

Many more deleterious effects, and some potentially beneficial effects, of oxidized lipids have been reported. Cancer, membrane effects, enzyme effects, mutagenicity, and cytotoxicity are suggested. In many cases, the foregoing effects are interrelated: atherogenicity may be based on cytotoxicity which, in turn, is possibly caused by a combination of membrane and enzyme disturbances. In addition, numerous other related phenomena complicate these issues. As is true for most food chemical toxicological issues, the nutritional status of the subjects being studied is important—especially with regard to antioxidant nutrients. Therefore, yet another complicating issue (nutrition) is superimposed on the question of lipid oxidation products and health. Other factors which complicate the determination of the health effect of lipid oxidation products include:

Potential problem foods

A 'problem food' is defined here as any food which is currently, or has been in the past, responsible for the exposure of a large segment of the population to significant levels of lipid oxidation or degradation products. Inherent in this definition are the concepts of high frequency of consumption and relatively high levels of lipid oxidation (i.e. $\geqslant 10$ ppm vs. trace) in the food. Of course, at this time it is impossible to be sure that a 'problem food' actually exists, let alone try to identify it. Much further research on food analysis, absorption by humans and toxicology is needed. Our main purpose is to suggest as future research areas foods (and processes) which appear to be potential problems. There is yet another way to view problem foods as discussed here. The AHA and NHLBI have done much to promote a 'prudent diet', which limits consumption of eggs, meat and dairy products. Based on the research reviewed in this chapter, limitations on 'problem foods' makes at least as much sense as limiting consumption of fresh meats, for example, in a prudent diet. Finally, it must be realized that problem foods of yesterday are not necessarily problem foods of tomorrow. There is some evidence that changes have been made by the industry to lessen the exposure of consumers in some cases.

In our opinion, there are two major categories of 'problem foods' which should receive the highest priority for future research: heated fats and powdered eggs. In the case of heated fats it matters little whether a vegetable shortening, an animal fat or a combination of the two is used because the primary products formed would be fatty acid degradation products, cholesterol oxides and a mixture, respectively.

Heated fats

Health concerns have been raised about heated fats since the inception of deep-frying. Both saturated and unsaturated triacylglycerols undergo both oxidative and nonoxidative (thermolytic) degradation (Nawar and Witchwoot, 1980). Saturated fats are more stable, but potentially significant thermolytic and oxidative products can occur, particularly in abused oil. Unsaturated fats degrade rapidly, although hydrogenated fats somewhat less so. Some of the compounds formed in heated oils are shown in Fig. 5.3. Cholesterol present in heated tallow degrades initially to 7-ketocholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol and α-epoxide (Park and Addis, 1986a), and subsequently to derivatives which have not yet been identified. Antioxidants (α-tocopherol plus ascorbyl palmitate) slow but do not stop the cholesterol degradation reactions (Park and Addis, 1986b). Bascoul *et al.* (1986) reported that heated tallow formed all the products found by Park and Addis (1986a) plus β-epoxide, cholestanetriol and 20- and 25-hydroxycholesterol. Unfortunately, Basoul *et al.* (1986) used hot saponification and reported the artifact 3,5-cholestadiene-7-one. Variable but significant

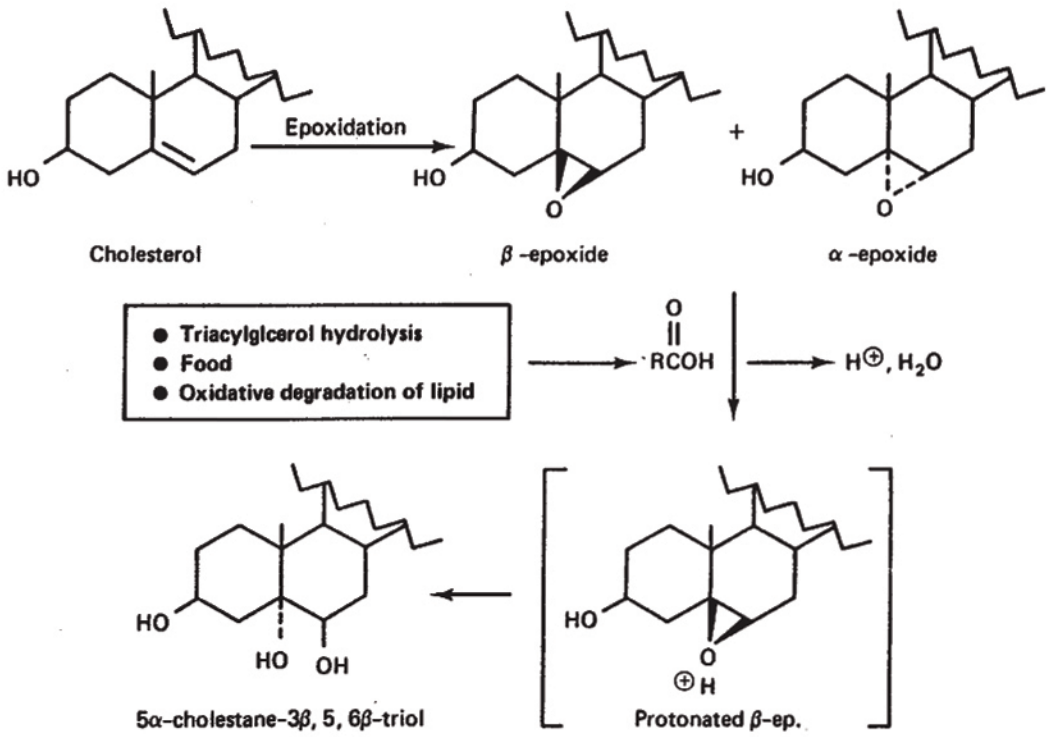

Figure 5.4 Formation of cholestane-3β, 5, 6β-triol. The suggested reaction probably occurs in deep-fat fryers in restaurants and could result in contamination of French fries with triol. The structure of cholestanetriol was confirmed by mass spectroscopy.

levels of cholesterol oxides in French fries from fast-food restaurants. In one restaurant, total cholesterol oxides reached 50 ppm, α- plus β-epoxides reaching 42 ppm. In another restaurant, variable levels were also seen, but it is perhaps significant that the highly atherogenic cholestanetriol was noted fairly consistently in daily samples.

Restaurants are reluctant to use the available quality-control devices to monitor oil quality. According to Gray and Morton (1981) the primary end-point selected as an indicator that the oil has reached the end of its 'fry-life' is foaming, a characteristic of heated fats that occurs with extensive polymer formation. Because polymer formation is preceded by extensive chemical degradation of the oil, a large build up of potentially toxic compounds can occur. Recent studies emanating from the authors' laboratory indicated that measurements of polarity are highly significantly correlated to the cholesterol oxide levels of a 90/10 tallow/cottonseed shortening used to fry French fries under laboratory conditions.

Based on the extensive degradation of cholesterol in heated tallow, both in restaurant and in laboratory investigations, one can only imagine the complex mixture of potentially toxic compounds formed by hydrogenated vegetable shortenings and, even worse from the oxidation standpoint, lightly hydrogenated vegetable oils. Indeed, some highly complicated triglyceride polymers have been reported in heated fats (Ohfuji and Kaneda, 1973). Clearly there

is an urgent need for investigations, using the improved analytical and toxicological methods available, of the heated-fats and -oils issue.

The literature on the toxicology of heated fats is extensive but much of the research conducted to date has at least two important shortcomings, especially the early research on the subject. Toxicological end-points were often crude and almost never included atherosclerosis. The possible link between consumption of heated fats and CHD must be vigorously explored. Secondly, heated fats used for early studies were often heated far more extensively than would occur in a restaurant. On the surface, one might assume that such a practice would tend to overestimate the problem of heated-fat toxicity, but in fact just the opposite might be true. As oils are heated, polymer formation occurs following the formation of oxidation products and these polymers are not well absorbed. Therefore, in the absence of untoward effects on the intestinal mucosa they are probably minimally toxic. This is clearly not the case for the aldehydes, hydroperoxides, lipid peroxides, dimers, cyclic compounds and, in particular, malonaldehyde, which are formed prior to polymers. For tallow, the analogous situation may exist. Cholesterol oxides, shown to be toxic in many studies, form fairly rapidly but eventually break down to other unknown and possibly less (or more) toxic compounds (Park and Addis, 1986a,b). Several early studies are recommended to the reader, including Morris *et al.* (1944), Lane *et al.* (1950), Crampton *et al.* (1956), Nishida *et al.* (1958), Perkins and Kummerow (1959) and Poling *et al.* (1969). Various forms of toxicity and some carcinogenicity were reported by the authors and loss of nutritional values was consistently reported, but at least one group (Poling *et al.*, 1969) concluded that levels of exposure of humans to toxic compounds in heated fats were insignificant.

Alexander (1983) summarized the biological effects of heated fats in experimental animals to include 'depression of growth, diminished feed efficiency, increased liver size, fatty necrosis of the liver and numerous other organ lesions'. Heated oils were found to induce damage to interstitial tissues and blood vessel walls, including vascular endothelium.

A more recent study by Alexander *et al.* (1987) confirmed the deleterious effects of heated oils in the rat. Male weanling rats were given diets of 15% fresh or laboratory-heated corn oil or fresh, laboratory-heated, or 'commercial-pressure deep-fry peanut oil'. The rats given heated corn oil or heated peanut oil exhibited diarrhoea, dermatitis, seborrhoea and hair loss. Commercial-pressure deep-fry peanut oil caused liver damage, toxicity to the thymus, and to testes and epididymis, the latter effects causing complete cessation of spermatogenesis.

Powdered eggs

The other major problem food is, and has been for some time, powdered eggs. Interestingly, as this is written, the importance of powdered eggs as a source of cholesterol oxides in the human diet may be decreasing for a number

of reasons. Powdered eggs are being used with less frequency in traditional products because of the cholesterol controversy and perhaps because of the cholesterol oxidation product problem. In addition, powdered eggs are possibly produced with less cholesterol oxidation product formation as processors nowadays change methods of spray-drying of eggs and yolks.

Several studies have been completed on cholesterol oxides in powdered eggs. These include the studies reported by Tsai and Hudson (1985), Missler *et al.* (1985), Morgan and Armstrong (1987) and Sander *et al.* (1989a). In general, gas-fired dryers are more detrimental than steam-injected dryers. Cholesterol oxidation increases with time and H_2O_2 treatment increased cholesterol autoxidation. Sander *et al.* (1989a) reported >150 ppm total epoxides in some powdered-egg samples but little or no triol and 25-hydroxycholesterol was seen.

Powdered eggs are a frequently used source of cholesterol for the inducement of atherosclerosis in animals. The atherosclerosis seen in animals from such experiments is invariably attributed to hypercholesterolaemia, which in turn is attributed to dietary cholesterol. The results are then applied to humans to make dietary recommendations.

However, the following questions could be asked. Is the atherosclerosis seen as the result of hypercholesterolaemia or arterial injury or both? Is it cholesterol of cholesterol oxides or both that are the atherogenic factors? Finally, how applicable are these findings to humans, who are not as responsive to dietary cholesterol in terms of developing hypercholesterolaemia as rabbits? Clearly more research is needed to answer these questions.

Other problem foods

In our opinion, deep-fat-fried foods and powdered eggs are in a class by themselves as far as lipid oxidation products are concerned. However, other areas of concern exist.

Freeze-dried meats, dehydrated cheddar, blue, parmesan and romano cheese powders, as well as sour cream and butter powders, all contain variable but possibly significant quantities of cholesterol oxides (Sander *et al.*, 1989a). Fresh dairy products contain minimal cholesterol oxides however. Long-term heat treatment of butter oil can cause extensive oxidation of cholesterol (Sander *et al.*, 1989b). Precooked intact beef muscle contain little or no cholesterol oxides; but approximately 2% of the cholesterol in comminuted precooked beef has been noted to be oxidized (Park and Addis, 1987). In comminuted precooked turkey however, the amount of cholesterol that had undergone oxidation was approximately 3%. As rancidity development advanced in comminuted and cooked meat during storage, the oxidation of cholesterol became apparent (Park and Addis, 1987).

In addition to cholesterol, the structural membranes of muscle foods contain appreciable quantities of polyunsaturated fatty acids (PUFA). The TBA test, as discussed in Chapter 4, has been adapted by food scientists to

appear to escape the disease. The role of smoking, perhaps the single most important risk factor in CHD, may be linked to *in vivo* oxidation of blood and tissue lipids. Research is urgently needed on this point.

Lipid oxidation products in foods represent 'process-induced' toxicants and, therefore, are contrasted to the usual xenobiotic contaminant. Because such oxidation products are endogenously produced, as opposed to being derived from exogenous sources, the regulatory status is uncertain in the USA. Nevertheless, the FDA has established a program in 'process-induced' phenomena, indicating possible future regulatory interest. Heated oils would probably be targeted for early consideration of regulatory activity based on activities in Europe. Interestingly, the recent popularity of a more polyunsaturated-type of frying oil will likely produce greater oxidation problems than use of tallow or shortening.

There may be a connection between dietary lipid oxidation products and mLDL and between both of these and the consumption of dietary antioxidants. The potential benefits of antioxidants would appear to be very great if the conversion of LDL to mLDL can be slowed by antioxidants.

Future research needs

The future research needs of the health effects of lipid oxidation products are very great. Because of the highly significant new findings in CHD research as well as the highly specific and sensitive procedures now available for the quantification of lipid oxidation products, some extremely important advances are anticipated in CHD research. It is clear that much more data are needed on the absorption and transport of lipid oxidation products in both animal models and humans. Are fatty acid hydroperoxides absorbed? What percentages of the various oxysterols are absorbed and what factors influence the efficiency of absorption of oxysterols? Once absorbed, what factors influence the rate of clearance of oxides from the circulation and is there any connection to atherosclerosis? Is the rate of formation of mLDL influenced by dietary lipid oxidation products? Research is urgently needed to understand the complete metabolism of oxysterols, including absorption, transfer among lipoproteins in the blood and the assimilation into various tissues.

Oxysterols appear to raise serum cholesterol to a greater extent than cholesterol itself. Are other oxidation products also more hypercholesterolaemic than their native counterparts? Fatty acid degradation products inhibit prostacyclin synthesis, a factor favouring thrombosis/spasm. Do cholesterol oxides have similar effects?

French-fried foods are a major source of dietary oxidation products. Clearly, more research is needed on the detailed content of all the various lipid oxidation products in such foods. An assessment is needed of the toxicology

of these oxidation products using appropriate animal models, relevant combinations and levels of oxides and appropriate experimental treatment durations. In addition, a similar assessment is needed of the dimers, trimers and polymers produced by the thermal degradation of fats. There would also appear to be an urgent need for research on improved methods of monitoring oil quality and improved handling and filtration (clear-up) of oils and shortenings used for deep-frying.

From the standpoint of public health (CHD) a major question is related to whether or not it is advisable to change to unhydrogenated or lightly hydrogenated vegetable oils (substituted for animal fats) which are very susceptible to degradation. This question is especially pertinent in the USA where essentially no laws regarding oil quality exist and where effective methods for oil-quality assessment are largely ignored.

The other health-related research in relation to lipid oxidation products includes studies on the possible carcinogenic effects, the long-term effects on membranes, the possible accumulation in tissues, the enzyme effects and the possible clinical manifestation of these phenomena. Progress will likely be slower than in CHD research because less is known about cancer, ageing and other diseases which might be adversely affected by lipid oxidation products.

Much research is needed on improvements in methods for retarding the rancidity in foods. Powdered eggs should receive top priority in this regard. Also, fats and oils used for frying and deep-frying, precooked (uncured) meats and powdered products of all types need scrutiny. Freeze-dried products need much research.

In terms of broad scientific interest, the potential health effects of dietary lipid oxidation products are not going to be elucidated by the efforts of a single scientific field such as food chemistry or medicine. Only through cooperative research investigations among nutritionists, toxicologists, medical researchers and food chemists can truly significant advances be made. It is our hope that this treatise has helped to stimulate such cooperation.

Acknowledgements

Published as Paper No. 17,961 of the contribution series of the Minnesota Agricultural Experiment Station based on research conducted under Project No. 18-23, supported by Hatch funds. The authors thank R. J. Epley and C. A. Hassel for their helpful suggestions.

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POTENTIAL EFFECT OF FRYPOWDER ON POLYCYCLIC AROMATIC COMPOUNDS DURING DEEP FAT FRYING

Report prepared by Roman Bielska Decenber 1999

The literature data show that polycyclic aromatic compounds (PAC) including polycyclic aromatic hydrocarbons (PAH) are not (!) formed during careful heating of oils below their smoking point. To the contrary, numerous authors prove that heating at moderate temperatures decreases the quantities of PAHs in oil had they been present. Thus, during heating at lower temperatures the rate of PAHs formation is lower than the rate of PAHs removal from oil due to evaporation and steam distillation.

Polycyclic aromatic hydrocarbons are formed in a free radical process. Its rate increases with temperature. Somewhere between 200 and 300 °C the rate of (free radical) formation of PAHs becomes higher than that of evaporation.

The use of the Frypowder oil stabilizer makes it unnecessary to heat oils above 171 °C (340 °F). Therefore, its use enables further decrease in already minimal rate of formation of the carcinogenic and mutagenic compounds.

1. POLYCYCLIC AROMATIC COMPOUNDS

Polycyclic aromatic compounds (PACs) are ubiquitous environmental pollutants, and although they are formed from both natural and anthropogenic sources, the latter are by far major contributors [1]. PACs include heterocyclic aromatic amines (HAAs) and polycyclic aromatic hydrocarbons (PAHs). Since many of these compounds are carcinogenic, mutagenic and cytotoxic [2,3,4,5] the presence of each of these categories in food is of serious concern. In particular, PAHs have been given huge attention due to their occurrence not only in food but also in air (smoke), water and soil. However, the risk to humans of dietary ingestion of PAHs is uncertain as this route of exposure is inefficient in inducing carcinogenesis in laboratory animals [6].

The most analyzed polycyclic aromatic hydrocarbon is benzo(a)pyrene (BaP), one of the most potent PAH carcinogens [7]. Substantial effort was put into the research explaining the mode of action of BaP [6]. It must be metabolically activated to yield the ultimate carcinogen [8,9]. First, BaP is epoxidized by cytochrome P450 1A1 and, then, hydrolyzed. The second epoxidation catalyzed predominantly by cytochrome 450 3A1 yields the ultimate carcinogen, BP-7,8-dihydrodiol-9,10-epoxide.

2. PAHs IN FATS, OILS AND MEAT

The presence of polycyclic aromatic hydrocarbons including benzopyrene in vegetable oils has

been reported by various investigators [10]. Particularly high levels of PAHs have been found in crude coconut oils. Some researchers suggest that margarine is a major dietary source of PAHs [11]. Different routes of contamination of vegetable oils have been suggested [10]:

- Uptake by the oil plants from contaminated soils,
- Atmospheric pollution of the oil plants,
- Direct drying of the oilseeds with combustion gases,
- Uptake from petroleum-based solvents used in the extraction of the oils from oilseeds.

The crude oils undergo various treatments. One of the purposes of these treatments is to reduce the polycyclic aromatic hydrocarbons content. Most of these treatments include the use of elevated temperatures or passing of hot air. Finnish researchers [12] determined the PAH content in margarines, butter and vegetable oils. Larsson's [10] and Pyysalo's [12] results clearly show the effectiveness of deodorization process in decreasing the total PAHs levels.

More than thirty years ago it has been noticed that the amounts of PAHs in some foods processed at elevated temperatures are unexpectedly high. As early as in 1967 Lijinsky and Ross [13] investigated the effect of variations in methods of cooking on the content of benzo(a)pyrene and other PAHs in meat. They determined that the production of PAH in charcoal broiling was dependent on the fat content and the proximity of the food to the heat source. The authors conclude that to minimize production of carcinogens, the contact of the food with flames should be avoided, the food should be cooked for longer periods at lower temperature (!) and the meat used should have a minimum of fat.

Fritz [14] found that roasting or frying resulted in negligible quantities of endogenous carcinogens, while exogenous treatment with flue gas increased the concentration of aromatic hydrocarbons, especially benzo(a)pyrene. Another form of food processing leading to substantial quantities of PAH is smoking [7]. Also, cooking oil fumes can introduce into atmosphere (and thus, into food products) amounts of PAH of concern [15] during deep fat frying at high temperature of 220-240^{o} C. In charcoal-broiled meats PAHs can be present in concentrations as high as 200 ppb (parts per billion - equivalent to nanograms per kilogram and micrograms per gram) [3].

Recently, Chen et al. [16] analyzed polycyclic aromatic hydrocarbons in meat products using liquid chromatography. They found that stewed pork, stewed chicken breast, stewed chicken wing contain no detectable amounts of carcinogenic PAHs. Smoked pork and grilled chicken and duck contain significant amounts of these compounds.

Many authors' results [2,10,17] prove that the PAH concentrations increase with the cooking temperature. Of particular interest are results of Knize et al. [2] who show that propane grilled and charcoal grilled hamburgers contained significant quantities of PAHs, while no detectable amounts of PAHs could be found in pan fried hamburgers. The same authors conclude that "reducing the cooking temperature seems to be the most practical way to reduce HAA content, but avoiding the conditions where the temperatures are below those needed to kill harmful bacteria is essential. The formation of PAH can be reduced by not exposing the food directly to

the heat source and resulting smoke when grilling foods". The statement about avoiding too low temperature is very important. A recently published report shows evidence that inadequate deep-fat frying was responsible for an outbreak of salmonella enteriditis phage type 4 food poisoning at a hospital for mentally handicapped people in 1990 in Wales [18].

3. DEEP FAT FRYING

Deep fat frying is one of the many methods of food processing. However, it is the most common method of "high" temperature treatment. It is a popular preparation method because it produces desirable fried food flavor, golden brown color and crisp texture [19]. Moreover, frying has little or no impact on the protein or mineral content of fried food, the dietary fibre content of potatoes is increased after frying and fried foods are generally a good source of vitamin E, C and thiamine [20]. It should be added that no pyrolysis caused by dripping of oil onto very hot surfaces can take place during deep fat frying

Most widely used frying temperatures are at a range of 325-375°F (163-191 °C). Limited oxidation and polymerization can take place at these temperatures. These reactions are accelerated by frying at too high a temperature, the presence of oxygen, the use of a poor-quality frying fat, and poor frying practices. Temperatures close to 400°F (204°C) ordinarily produce a browned surface before the inside is completely done. Before the inside is properly cooked, the food is overcooked on the outside [21].

Warner [19] describes chemical processes that take place during deep fat frying at 190°C. The primary oxidation products - hydroperoxides - are rapidly formed and cleave to alkoxy and hydroxy free radicals that are unstable at 190°C. Free radicals will react with other compounds to form secondary oxidation products such as aldehydes, alcohols, ketones and hydrocarbons. As water is added to the oil, usually through the addition of food, hydrolysis occurs. Free fatty acids are formed in addition to di- and monoglycerides as the triglyceride decomposes. Polymerization of the oil takes place with the formation of many compounds including dimers, trimers and polymers. It must be strongly emphasized that some of these reactions are highly desirable.

As it was presented earlier the use of Frypowder oil stabilizer enables frying at lower temperatures. This ability of Frypowder is critical in controlling the frying oil degradation.

4. INFLUENCE OF FRYPOWDER ON PAHs CONTENT IN OIL DURING DEEP FAT FRYING

The exact mechanism of PAHs formation is not well understood [22]. Some authors postulated that they might be formed through free radical reaction, intramolecular addition or polymerization of small molecules [22]. Also, hydrogen abstraction at allylic position outside pentadiene system has been speculated to be responsible for products such as aromatic compounds from linoleate ester [23].

It is more than very likely that the reaction (PAHs formation) proceeds via free radicals. It should be emphasized that any polymerization of small molecules is almost unavoidably free

radical in this case. Moreover, small molecules had to be formed in free radical transformations. Furthermore, even if intramolecular addition takes place it very well may be a free radical process. The fact that PAHs are not formed (or formed at low rate) at lower temperatures but their rate of formation becomes very significant at increased temperatures suggests very strongly that the rate determining step is a formation of free radicals. This statement is very strongly supported by the data showing that no PAHs were detected in oils heated to 300 °C (absence of air) and very low levels were detected in oils heated to 400°C. However, very substantial (100 μg/kg) quantities of PAHs were formed at 700°C [7,23]. Pyrolysis, responsible for formation of PAHs, is undoubtedly a free radical process.

The boiling points of PAHs are significantly lower than those for majority of lipids, the reason being that molecular masses of PAHs are much lower. For example, BP contains only 20 carbon atoms and 12 hydrogen atoms (Mw = 252 amu), and its boiling point is 496°C. Molecular masses of fats depend on the acyl part of the glycerol ester. For example, tristearyl ester of glycerol ($C_{57}H_{110}O_6$; Mw = 890 amu) decomposes before reaching the boiling point even when heated under vacuum. Additionally, some of the PAHs when heated in the presence of water will undergo steam distillation i.e. will exit the system much below their boiling point. Therefore, one can expect that prolonged heating of oils below the free radicals formation temperature should decrease the PAHs content in an oil. This thesis is supported by the observation [12] that steam distillation is very effective in removing PAH compounds. Also, it has been shown that heating destroyed about 70% of the PAHs originally present in the oils [7,24]. Dennis at al. [11] claim that PAHs can be reduced during drying of seeds and refining of crude food oils.

Hence, there are very good reasons to believe that careful heating of oils below certain (pyrolysis of oils starts at above 200 °C [) temperature not only does not cause accumulation of mutagenic and carcinogenic polycyclic aromatic hydrocarbons and heterocyclic amines but it decreases original content of these compounds had they been present. One can argue that this temperature should be as significantly as practical below a smoking point of given oil. The method of deep frying seems to be particularly useful to minimize the PAHs content in the processed meat. It allows to avoid the contact of the food with very hot parts and with the smoke produced during heating.

The collected data suggest that frying or deep frying at moderate temperatures does not produce PACs. On the contrary, the quantities of these compounds decrease. The temperature of frying is absolutely crucial. The higher the temperature the lower the oxygen solubility. One can speculate that at the absence of oxygen undesired pyrolytic reactions take place.

The use of the Frypowder oil stabilizer makes it unnecessary to heat the fat to temperatures above 340 °F (171 °C). It has various advantageous consequences for the processed food (meat). Additionally, the use of Frypowder seems to be particularly beneficial because it decreases the frying temperature, and thus, the potential for the free radical formation and accumulation of benzopyrenes and other carcinogenic hydrocarbons. Additionally, employment of Frypowder requires periodic filtration of the liquid product. Therefore it enforces the removal of food particles that remain in oil during frying. Moreover, the use of Frypowder while enabling to maintain the relatively low oil temperature and still allows for relatively high heat delivery to

fully cook a food center.

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MIROIL

Addendum C

Acrylamide

Acrylamide has always been present in fried and other foods.We just were not aware. Acrylamide is a known neurotoxin. Like other unhealthy substances it may be abated by good frying practice.

Addendum C contains:

1. A few of many press releases to inform the public of acrylamide.
2. FDA report - Exploratory Data on Acrylamide in Foods.
   This shows the wide range of acrylamide present in a sampling of some fast food restaurants.
3. Report of Zurich Cantonal Laboratory & BELVOIRPARK Chefs College
   The highlight is a conclusion that acrylamide forms according to:
   a. temperature b. time at temperature c. Maillard or browning effect
   All 3 causes are inter related. Frying at 356°F, 18°F higher than 338°F, there was 7 times as much acrylamide in the food.
   The report confirms the potatoes cook much faster at 320°F temperatures with *Frypowder®* and *fryliquid™* - and with 36% less acrylamide than at 338°F. MirOil markets both *Frypowder®* and *fryliquid™*. Both are effective antioxidants.
4. Research paper in Journal of Food Chemistry reporting acrylamide forms from acrolein as well as asparagine. Acrolien is formed in the oil by oxidation. This means there are other souces of acrylamide beside those reported by the Zurich Laboratory.
   Accompanying this research paper is a report by an Italian Health Authority which shows LIFE® powder reduces acrolien and peroxide in the oil by 80%. (LIFE® powder is the same product as *Frypowder®*)

Suspected carcinogen found in foods

Varying amounts of acrylamide are in many baked, fried products.

By Marc Kaufman
Of The Washington Post

WASHINGTON | The Food and Drug Administration on Wednesday reported it had found high levels of the potentially cancer-causing substance acrylamide in a wide range of fried and baked products, particularly in french fries, potato chips and crackers.

The high levels discovered represent the first detailed American confirmation of earlier surprise findings from Europe, and have led to a broad FDA effort to determine whether acrylamide poses a cancer risk that requires changes in how foods are cooked and consumed.

So far, officials say, they have not found acrylamide risks great enough to recommend that consumers avoid any groups of food or specific products. It remains uncertain whether people consume enough acrylamide in their food for it to be harmful and whether the substance — which causes cancer in laboratory animals at high doses — is similarly hazardous to people, they said.

But Terry Troxell of the FDA's Center for Food Center and Applied Nutrition said Wednesday, at a two-day advisory committee meeting on acrylamide, that the agency agreed with the World Health Organization's conclusion that the discovery of acrylamide in many foods is a "major concern" and needs to be aggressively researched.

The new FDA findings are included in a report on 300 common products the agency has tested for the chemical since Swedish researchers announced their discovery of acrylamide in many foods seven months ago. The FDA list showed predictably high acrylamide levels in most potato chips and french fries, but also significant levels in some breads, cocoas, almonds, coffees and crackers.

In almost all categories the acrylamide levels highly varied. Popeyes french fries, for instance, had significantly higher levels than Burger King fries. The FDA also found great variations in acrylamide levels between bags of the same Lay's potato chips, even those produced on the same lines of the same factories.

Wednesday's meeting was to review the agency's overall action plan for acrylamide research. FDA officials said their recommendations will come later. They plan to test another 300 foods and want acrylamide to be deemed a "priority" issue for the agency next year.

Troxell and other speakers stressed the complexity of the acrylamide situation — saying that researchers don't even know how acrylamide is formed in food or whether it has any biological effect on people who consume it. But they said that because it is a suspected carcinogen — the WHO's International Agency for Research on Cancer has determined that it is "probably carcinogenic to humans" — its presence must be treated seriously, especially since it is sometimes found at levels considered quite high.

As part of its assessment Wednesday, the FDA reported that acrylamide is most commonly produced in the cooking process of starchy foods, and that its levels increase as the cooking process gets longer and hotter. The FDA list, for instance, shows that Ore Ida Golden Fries contained 107 parts per billion (ppb) of acrylamide, while the same baked Ore Ida fries had 1,098 ppb.

Acrylamide has been used to purify drinking water and to produce plastics and dyes, and has been highly regulated because of its dangers. Swedish scientists began testing for it in tunnel workers who had been exposed to it through water contaminated by an acrylamide-based solvent used to repair leaks. To their surprise, researchers found high acrylamide levels in the red blood cells of both the tunnel crew and others with no known exposure to the chemical.

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Food acrylamide mystery solved

Frying and baking explain potential carcinogen in crisps and bread.

1 October 2002

Charlotte Westney

Acrylamide, a compound that causes cancer symptoms in animals, is formed during frying and baking, two studies now show.

The Maillard reaction accounts for acrylamide in crisps.
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The discovery solves a mystery that had caused public alarm. In April a Swedish study found the chemical in crisps and biscuits, but not raw food, at levels higher than the World Health Organisation (WHO) recommends for drinking water[1].

"I haven't known as much interest in a topic in many years," says Don Mottram, who studies food chemistry at the University of Reading, UK. Not knowing where the chemical was coming from was "a very big problem", he explains.

Baked bread tastes better than raw dough, and fried chips are tastier than boiled, because of the Maillard reaction. As long as there's sugar around, high temperature breaks proteins down to give food more flavour and a golden brown colour.

The Maillard reaction also produces acrylamide, Mottram has found, as has Richard Stadler of the Nestlé Research Centre in Lausanne, Switzerland, in independent experiments[2,3]. Potatoes and some cereals contain large amounts of the amino acid asparagine, which is similar to acrylamide. In the lab, heating asparagine with sugar at 185 ºC turns much of it into acrylamide, Motram and Stadler have found.

"During cooking, many complex chemical reactions take place," says Stadler: other amino acids change their form repeatedly, also producing acrylamide. More tests are needed on different types of food to see how acrylamide forms, he says, and to understand the effects of different cooking techniques.

Exposing more of a food to higher temperatures, as in thin potato crisps, generates more acrylamide. So too does cooking food for longer. No acrylamide has been found so far in boiled foods, probably because of their lower cooking temperature.

Hot topic

In rats and fruitflies, acrylamide causes cancerous changes, at concentrations 1,000 times higher than those found an average diet. There is no direct evidence for acrylamide having a similar impact on humans, but the International Agency for Research on Cancer nevertheless classified it as "probably carcinogenic" in 1994[4].

Rats don't eat heated food. But as humans have been doing so for thousands of years, we may be more tolerant to acrylamide, Mottram suggests. Obesity, diabetes and a lack of fruit and vegetables in Western diets are more serious health threats than acrylamide, he adds.

Finding the mechanism at work is important, but still just "one of many missing points", says Jorgen Schleudt, head of WHO's food-safety programme. He is calling for more research into the effects of acrylamide on humans.

Indeed, next week the United Nations Food and Agriculture Organization and WHO are launching a web-based network to coordinate acrylamide research. It should encourage a "global exchange of information", Schleudt explains.

raised much concern, but... Nature419, 449 - 450 (03 Oct 2002) DOI: 10.1038/

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U.S. Department of Health and Human Services
U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
December 4, 2002

Exploratory Data on Acrylamide in Foods

On April 24, 2002, researchers at the Swedish National Food Administration and Stockholm University reported finding the chemical acrylamide in a variety of fried and oven-baked foods. The initial Swedish research indicates that acrylamide formation is particularly associated with traditional high temperature cooking processes for certain carbohydrate-rich foods. In response to concerns about the potential risk of food-borne acrylamide based on known toxicity of acrylamide at much higher doses than those seen in foods, the FDA began to analyze a variety of U.S. food products for acrylamide. The data presented below are initial and partial results from an ongoing exploratory survey of foods for acrylamide. FDA's action plan and full research agenda are presented at http://www.cfsan.fda.gov/~lrd/pestadd.html#acrylamide. We are presenting this incomplete data set now to inform the public of FDA's progress and to help stimulate research into the formation of acrylamide in foods.

At a public meeting held on September 30, 2002, FDA discussed preliminary findings on acrylamide in a select group of food products to illustrate initial conclusions concerning variability that can be drawn from the early stages in the exploratory survey. Since the public meeting, FDA has continued to study acrylamide in a wide variety of foods, including breads, cereals, and snack foods in an effort to understand the occurrence of this chemical in the U.S. food supply. FDA will continue to investigate how acrylamide is formed in food, seek to identify ways to reduce acrylamide levels, and study the human health risk of consuming acrylamide in foods. FDA is collaborating with other federal public health agencies, international partners, academia, consumers, and the food-processing industry to coordinate efforts related to acrylamide in foods.

- These data include the data that were the basis for the graphs presented at the September 30, 2002, public meeting as well as additional data collected through November 15, 2002.
- These results indicate acrylamide levels in individual purchased food products.
- These data are exploratory and should not be taken to indicate the distribution of acrylamide levels in U.S. foods, or as an indicator of food product choices by consumers.
- In relation to the level of sampling that is needed to understand exposure and risk, the data cover a limited number of food categories, a limited number of products in those categories, and a limited number of brands.
- The data generally do not address unit to unit variation or lot to lot variation.
- Also, differences in acrylamide levels between foods or even between brands at this early point in the survey do not necessarily indicate differences in exposure or potential risk that would be experienced by consumers. When estimating exposure and potential risk it is important to

consider the amount of the food consumed and the day to day variation in levels, in addition to the level of acrylamide measured at a particular time.

Notes:

(1) ND = nondetect
(2) The limit of quantitation (LOQ) is 10 ppb. Values below the LOQ but above 0 are reported as <10 ppb.
(3) Dried and/or powdered products (baking cocoa, dried potatoes, coffee, certain cereals, powdered formulas, and gravy and seasoning mixes) were tested as purchased.
(4) Baked products were baked according to the manufacturers' directions. Toasted products were not cooked in a standardized fashion. Fried products (tortillas) were fried for 1 minute at 155 °C. Some products are intended to be eaten without further cooking (e.g., bread); other products, although precooked by the manufacturer, are intended to be eaten only with further cooking (e.g., chicken pieces and frozen french fries). The graphs presented at the September 30, 2002, public meeting on acrylamide levels in food contain values for products as commonly eaten only, not both uncooked and cooked values.

Table 1: Acrylamide values in food product samples

Product		Acrylamide (ppb)
Baby food	Beech Nut Stage 2 Apples & Cherries	ND
	Gerber 2nd Foods Apples & Cherries	ND
	Beech Nut Stage 2 Butternut Squash	22
	Gerber 2nd Foods Squash	ND
	Beech Nut Stage 2 Carrots & Peas	17
	Gerber 2nd Foods Carrots & Sweet Peas	39
	Beech Nut Stage 1 Oatmeal Cereal for Baby	ND
	Carnation Baby Cereal with Formula Oatmeal	ND
	Gerber Single Grain Oatmeal Cereal for Baby	ND
	Beech Nut Rice Cereal for Baby	<10
	Carnation Baby Cereal with Formula Rice	<10
	Beech Nut Stage 2 Tender Golden Sweet Potatoes	37
	Gerber Tender Harvest Organic Sweet Potatoes (lot 1)	62
	Gerber Tender Harvest Organic Sweet Potatoes (lot 2)	121
	Gerber 2nd Foods Sweet Potatoes	68
	Beech Nut Stage 2 Vegetables & Chicken	75
	Gerber 2nd Foods Vegetable Chicken Dinner	30
	Gerber 2nd Foods Green Beans	26
	Gerber Finger Foods Biter Biscuits	130
	Gerber Finger Foods Fruit Wagon Wheels	20
	Gerber Graduates for Toddlers Animal Crackers	60
	Gerber Mixed Cereal for Baby	ND
	Nabisco Arrowroot Biscuit (baby food)	113
	Nabisco Zwieback Toast (baby food)	20
These data are exploratory and do not show the distribution of acrylamide in foods. They cover a limited number of food categories, products, and brands. They generally do not address unit to unit variation or lot to lot variation.		
French fries	Arby's french fries	252
	Burger King french fries, location 1	197
	Burger King french fries, location 2	220
	Burger King french fries, location 3	369

Checkers french fries, location 1	257
Checkers french fries, location 2	407
Chick-fil-A french fries	389
Fuddruckers french fries, location 1	452
Fuddruckers french fries, location 2	346
KFC french fries, location 1	313
KFC french fries, location 2	270
KFC french fries, location 3	162
KFC french fries, location 4	117
McDonald's french fries, location 1	193
McDonald's french fries, location 2	328
McDonald's french fries, location 3	155
McDonald's french fries, location 4	326
McDonald's french fries, location 5	245
McDonald's french fries, location 6	270
McDonald's french fries, location 7	497
Popeyes french fries, location 1	301
Popeyes french fries, location 2	484
Popeyes french fries, location 3	1030
Popeyes french fries, location 4	610
Wendy's french fries, location 1	302
Wendy's french fries, location 2	157
Wendy's french fries, location 3	254
Wendy's french fries, location 4	260
Wendy's french fries, location 5	169
McCain Crinkle Cut french fries (not baked)	49
McCain Crinkle Cut french fries (baked)	356
Ore Ida Golden Crinkles (not baked)	74
Ore Ida Golden Crinkles (baked)	441
Lamb Weston Inland Valley French Fries (not baked)	212
Lamb Weston Inland Valley French Fries (baked)	798
Ore Ida Golden Fries (not baked)	107
Ore Ida Golden Fries (baked)	1098
Richfood French Fried Potatoes (not baked)	21
Richfood French Fried Potatoes (baked)	438

	Lamb Weston Inland Valley Fajita Fries (not baked)	200
	Lamb Weston Inland Valley Fajita Fries (baked)	1325
	Ore Ida Zesties! (not baked)	67
	Ore Ida Zesties! (baked)	572
	Linden Farms French Fries Shoestring Style (not baked)	70
	Linden Farms French Fries Shoestring Style (baked)	1036
	Ore Ida Fast Food Fries (not baked)	79
	Ore Ida Fast Food Fries (baked)	332
	Ore Ida Crispers! (not baked)	218
	Ore Ida Crispers! (baked)	616
	Ore Ida Golden Twirls (not baked)	20
	Ore Ida Golden Twirls (baked)	119
	Ore Ida Tater Tots (not baked)	199
	Ore Ida Tater Tots (baked)	255
These data are exploratory and do not show the distribution of acrylamide in foods. They cover a limited number of food categories, products, and brands. They generally do not address unit to unit variation or lot to lot variation.		
Potato chips	Good Health Natural Foods Olive Oil Potato Chips Plain	385
	Herr's Crisp 'N Tasty Potato Chips	468
	Lay's Classic Potato Chips, code date Oct. 15	249
	Lay's Classic Potato Chips, code date Oct. 29	318
	Lay's Classic Potato Chips, code date Nov. 5, bag 1	319
	Lay's Classic Potato Chips, code date Nov. 5, bag 2	398
	Lay's Classic Potato Chips, code date Nov. 5, bag 3	338
	Lay's Classic Potato Chips, code date Nov. 5, bag 4	337
	Lay's Classic Potato Chips, code date Nov. 12, bag 1	432
	Lay's Classic Potato Chips, code date Nov. 12, bag 2	462
	Lay's Classic Potato Chips, code date Nov. 12, bag 3	462
	Lay's Classic Potato Chips, code date Nov. 19, bag 1	280
	Lay's Classic Potato Chips, code date Nov. 19, bag 2	301
	Lay's Classic Potato Chips, code date Nov. 19, bag 3	283
	Lay's Classic Potato Chips, code date Nov. 19, bag 4	258
	Lay's Classic Potato Chips, code date Nov. 26, bag 1	257
	Lay's Classic Potato Chips, code date Nov. 26, bag 2	262
	Lay's Classic Potato Chips, code date Nov. 26, bag 3	275

	Lay's Classic Potato Chips, code date Nov. 26, bag 4	303
	Lay's Classic Potato Chips, code date Dec. 3, bag 1	343
	Lay's Classic Potato Chips, code date Dec. 3, bag 2	333
	Lay's Classic Potato Chips, code date Dec. 3, bag 3	291
	Lay's Classic Potato Chips, code date Dec. 3, bag 4	336
	Lay's Classic Potato Chips, code date Dec. 10, bag 1	425
	Lay's Classic Potato Chips, code date Dec. 10, bag 2	463
	Lay's Classic Potato Chips, code date Dec. 10, bag 3	490
	Lay's Classic Potato Chips, code date Dec. 10, bag 4	549
	Utz Crisp All Natural Potato Chips, lot 1	879
	Utz Crisp All Natural Potato Chips, lot 2	433
	Grandma Utz's Handcooked Potato Chips	146
	Kettle Chips Lightly Salted Natural Gourmet Potato Chips	1265
	Lay's Kettle Cooked Mesquite BBQ Flavored Potato Chips	198
	Utz's Home Style Kettle-Cooked Potato Chips	117
	Lay's WOW! Original potato chips	415
	Ruffles WOW! Original potato chips	270
	Ruffles Original potato chips	292
	Wavy Lay's Original Potato Chips	198
	Baked! Lay's Original Naturally Baked Potato Crisps	1096
	Terra Sweet Potato Chips	767
	Route 11 Sweet Potato Chips	2762
These data are exploratory and do not show the distribution of acrylamide in foods. They cover a limited number of food categories, products, and brands. They generally do not address unit to unit variation or lot to lot variation.		
Infant formulas	Carnation Good Start Milk-Based Infant Formula (liquid)	ND
	Carnation Good Start Milk-Based Infant Formula (powdered)	ND
	Enfamil Milk-Based Infant Formula with Iron (liquid)	ND
	Enfamil Milk-Based Infant Formula with Iron (powdered)	<10
	Similac Infant Formula with Iron (liquid)	ND
	Similac Infant Formula with Iron (powdered)	<10
	Carnation Alsoy Soy Infant Formula (liquid)	ND
	Carnation Alsoy Soy Infant Formula (powdered)	ND
	Enfamil ProSobee Soy Formula (liquid)	ND
	Enfamil ProSobee Soy Formula (powdered)	ND

	Isomil Infant & Toddler Soy Formula with Iron (powdered)	ND
	Isomil Soy Formula with Iron (liquid)	ND
Protein foods	Checkers Chicken Pieces	22
	Tyson Crispy Chicken Strips (not baked)	32
	Tyson Crispy Chicken Strips (baked)	35
	Gorton's Tenders Extra Crunchy fish fillets (not baked)	25
	Gorton's Tenders Extra Crunchy fish fillets (baked)	30
	Mrs. Paul's Crispy Fish Fillets (not baked)	13
	Mrs. Paul's Crispy Fish Fillets (baked)	12
	Van de Kamp's Crunchy Fish Sticks (not baked)	ND
	Van de Kamp's Crunchy Fish Sticks (baked)	ND
	Pastene Fancy Light Tuna in Olive Oil	ND
	Progresso Light Tuna in Olive Oil	ND
	Boca Burgers Grilled Vegetable burgers (not baked)	58
	Boca Burgers Grilled Vegetable burgers (baked)	116
	Boca Nuggets Original Chik'n (not baked)	<10
	Boca Nuggets Original Chik'n (baked)	<10
	Morningstar Farms Breakfast Patties (not baked)	ND
	Morningstar Farms Better 'n Burgers (not baked)	ND
	Morningstar Farms Better 'n Burgers (baked)	ND
	Worthington Veja-Links (uncooked)	ND
	Worthington Veja-Links (microwaved)	ND
	Worthington Veja-Links (toasted)	ND
These data are exploratory and do not show the distribution of acrylamide in foods. They cover a limited number of food categories, products, and brands. They generally do not address unit to unit variation or lot to lot variation.		
Breads and bakery products	Pepperidge Farm Dark Pump Pumpernickel (not toasted)	34
	Pepperidge Farm Dark Pump Pumpernickel (toasted)	364
	Pepperidge Farm Natural Whole Grain Whole Wheat (not toasted)	ND
	Pepperidge Farm Natural Whole Grain Whole Wheat (toasted)	59
	Pepperidge Farm Original White Bread (not toasted)	36
	Pepperidge Farm Original White Bread (toasted)	216
	Sara Lee Honey Wheat Bagels (not toasted)	27
	Sara Lee Honey Wheat Bagels (toasted)	57
	Sara Lee Plain Mini Bagels (not toasted)	58

	Sara Lee Plain Mini Bagels (toasted)	343
	Contadina Bread Crumbs Three Cheese	39
	Super G Bread Crumbs Regular Style	42
	Shoppers Food Warehouse Cake Doughnut	24
	Shoppers Food Warehouse French Twirl Doughnut	ND
	Shoppers Food Warehouse Plain Doughnut	14
	Indian flat bread (from local restaurant)	125
	Boboli Italian Pizza Crust (not baked)	33
	Boboli Italian Pizza Crust (baked)	24
	La Banderita Corn Tortillas (not fried)	10
	La Banderita Corn Tortillas (fried)	13
	La Banderita Flour Tortillas (not fried)	<10
	La Banderita Flour Tortillas (fried)	15
These data are exploratory and do not show the distribution of acrylamide in foods. They cover a limited number of food categories, products, and brands. They generally do not address unit to unit variation or lot to lot variation.		
Cereals	General Mills Cheerios	266
	General Mills Cinnamon Toast Crunch	61
	General Mills Honey Nut Cheerios	146
	General Mills Lucky Charms	176
	Kellogg's Corn Flakes	77
	Kellogg's Corn Pops	71
	Kellogg's Frosted Flakes	52
	Kellogg's Frosted Mini-Wheats	78
	Kellogg's Raisin Bran	156
	Kellogg's Rice Krispies	47
Snack foods (other than potato chips)	Chifles Fried Pork Rinds Smokehouse Flavored	12
	Gen Soy Zesty Barbeque Soy Crisps	17
	Super G Cheddar Cheese Corn Twists	133
	Super G Microwave Popping Corn (popped)	181
	Orville Redenbacher's Gourmet Popping Corn Movie Theater Butter (popped)	157
	Good Health Natural Foods Honey Dijon Mustard Julienne Potato Stix	1168
	Herr's Bite Size Dippers Tortilla Chips	117
	Herr's Extra Thin Pretzels	309

	Utz Unsalted Sourdough Specials	70
	Snyder's of Hanover Veggie Crisps	832
	Terra Stix	990
	Utz White Corn Tortillas	111
These data are exploratory and do not show the distribution of acrylamide in foods. They cover a limited number of food categories, products, and brands. They generally do not address unit to unit variation or lot to lot variation.		
Gravies and seasonings	Heinz Home Style Savory Beef Gravy (canned)	ND
	Heinz Home Style Classic Chicken Gravy (canned)	ND
	Butterball Brown Gravy Mix	ND
	McCormick Mushroom Gravy Mix	ND
	McCormick Turkey Gravy Mix	ND
	Kame Dark Soy Sauce	ND
	Kikkoman Soy Sauce	ND
	Colgin Natural Hickory Liquid Smoke	54
	Colgin Natural Pecan Liquid Smoke	151
	Stubb's Mesquite Liquid Smoke	38
	Accent Flavor Enhancer	ND
	Char Crust Roto Roast Dry-Rub Seasoning	ND
	Wyler's Shakers Beef & French Onion Flavor Instant Bouillon	ND
Nuts and nut butters	Blue Diamond Roasted Salted Almonds	236
	Blue Diamond Smokehouse Almonds	457
	Planters Salted Almonds	249
	Planters Smoked Almonds	339
	Planters Halves and Pieces Lightly Salted Cashews	ND
	Super G Dry Roasted Peanuts Unsalted	28
	Super G Honey Roasted Peanuts	ND
	Super G Party Peanuts	ND
	Arrowhead Mills Crunchy Peanut Butter	114
	Jif Creamy Peanut Butter	64
	Peter Pan Plus Creamy Peanut Butter	89
	Richfood Creamy Peanut Butter	79
	Smucker's Natural Creamy Peanut Butter	125
These data are exploratory and do not show the distribution of acrylamide in foods. They cover a limited number of food categories, products, and brands. They generally do not address unit to unit variation or lot to lot variation.		

Crackers	Red Oval Farms Mini Stoned Wheat Thins	26
	Dare Breton Thin Wheat Crackers	300
	Super G Unsalted Tops Crackers	41
	Keebler Town House Crackers Reduced Fat	130
	Pepperidge Farm Cheddar Goldfish	57
	Streit's Lightly Salted Matzos	182
	Wasa Original Crispbread Fiber Rye	504
Chocolate products	Droste Cocoa	ND
	Ghirardelli Unsweetened Cocoa	316
	Hershey's Cocoa	909
	Hershey's European Style Dutch Processed Cocoa	58
	Baker's Bittersweet Baking Chocolate Squares	104
	Ghirardelli Bittersweet Chocolate Baking Bar	93
	Hershey's Milk Chocolate Bar	ND
	Hershey's Chocolate Milk Mix	ND
	Nestle Nesquik Chocolate Flavor	45
	Land O Lakes Cocoa Classics Chocolate Supreme Artificially Flavored	<10
	Super G Hot Cocoa Mix Milk Chocolate Flavor	24
	Swiss Miss Milk Chocolate Flavor Hot Cocoa Mix	ND
	Jell-O Chocolate Flavor Instant Pudding & Pie Filling	15
	Super G Chocolate Flavor Instant Pudding & Pie Filling	17
Canned fruits and vegetables	Mott's Apple Sauce	<10
	Richfood Apple Sauce Cinnamon	<10
	B&M Original Brick Oven Baked Beans	83
	B&M Vegetarian 99% Fat Free Brick Oven Baked Beans	70
	Green Giant Sliced Mushrooms Broiled in Butter	ND
	Super G Mushrooms Stems and Pieces	ND
	Cedar Chick Peas	ND
	Libby's Pumpkin	25
Cookies	Archway Oatmeal Cookies	36
	Nabisco Chips Ahoy! Chewy Chocolate Chip Cookies	97
	Nabisco Chocolate Teddy Grahams	199
	Stella D'Oro Anisette Toast Cookies	107

These data are exploratory and do not show the distribution of acrylamide in foods. They cover a

limited number of food categories, products, and brands. They generally do not address unit to unit variation or lot to lot variation.		
Coffee	Maxwell House Slow Roast (ground, not brewed)	209
	Starbucks Coffee Columbia Ground (ground, not brewed)	175
	Super G Instant Coffee (powdered, not brewed)	188
	Folgers Classic Decaf Coffee Crystals (crystals, not brewed)	351
	Maxwell House Instant Coffee (powder, not brewed)	263
	Medaglia D'Oro Caffe' Espresso Coffee (ground, not brewed)	179
Frozen vegetables	Hanover Premium Petite Asparagus Spears	<10
	Hanover Blue Lake French Style Green Beans	<10
	Hanover Premium Petite Whole Carrots	<10
	Super G Cooked Squash	<10
Dried foods	Knorr Taste Breaks Soup Chicken Noodle Flavor	22
	Maruchan Instant Lunch Ramen Noodles with Vegetables Chicken Flavor	52
	Nissin Cup Noodles Chicken Flavor	136
	Lipton Noodles & Sauce Creamy Chicken	17
	Lipton Asian Side Dishes Teriyaki Noodles	34
	Kraft Macaroni & Cheese Dinner	11
	Super G Macaroni & Cheese Dinner	12
	Lipton Recipe Secrets Onion Soup & Dip Mix	1184
	Super G Onion Recipe Soup Mix	90
	Lipton Rice & Sauce Herb & Butter	<10
These data are exploratory and do not show the distribution of acrylamide in foods. They cover a limited number of food categories, products, and brands. They generally do not address unit to unit variation or lot to lot variation.		
Dairy	Grace Sweetened Condensed Milk	ND
	Carnation Malted Milk Original	43
	Carnation Insant Nonfat Dry Milk	11
	Saco Cultured Buttermilk Blend	<10
Miscellaneous	Fuddruckers Onion Rings	13
	General Mills Lucky Charms marshmallows	ND
	Idahoan Butter & Herb Mashed Potatoes	ND
	Jell-O Gelatin Dessert Raspberry Artificial Flavor	<10
	Super G Raspberry Artificial Flavor Gelatin Dessert	<10
	KFC Mashed Potatoes	<10

	Kraft Quick Cooking Minute Tapioca	<10
	Mrs. Richardson's Butterscotch Caramel Topping	ND
	Idaho Spuds Mashed Potatoes	ND
	Super G Non-Dairy Coffee Creamer	<10

These data are exploratory and do not show the distribution of acrylamide in foods. They cover a limited number of food categories, products, and brands. They generally do not address unit to unit variation or lot to lot variation.

Acrylamide in Foods

Backgrounds for the tips for French fries with minimum acrylamide content

This evaluation was carried out by the Belvoirpark School for Hotel Management in Zurich and the Zurich Cantonal Laboratory. Professional cooks produced and rated the samples. The analysis was made in the Cantonal Laboratory.

Frying right

Frying is a technique of food preparation that often has negative headlines. In most cases the frying technique does not produce unhealthy products. A lack of knowledge about recent frying developments is responsible for criticism about fried food.

The advantages of frying are fascinating. Careful frying brings results which are “up to date” in the view of many culinary and nutritional physiologic. Frying causes a quick closure of the pores of the fried food, so that only little fat can enter into the food. The cooking is quick, the nutrition materials are treated carefully, the fried food keeps the aromatic taste, and the formation of acrylamide can be limited.

A basic rule of a healthy nutrition is to eat fried food in reasonable amounts. The central point of the recommendations of the Federal Office for Health (BAG) aims in the same direction for balanced nutrition (http://www.bag.admin.ch/verbrau/aktuell/Acrylamid-Empfehlungen-nov02.pdf).

Short introduction into formation of Acrylamid

Asparagin and reducing sugars

Acrylamide forms by thermal decomposition of the free amino acid asparagin that is not bound to protein. Acrylamide forms only after dehydration, i.e. in a crust. Cooking alone does not form acrylamide even in a steamer at about 120°C. For acrylamide to form it is now obvious that a mostly dry medium is required. For French fries acrylamide is found only on the exterior surfaces and not in the humid centers.

Asparagin must react first with a substance, which makes the molecule unstable. Usually these are the “reducing sugars” of fructose and the somewhat less effective glucose. Saccharose, a “normal” sugar, is not involved in this reaction. Temperatures must be from about 100°C and up to initiate the reaction.

Potatoes contain a quantity of asparagin for storing nitrogen. This is about 3% in relation to the fresh weight. As a comparison, cereals contain about 100 times less asparagin (in proportion to the dry weight of the potatoes). The content of asparagin is similar for all potatoes so there is not an option to reduce the formation of acrylamide by the selection of potatoes species with less asparagin.

The concentration of reducing sugars varies greatly so a fresh potato may contain between 0.01 and 3% fructose depending on the specie and on the conditions of growth. It is therefore possible to make products containing considerably less acrylamide by the selection of potatoes with less reducing sugars.

Maillard reaction

Baking, frying, roasting or grilling creates a browning effect. Many chemical reactions that develop not only the color but components of taste and smell are responsible. They are collectively called the “Maillard reaction”. This reaction is based as a first step on the reaction of reducing sugars (fructose and glucose) with free amino acids. The formation of acrylamide belongs to this class of reactions.

This explains why the formation of acrylamide is directly related with the browning effect. The browner the French fries, the higher the content of acrylamid.

Different classes of foods cannot be compared. Bread with a very black surface contains less acrylamide when compared with French fries with a darker finish because flour does not contain much asparagin (i.e. it has mostly other amino acids). This perception is useful because the cooking action can be controlled with it. A large formation of acrylamide starts only with the browning of the French fries. French fries with a golden (yellow) surface contain less than 100 µg/kg acrylamide while fries with a darker brown finish will quickly form over 1000 µg/kg.

Tolerable ACRYLAMIDE concentration

We don't know how many cancer cases are caused by acrylamid. These tumors develop from chronic effects. The quantity of a single portion of French fries is not important by itself. The total of acrylamide intake from all foods and stress from all sources is the crucial factor.

The actual toxicology is not able to indicate which amount of acrylamide will be only a little risk. The laboratory assessment can therefore only measure the improvement (reduction) in acrylamide content. With lower content, a point may be reached where the acrylamide contamination in food will irrelevant.

"Classic" French fries contain 200-700 µg/kg acrylamide. Fries with a darker brown finish contain as much as 1500 µg/kg acrylamid. These are about 50 to175µg/kg acrylamide. This is 375 µg/kg acrylamide in a portion of 250 g. In comparison with this, 1000-5000 µg/kg acrylamide are found in Rösti (hashed potatoes with a darker browned finish). This is1000 µg/kg for a single portion.

100 g crisps contain about 100 µg, a cup coffee has about 3 µg and a portion of 200 g dark bread has about 10 µg while 100 g of highly contaminated gingerbread has close to 100 µg. This is evidence that frequent consumption of French fries may be a large part of acrylamide consumed by an individual and that efforts for minimizing the acrylamide content of French fries are important.

As shown below, the concentrations of acrylamide in French fries can easily be dropped below 100 µg/kg. With 70 µg/kg, a portion contains less than 20 µg. This is 3 to 10 times less than normal. The total contamination is reduced by more than a factor of 21 for someone who loves fries. Such an improvement is worthwhile since it can be achieved without affecting food quality.

We evaluated only French fries which are rated by chefs for recognized restaurants as good or optimal.

Right potatoes Which choice for production?

The suitability of a choice of potato depends on various factors. The potato should have some yellow color so that the product looks golden yellow and not gray (the choice of Agria, Granola and Ditta are therefore more appropriate than Urgenta). They also should have high starch content so that the French fries don't have cavities from the migration of water. This suggests the choice of Agria and against Sirtema.

French fries become crispy before they brown. Brown should be as late as possible. It is undesirable when the browning occurs randomly. The 3 to 5 mm ends of the potato brown quickly. This is where the sugar concentration is the highest. Potatoes with a low content of reduced sugars have been selected for many years to meet these requirements by the producers of factory prepared frozen French fries. Without knowing about acrylamide, the producers used potatoes with a low potential for the formation of acrylamid. Agria is on that score a very suitable and often used choice.

Storage

Almost all of the sugar that is produced by photosynthesis converts to starch. The reducing sugars in the potato represent only a small residue that was not converted into starch or not released for the germination. Potatoes which are ripe and before the onset of germination contain the least amount of fructose and glucose. Therefore they produce the least acrylamide.

Potatoes also utilize the reducing sugars for protection from winter frost. A temperature of lower than about 8 °C has the effect of a signal that induces a release of reducing sugars from starch. Around the freezing point the sugar content escalates rapidly and the potato becomes sweet and inedible.

A longer storage at 4 °C does not cause significant sweetening so this temperature is considered optimum for preservation and for retardation of germination. This temperature is an alternative for chemical retardation of germination.

However the content of glucose and fructose increase by a factor of from 5 to 50. This is especially higher in potatoes that originally had a lesser sugar content. The formation of acrylamide (measured as a "potential for acrylamide formation") rises with the similar frying conditions by this factor because the browning also happens more quickly. The producers of factory prepared French fries or potato crisps have always avoided refrigerating lower than about 8 °C.

Potatoes sold after November are nearly all from refrigeration storage at 4°C (at least still in winter 2002/2003). Producers of French fries who purchase potatoes will often experience a faster browning and more acrylamide content. Potatoes from storage at lower temperatures may break down after 1 to 3 weeks of storage. This damage is only partly reversible.

Refrigeration at 4 °C produces content of fructose and glucose that are substantially greater and are the least favorable choice. **The rule that potatoes should never be refrigerated lower than 8°C is therefore more important than the selection of the species.** Data to this topic are to be found in other articles of the homepage of the Cantonal Laboratory of Zurich.

Pretreatment of the cut potatoes

Industrially blanched and frozen French fries are kept in hot water for some minutes. This has the effect that the reducing sugars are washed out of the exterior layer of the cut potatoes. This is a positive effect that slows browning and prevents brown spots. Simultaneously asparagin is also washed out. Both lead to a reduction of acrylamide formation.

The "wetting" or soaking of the potato sticks also influences the frying process. The following is some experimental data for this.

Measurement of extracted sugar and asparagin

The effectiveness of various extraction methods was analyzed with potato slices of 1.3 mm thickness (sort Ditta). Contents of asparagin, fructose and glucose were measured in the water as well in the slices before and after extraction. The slices showed, how effectively asparagin is extracted from both sides a 0.65mm thick layer of thinly sliced potato. Chart 1 shows washing with cold water only extracts about 10%. This is barely more than rinsing of the cut cells. With hot water (after the water was cooled by the potato sticks to about 45 °C) somewhat more extraction of asparagin, fructose and glucose was achieved in half of the time. The extraction from the intact cells was minimal.

With higher temperatures a jump can be noticed: With only 2 min in water of 80 or 100 °C more than half of the asparagin and sugar was washed out. The potato is cooked in this process. This destroys the cell structures and makes the fiber porous.

A similar extraction of asparagin, fructose and glucose can be expected from the surface depth of 0.65 mm may be applied to cut potatoes (sticks). The conclusion may be that the raw cut potatoes should be kept a short time in boiling water. However this final conclusion is premature.

Extraction (%)

	Asparagin	Fructose	Glucose
Cold water, 30 min	10	12	6
Hot boiler water, 15 min	14	14	8
Water at 80 °C, 2 min	53	54	51
Boiling water, 2 min	63	61	61

Complexity of the frying process

The chefs quickly established that the French fries from cut potato sticks cooked 2 min do not become crispy. Laboratory tests also showed that the acrylamide content may even be higher than in French fries made of raw cut potato sticks. This contradicts the results described above.

Crispness requires dehydration of the surface layer so more water must evaporate from the potato's surface than is delivered from the interior. Since the fiber of the potato "cooked" in hot water is more porous more water is delivered to the surface faster than with the raw potato. Another effect is the water that flows from the more porous fiber just below the surface also contains the sugar and the asparagin that was retained in the potato. This is converted to acrylamide as it reaches the surface. The migration of asparagin and sugars from the interior makes the previous extraction from the outer layer pretty useless.

The processes are more complicated. The development of the crust isn't only a drying process. The crust must develop before the interior is cooked and the cell structures are destroyed by heating. The crust must not be a barrier to prevent water from the potato stick escaping and evaporating. A superior crust without porosity will cause eruptions at the potato surface. The temperatures never exceed 100 °C in the moist interior so development of acrylamide is minimal except at the surface of the potato.

Studies with frying

Chart 1 shows when water is used for extraction of asparagin and sugars from the potato sticks the formation of acrylamide is reduced by a considerable factor. After 8 min of frying the values were only between 10 and 30 µg/kg instead of 100 µg/kg.

However, the extraction also influenced the sensory nature of the French fries. The product from raw potatoes was at 8 min with 170 °C of good quality, crispy with significant browning of the ends. The French fries from extracted sticks had an unfinished pale appearance, minimal crispness and weakly developed flavor.

Even after 10 min, the sticks extracted in boiling water produced French fries with unsatisfactory quality although their content of acrylamide was now the highest among the products from extracted potatoes. This confirms previous experiments and conclusions.

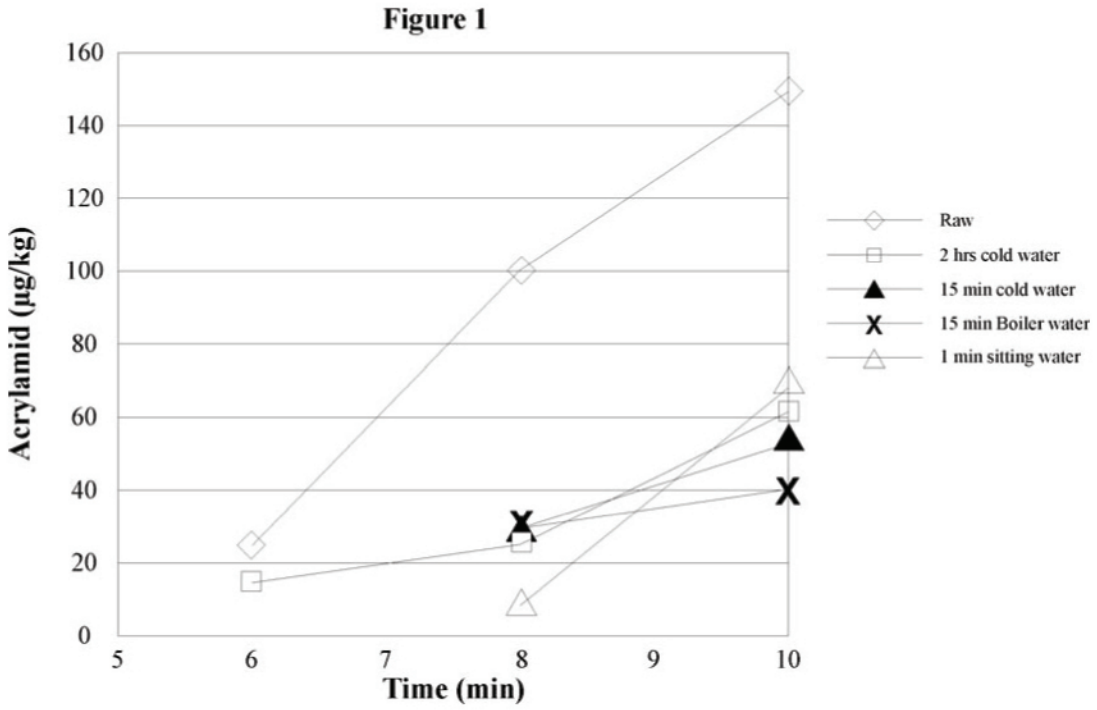
The other 3 treatment variations (cold extraction for 15 min or 2 h and extraction with hot boiler water) resulted after 10 min in good crispy fries, brighter than those from raw potatoes and still half content of

acrylamide (40-60 µg/kg: comparison with raw potatoes fried for 8 min). The water had an initial temperature of 60°C, instantly cooled by the potatoes to 45°C and after 15 min to 35°C.

It was determined from this data that a treatment in boiling (hot) water is undesirable while the other extraction methods resulted in French fries with a preferred finish and with less acrylamide.

The extraction with boiler water reduced the acrylamide content most effectively, but e product was not as good. Perhaps longer frying time could compensate for less desirable finish.

See chart 1: Contents of acrylamide in French fries from raw potatoes with asparagin and sugars extracted after 6 to 10 min frying at 170 °C.



Stagnant or agitated water?

Another experiment tested whether stagnant or agitated water has better results. With stagnant water less starch is washed out. Starch is visible as clouding while sugar and asparagin are dissolved in the water. There is a hypothesis that starch on the surface contributes to crispness or crust because of its fast adhesion in the initial frying process. This suggests that sugar and asparagin are washed off and minimal starch is lost.

The experiment was by Bintje with potatoes from the market (January 2003). They probably came from cold storage because the 120°C measurement of “potential for formation of acrylamide” was high;1340 µg/kg. For comparison Bintje stored potatoes at 8 to 10 °C in December had a “potential for formation of acrylamide” of 400 µg/kg (T. Amrein et al., 11 samples). Those Agria used for the above described experiment had 110 µg/kg. The use of these specific potato had the advantage to provide results that are easier to be quantified but also showed the influence of the potato character. This is that potatoes with too high a sugar content will not produce optimum fries even with very best frying technique.

The French fries were not fried over a constant time but to a comparable sensory end point because of the influences of extraction potatoes require longer frying time. As the chart 2 shows: the probe 2 contained only 130 instead of 240 µg/kg acrylamide (compared with probe 1); the sample 4 had only 150 instead of 220 µg/kg. Conclusion - stagnant cold water reduced the content of acrylamide more

than running water. By the extraction with boiler water a sample (8) was compared with one that was several times stirred manually (7). Again the acrylamide of the left one was lower (160 instead of 240 µg/kg). The improved crust development by the starch on the surface presumably allows less water to evaporate from the surface.

Chart 2. French fries from Bintje potatoes with high potential for acrylamide formation; influences of stagnant or agitated extraction and prefrying (blanching) at 140 °C. Times are in min and sec and acrylamide is in µg/kg.

	Pretreatment	Prefrying	Frying	Acrylamid	Judgement
1	2 h, cold running	no	171 °C, 5:15	240	servable, quickly mushy
2	2 h, cold stagnant	no	169 °C, 5:05	130	servable, quickly mushy
3	2 h, cold running	140 °C, 2:30	170 °C, 2:51	220	good, puffed up, good taste
4	2 h, cold stagnant	140 °C, 2:30	170 °C, 2:39	150	good, puffed up, crispy
5	15 min cold running	no	169 °C, 3:46	90	short, servable
6	15 min cold running	140 °C, 2:30	172 °C, 1:24	140	good, puffed up, crispy
7	15 min boiler stirred	140 °C, 2:30	169 °C, 4:19	240	short, tasteless, servable
8	15 min boiler stagnant	no	171 °C, 5 :08	100	good, somehow spotted

Prefrying (blanching) in oil

In the professional kitchen the French fries are often prefried to provide quicker production during busy times. Here the question is whether this brings benefits or disadvantages for the quality of the fries.

Prefrying brought a clear improvement for the crispness of French fries. The increased “blow out” of the sticks confirmed the development of a stronger, less porous outside skin. There was not a significant difference in the content of acrylamide. The results of the samples 3 and 4 did not differ significantly from the samples 1 and 2. The content of the pre-fried sample 6 was higher, but the compared product 5 was fried too dark very quickly. The same is valid for the samples 8 and 9.

The product 9 (extraction of asparagin in stagnant warm water and prefrying) was clearly the best of the series concerning quality. The content of160 µg/kg is moderate in comparison with the others. It may be this high only because the potential for forming acrylamide of the potato was 10 times too high. This product may also serve as an argument to give priority to the extraction of asparagin and sugars by boiler water compared with cold water. However the differences are so little that we put extraction by boiler water in the same category with that of cold water.

Industrially prefabricated French fries are already pre-fried. They can be fried directly at 170°C.

Frying in oil

Chart 3 shows the results from the French fries of the old school which were fried to a darker finish than necessary. They contained proportionally more acrylamid. Potatoes of the specie Urgenta with a high “potential for formation of acrylamide” (also not preferred for color reasons) were cut and rinsed for 2 h in running cold water; then prefried at 137°C in oil and finally finished at 170 or 180°C. The first 4 samples were produced with 100 g potato / liter oil. 180 °C / 165 sec and 170 °C / 180 sec resulted in products of similar quality as that of 170°C with a clearly lower content of acrylamid.

Two further samples were produced with 150 and 50 g respectively of potato sticks per liter oil. This reduced the oil temperature differently. When a lower quantity of potato was cooked a substantially higher acrylamide content was measured. The cause is a known problem while frying.

The 10% rule

Most acrylamide forms in the last seconds of frying. Only at this moment is the surface of the potato stick sufficiently dehydrated and heated. Therefore the temperature towards the end of frying is important and not the beginning temperature. The ending temperature is usually considerably lower than the starting temperature. The evaporation of water from the cold potatoes removes more heat from the oil. The result is cooling which for 100 g potato/liter oil (10%) is 20 to 35°, depending on the fryer. The heating starts immediately. But the fryer does not deliver enough energy in the short cooking time. Even with a good working fryer the input of temperature is insufficient in the crucial moments.

The effective temperature towards the end of the frying process depends on the quantity of potato sticks that is cooked. With 200 g / liter (20%) the temperature drops in most cases lower than 130°C. The French fries stay soft and absorb much oil. One should only fry as much food so the temperature does not drop lower than 140°C.

The higher acrylamide content for the test with only 5% potato input are the result of a higher oil temperature towards the end of the frying process from less cooling. This does not mean that frying a smaller quantity of food causes higher acrylamide content. It means that the French fries should have been finished earlier! Shorter frying time diminishes the content of acrylamide just like lower temperatures. Or a lower beginning oil temperature should have been sufficient.

The temperature drop with frying is seen as the most frequent cause for bad results. It is inevitable even different with different fryers. This means that the critical temperature towards the end of the frying process is different - even with same starting temperature and same potato input with different fryers. Strict rules with temperature and time indication are therefore unreliable.

Chart 3. French fries from cold watered, raw potato sticks, with pre-frying at low temperatures

Dimension	Prefrying	Potato per oil (%)	Start Temp. (°C)	Finishing period (s)	Judge-ment	Acrylamide (µg/kg)
7 x 7 mm	137 °C, 3 min	10	180	180	good	610
7 x 7 mm	137 °C, 3 min	10	180	165	optimal	530
7 x 7 mm	137 °C, 3 min	10	180	150	good	550
7 x 7 mm	137 °C, 3 min	10	170	180	optimal	320
7 x 7 mm	137 °C, 3 min	15	180	165	optimal	400
7 x 7 mm	137 °C, 3 min	5	180	165	optimal	710
20 x 20 mm	137 °C, 4.5 min	10	180	165	optimal	410
20 x 20 mm	137 °C, 4.5 min	10	170	180	optimal	210
20 x 20 mm	137 °C, 4.5 min	10	180	180	good	410
20 x 20 mm	137 °C, 4.5 min	10	180	150	good	310

A potato load of 10% is considered to be a realistic compromise, which prevents the inevitable sudden temperature drop from getting out of control. If larger quantities of French fries must be fried in a small fryer they should be fried in portions. Between frying portions the oil must have enough time to come back to initial temperature.

Dimension of the sticks

The essentially thicker French fries (”pont-neuf”), 20 x 20 mm resulted in a somewhat lower acrylamide content. This goes back to the smaller content of crust. The differences were however not large because thicker sticks have to be fried longer (in this trial over longer pre-frying). On the other hand, the smaller sticks showed that it is more difficult to fry them from the point of view of quality because they get brown within seconds and that the acrylamide content shoots up.

The fine ends and edges of the potato are a problem: The ends and edges reach the optimal condition earlier than the regular sticks and brown more quickly. The sharp ends are a special danger area as they may quickly contain over 1000 µg/kg acrylamid. The problem is aggravated as in these areas the sugar content is higher. That is why such ends and thin cuts should be removed before frying.

For the same reasons picking out darker brown parts after frying is effective. The acrylamide content of the whole portion may be easily dropped in half by the removal of some very dark brown pieces.

Factory Prepared Potatoes

The use of factory prepared potatoes, in most cases frozen, is both convenient and contributes to low acrylamide content. The potatoes are selected so that they have a little sugar content and have suitable hot water extraction. The peripheral fine cuts are eliminated. A comparative result obtainable in the household has additional acrylamide as described above.

Chart 4 shows data from deep frozen, factory prepared French fries commonly used in restaurants. They were fried for optimum sensory quality defined as a light crisp surface with minimal browning. All acrylamide contents are clearly shown to be lower than 100 µg/kg. This is 5 to10 times lower than

usual. The improvement has its origin from the lower oil temperature and the careful choice of the frying time.

Chart 4. French fries prepared for optimum quality from prefabricated, deep frozen raw material, as they are commonly used in gastronomy.

Dimension	Temperature (°C)	Time (s)	Acrylamide (µg/kg)
7 x 7 mm	176	255 s	60
7 x 7 mm	170	315 s	75
8 x 8 mm	170	255 s	65
8 x 8 mm	170	315 s	55
9 x 9 mm	177	255 s	60
9 x 9 mm	170	315 s	50

With increasing thickness of the sticks (7 to 9 mm in chart 4) the contents of acrylamide was only a little lower. In the experiment, the frying time was fixed. Probably the thicker sticks would have been fried somewhat longer. Otherwise what leveled out the differences?

Acrylamide in the last moments

Acrylamide forms in the last moments of a frying, roasting or baking process. Chart 2 shows contents of acrylamide in French fries, which were produced in a household fryer from factory prepared frozen potato sticks of 8 x 8 mm at 170 and 180 °C oil temperature. The available fryers had very inaccurate temperature regulation so the oil temperatures were set with a laboratory thermometer.

Frying time	3 min	4 min	5 min	6 min	7 min	8 min	Temp.
Acrylamid	50 µg/kg	80 µg/kg	100 µg/kg	320 µg/kg	500 µg/kg		**180 °C**
Acrylamid		40 µg/kg	40 µg/kg	40 µg/kg	80 µg/kg	220 µg/kg	**170 °C**

Chart 2. Acrylamide contents in French fries, fried at 170 or 180 °C with different frying time. Optimum products at 4.5 min (180°C) and 6 min (170°C).

Optimum French fries were produced after 4 to 5 min and a frying temperature of 180°C. They were crispy with slightly browned ends. After an additional minute a general browning started with a steep increase of the acrylamide content from 80 to 320 µg/kg. At 170°C the same optimum result was achieved only after 6 min. The acrylamide content was low, still at 40 µg/kg. The general browning and the advance growth in acrylamide formation started only 2 min later. Pictures of the chosen samples are found in the attachment of this text.

Three conclusions

1. The acrylamide develops only in the last moment of frying.
   The frying process should end at the right moment.
2. 170 °C is verified to be the more favorable beginning temperature. The acrylamide for comparable French fries is lower and extending the frying time for one minute did not show a strong increase.

3. The measurements of the oil showed that the household fryers controlled the temperatures with a difference of 10 to 40°C (They were mostly too low). No controlled frying would have been possible without temperature measurements in the oil with all three fryers.

Frying additives?

Additives for frying oils are on the market, which remove, inactivate or hold back breakdown substances from the oil. **"Frypowder" allows 10°C lower temperature to be sufficient for speedy cooking. This protects the oil against breakdown reactions and diminishes the formation of acrylamid.** The lower temperature has the same result only when the heat transfer to the food is accelerated by the additive. Acrylamide cannot be very different if heat delivery is not improved at the lower temperature.

The heat transfer from oil to the potato stick is impeded by the formation of steam. The potato stick is surrounded by a steam blanket which prevents the direct contact with the oil and acts like an insulation layer. The dimension and the movement of the steam bubbles depend from the surface tension of the oil. That is why the frying process is different in different oils. Frying additives influence these properties as well.

The results shown in chart 5 come from Agria potatoes which were cooled to 4°C during 12 hours to increase the content of sugar for easier measurement. The raw 7 x 7 mm stocks (cut ends removed) were washed in running water during 2 h and pre-fried at 2:30 min at 140°C in oil. Then they were produced in a restaurant fryer mostly at 170°C and during the indicated times. Frying in the area of 3:40 to 4:13 resulted in a good until optimum quality with acrylamide contents less than 100 µg/kg. This confirms that optimum French fries of lower than 100 µg/kg acrylamide can be produced without industrial blanching.

Chart 5. French fries from watered Agria potatoes, pre-fried, then produced as indicated, partially with oil additive.

Temperature °C	Time (min)	Acrylamide (µg/kg)	Result
Without additive:			
170	04:13	90	very crisp, browned ends
170	04:00	83	very crisp, golden yellow
170	03:40	45	just crisp, yellow
170	03:20	60	little crispness, bright
170	02:40	35	not crispy enough
170	03:00	35	insufficient
With additive			
170	04:00	80	very crispy
160	04:00	45	crispy
170	03:40	70	crispy

With the liquid additive (*fryliquid*™) and frying at only 160°C / 4:00 the result was still good and with clearly a lower acrylamide content. With the liquid additive *fryliquid*™ (MirOil) the frying gave a similar positive finished product result as frying without an additive. Using the additive at 170°C / 3:40 brought an improvement of the product tied with a slightly higher acrylamide contamination that could result from an increase in heat transfer.

Other tests with the additive in powder form or liquid confirmed the assumption, that the heat transfer to the center of the food is accelerated and that with this a good result at about 10°C lower temperature is achieved. Compared with French fries of similar quality the contents of acrylamide could be a bit lower, however in our trial series the differences were always only little significant.

Oven fries

Comparison with frying in oil

Part of factory prepared frozen 7 x 7 mm oven fries was baked for 14 min at 200°C in the oven. The other part was fried at 176°C initial temperature during 165 sec to a similar quality. Both products contained 90 and 80 µg/kg acrylamide respectively. The result shows that fries produced with the same raw material contain comparable acrylamide whether they were heated in oil or in the oven. Of course, the temperature and the heating time are very different for a comparable result.

Quick increase at the end

Charts 3. and 4. show acrylamide content in oven fries which were produced in different ovens and from different frozen raw materials. Measurements showed that both ovens regulated the temperature inexactly. In empty ovens these varied from the set values (by 20 to 30°C higher). With the French fries in the oven the temperature fell most of the time much below set temperature. There is much reservation to apply these results to other ovens.

The package instructions for the source material (potatoes) for chart 3 recommended an oven temperature of 250°C with time in the preheated oven of 16 to18 min. After 12 min in the oven 1 without circulated air the fries were still soft. After 16 min they were just optimal: crispy with light browning of the ends and the acrylamide content was 50 µg/kg. After 20 min the browning increased and the ends were dark. The acrylamide content increased in only 4 min by a factor 7 to 370 µg/kg because of the greater dark areas. After 24 min the content reached 1170 µg/kg. The French fries were now fully brown with almost all black ends. This is barely darker than what would have been served up to now. The acrylamide content in light and dark French fries (regarded as just acceptable) covered a factor of more than 20 to 1!

When the oven temperature was dropped to 220°C the optimal result was reached after 20 min, The content of acrylamide remained at 20 µg/kg. The product after 24 min had exceeded the optimum finish slightly with light browning and had 100 µg/kg acrylamid. As compared to 250°C, at 220°C even after 28 min there were no dark parts developed. The browning was much more consistent and the acrylamide content was 230 µg/kg (far below the values from 250°C).

Chart 3. Acrylamide contents in oven fries from an oven without circulated air. The oven temperature is 250°C corresponding to the recommendations of the producer.

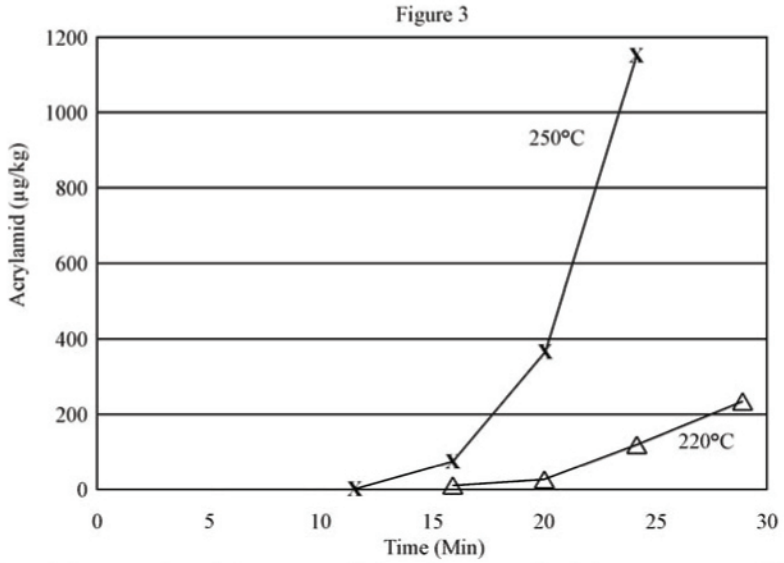
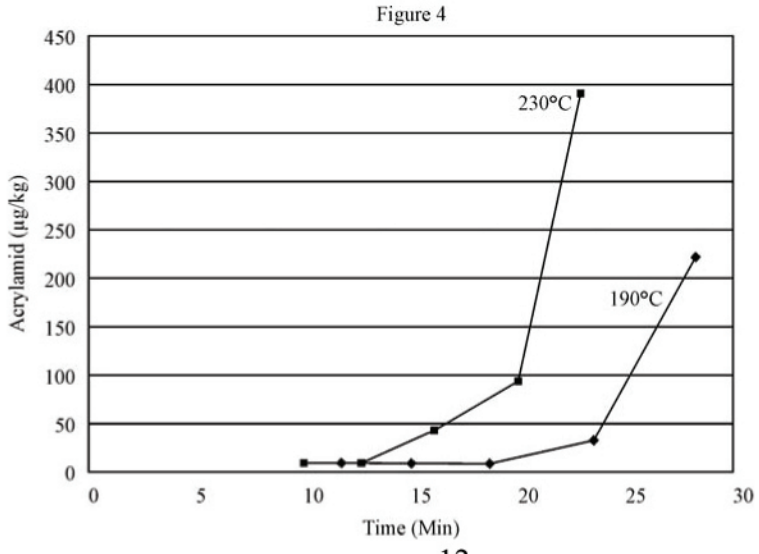


Figure 4. Acrylamide content in oven fries from a baking oven with circulated air.



Even with a 20 to 30°C lower temperature in oven 2 with circulated air there was quicker baking than with oven 1 (chart 4). The recommended oven temperature of the frozen source product was 230°C (without specification whether with or without circulated air); the recommended baking time without preheating 15 to 20 min plus 5 to10 min after turning upside down or 12 to 15 min in a preheated oven. Again it appeared that the French fries with optimum baking at 230°C (16 min) and even the somewhat over baked (20 min) French fries contained less than 100 µg/kg acrylamide (40 respectively 80 µg/kg).

A barely optimal product was obtained with only 11 µg/kg acrylamide after 18 min with an oven temperature of 190°C. After 23 min the content was only 36 µg/kg.

Both charts show that virtually no acrylamide is formed during the first 10 min of the baking process. This is followed with a slow increase up to100 µg/kg. Finally there is a quick sharp increase on content. This final sharp increase should be prevented. At higher temperatures a few minutes cooking time is the difference between whether the acrylamide content remains lower than 100 µg/kg or increases up to 1000 µg/kg. In a single minute the acrylamide content can double.

Because the quantity of acrylamide that forms in the last minutes is very high (with frying in oil it is very high in the last seconds) the cooking process must be observed closely near the end of the cooking time and be interrupted in the right moment. It is overlooked that a major portion of the acrylamide contamination happens in this context when the product is “forgotten” in the oven or in the fryer.

Baking “paper”?

There is a good argument for putting French fries on an oven “paper”. The potatoes that lay on the baking tray may darken more as more heat is transferred by the direct contact with the tray than from the hot air. These potatoes develop acrylamide more quickly before the other surfaces get crispy. The heat conductivity of the “paper” is low so it acts like insulation.

Half of the baking tray was covered with oven “paper” in a test. Relatively dark French fries were produced using frozen product. This made the differences more significant and easier to observe. The acrylamide concentrations amounted to 1380 µg/kg with direct contact on the tray and 1050 µg/kg with product on the “paper”. The additional browning of the contact surfaces was weak. This result may not be typical for all situations because the sticks were solid and the contact surfaces between the potatoes and the surface was small. For soft, thick sticks the differences may be significantly higher.

Conclusions

The experiments show that optimum French fries with lower than 100 µg/kg can be produced with care and attention simple rules. This is about 5+ times lower than typical values currently produced.

The most important rule:

- **Never fry or bake longer than necessary!**
- **Cook at lower temperatures!**
  Lower temperatures help in two ways:
  a) the food has small areas where very high acrylamide content have formed
  b) the increase of acrylamide content is less intense when the food is cooked longer than the optimum cooking time.

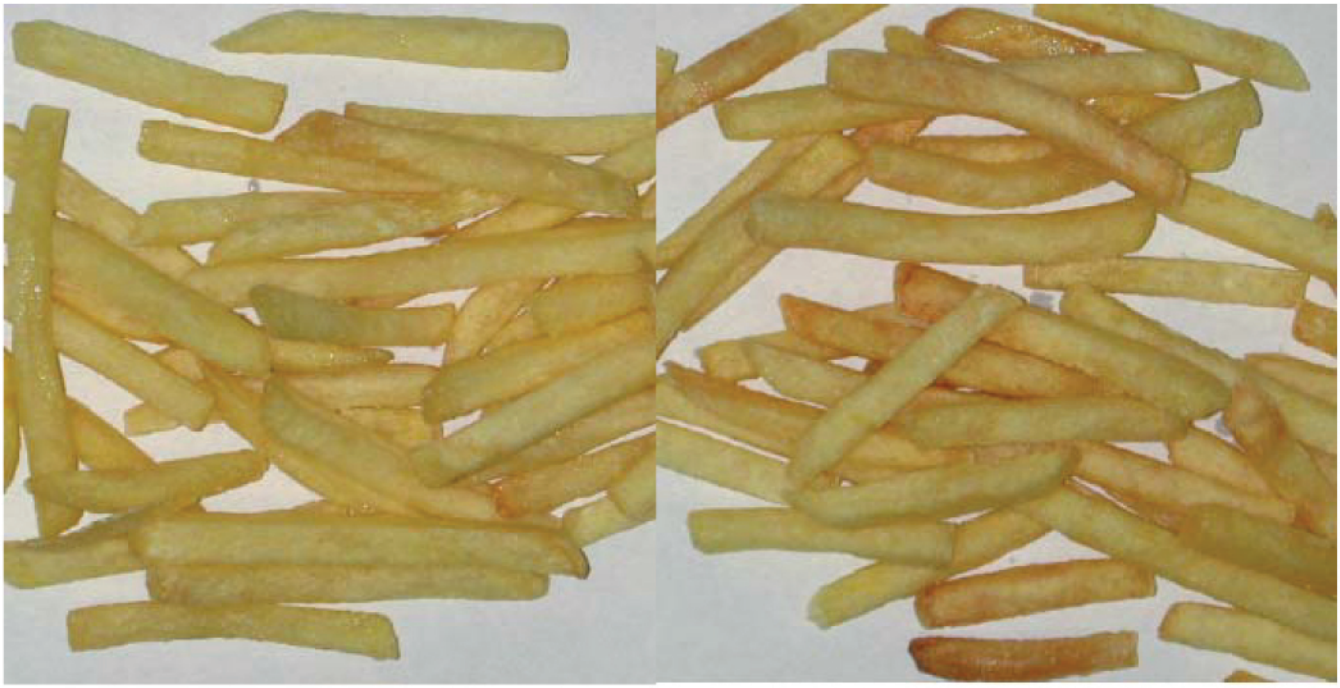
BelvoirPark Zurich

Cantonal Laboratory Zurich

January 2003

40 ppb, 6 Min. 170 °C

220 ppb, 8 min 170 °C



70 ppb, 7 min 170 °C

500 ppb, 7 min 180 °C

Figur 5. Pommes frites aus vorgefertigtem tiefgekühltem Material mit steigendem Bräunungsgrad und Acrylamidgehalt (Produkte aus dem Versuch mit Daten in der Figur 2).

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. 2003, 51, 3999−4003 3999

Gas Chromatographic Investigation of Acrylamide Formation in Browning Model Systems

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Acrylamide formed in browning model systems was analyzed using a gas chromatograph with a nitrogen−phosphorus detector. Asparagine alone produced acrylamide via thermal degradation at the level of 0.99 μg/g of asparagine. When asparagine was heated with triolein—which produced acrolein at the level of 1.82 ± 0.31 (n = 5) mg/L of headspace by heat treatment—acrylamide was formed at the level of 83.6 μg/g of asparagine. When acrolein gas was sprayed onto asparagine heated at 180 °C, a significant amount of acrylamide was formed (114 μg/g of asparagine). On the other hand, when acrolein gas was sprayed onto glutamine under the same conditions, only a trace amount of acrylamide was formed (0.18 μg/g of glutamine). Relatively high levels of acrylamide (753 μg/g of ammonia) were formed from ammonia and acrolein heated at 180 °C in the vapor phase. The reaction of acrylic acid, which is an oxidation product of acrolein and ammonia, produced a high level of acrylamide (190 000 μg/g of ammonia), suggesting that ammonia and acrolein play an important role in acrylamide formation in lipid-rich foods. Acrylamide can be formed from asparagine alone via thermal degradation, but carbonyl compounds, such as acrolein, promote its formation via a browning reaction.

KEYWORDS: Acrylamide; acrolein, asparagine; browning reaction; gas chromatography

INTRODUCTION

The presence of acrylamide in foods has been recently reported by European researchers (*1*, *2*). After these papers, many institutions have begun to analyze acrylamide in food products. For example, the U. S. Food and Drug Administration reported the analysis of acrylamide in 286 commercial food products (*3*). Their results ranged from none detected to 1184 ppb (Lipton Recipe Secrets Onion Soup and Dip Mix).

The method commonly used for acrylamide analysis is the high-performance liquid chromatography (HPLC)/mass spectrometry (MS) method. A commercial LC/MS instrument became available in 1980. However, it is still a very expensive instrument. The price of LC/MS today ranges from $200,000 to $300,000. Therefore, it is difficult to study acrylamide in food products in individual laboratories. In the present study, a less expensive gas chromatographic method for acrylamide analysis was developed using browning mode systems.

Significant amounts of acrylamide (221 mg/mol of amino acid) formation were reported in a browning model system that consisted of an equimolar of asparagine and glucose heated at 185 °C (*4*). Tremendous numbers of chemicals are formed in a browning reaction, which occurs from the interaction between carbonyl compounds (aldehydes, ketones, sugars, carbohydrates, and lipids) and amine compounds (ammonia, alkylamines, amino acids, proteins, peptides, and phospholipids) upon heat treatment (*5*, *6*). Some of these browning reaction products, which contain a three carbon unit (e.g., acrolein, propionaldehyde, propanenitrile, propioamide, and methylglyoxal), can be precursors of acrylamide. In addition, a series of alkyl amides (e.g., propionamide—which are closely related to acrylamide in their structure) have been found in many sugar/amino acid browning model systems. For example, *n*-alkylamides and N-alkyl *n*-amides were formed in beef fat heated with glycine at 200 °C (*7*). In the present study, therefore, simple chemicals such as acrolein and ammonia, in addition to asparagine and triolein, were used for the preparation of browning reaction mixtures.

MATERIALS AND METHODS

Chemicals and Reagents. Triolein, L-asparagine monohydrate, L-(+)-glutamine, glycerol, and acrylic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acrylamide and acrolein were bought from Tokyo Kasei Kogyo, Co., Ltd. (Tokyo, Japan). Ammonium solution (special reagent grade) was from Kanto Kagaku, Co., Ltd. (Tokyo, Japan).

Measurement of Detection and Quantitative Limits of Acrylamide. A standard solution of acrylamide was prepared with ethyl acetate (1 mg/L). Detection and quantitative limits were determined using a

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10.1021/jf0300947 CCC: $25.00
Published on Web 06/10/2003

method previously reported (*8*). A standard solution of 1 μL of acrylamide (1 ng of acrylamide) was injected into GC. The injection was duplicated six times.

Recovery Tests on Acrylamide from Silica Gel Column Chromatography. Silica gel chromatography was used to clean up samples. Acrylamide (400 μg) in 1 mL of dichloromethane was placed in a glass column (185 mm × 15 mm o.d.) packed with 10 g of silica gel and subsequently developed with 100 mL each of dichloromethane, methanol/ethyl acetate (1/19, v/v), and methanol/ethyl acetate (1/19, v/v) in series. Acrylamide was analyzed by a gas chromatograph/mass spectrometer (GC/MS).

Recovery Tests on Acrylamide from Triolein. Triolein (5 g) spiked with acrylamide (100 μg) was placed in a 300 mL separatory funnel with 130 mL of a hexane/methanol (10/3) solution, and then, the solution was shaken for 10 min. After the methanol layer was removed, the residual hexane solution was extracted with 30 mL of methanol. The methanol layer was added to the original methanol solution. After the methanol solution was filtered with a glass fiber, the methanol filtrate was condensed with a rotary evaporator under reduced pressure. The methanol was removed further with a purified nitrogen stream. The brown viscous material obtained was dissolved into 100 mL of dichloromethane. This dichloromethane solution was transferred into a glass column (185 mm × 15 mm o.d.) packed with 10 g of 75−150 μm mesh silica gel (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and anhydrous sodium sulfate (1 g) was placed on the top of the silica gel. After all of the dichloromethane was eluted, acrylamide was eluted with a 100 mL methanol/ethyl acetate (5/95) solution. The eluate was condensed with a rotary evaporator, and then, it was condensed further with a purified nitrogen stream. The residual brown viscous material was dissolved in ethyl acetate, and then, the volume of the sample solution was adjusted to exactly 5 mL with ethyl acetate. The ethyl acetate solution was analyzed for acrylamide by a GC/nitrogen phosphorus detector (NPD).

Recovery Tests on Acrylamide from Solid Matrix. Soy protein (50 g), starch (50 g), and acrylamide (1 mg) were mixed well with 125 mL of water in an aluminum pan (245 mm × 180 mm × 30 mm depth). After water was removed from the mixture in an electric dryer at 200 °C, a part of the residual solid material (10 g) was ground and then added into 50 mL of methanol in a 100 mL flask. The methanol solution was sonicated for 20 min. After the solution was filtered, methanol was removed from the filtrate by a rotary evaporator. Methanol was further removed by a purified nitrogen stream. The residual material was treated with silica gel column chromatography and analyzed for acrylamide as described above. The blank sample was prepared with the same mixture without acrylamide. The recovery values were adjusted to the blank values.

Sample Preparation of Reaction Mixtures from Thermal Degradation of Asparagine and Glutamine. Two grams of asparagine monohydrate (1.76 g as asparagine) was heated in a 50 mL recovery flask (unsealed) at 180 °C for 30 min in an oil bath. Glutamine (2 g) was placed in a watch glass in a stainless steel beaker and then heated at 180 °C for 30 min in an oil bath—a watch glass was used for glutamine because the solid formed after heating was very hard and difficult to remove from a flask. The reaction mixture became solid after it was cooled to room temperature. The solid materials were transferred into a mortar and then ground into powder.

After 50 mL of methanol was added to the reaction mixture (from asparagine) or the powder (from glutamine), the solution was sonicated for 20 min. After the methanol solution was filtered, the methanol filtrate was condensed with a rotary evaporator under reduced pressure. The methanol was further removed with a purified nitrogen stream. The brown viscous material obtained was treated with silica gel column chromatography as described above for acrylamide analysis.

Analysis of Acrolein Formed in Headspace of Heated Triolein. Triolein (5 g) was heated in a three-necked round bottom flask at 180 °C for 30 min. Acrolein formed in the headspace was collected by a 100 μL gastight syringe (Supelco, Bellefonte, PA), and then, 10 μL of the headspace gas was injected into a GC/flame ionization detector.

Sample Preparation of Reaction Mixtures of Triolein and Amino Acids. Triolein (5 g) and 2 g of asparagine or 2 g of glutamine were mixed in a 50 mL recovery flask. The mixture was heated at 180 °C for 30 min in an oil bath. The reaction mixture was transferred into a 300 mL separatory funnel with a 130 mL hexane/methanol (10/3) solution. The solution was subsequently treated in exactly the same way as the method described for Recovery Tests on Acrylamide from Triolein.

Sample Preparation of Reaction Mixtures of Amino Acids and Ethylene Glycol or Glycerol. Asparagine or glutamine (2 g each) and 5 mL each of ethylene glycol or glycerol were mixed in a 50 mL recovery flask, which was subsequently heated at 180 °C for 20 min. The reaction mixtures (1 mL each) were treated with silica gel column chromatography as described above for acrylamide analysis.

Sample Preparation of Reaction Mixtures of Amino Acids and Acrolein. Amino acid (2 g of asparagine or glutamine) was heated to 180 °C in a 50 mL recovery-flask, into which acrolein gas was subsequently sprayed for 30 min using a gas washing bottle purging with a highly purified nitrogen stream (acrolein concentration in a gas phase = 117 mg/L). After 50 mL of methanol was added to the reaction mixture, the solution was sonicated for 20 min. The solution was filtered and then treated with silica gel chromatography as described above for acrylamide analysis.

Sample Preparation of Reaction Mixtures of Acrolein and Ammonia. Silica gel (2 g) in a 50 mL recovery flask was sprayed with ammonia gas using a gas washing bottle purging with air, and simultaneously, it was sprayed with acrolein gas using the method described above at various temperatures in an oil bath for 30 min. After 50 mL of methanol was added to the flask, the samples were treated using the procedure described above for acrylamide analysis. A final sample obtained from this experiment was also analyzed for acrylamide using LC/MS to confirm the GC/MS method.

Sample Preparation of Reaction Mixtures of Acrylic Acid and Ammonia. Silica gel (2 g) and 2 g of acrylic acid in a recovery flask was sprayed with ammonia gas using the method described above for 30 min at 180 °C in an oil bath. After 50 mL of methanol was added to the reaction mixture, the samples were treated using the same procedure as described above for acrylamide analysis.

Sample Preparation for LC/MS Analysis. A 1 g aliquot of the ethyl acetate sample prepared above was weighed into a 15 mL disposable Corning centrifuge tube (concurrent recoveries were fortified at this point with D_3-acrylamide), and 10 mL of water was added. The sample was placed on a platform shaker for 20 min at 150 rpm, followed by centrifugation for 30 min at about 2800 rpm. The supernatant was removed (5 mL) and filtered through a Whatman 5.0 μm nylon with glass fiber disposable filter into a 12 mL tube.

An Oasis HLB solid phase extraction (SPE) cartridge (0.2 g/6 mL, Waters Corp, Milford, MA) was conditioned with 1 column volume of methanol followed by 1 column volume of water. Each conditioning solvent was allowed to flow by gravity. An aliquot of the sample (0.2 g) was then cleaned with the SPE. The eluant was discarded. Acrylamide residues were eluted with 3 mL of water into a 15 mL graduated centrifuge tube. The final sample volume was adjusted, and an aliquot was filtered through a 0.2 μm Acrodisc (Pall Corp., Ann Arbor, MI) into an autosampler vial.

Acrylamide Analysis by GC/NPD and GC/MS. A Hewlett-Packard (HP) 5890A GC equipped with a 30 m × 0.25 m i.d. (d_f = 0.25 μm) DB-WAX fused silica capillary column (J & W Scientific, Folsom, CA) and a NPD was used for routine acrylamide analysis. The oven temperature was held at 50 °C for 1 min and then programmed to 180 °C at 30 °C/min and to 210 °C at 5 °C/min. Injector and detector temperatures were 250 and 280 °C, respectively. Linear velocity of helium carrier gas was 27 cm/s in splitless mode.

An Agilent 6890 Series GC interfaced to an Agilent MS with an Agilent 5973 N mass selective detector was used to confirm acrylamide in samples. The GC was equipped with a 30 m × 0.25 mm i.d. (d_f = 0.30 μm) DB-FFAP (J & W Scientific). The oven temperature was held at 40 °C for 2 min and then programmed to 250 °C at 5 °C/min and held for 10 min. The injector temperature was 280 °C. The linear velocity of helium carrier gas was 36 cm/s in splitless mode.

Acrylamide Analysis by LC/MS Sample Analysis. Sample analysis was conducted with a Perkin-Elmer Series 200 autosampler and micropump (Perkin-Elmer, Shelton, CT) coupled to a PE Sciex API 2000 tandem mass spectrometer via an atmospheric pressure chemical

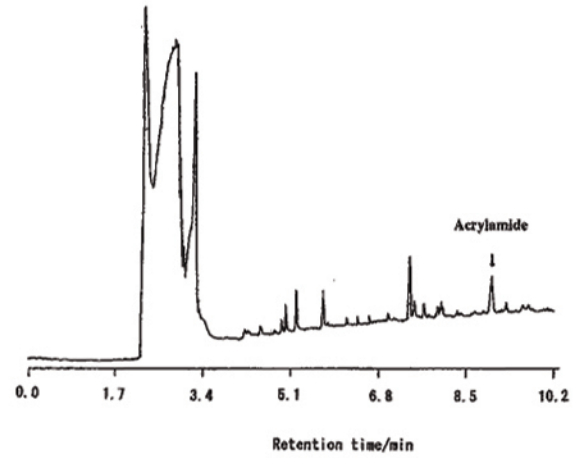

Figure 1. Typical gas chromatogram of a sample obtained from asparagine heated at 180 °C. Refer to the Materials and Methods for GC conditions.

ionization (APCI) source (PE Biosystems, Walnut Creek, CA). The APCI source was operated in positive ionization mode at 475 °C with nitrogen gas. The MS was operated in multiple reactant monitoring mode to observe the transition of *m/z* 72 to *m/z* 55 for acrylamide and *m/z* 75 to *m/z* 58 for deuterated acrylamide (via collision-induced dissociation with nitrogen gas). Chromatographic separation was accomplished with a Thermo Hypersil-Keystone Hypercarb (catalog no. 35005-052131, 50 mm × 2.1 mm i.d., 5 μm particle size). The autosampler was programmed to inject 5 μL. The mobile phase condition was isocratic at 99/1 acetic acid (0.1%)/methanol with a flow rate of 400 μL/min.

RESULTS AND DISCUSSION

Because acrylamide is highly soluble in water (215.5 g/100 mL) and less soluble in organic solvents (155 g/100 mL methanol, 12.6 g/100 mL ethyl acetate, and 0.0068 g/100 mL hexane), sample preparation steps for GC analysis are significantly difficult. Therefore, many methods were tested for sample preparations and the method reported in the Materials and Methods was found to be the best method at this point.

The detection and quantitative limits of GC/NPD were 0.20 and 0.67 ng, respectively, in the present study. **Figures 1** and **2** show typical gas chromatograms of samples obtained from asparagine alone and from an asparagine/triolein model system, respectively.

The recovery of acrylamide from silica gel chromatography was 0% by 100 mL of dichloromethane (fraction 1), 100% by 100 mL of methanol/ethyl acetate (1/19, v/v; fraction 2), and <0.01% by methanol/ethyl acetate (1/19, v/v, fraction 3). Therefore, the optimum condition for silica gel cleanup was to wash a column with 100 mL of dichloromethane and then to elute acrylamide with 100 mL of methanol/ethyl acetate (1/19, v/v).

Recovery efficiencies of acrylamide from triolein and solid matrix were 90 and 85%, respectively. The results indicate that the method developed in the present study is satisfactory.

Table 1 shows the results of acrylamide analysis in various browning model systems. The values are average of two experiments. It was hypothesized that asparagine and glutamine alone produced acrylamide upon heat treatment because they contain an amide moiety in their molecule. Asparagine alone produced acrylamide (0.99 μg/g of asparagine) upon thermal degradation, while glutamine produced 0.17 μg/g under same conditions. When asparagine was heated at 180 °C with glucose, a large amount of acrylamide (1200 μg/g of asparagine) was formed. The result was similar to the previously reported value

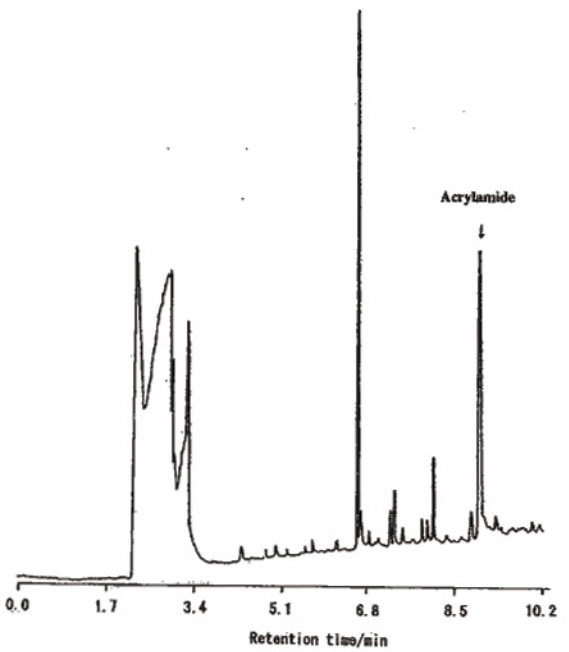

Figure 2. Typical gas chromatogram of a sample obtained from an asparagine/triolein model system. Refer to the Materials and Methods for GC conditions.

Table 1. Results of Acrylamide Analysis in Browning Model Systems

reactants			
amines (amount)	carbolys (amount)	temp (°C)	amount of acrylamide (μg/g of amine)
L-asparagine (1.76 g)		180	0.99
L-asparagine (1.76 g)	triolein (5 g)	180	88.6
L-asparagine (1.76 g)	glucose	180	1200
L-(+)-glutamine (2 g)		180	0.17
L-(+)-glutamine (2 g)	triolein (5 g)	180	3.53
ammonium chloride (1 g)	triolein (5 g)	180	0.51
L-asparagine (1.76 g)	ethylene glycol (5 g)	180	<0.01
L-asparagine (1.76 g)	glycerol (5 g)	180	4.42
L-asparagine (1.76 g)	acrolein (0.878 g)[a]	180	114
L-(+)-glutamine (2 g)	glycerol (5 g)	180	0.34
L-(+)-glutamine (2 g)	acrolein (0.878 g)	180	0.18
ammonium chloride (2 g)	acrolein (0.878 g)	180	<0.01
ammonia (0.329 g)	acrylic acid (2 g)	180	190 000

[a] Acrolein was supplied as a gas phase, whose concentration was 117 mg/L in nitrogen, at a flow rate of 250 mL/min for 30 min. [b] Ammonia was supplied as a gas phase, whose concentration was 73.0 mg/L in air, at a flow rate of 150 mL/min for 30 min.

(1672.7 μg/g of asparagine) (*4*), suggesting that acrylamide formation was promoted by glucose via the browning reaction. As mentioned above, the browning reaction occurs from interaction between an amine and a carbonyl compound. Many cooking practices involve the use of oils, which can be a carbonyl source for the browning reaction. Therefore, triolein was used as one of the carbonyl reactants in the present study. When asparagine was heated with triolein at 180 °C, acrylamide formed at the level of 88.6 μg/g of asparagine. Formation of ammonia and glycerol was observed in this reaction mixture. It is well-known that α-amino acid produces ammonia via Strecker degradation in the presence of a carbonyl compound (*9*). **Figure 3** shows the hypothesized formation mechanisms of acrylamide from amino acids and lipids. When asparagine was reacted with glycerol, acrylamide formed at the level of 4.42 μg/g of asparagine, whereas asparagine did not produce

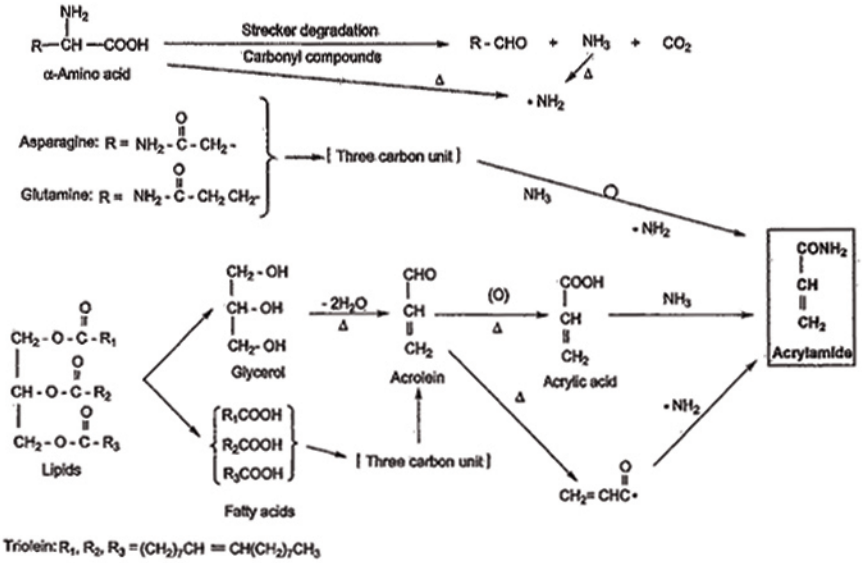

Figure 3. Hypothesized formation mechanisms of acrylamide from an amino acid and a lipid.

detectable amounts of acrylamide with ethylene glycol. The results suggest that the acrylamide formation is accelerated by a carbonyl compound and that a three carbon unit, such as glycerol, is required for acrylamide formation. Also, when ammonium chloride was heated with triolein as an ammonia source, acrylamide formed at the level of 0.51 μg/g of ammonium chloride. From these results, it is hypothesized that glycerol produced from lipids, such as triolein, forms acrolein via a dehydration reaction. Acrolein was oxidized to give acrylic acid, which subsequently reacted with ammonia from asparagine to yield acrylamide (*10*).

It is well-known that lipids (triglycerides) produce a large amount of acrolein by heat treatment. For example, when various cooking oils were heated at 300 °C for 2 h, large amounts of acrolein formed (*11*). Acrolein in the headspace of heated corn oil increased considerably when the oil was heated above 200 °C (*12*). When triolein was heated at 180 °C for 30 min, acrolein formed at the level of 1.82 ± 0.31 (n = 5) mg/L of headspace gas in the present study. The reaction of acrylic acid and ammonia produced a great amount of acrylamide (190 000 μg/g of ammonia), suggesting that ammonia and acrolein play an important role in acrylamide formation in lipid-rich foods upon heat treatment.

When acrolein gas was sprayed onto asparagine heated at 180 °C, significant amounts of acrylamide were formed (114 μg/g of asparagine). On the other hand, when acrolein gas was sprayed onto glutamine under the same conditions, acrylamide was formed only at the level of 0.18 μg/g of glutamine. Even though both amino acids contain an amide moiety, asparagine produced much more acrylamide than glutamine did in the model systems used in the present study. 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.

Figure 4 shows the amounts of acrylamide formed from the reaction of ammonia and acrolein at various temperatures. Acrylamide formed even at room temperature. Acrylamide formation increased with temperatures up to 180 °C and then reduced at 200 °C. The results suggest that acrylamide formation does not require very high temperatures. It is also reported that acrylamide formation from a reaction of asparagine and glucose reached its highest at a temperature around 170 °C (*4*).

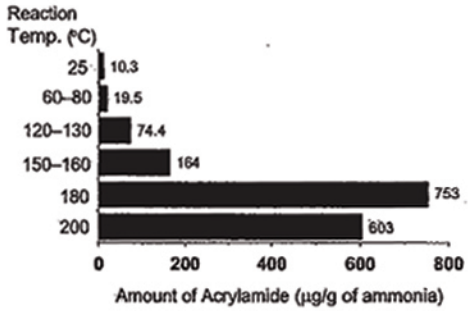

Figure 4. Acrylamide formed from ammonia and acrolein at various temperatures.

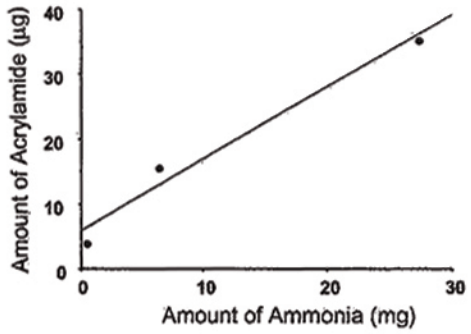

Figure 5. Amount of acrylamide formed from different amounts of ammonia in an ammonia/acrolein model system.

Figure 5 shows the amount of acrylamide formed from different amounts of ammonia (0.42, 6.56, and 27.0 mg) in an ammonia/acrolein model system. The values are the average of two experiments. In this system, the amount of acrolein (100 mg) was kept constant. The total amount of acrylamide formed was proportional to the amount of ammonia reacted. The relationship between the amount of ammonia (X, mg) and the amount of acrylamide formed (Y, μg) was written as $Y = 1.107X + 5.8846$, $R^2 = 0.971$.

The results obtained in the present study suggest the following formation pathways for acrylamide (refer to **Figure 3**): (i) Acrylamide forms from an amino acid alone upon thermal degradation. In this case, a three carbon unit is provided by an

amino acid. (ii) Ammonia produced from α-amino acids via Strecker degradation in the presence of carbonyl compounds reacts with acrylic acid, which is produced from acrolein, to give acrylamide. Acrolein forms from lipids (triglyceride) at high temperature treatment. (iii) An acrylic radical formed from homolytic fusion of acrolein at high temperatures absorbs an amine radical formed from amino acid at a high temperature treatment to yield acrylamide. In the case of ii and iii, acrolein also forms from a compound with a three carbon unit.

α-Amino acids, in particular asparagine, may play an important role in acrylamide formation in foods (*4*, *13*). However, acrylamide can also form from many different food constituents in addition to amino acids. Many foods and beverages that have undergone certain heat treatments may produce acrylamide via nonenzymatic browning reactions. It should be noted that browning reactions involve numerous types of chemical reactions, such as oxidation, reduction, dehydration, hydrolysis, and dehydrogenation as well as radical reactions. Also, it is very important to consider that formation of acrylamide in foods is at extremely low levels. Therefore, it may not be possible to explain every acrylamide formation by conventional organic reaction mechanisms.

When the sample obtained from an ammonia/acrolein model system heated at 180 °C was analyzed for acrylamide by LC/MS, a level of 507 μg/g of ammonia was found. This level was lower than that obtained by GC. This may be due to additional cleanup performed with the SPE. The result indicates that a GC/NPD method was comparable to a LC/MS method. The gas chromatographic method provided satisfactory results on acrylamide analysis in the present study.

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Received for review February 6, 2003. Revised manuscript received May 12, 2003. Accepted May 13, 2003.

JF0300947