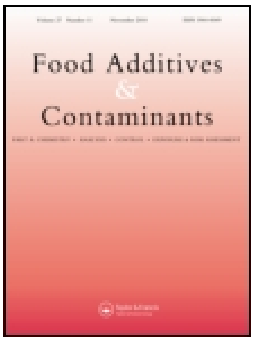

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Acrylamide concentrations in potato crisps in Europe from 2002 to 2011

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A dataset of manufacturers' measurements of acrylamide levels in 40,455 samples of fresh sliced potato crisps from 20 European countries for years 2002 to 2011 was compiled. This dataset is by far the largest ever compiled relating to acrylamide levels in potato crisps. Analysis of variance was applied to the data and showed a clear, significant downward trend for mean levels of acrylamide, from 763 ± 91.1 ng g^{-1} (parts per billion) in 2002 to 358 ± 2.5 ng g^{-1} in 2011; this was a decrease of 53% ± 13.5%. The yearly 95th quantile values were also subject to a clear downward trend. The effect of seasonality arising from the influence of potato storage on acrylamide levels was evident, with acrylamide in the first 6 months of the year being significantly higher than in the second 6 months. The proportion of samples containing acrylamide at a level above the indicative value of 1000 ng g^{-1} for potato crisps introduced by the European Commission in 2011 fell from 23.8% in 2002 to 3.2% in 2011. Nevertheless, even in 2011, a small proportion of samples still contained high levels of acrylamide, with 0.2% exceeding 2000 ng g^{-1}.

Keywords: LC-MS/MS; statistical analysis; acrylamide; potatoes; snack products

Introduction

The formation of acrylamide during the high-temperature cooking and processing of foods made from potatoes, cereals and other crops has become one of the most intractable problems facing the food industry since the discovery of acrylamide in food in 2002 (Tareke et al. 2002). Acrylamide is classified by the World Health Organization and the International Agency for Research on Cancer as a Group 2a carcinogen ("probably carcinogenic to humans"), and it also has effects on neurological and reproductive systems at high doses (Friedman 2003).

The FAO/WHO Joint Expert Committee of Food Additives (JECFA) recently concluded that the margins of exposure for acrylamide indicate that dietary intake is a human health concern and that epidemiological studies using haemoglobin adducts of acrylamide itself or its primary epoxide metabolite, glycidamide, as a measure of exposure were required to estimate the risk (Joint FAO/WHO Expert Committee of Food Additives 2011). However, the JECFA conceded that to date the results of epidemiological studies have been inconsistent and that "overall the epidemiological studies do not provide any consistent evidence that occupational exposure or dietary exposure to acrylamide is associated with cancer in humans", though it noted that some studies indicated an association with hormone-related cancers in women, which needed confirmation (Joint FAO/WHO Expert Committee of Food Additives 2011). Another review of the epidemiological data published in recent years concluded that the sporadically and slightly increased and decreased risk ratios seen in the studies suggested the pattern one would expect to find for a true null hypothesis (Lipworth et al. 2012). However, more recently, a Danish study found a link between acrylamide exposure and breast cancer–specific mortality (Olsen et al. 2012), and another showed a link between haemoglobin adducts of acrylamide and glycidamide in umbilical cord blood (reflecting exposure in the last months of pregnancy) and low birth weight and head circumference in babies (Pedersen et al. 2012).

Given the low margins of exposure and the uncertainty regarding the human health risk from acrylamide in the diet, the JECFA and other risk assessment bodies have recommended that acrylamide levels in food be reduced as a matter of priority and the European Commission issued "indicative" levels for acrylamide in food in early 2011 (http://ec.europa.eu/food/food/chemicalsafety/contaminants/recommendation_10012011_acrylamide_food_en.pdf). Indicative values are not safety thresholds but are intended to indicate the need for an investigation into why the level has been exceeded. Fried potatoes (such as French fries) and potato crisps are important contributors to dietary intake across Europe (European Food Safety Authority

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2011), along with bread, coffee, biscuits and, in some countries, rye crisp-breads, and the manufacturers of these foods have devised many strategies for reducing acrylamide formation by modifying food processing. These strategies have been compiled in a "Toolbox" produced by FoodDrinkEurope (http://ec.europa.eu/food/food/chemicalsafety/contaminants/ciaa_acrylamide_toolbox09.pdf); they include modification of time/temperature conditions during processing, lowering the pH by addition of citric acid, pre-soaking in water, addition of antioxidants and addition of divalent cations, such as calcium chloride. The addition of asparaginase to reduce asparagine concentration prior to cooking has been successful in some products but is not applicable to all foods. Further progress may be possible by variety selection (Amrein et al. 2003, 2004; Becalski et al. 2004; Olsson et al. 2004; Halford, Muttucumaru et al. 2012) and optimising crop management (Kumar et al. 2004; De Wilde et al. 2005, 2006; reviewed by Halford, Curtis et al. 2012). For potatoes, particular care has been taken to optimise harvesting, transportation and storage practices, with industry going to great lengths to keep potatoes stable during storage to prevent cold sweetening (the accumulation of reducing sugars that occurs when potatoes are stored at low temperature (Sowokinos 1990)).

The publication of European data on acrylamide levels in a variety of foods from 2007 to 2010 (European Food Safety Authority 2012) has been used as a measure of whether the strategies identified within the Toolbox approach have been successfully implemented by the European food industry. This study reported the analysis of 13,162 samples in 25 countries over the 4-year period, covering 10 different food categories. For potato crisps, only 293, 532, 414 and 242 samples were analysed in years 2007, 2008, 2009 and 2010, respectively, making a total of 1481. However, the study reported no significant trend in acrylamide levels in these crisps. Evidence or lack of evidence of the efficacy of the Toolbox approach taken by the food industry is important because it will inform future European member state discussions on their approach to the acrylamide issue.

In the present study, we report the analysis of a much larger dataset for acrylamide concentrations in potato crisps from 2002 to 2011 and show that, in contrast to previous findings, a general trend of decreasing acrylamide levels over time was sustained throughout that period.

Materials and methods

Acrylamide determinations

Data on acrylamide levels in samples of potato crisps were supplied by the European Snacks Association. All analyses had been performed using procedures based on LC-MS/MS (Roach et al. 2003), and all of the laboratories that conducted the analyses had carried out validations on the methods used. The methods were compatible with the Comité Européen de Normalisation (European Committee for Standardisation) standard method, which is due to be published under Mandate M463 at the end of 2013 (https://www.cen.eu/cen/Sectors/Sectors/Food/Documents/M_463.pdf).

Statistical analyses

The GenStat (2011, 14th edition, © VSN International Ltd., Hemel Hempstead, UK) statistical package was used for the calculation of summary statistics, and analysis of variance (ANOVA) was used to test the overall significance (*F*-test) of differences between years using all the data. The SigmaPlot (2011, version 12, © Systat Software, Inc., Richmond, CA, USA) package was used for graphs except histograms for which GenStat was used. Excel (2010, © Microsoft Corporation, USA) was used to store the data.

Results

Acrylamide levels in potato crisps from 2002 to 2011

Data on acrylamide levels in samples of potato crisps were supplied to the European Snacks Association by European manufacturers, having been collected over 10 years from 2002 to 2011. The data concerned crisps made from fresh, sliced potatoes, rather than from potato dough (formed or stacked crisps). Only those data for which acrylamide analyses had been carried out following authenticated analytical procedures using LC-MS/MS were included in the study. The dataset comprised 40,455 observations from crisps produced in 20 countries, making it by far the largest dataset to be analysed to date, and the numbers of observations for each year are shown in Table 1. Note that the number of observations per year prior to 2006 was relatively low, with 2002 having data only from May to November.

Reduction in acrylamide over time

The mean, standard error of the mean, variance, median (Q50), 95% quantile (Q95) and minimum and maximum values for acrylamide for each year are given in Table 1. The data were subjected to ANOVA. On the (natural) log (to base *e*) scale, the data were found to be distributed as Normal (Table 2, Figure 1). Analysis of residuals (Supplementary File S1; available via the article webpage) showed reasonable conformation to the assumptions of ANOVA (Normal distribution, constant variance across factor levels and additivity of effects). A statistical assessment was made of the year-to-year differences, based on the estimated standard error of the difference (SED) between pairs of means, which was calculated from the

Table 1. Number of observations (*n*), mean (ng g^{-1}), standard error of mean (SE), variance, minimum, median (Q50), 95% quantile (Q95) and maximum values for acrylamide in samples of potato crisps from 2002 to 2011.

Year	*n*	Mean	SE	Variance	Minimum	Q50	Q95	Maximum
2002	42	763	91.1	348,149	70	493	2080	2500
2003	136	573	27.3	101,440	160	475	1118	2080
2004	321	624	26.6	227,186	130	490	1580	4450
2005	230	621	21.3	103,875	100	565	1210	1780
2006	1151	577	11.9	163,776	22	460	1350	2830
2007	3206	570	7.0	157,220	30	476	1270	5900
2008	5692	472	5.1	145,005	30	367	1170	4300
2009	6493	500	5.8	215,229	40	350	1400	6000
2010	10,971	435	4.1	182,087	22	330	1020	12,000
2011	12,213	358	2.5	74,727	17	280	870	3090

Table 2. Mean acrylamide (ng g^{-1}) on the natural log (to base *e*) scale in samples of potato crisps for each year from 2002 to 2011, with number of observations (*n*).

Year	Mean (on $\log_e$ scale)	*n*	Year	Mean (on $\log_e$ scale)	*n*
2002	6.351	42	2007	6.148	3206
2003	6.217	136	2008	5.908	5692
2004	6.250	321	2009	5.899	6493
2005	6.286	230	2010	5.833	10,971
2006	6.158	1151	2011	5.654	12,213

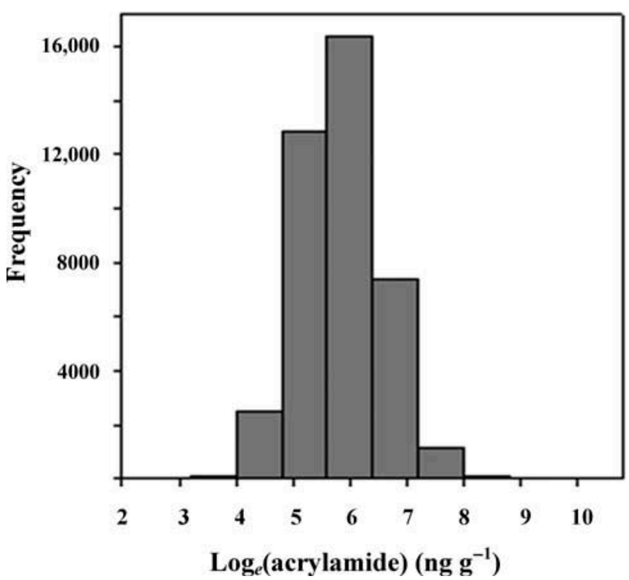

Figure 1. Distribution (histogram) of the $\log_e$(acrylamide) values (ng g^{-1}) in samples of potato crisps.

residual variance in the data (s^2). Because the year-to-year replication was unequal, there was one SED for each comparison. Corresponding least significant difference (LSD) values were then calculated by multiplying the SED by the upper 97.5% critical value of a *t*-distribution on the residual degrees of freedom from the ANOVA (Table 3). Pairs of means on the log (to base *e*) scale (Table 2) that differ by more than the corresponding LSD (Table 3) are significantly different at the 5% ($p < 0.05$) level.

The analysis revealed a highly significant effect of years ($F_{9,\ 40,445} = 242.75$; $p < 0.001$, noting that there are nine degrees of freedom for years and 40,445 residual degrees of freedom for the *F*-test, giving 40,454 degrees of freedom in total with 40,455 observations), with a general trend of decreasing acrylamide levels from 763 ± 91.1 ng g^{-1} in 2002 to 358 ± 2.5 ng g^{-1} in 2011 (Table 1), an overall significant ($p < 0.05$, LSD) reduction of 53% ± 13.5%. The trend in the Q95 values was also downward (Table 1), from 2080 ng g^{-1} in 2002 to 870 ng g^{-1} in 2011. 2011 was the first year in which the Q95 was below the 1000 ng g^{-1} indicative level for potato crisps set by the European Commission. Nevertheless, the range of acrylamide levels in the samples was wide, as can be seen from the standard errors, minima, maxima and variances (Table 1).

The overall trends are shown graphically in the plot of mean and Q95 acrylamide values over time in Figure 2. The first statistically significant ($p < 0.05$, LSD) year-to-year reduction in acrylamide was for 2005–2006, but this may in part be due to the low sample number in years 2002–2005, and the lack of samples from early 2002. There were also significant ($p < 0.05$, LSD) year-to-year reductions for 2007–2008, 2009–2010 and 2010–2011.

Seasonality

The mean, median (Q50) and Q95 acrylamide levels for potato crisps produced each month showed clear seasonality (Table 4). This was evident when the mean was plotted monthly over time (Figure 3a) and when the overall mean and Q95 values for each month were plotted (Figure 3b). Note that the lower standard errors on the monthly means from 2006 onwards (Figure 3a) arise from the greater numbers of observations that were

Table 3. Least significant difference (LSD) values on 40,445 degrees of freedom at the 5% ($p < 0.05$) level of significance, from analysis of variance of acrylamide in samples of potato crisps from 2002 to 2011.

2003	0.2367								
2004	0.2200	0.1372							
2005	0.2250	0.1451	0.1158						
2006	0.2107	0.1216	0.0846	0.0969					
2007	0.2083	0.1174	0.0785	0.0915	0.0461				
2008	0.2077	0.1164	0.0769	0.0902	0.0433	0.0296			
2009	0.2076	0.1162	0.0767	0.0900	0.0429	0.0289	0.0243		
2010	0.2073	0.1157	0.0759	0.0893	0.0415	0.0269	0.0219	0.0210	
2011	0.2073	0.1156	0.0758	0.0892	0.0413	0.0266	0.0215	0.0206	0.0176
	2002	2003	2004	2005	2006	2007	2008	2009	2010

Note: The value for a particular comparison of the means in Table 2 is given by reading along the appropriate row and up the appropriate column.

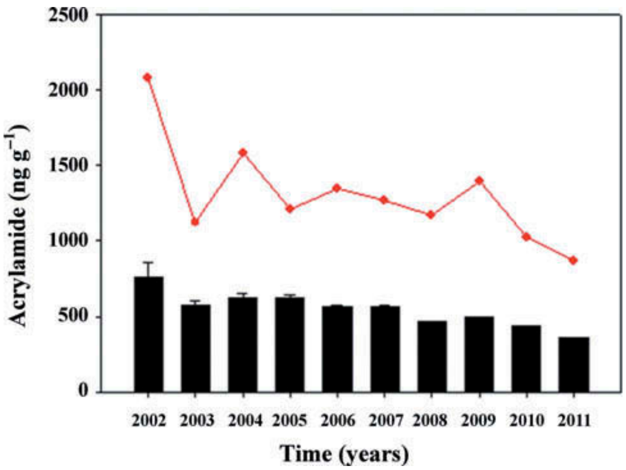

Figure 2. Overall mean acrylamide levels (ng g^{-1}) in samples of potato crisps shown over years from 2002 to 2011, with standard errors and with trend in 95% (Q95) quantiles (red).

Table 4. Number of observations (n), mean (ng g^{-1}), standard error of mean (SE), median (Q50) and 95% quantile (Q95) values for acrylamide in samples of potato crisps for each month using data from 2002 to 2011.

Month	*n*	Mean	SE	Q50	Q95
January	2561	554	8.0	453	1285
February	2543	540	7.8	422	1300
March	3689	546	7.4	434	1310
April	3258	519	7.2	420	1200
May	3615	551	9.2	419	1375
June	3377	462	6.8	354	1200
July	3191	302	5.2	220	763
August	3279	318	5.1	233	800
September	3779	314	4.8	230	820
October	3339	395	5.2	316	970
November	4088	434	5.4	340	1060
December	3736	450	5.4	360	1040

taken in more recent years and the resulting greater precision. The data show the acrylamide level in potato crisps to be at its lowest in July when new season potatoes start to be used in production in most European countries. The level remains low in August and September before increasing from October until January, then remaining relatively high until the cycle starts again in July.

Samples containing high levels of acrylamide

The number and percentage of observations that were greater than 1000 or 2000 ng g^{-1} in each year are given in Table 5, and the percentages are displayed in Figure 4 to show the trend. In 2002, 23.8% of the observations exceeded 1000 ng g^{-1}, the indicative level set for potato crisps by the European Commission in 2011, but the proportion had fallen to 5.2% by 2010 and 3.2% by 2011. Indeed, these last 2 years were clearly the best so far for meeting the criteria of reducing acrylamide in potato crisps to less than 1000 ng g^{-1}, although variability remained. The proportion of observations exceeding 2000 ng g^{-1} also showed a fall, from 4.8% in 2002 to 0.2% in 2011. This included a small number of samples with very high levels of acrylamide (see maxima in Table 1), which resulted in the data being positively skewed on the raw scale, with the mean higher than the median (Table 1).

Discussion

This study shows a clear downward trend in acrylamide levels in potato crisps in Europe that predated both the introduction of European-wide monitoring and indicative values, and was sustained right up to 2011 (the last year for which data were available), with significant year-on-year reductions from 2005 to 2006, 2007 to 2008, 2009 to 2010 and 2010 to 2011. The decrease from 763 ng g^{-1} in 2002 to 358 ng g^{-1} in 2011 represented an overall significant ($p < 0.05$, LSD) reduction of 53%. It is important to note that the study was European-wide and the situation may be different in some individual countries because acrylamide-forming potential in potato may be influenced by environmental factors. However, overall the study demonstrates the

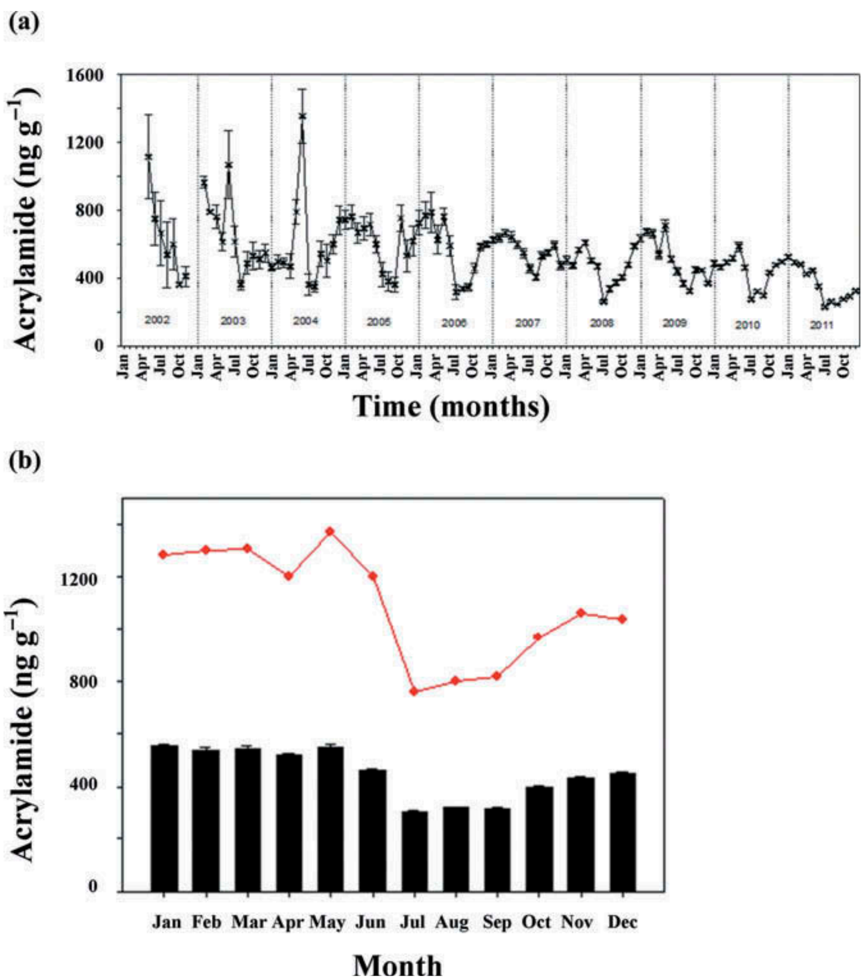

Figure 3. Seasonality in acrylamide levels in samples of potato crisps from 2002 to 2011. (a) Mean acrylamide levels over time (2002–2011) with standard errors, plotted monthly. (b) Mean acrylamide levels per month over all years with standard errors and with trend in 95% (Q95) quantiles (red).

Table 5. Number and percentage of potato crisp samples containing more than 1000 or 2000 ng g^{-1} acrylamide for each year from 2002 to 2011.

Year	Total number	Number > 1000 ng g^{-1}	Percentage > 1000 ng g^{-1}	Number > 2000 ng g^{-1}	Percentage > 2000 ng g^{-1}
2002	42	10	23.8	2	4.8
2003	136	11	8.1	1	0.7
2004	321	41	12.8	4	1.2
2005	230	31	13.5	0	0.0
2006	1151	130	11.3	15	1.3
2007	3206	320	10.0	28	0.9
2008	5692	429	7.5	44	0.8
2009	6493	706	10.9	97	1.5
2010	10,971	566	5.2	105	1.0
2011	12,213	386	3.2	27	0.2

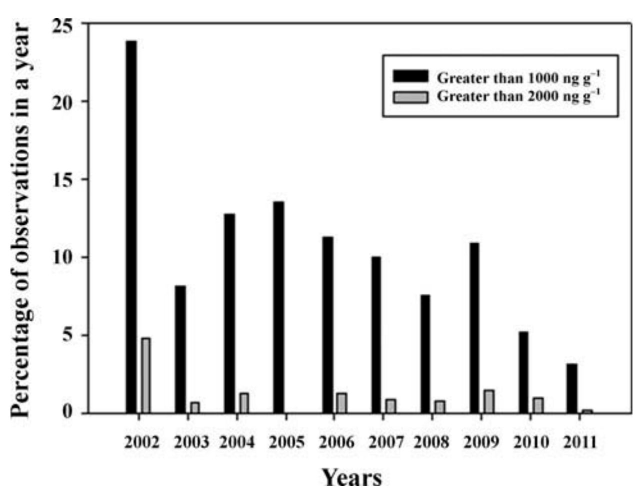

Figure 4. Percentage of samples of potato crisps in each year from 2002 to 2011 containing >1000 or 2000 ng g^{-1} acrylamide.

effectiveness of the approaches taken by potato crisp manufacturers to reduce acrylamide levels in their products, as compiled in the FoodDrinkEurope Acrylamide "Toolbox".

A continuation of the downward trend in acrylamide levels in potato crisps from 2007 onwards was not evident in data compiled by the EFSA for potato crisps (European Food Safety Authority 2012). The EFSA reported a mean acrylamide level of 551 ng g^{-1} in potato crisps in 2007, slightly lower than the 570 ng g^{-1} reported here, but showed no significant trend from 2007 to 2010, the latest year for which data were included in their report. In fact, acrylamide levels in 2010 were reported as having risen slightly (although not significantly) to 675 ng g^{-1}, whereas this study showed a continued reduction to 435 ng g^{-1} in 2010 and 358 ng g^{-1} in 2011. The decrease from 570 ng g^{-1} in 2007 to 358 ng g^{-1} in 2011 represents a reduction of 37%. There is no obvious reason why the results of the two studies should differ except that the dataset compiled for the present study was very much larger than the EFSA dataset and therefore more likely to be representative of the real situation. For 2010, for example, the EFSA dataset included only 242 potato crisp samples, whereas the analysis reported here was based on 10,971 samples. It was notable from the EFSA report that the number of samples being analysed has declined in recent years, from a total for all food types of 3889 in 2008 to 2071 in 2010, and for potato crisps of 532 in 2008 to 242 in 2010, a decrease of 54%. The EFSA relies on national organisations to sample foods and submit data for inclusion in its analysis, and its report states that the decline "may reflect the challenges that countries have had in submitting requested acrylamide data in a standardised way" (European Food Safety Authority 2012).

The range of acrylamide levels in the samples was wide, as can be seen from the minima, maxima and variances. The fact that the Q95 value was shown to be falling over time is therefore another important indicator of progress. However, even in 2011, which was the first year in which the Q95 was below the European Commission's indicative value of 1000 ng g^{-1} for potato crisps, 3.2% of the samples contained levels of acrylamide that exceeded the indicative value. The wide range of acrylamide levels probably reflects variability in the raw material and the difficulty of processing such a variable raw material to give a consistently low acrylamide level in the product. Potato tuber composition differs significantly between varieties (Amrein et al. 2003, 2004; Becalski et al. 2004; Olsson et al. 2004; Halford, Muttucumaru et al. 2012) and is affected by crop management practice (Kumar et al. 2004; De Wilde et al. 2005, 2006; reviewed by Halford, Curtis et al. 2012) and, almost certainly, by environmental factors such as weather conditions that are beyond the control of growers. It is also notoriously difficult to control during storage (Olsson et al. 2004; Matsuura-Endo et al. 2006; Halford, Muttucumaru et al. 2012). Reducing sugars, for example, which are precursors for acrylamide, accumulate rapidly in stored potato tubers in response to the temperature falling below approximately 8°C (cold sweetening; Sowokinos 1990), to sprouting (dormancy break) and to extended storage beyond the normal storage window, which is different for each variety (Halford, Muttucumaru et al. 2012). Potatoes are, therefore, usually stored at 8–10°C to prevent cold sweetening, while sprouting, which would occur at this temperature, is controlled through the use of sprout suppressants such as chlorpropham. The problem of keeping potatoes stable during storage was reflected in the seasonality of acrylamide levels shown in the study, with levels being lowest in July to September when potatoes are being harvested and highest in the early months of the calendar year when potatoes have to be used from long-term storage.

Improving the storage characteristics of potatoes, reducing the concentrations of free asparagine, the other precursor for acrylamide, and reducing sugars and making the concentrations of these metabolites less responsive to environmental factors is being addressed by potato breeders. Potato breeding is a slow process, however, and so food processors will have to be patient as they wait for improved varieties that will enable them to reduce acrylamide levels further without additional changes to processes. Nevertheless, it is important that potato breeders be encouraged to make reduced acrylamide-forming potential a priority and that they have sufficient information on how to achieve that target.

Although some genetic investigation has been done (Shepherd et al. 2010), identifying targets for breeders that will result in reduced acrylamide-forming potential in potato is not simple. The predominant route for acrylamide formation is through the Maillard reaction (Mottram et al. 2002; Stadler et al. 2002; Zyzak et al. 2003), a complex series of non-enzymic reactions between amino groups and reducing sugars that occur at high temperatures, such as those that arise during frying, baking and roasting. Acrylamide forms when asparagine participates in the final stages of the reaction, but the Maillard reaction also results in the formation of a plethora of compounds that impart colour, aroma and flavour (Nursten 2005; Mottram 2007; Halford et al. 2011) and, therefore, give particular products and brands their defining characteristics. Reducing acrylamide formation while not affecting the qualities that are demanded by consumers is therefore very difficult, and it is important that breeders take this into account as they bring about changes in potato composition.

The other problem for breeders is that the relationship between the concentrations of reducing sugars and free asparagine in potatoes and acrylamide formation during cooking and processing is complicated. Different studies, for example, have concluded that reducing sugar concentration, free asparagine concentration or free asparagine concentration as a proportion of the total free amino acid pool could be the determining factors (Amrein et al. 2003, 2004; Becalski et al. 2004; Elmore et al. 2007, 2010). In another study clear correlations were shown between reducing sugar concentration and acrylamide-forming potential in nine varieties of potatoes grown commercially in the United Kingdom (Halford, Muttucumaru et al. 2012), but free asparagine and total free amino acid concentration also correlated significantly with acrylamide-forming potential in French fry varieties, which contain higher concentrations of sugars than do crisping varieties. Another recent study modelled the kinetics of acrylamide formation in French fry production and concluded that reducing the ratio of free asparagine to total free amino acids could be the best strategy because it would decrease acrylamide formation without affecting quality (Parker et al. 2012).

Variability in the raw material from tuber to tuber may explain why some of the acrylamide levels in the study were over the 1000 ng g^{-1} indicative level, but it is difficult to understand how levels in a small number of samples were much higher than this. As discussed above, colour in crisps forms via similar pathways to acrylamide and a crisp containing such high levels of acrylamide would be a very dark colour. Such dark crisps are normally removed during standard industry quality control procedures and would not reach the market. Assuming that they have been measured accurately, these high acrylamide concentrations, therefore, could indicate a breakdown in either variety selection or processing, together with a failure in post-production quality control. They show that there is still room for improvement, and the cause of such high acrylamide levels would certainly require further investigation by the manufacturer. However, these samples represented a tiny fraction of the total. Indeed, by 2011, the proportion of samples exceeding 2000 ng g^{-1} was only 0.2% (27 observations out of 12,213).

In conclusion, this study represents the statistical analysis of the largest dataset ever compiled of acrylamide levels in potato crisps. It shows a clear, significant downward trend for mean levels of acrylamide from 2002, when acrylamide was first discovered in food, to 2011, suggesting that the "Toolbox" approach co-ordinated by FoodDrinkEurope has been effective.

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