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Influence of variety and processing conditions on acrylamide levels in fried potato crisps

J.S.E. Williams

Campden and Chorleywood Food Research Association, Chipping Campden, Gloucestershire GL55 6LD, UK

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Abstract

An investigation was carried out to determine the factors of greatest significance for the level of acrylamide formation in fried potato crisps. Factors under investigation were potato variety, the inclusion of a water soak prior to frying, cooking oil type, cooking temperature and cooking time. Data showed that cooking time and temperature had the greatest influence on acrylamide formation. Cooking oil type and soaking were found to be insignificant. Potato variety had a significant effect, with acrylamide levels found to be controlled by the levels of reducing sugars rather than asparagine. In addition, there were indications that the condition of the cooking oil (as indicated by peroxide value) did not affect acrylamide levels.

1. Introduction

The possibility that acrylamide, a known human toxigen, may be formed in the heat-processing of foods was first postulated in Sweden in 2000 (Tareke, Rydberg, Karlsson, Eriksson, & Toernqvist, 2000). The observation went largely unnoticed until the same authors published a study showing relatively high levels of acrylamide in many heat-processed foods (Tareke, Rydberg, Karlsson, Eriksson, & Toernqvist, 2002). This publication aroused intense international interest, leading to the initiation of a significant research effort around the world (Friedman, 2003).

Early investigations of the reaction pathways associated with the formation of acrylamide reached a consensus that the process is initiated with the reaction between a reducing sugar and asparagine, thus suggesting the Maillard reaction as a source of acrylamide (Stadler et al., 2002; Mottram, Wedzicha, & Dodson, 2002; Yaylayan, Wnorowski, & Perez Locas, 2003). However, subsequent studies (Becalski, Lau, Lewis, & Seaman, 2003; Yasuhara, Tanaka, Hengel, & Shibamoto, 2003; Zyzak et al., 2003) have suggested that the Maillard reaction may represent only one of a number of pathways to acrylamide formation, although it is still accepted that the presence of asparagine is a crucial factor in the formation of acrylamide in food.

Potato products have been associated with some of the highest levels of acrylamide, partly due to relatively high levels of suspected acrylamide precursors. Although acrylamide is not present in raw potatoes, or formed during boiling, high levels of acrylamide may be formed at the higher temperatures associated with frying and oven-baking (Ahn et al., 2002). The fact that most commercially produced potato products are subjected to high temperature processing, at some stage, means that potato products have become associated with high acrylamide levels. For that reason, potatoes and potato-like model systems have been the subject of numerous studies of acrylamide formation (Weisshaar & Gutsche, 2002).

A study of the formation of acrylamide in a range of fried foods found the highest levels in potato products (Becalski et al., 2003). The study found that the type of

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cooking oil used could affect the level of acrylamide, with olive oil giving rise to the highest levels. The possible role of acrolein in acrylamide formation was also investigated, although no evidence was obtained to suggest that this is an important synthetic pathway.

Another study investigated the relationship between potato variety and acrylamide formation (Amrein et al., 2003). It was found that asparagine was more abundant in potatoes than reducing sugars, although the concentrations of glucose and fructose varied more than that of asparagine between varieties. Very little correlation was found between asparagine content alone and acrylamide formation. It was suggested that the acrylamide potential of a potato was equal to 0.5×[glucose]×[fructose]×[asparagine]. The principles of this study were applied to develop an approach for the production of french fries with an acrylamide concentration of less than 100 μg kg^{-1} (Grob et al., 2003).

In response to scientific developments, national and international authorities have been concerned with monitoring levels of acrylamide in different food types, and with methods for the reduction of acrylamide in a typical diet. For example, the German government issued a set of practical tips aimed at minimisation of acrylamide in domestic potato and cereal products. This included a suggestion that potatoes should be soaked in water prior to cooking, and that cooking temperatures should be reduced.

The objective of this study was to determine which parameters associated with the production of potato crisps are of greatest significance for the level of acrylamide formation. In conjunction with companies in the industry, a list of factors associated with the composition of potatoes and the process of crisp manufacture was compiled. Work was carried out to investigate the significance of these factors for acrylamide formation. Five different varieties of potato were studied in order to determine whether acrylamide minimisation strategies can be universally applied, or whether significant interactions between effects mean that different approaches are appropriate for different varieties.

2. Materials and methods

2.1. Potato samples

Samples of five potato varieties were supplied by Higgins Agriculture: Courage, Estima, Hermes, Maris Piper and Saturna. Courage tubers were soft and would be considered unfit for normal production. All other varieties were in sound condition. Estima samples had been held in a cold store (+3 °C) for approximately 1 month, while all other varieties had been stored between 8 and 12 °C for less than 2 months.

2.2. Chemicals

Acrylamide-2,3,3-d_3 (D > 98%) was purchased from Cambridge Isotope Laboratories, Andova, MA, USA. Hydrobromic acid (48 wt. % in water) was purchased from Aldrich. Bromine water was prepared by adding excess bromine (>99.5%), purchased from BDH, to distilled water. Potassium bromide (>99.5%) and sodium thiosulphate (>99.5%) were purchased from BDH. Ethyl acetate (>99.5%), sodium sulphate (>99.5%) and hexane (95%) were purchased from Fisher Scientific UK. All chemicals used for other analyses were of analytical grade.

2.3. Determination of amino acid content of potatoes

Determination of free amino acid content was carried out by Alta Bioscience (Alta Biosciences, The University of Birmingham, Birmingham, UK) using ion-exchange chromatography (Spackman, Stein, & Moore, 1958).

2.4. Determination of sugar contents of potatoes

Glucose, fructose, sucrose, lactose and maltose were quantified in potato samples by extraction into hot water and analysis by HPLC. The homogenised sample (5 g) was mixed with hot water (80 °C, 100 ml) for sufficient time to allow dissolution of sugars. After cooling, the mixture was made up to 250 ml and filtered. For samples with high sugar levels, further dilutions were made with water. Samples were analysed by HPLC using a Dionex Carbopac PA1, with an injection volume of 10 μl and an eluent solution of 0.16 M sodium hydroxide and 0.125 mM zinc acetate at a flow rate of 1 ml/min. Analytes were detected using a Pulse Amperometric Detector and quantification was made using the method of standard addition.

2.5. Determination of cooking oil composition

Fatty acid profiling was carried out using the standard IUPAC method (IUPAC, 1987). Free fatty acids were determined using the standard method prescribed in British Standard BS EN ISO 660:2000 (BSI, 2000). Peroxide values were determined using the standard method prescribed in British Standard BS 684 (BSI, 2001).

2.6. Preparation of fried potato crisps

Potato tubers were placed in a carborundum peeling device and rumbled for sufficient time until at least 90% of the peel was removed. For some samples, the tubers were soaked in water for 2 h at room temperature. The tubers were washed and sliced to a thickness of 135 ± 20 μm. 300 g of slices (3–4 tubers) and were fried

using a Bartlett mini-fryer at either 150 or 175 °C for 3 or 5 min.

2.7. Experimental design and statistical analysis

Samples were prepared in accordance with a full factorial experimental design. The design parameters were: potato variety (5 levels: Estima, Hermes, Saturna, Maris Piper & Courage), cooking temperature (150 and 175 °C), cooking time (3 and 5 min), soak (0 and 2 h), cooking oil (Vegetable & Palm). Statistical analysis was carried out using a Minitab general linear model.

2.8. Determination of acrylamide content of fried potato crisps

2.8.1. General

Acrylamide was quantified in potato crisps using a method adapted from a previously published method (Castle, 1993). For 21 of the 80 samples, duplicate analyses were carried out, with the mean result used for interpretation. In cases where the difference between duplicate results was greater than 10% of the mean result of duplicates, the analyses were repeated.

The analytical method had previously been successfully validated with replicate ($n=6$) analyses of a crispbread sample used in a UK FAPAS ring trial (Central Science Laboratory, UK; Ref: Series 30 Round 1, T3001, robust mean value 1213 μg kg^{-1}). The mean concentration of acrylamide was 1270 μg kg^{-1}, with a standard deviation of 94 μg kg^{-1}.

2.8.2. Extraction and derivatisation of acrylamide from samples

Sample (10 g) was homogenised with water (100 ml) and a 1 mg l^{-1} aqueous solution of acrylamide-d_3 (2 ml) for 1 min. After standing for 5 min, the top 'foam' layer was removed and the slurry was centrifuged at 5000 rpm for 15 min. An aliquot (25 ml) of the liquid was mixed with potassium bromide (7.5 g), hydrobromic acid (0.3 ml) and saturated bromine water (10 ml), and stored overnight at 4 °C. Excess bromine was removed by addition of sodium thiosulphate solution (0.1 M), dropwise, until discoloration was achieved. Into the mixture, sodium sulphate (7.5 g) was mixed and dissolved. The analyte was extracted into ethyl acetate/hexane (1:4 v/v) with two portions (40 ml) of the organic solvent. The organic extract was passed through sodium sulphate (1 g) and reduced to approximately 100 μl using a rotary evaporator at 40 °C.

2.8.3. Derivatisation of acrylamide calibration standards

Aqueous standard solutions, containing acrylamide-d_3 at 5 mg l^{-1} and acrylamide of concentrations 0.05, 0.25, 0.5, 1, 2.5 and 5 mg l^{-1}, were analysed for quantification purposes. The procedure used was identical to the extraction and derivatisation of samples with the omission of the initial extraction into water and centrifugation stages. The final organic extract was reduced to 1ml, rather than 100 μl.

2.8.4. Analysis of extracts by GC/MS

Analyses were carried out using a Hewlett Packard 6890 gas chromatograph connected to a Hewlett–Packard 5890 mass spectrometer. An injection volume of 2 μl was made using a splitless injector heated to 250 °C. The GC column was a Varian 60 m×0.25 mm fused silica capillary with a 0.25 μm VF-5MS phase. The column was held at 50 °C for 2 min, before being heated to 340 °C at a rate of 10 °C/min. Detection was in selected ion monitoring mode, monitoring ions 106, 108, 150 and 152 for acrylamide, and 109, 111, 153 and 155 for acrylamide-d_3 Ion 150 was used to quantify acrylamide, and ion 155 was used to quantify acrylamide-d_3.

3. Results and discussion

3.1. Composition of potatoes and cooking oils

Data from compositional analyses of each potato variety showed a number of differences (Table 1). For free amino acids, the largest variations between varieties were observed for alanine, arginine and glutamine. Notably, some differences were found between varieties in terms of asparagine concentration. The highest total levels of free amino acids were detected in the Hermes variety, with the lowest levels detected in Maris Piper.

The greatest overall differences between the varieties were in the concentrations of glucose and fructose. The highest levels of these reducing sugars were found in the Estima variety, presumably a consequence of cold storage.

Substantial differences were observed between the two cooking oils in terms of fatty acid profile, although the composition did not significantly alter during the frying of the 40 batches of crisp samples in each (Table 2). The profile of individual fatty acids was also unchanged by the frying of samples. Similarly, no change was observed in the content of free fatty acids through frying. However, peroxide value did increase substantially.

3.2. Acrylamide levels in samples

The concentration of acrylamide was found to range from 85 μg kg^{-1} to levels exceeding the range of the calibration curve. Although this increased the probability of inaccurate determination of acrylamide in some samples, it was considered that the data were suitable for the purpose of identifying overall trends.

Table 1
Data from compositional analyses of each potato variety

	Hermes (mol/kg)	Saturna (mol/kg)	Courage (mol/kg)	Maris Piper (mol/kg)	Estima (mol/kg)
Asoartic acid	1.8	1.7	1.6	1.75	2.2
Threonine	0.71	0.22	0.53	0.37	0.53
Serine	0.76	0.29	0.71	0.73	1.1
Glutamic acid	1.1	1.2	1.3	0.85	1.2
Asparagine	16	11	9	5.6	9.5
Glutamine	8.9	2.6	6.8	5	4
Proline	n.d.	n.d.	n.d.	n.d.	n.d.
Glycine	0.28	0.08	0.19	0.2	0.16
Alanine	0.91	0.22	0.63	0.36	0.44
Valine	1.3	0.73	1.1	0.88	1.7
Cystine	n.d.	n.d.	n.d.	n.d.	n.d.
Methionine	0.38	0.17	0.38	0.35	0.28
Isoleucine	0.62	0.47	0.48	0.42	0.53
Leucine	0.32	0.22	0.23	0.2	0.33
Tyrosine	0.27	n.d.	0.22	0.21	0.21
Phenylalanine	0.69	0.29	0.52	0.36	0.6
G aminobutyric acid	3.9	1.9	2.5	3	3.1
Lysine	1.3	0.71	1.2	0.76	0.94
TryDtoohan	0.23	0.2	0.23	0.07	0.12
Histidine	0.43	0.34	0.43	0.23	0.35
Arginine	1.9	1.3	3.1	1.1	1.2
Glucose	2.78	2.22	3.33	8.33	48.33
Fructose	3.33	2.22	4.44	8.33	37.78
Lactose	n.d.	n.d.	n.d.	n.d.	n.d.
Sucrose	2.92	1.75	4.1	3.22	5.26
Maltose	0.29	n.d.	n.d.	0.29	n.d.

n.d. = Not detected.

Table 2
Data from the compositional analysis of cooking oils before and after the frying of all samples

	Vegetable oil		Palm oil	
	Before frying	After frying	Before frying	After frying
Saturated (%)	15.9	16.1	50.5	50.1
Monounsaturated (%)	22.5	22.9	39.0	39.4
Polvunsaturated (%)	61.5	60.8	9.9	10.1
Trans-fattv acids (%)	0.14	0.12	0.58	0.55
Peroxide value (meq/kq)	4.00	8.90	2.50	10.20
Free fatty acids	0.11 (% oleic)	0.13 (% oleic)	0.09 (% palmitic)	0.1 4 (% palmitic)

Interpretation of the data was carried out using an ANOVA general linear model to evaluate the significance and magnitude of main effects and interactions. It was found that more normal distributions of the data itself and the residuals of the model were obtained when log([acrylamide]) was used as the response. This indicated that the distribution of acrylamide concentration was skewed, due to the inclusion of a small number of samples of very high acrylamide concentration in the data set. The use of log values also had the benefit of reducing the impact of inaccuracies at acrylamide levels above the calibration range.

Plots of the main effect of each factor considered in the experimental design are shown in Fig. 1. The influence of cooking temperature and time were found to be highly significant ($P < 0.001$), reflecting that the extent of formation of acrylamide in heated foods is dependent on the severity and duration of heat exposure.

The effect of soaking in water before frying was not found to be significant ($P = 0.180$). This does not support the proposal that acrylamide levels may be reduced in fried potato products through the leaching out of precursors into water. It is possible that acrylamide levels may be reduced by parboiling prior to frying. However, that reduction may be due to the consequent reduction in the required extent of frying as much as any loss of reactants into water.

Significant differences were observed between the potato varieties. A study of the relationship between compositional parameters for each variety and acrylamide concentration revealed that the concentration of reducing sugars showed the greatest correlation to

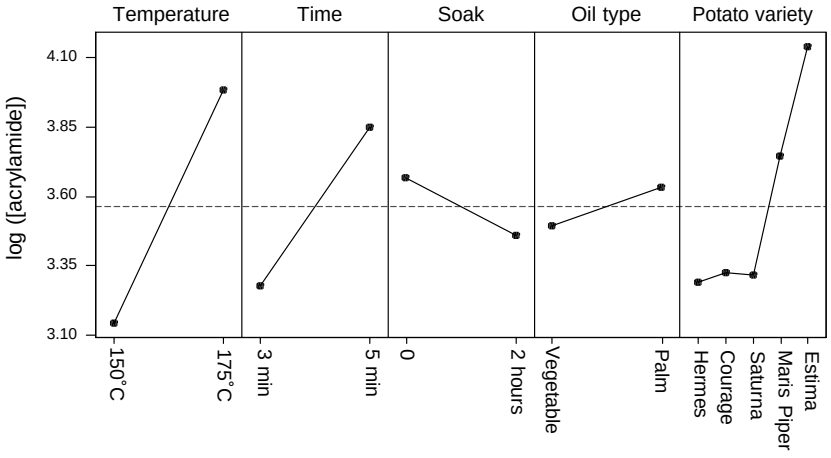

Fig. 1. Plots of the effect of factors under investigation on log([acrylamide]).

acrylamide formation (Fig. 2). Glucose and fructose concentrations in the raw potatoes both showed correlation with acrylamide level in the fried crisps of $r^2 = 0.97$. No correlation was found between amino acid content, including asparagine ($r^2 = 0.1$), or non-reducing sugars, such as sucrose ($r^2 = 0.6$) and acrylamide concentration. This finding indicates that, as long as asparagine is present, the concentration of reducing sugars is the limiting factor in the generation of acrylamide in food. This is in agreement with previously published findings (Amrein et al., 2003).

Differences were observed in the composition of the cooking oils, although the effect of oil type was not found to be significant ($P = 0.364$). Therefore, it appears that the nature of the lipid present, in terms of chain length and content of unsaturated fats, does not influence the formation of acrylamide.

Although changes were observed between the peroxide value of the cooking oils before and after cooking all samples, it was not possible to identify whether the observed changes occurred at a similar rate throughout the frying of the samples. Thus, it was not possible to directly study the correlation between oil decomposition and acrylamide concentration. However, a plot of residuals from the modelling of all main effects against the sequence in which samples were fried (fry number) showed no correlation (Fig. 3). This indicated that there was no link between oil condition and acrylamide formation provided that the oils were used according to normal recommended guidelines.

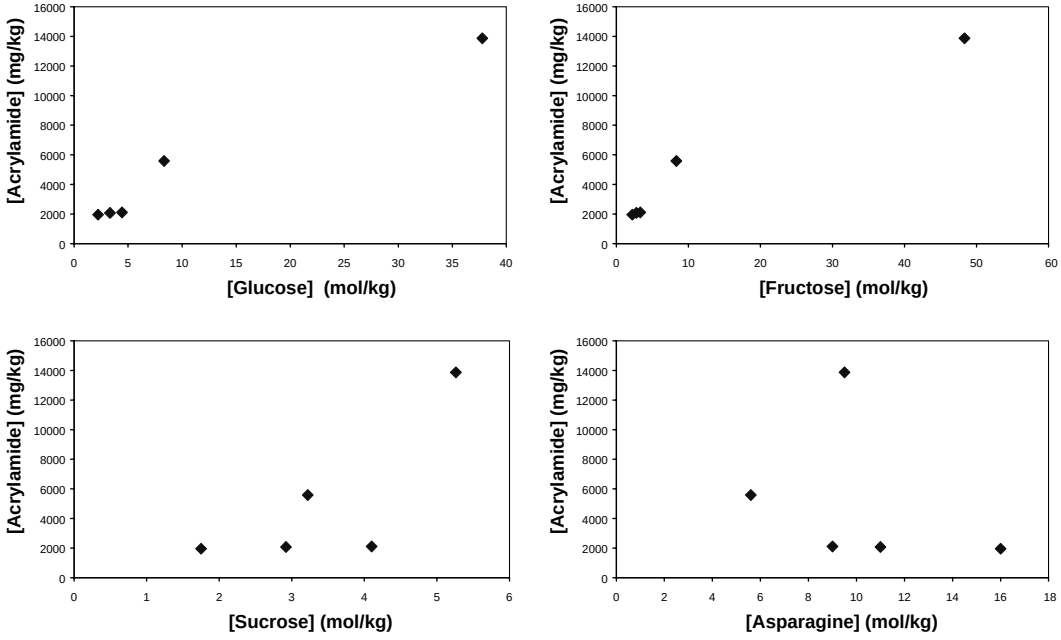

Fig. 2. Plots of the effects of the contents of sugars and amino acids in potatoes on [acrylamide].

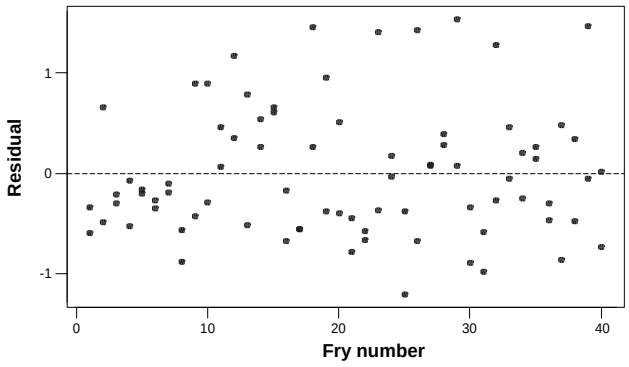

Fig. 3. A plot of residuals from the model of main effects against fry number.

Investigation of the influence of factor interactions found that only the interaction between cooking temperature and cooking time was significant ($P < 0.01$). It was observed that the difference between acrylamide levels formed at 150 and 175 °C was smaller after 5 min than after 3 min. This may indicate that most acrylamide formation occurs during the early stages of frying at the higher temperature, while there are still sufficient quantities of precursors remaining after 3 min at the lower temperature for acrylamide formation to continue at a high rate. Alternatively, it is possible that acrylamide is consumed in subsequent reactions at the higher temperature.

4. Conclusions

Cooking temperature and time were found to have the greatest influence on the level of acrylamide formation in fried potato crisps. However, the approach to acrylamide minimisation of reducing cooking temperature and/or time is of limited use to the food industry as it would produce a negative impact on product quality. It would be prudent for manufacturers to search for a time/temperature combination at which acrylamide levels may be reduced without unduly affecting quality. Such solutions are likely to be product specific.

The findings were broadly in line with those of previously published studies (Amrein et al., 2003; Grob et al., 2003), which found that the extent of acrylamide formation in fried potato products is predominantly controlled by the level of reducing sugars in the raw potato and cooking temperature.

One implication of this observation is the suggestion that the most effective means for limiting acrylamide levels in fried potato products is to control reducing sugar concentration using suitable storage conditions. It appears that the control of asparagine concentration in raw potatoes, for example by using asparaginase, would be a less effective approach.

This study also indicated that normal degradation of cooking oils, as monitored by measuring peroxide values, did not influence the levels of acrylamide formed (at least within normal recommended use). No evidence was found to support the approach of soaking of potatoes in cold water, prior to frying, as an effective means of reducing acrylamide. However, the principle that soaking could reduce acrylamide if it removed a significant proportion of reducing sugars is unchallenged.

This and other studies have demonstrated that effective approaches may be applied to the minimisation of acrylamide formation during the frying of potatoes. However, further studies are necessary to ensure that the quality and wholesomeness of these products are not unduly reduced as a result of implimenting these approaches.

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