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Acrylamide levels in potato crisps in Europe from 2002 to 2016

Stephen J. Powers[a], Donald S. Mottram[b], Andrew Curtis[c] and Nigel G. Halford[d]

[a]Computational and Analytical Sciences Department, Rothamsted Research, Harpenden, UK; [b]Department of Food and Nutritional Sciences, University of Reading, Reading, UK; [c]European Snacks Association asbl, Brussels, Belgium; [d]Plant Science Department, Rothamsted Research, Harpenden, UK

ABSTRACT

European manufacturers' data on acrylamide in potato crisps from 2002 to 2016 were analysed. A previous study showed a 53% reduction in mean acrylamide levels from 763 ng g^{-1} in 2002 to 358 ng g^{-1} in 2011. Analysis of data from the longer period showed that since 2011 there has been a levelling off, with the mean level for 2016 being 412 ng g^{-1} (still a 46% reduction from 2002), suggesting that the most effective acrylamide reduction measures had been devised and implemented by 2011. There were similar trends in the 90th and 95th quantile values, with the 90th quantile values being below 1000 ng g^{-1} (the European Commission's current 'Indicative Value' for acrylamide in potato crisps) since 2010. The proportion of samples with acrylamide above 2000 ng g^{-1} fell from 4.8% in 2002 to 0.6% in 2016. Acrylamide levels showed marked seasonal variability, being highest in the first half of the year when potatoes were being used from storage, and lowest from July to September when potatoes were being harvested. Acrylamide levels were higher in thicker types of crisp in the early years of the study, but this difference disappeared in the later years, suggesting that manufacturers had acted to reduce acrylamide formation in these products. Higher values for acrylamide were recorded in north and east Europe than in the south and west up to 2013. Levels in the north and east declined in recent years, but remained higher in the north than in the other regions. The manufacturers' data were compared with a much smaller dataset provided by the European Food Safety Authority (EFSA). Levels of acrylamide in the EFSA dataset were consistently higher than in the manufacturers' data, possibly due to uneven sampling through the year and the seasonality of acrylamide levels.

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Introduction

Acrylamide (C_3H_5NO) is a processing contaminant produced predominantly in the Maillard reaction at the high temperatures generated by frying, baking, roasting or high-temperature processing (Nursten 2005; Mottram 2007; Halford et al. 2011). It is classified as a Group 2A, 'probably carcinogenic to humans', carcinogen and the latest report from the European Food Safety Authority (EFSA) Expert Panel on Contaminants in the Food Chain (CONTAM) stated that the margins of exposure for dietary acrylamide indicated a concern for neoplastic effects based on animal evidence (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2015). Potato, coffee and cereal products are the major contributors to dietary acrylamide intake. For potato products, the majority of surveys conducted in Europe have found potato crisps to account for 0 – 5% of dietary acrylamide intake for adults and the elderly, and 0 – 5% or 5 – 10% for children and adolescents, depending on the survey, while French fries account for 10 – 25% or 25 – 50% of dietary intake for all age groups, with one survey putting the figure higher than 50% for adolescents (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2015).

The European Commission issued 'Indicative Values' for the presence of acrylamide in food in 2011, based on results reported to EFSA (European Food Safety Authority 2011), and revised them downwards for many product types in 2013 (European Commission 2013). Indicative Values are not regulatory limits or safety thresholds, but if a product is found to exceed the Indicative Value the relevant food safety authority should take action to ensure that the manufacturer addresses the problem. Currently the Indicative Values for potato crisps and

CONTACT Nigel G. Halford nigel.halford@rothamsted.ac.uk Plant Science Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

French fries are 1000 and 600 ng g^{-1}, respectively. Furthermore, at the time of writing the European Commission has just opened a public consultation on strengthened risk management measures including compulsory Codes of Practice and the renaming of Indicative Values as Benchmark Levels, with reduced Benchmark Levels of 750 ng g^{-1} for potato crisps and 500 ng g^{-1} for French fries (European Commission 2017).

Acrylamide forms principally via the deamination and decarboxylation of asparagine (Mottram et al. 2002; Stadler et al. 2002; Zyzak et al. 2003): free asparagine and reducing sugars can therefore be regarded as its precursors (in fact the carbon skeleton is derived entirely from asparagine). In the case of potato tubers, fructose and glucose account for almost all the reducing sugar content, with almost no maltose present. The relationship between precursor concentration and acrylamide formation in potato is complex, with reducing sugars being the major determinants of acrylamide-forming potential in most datasets but free asparagine contributing to the variance in some (Amrein et al. 2003, 2004; Becalski et al. 2004; De Wilde et al. 2005; Elmore et al. 2007, 2010; Shepherd et al. 2010; Halford et al. 2012b; Muttucumaru et al. 2013, 2014). Free asparagine concentration has more influence in French fry rather than crisping varieties, probably because French fry varieties contain higher concentrations of sugars (Halford et al. 2012b; Muttucumaru et al. 2014), and modelling of the relationship between the ratio of free asparagine to reducing sugars in potatoes with unusually low concentrations of free asparagine from a U.K. field trial identified a ratio of free asparagine to reducing sugars of 2.257 ± 0.149 as a tipping point below which free asparagine concentration could affect acrylamide formation (Muttucumaru et al. 2017). Another study modelled the kinetics of acrylamide formation in French fry production and concluded that both the fructose/glucose ratio and the ratio of asparagine to total free amino acids could affect acrylamide formation (Parker et al. 2012).

Melanoidin pigments and complex mixtures of compounds that impart the flavours and aromas that are associated with fried, baked and roasted foods, also form within the Maillard reaction. This makes acrylamide reduction more problematic because any measures that are taken are likely to affect the characteristics that define products and brands, and are demanded by consumers, as well as the concentration of acrylamide. Nevertheless, the food industry has devised many strategies for reducing acrylamide formation by modifying food processing and these have been compiled in a 'Toolbox' produced by FoodDrinkEurope, the latest update of which was published in 2013 (FoodDrinkEurope 2013) (this update did not include major changes to the advice on potato crisps). The success of this approach was demonstrated in an analysis of manufacturers' data on acrylamide levels in potato crisps in Europe from 2002 to 2011, which showed a statistically significant downward trend for mean levels of acrylamide from 763 (±91.1) ng g^{-1} in 2002 to 358 (± 2.5) ng g^{-1} in 2011, a decrease of 53% (Powers et al. 2013). That study also showed the effect of seasonality arising from the influence of potato storage on acrylamide levels, with acrylamide in the first six months of the year being higher (528 ± 3.2 ng g^{-1}) than in the second six months (372 ± 2.2 ng g^{-1}) (Powers et al. 2013). This was consistent with the results of several studies showing an effect of storage on the concentration of reducing sugars and potential for acrylamide formation (De Wilde et al. 2005; Halford et al. 2012b; Muttucumaru et al. 2014; Elmore et al. 2015). These studies led to advice being issued that potatoes should only be used for crisping, frying and roasting within their recommended storage window (Halford et al. 2012a), and highlighted the challenge faced by the food industry in processing such a variable raw material to give a level of acrylamide in the end-product that consistently complies with Indicative Values.

In the present paper, we analyse manufacturers' data from 2012 to 2016, together with the 2002 to 2011 data, to enable the trends over the whole period from 2002 to 2016 to be considered.

Materials and methods

Acrylamide determinations

Data on acrylamide levels in samples of potato crisps were supplied by the European Snacks Association, having been collected from European manufacturers over 15 years from 2002 to 2016, inclusive. The samples from 2002 to 2011 that had been analysed previously by Powers et al. (2013) were included to enable trends in the data to be considered for the full period since the presence of acrylamide in food was first reported (Tareke et al. 2002). All analyses were carried out using procedures based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) or gas chromatography mass spectrometry (GC-MS) and all 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) (CEN) standard methods, EN 16618:2015 (Food analysis – Determination of acrylamide in food by liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS)), or FprCEN/TS 17083 (Foodstuffs – Determination of acrylamide in food and coffee by gas chromatography-mass spectrometry (GC-MS) (draft)).

Statistical analyses

For 2012–16 there were 25,986 observations for LC-MS/MS and 7422 for GC-MS. Together with the 40,455 observations which all used LC-MS/MS for 2002–11, this gave 73,863 observations in total for 2002–16. Plots of the data as means, standard errors, 90% and 95% upper percentiles (quantiles) (Q90 and Q95) were produced to give a summary of the differences between years. Further graphical inspection of the data was undertaken to check on seasonal trends by considering monthly means as a time series over the 15 years and monthly means taken over all years. To assess the overall trends over years, analysis of variance (ANOVA) was applied to the natural log (to base *e*) of the acrylamide data to test for an overall change in mean acrylamide over years (F-test) and then to allow specific comparison of years using the least significant difference (LSD) value at the 5% level of significance ($p = 0.05$). The data on the $\log_e$ scale were found to be distributed as Normal and analysis of residuals showed reasonable conformation to the assumptions of ANOVA (Normal distribution, constant variance across factor levels and additivity of effects) (not shown). Further summary statistics were calculated and ANOVA applied to consider the differences between types of product (standard, thick cut and ridge/wave crisps), and between assigned geographical regions (north, south, east and west) from which samples derived, over years. Proportions of observations greater than 1000 and 2000 ng g^{-1} according to the different factors of interest were also calculated and graphed.

The GenStat (2015, 18th edition, © VSN International Ltd, Hemel Hempstead, UK) statistical package was used for all analyses. The SigmaPlot (version 13, © Systat Software Inc., San Jose, California, US) was used for graphs. Excel (2016, © Microsoft Corporation, US) was used to store the data.

Results

Acrylamide levels in potato crisps from 2002 to 2016

European manufacturers' data on acrylamide levels in potato crisps was provided for the period 2002 to 2016. For the purposes of the study, a potato crisp is defined as a thin slice of potato (*Solanum tuberosum*) tuber which has been fried to impart a desirable colour, 'crisp' texture and taste via the absorption of hot oils into the potato base and the reduction of moisture content to a specified level. The dataset included observations from every European Union country apart from Estonia, Luxembourg, Malta and Slovenia, as well as observations from Switzerland and Norway.

The data are compiled in Supplementary File S1. For 2012–16 there were 25,986 observations for LC-MS/MS and 7422 for GC-MS. Together with the 40,455 observations which all used LC-MS/MS for 2002–11, this gave 73,863 observations in total for 2002–16, comprising 66,441 that had been quantified by LC-MS/MS and 7422 by GC-MS. Since there was no scientific reason for differentiating between the LC-MS/MS and GC-MS methods, the full dataset of 73,863 observations was analysed.

Analysis of variance (ANOVA) was applied to the data and the means on the log (to base *e*) scale are given in Table 1a. The least significant difference (LSD) values at the 5% level of significance, on 73,848 degrees of freedom (Table 1b), can be used to compare means of interest (Table 1a) to see if there are statistically significant differences between them. Pairs of means on the $\log_e$ scale that differ by more than the corresponding LSD are significantly different at the 5% ($p < 0.05$) level. This analysis revealed significant ($p < 0.001$, F-test) differences between years. As reported previously (Powers et al. 2013), the first statistically significant ($p < 0.05$, LSD) year-to-year reduction in acrylamide was for 2005–2006,

Table 1. Analysis of variance of acrylamide data for samples of potato crisps from Europe 2002 to 2016 to show differences between years.

a. Mean acrylamide (ng g^{-1}) for each year on the natural log (to base *e*) scale and number of observations (*n*).

Year	Mean (on log$_e$ scale)	*n*	Year	Mean (on log$_e$ scale)	*n*
2002	6.3508	42	2010	5.8328	10,971
2003	6.2165	136	2011	5.6536	12,213
2004	6.2496	321	2012	5.8191	8656
2005	6.2855	230	2013	5.8147	5371
2006	6.1580	1151	2014	5.7999	5710
2007	6.1475	3206	2015	5.8454	6407
2008	5.9078	5692	2016	5.7959	7264
2009	5.8986	6493			

b. Least significant difference (LSD) values on 73,848 degrees of freedom at the 5% (p < 0.05) level of significance. The value for a particular comparison of the means in Table 1a is given by reading along the appropriate row and up the appropriate column.

LSDs (5%)

2002								
2003	0.23038							
2004	0.21414	0.13352						
2005	0.21899	0.14117	0.11274					
2006	0.20501	0.11833	0.08237	0.09426				
2007	0.20269	0.11426	0.07640	0.08909	0.04484			
2008	0.20211	0.11324	0.07487	0.08777	0.04218	0.02882		
2009	0.20202	0.11307	0.07462	0.08756	0.04174	0.02817	0.02370	
2010	0.20176	0.11260	0.07390	0.08695	0.04043	0.02620	0.02132	0.02043
2011	0.20172	0.11253	0.07379	0.08686	0.04024	0.02590	0.02094	0.02004
2012	0.20186	0.11278	0.07418	0.08719	0.04094	0.02698	0.02227	0.02143
2013	0.20216	0.11331	0.07499	0.08788	0.04239	0.02913	0.02483	0.02407
2014	0.20211	0.11323	0.07486	0.08777	0.04217	0.02880	0.02444	0.02368
2015	0.20203	0.11309	0.07464	0.08758	0.04178	0.02823	0.02377	0.02298
2016	0.20195	0.11295	0.07443	0.08740	0.04140	0.02767	0.02310	0.02229
	2002	2003	2004	2005	2006	2007	2008	2009

LSDs (5%)

2010							
2011	0.01717						
2012	0.01876	0.01834					
2013	0.02173	0.02137	0.02267				
2014	0.02130	0.02092	0.02225	0.02481			
2015	0.02052	0.02013	0.02151	0.02414	0.02375		
2016	0.01974	0.01934	0.02077	0.02349	0.02308	0.02237	
	2010	2011	2012	2013	2014	2015	2016

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. Since then there has been a levelling off. Indeed, the mean acrylamide level for 2012 was significantly different ($p < 0.05$, LSD) and greater than that for 2011. The mean values in the following years, 2013 and 2014, were not significantly different ($p > 0.05$, LSD) from each other, but remained significantly different ($p < 0.05$, LSD) and greater than the mean in 2011. Finally, the mean in 2015 was marginally significantly different ($p < 0.05$, LSD) and greater than the means in 2014 and 2016, but with the means in all three years (2014–2016) also being significantly different ($p < 0.05$, LSD) and greater than the mean in 2011. Therefore, the year when the lowest levels of acrylamide were achieved was 2011.

The raw mean, standard error of the mean, number of observations, 90% and 95% quantiles (Q90 and Q95), and maximum levels of acrylamide for each year, as well as the proportion of samples with greater than 1000 or 2000 ng g^{-1} are given in Table 2, while the means, standard errors, Q90 and Q95 are plotted in Figure 1. The overall, significant ($p < 0.05$, LSD) reduction in mean acrylamide achieved between 2002 and 2011 was 53% (±13.5), from 763 (± 91.1) ng g^{-1} in 2002 to 358 (± 2.5) ng g^{-1} in 2011 (Table 2; Powers et al. 2013), and while the mean in 2016 was higher at 412 (± 3.8) ng g^{-1}, it still represented a 46% reduction from 2002.

The trends in the Q90 and Q95 values were also downward from 2002 to 2011, followed by a levelling off to 2016 (Table 2 and Figure 1). The Q90 values have been below 1000 ng g^{-1}, the Indicative Value set for potato crisps by the European Commission in 2011 and reaffirmed in 2013 (European Commission 2013), since 2010, while the Q95 was below 1000 ng

Table 2. Acrylamide in samples of potato crisps from 2002 to 2016: Mean (ng g^{-1}), standard error of mean (SE), number of observations (*n*), 90% and 95% quantiles (Q90 and Q95), maximum levels, and proportion (%) of samples containing greater than 1000 or 2000 ng g^{-1}.

Year	Mean	SE	*n*	Q90	Q95	Max	% > 1000	% > 2000
2002	763	91.1	42	1581	2080	2500	23.8	4.8
2003	573	27.3	136	976	1118	2080	8.1	0.7
2004	624	26.6	321	1168	1580	4450	12.8	1.2
2005	621	21.3	230	1085	1210	1780	13.5	0.0
2006	577	11.9	1151	1100	1350	2830	11.3	1.3
2007	570	7.0	3206	1000	1270	5900	10.0	0.9
2008	472	5.1	5692	903	1170	4300	7.5	0.8
2009	500	5.8	6493	1037	1400	6000	10.9	1.5
2010	435	4.1	10,971	780	1020	12,000	5.2	1.0
2011	358	2.5	12,213	680	870	3090	3.2	0.2
2012	415	3.3	8656	784	1020	4500	5.2	0.2
2013	423	4.4	5371	830	1090	2635	6.2	0.3
2014	397	3.4	5710	740	920	2049	3.7	0.0
2015	422	3.8	6407	790	1020	2600	5.2	0.2
2016	412	3.8	7264	770	1018	3400	5.6	0.6

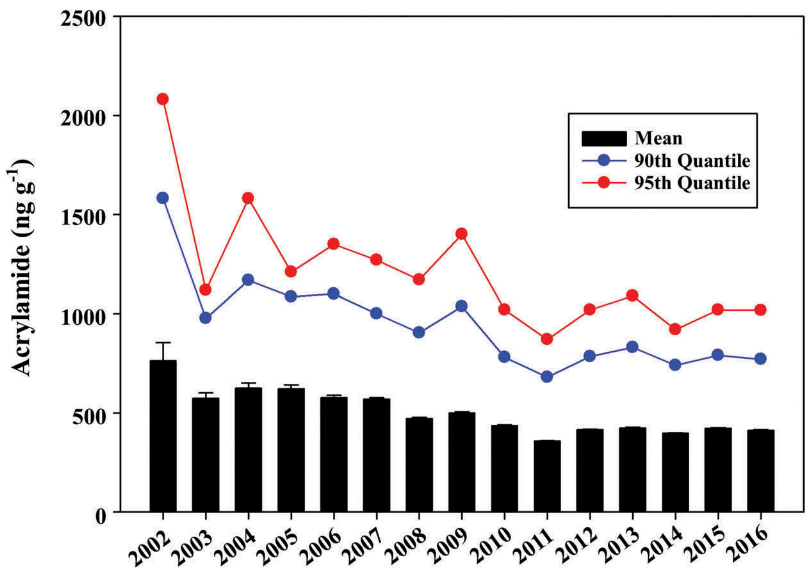

Figure 1. Overall mean acrylamide levels (ng g^{-1}) in samples of potato crisps shown over years from 2002–2016, with standard errors and with trend in 90% and 95% quantiles.

g^{-1} in 2011 (870 ng g^{-1}) but marginally above that mark in subsequent years, except for 2014 (920 ng g^{-1}). The proportion of samples containing more than 1000 ng g^{-1} acrylamide in each year is shown graphically in Figure 2. Almost a quarter of the samples for 2002 exceeded this level, but the proportion fell to 5.2% by 2010. The highest proportion in the years since then was 6.2% in 2013, while the lowest was 3.2% in 2011. The proportion in 2016 was 5.6%. The proportion of samples having very high acrylamide, above 2000 ng g^{-1}, or twice the Indicative Value, also fell dramatically, from 4.8% in 2002 to 1% in 2010 and below 1% after that (Table 2 and Figure 2). Indeed, the proportion of samples over 2000 ng g^{-1} from 2011 to 2015 was at most only three in a thousand, although the proportion in 2016 was slightly higher, at six in a thousand. There was also one year, 2005, when no samples were found to contain over 2000 ng g^{-1} acrylamide, and another, 2014, with just a single sample over 2000 ng g^{-1} acrylamide.

Seasonality

The seasonality of acrylamide levels in potato crisps was evident in the study of the 2002–2011 data (Powers et al. 2013). The larger dataset available to the present study was analysed in the same way, and the number of observations, mean, standard error of the mean, as well as Q90 and Q95 values for acrylamide for each

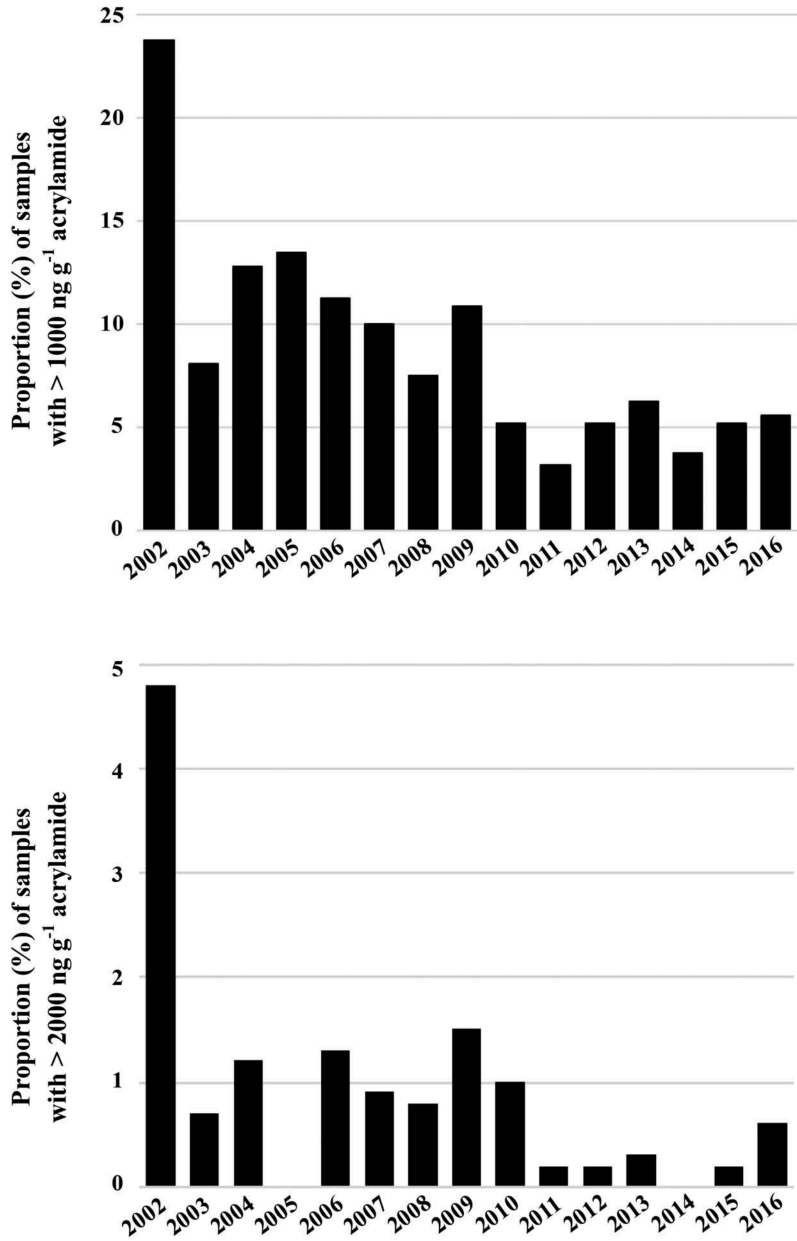

Figure 2. Proportion (%) of samples of potato crisps in each year from 2002 to 2016 containing more than 1000 (top) or 2000 ng g^{-1} (bottom) acrylamide.

month are given in Table 3. The mean and SE are plotted monthly for 2002–2016 in Figure 3, and the means with standard errors together with the Q90 and Q95 for each month are displayed separately.

The analysis showed that the trend that was evident in the 2002–2011 data of higher acrylamide levels in the first half of the year continued from 2012 to 2016, despite the lower overall yearly values for acrylamide. Acrylamide levels were at their lowest from July to September each year, before increasing again through October to December. Taking the data for all years together, the Q90 and Q95 also showed clear seasonality, with the Q95 above 1000 ng g^{-1} and the Q90 above 750 ng g^{-1} for the whole of the January to June period. This remained the case even for the years from 2011 to 2016 (Table 3), i.e. the period when overall acrylamide levels had flattened out.

Crisp types

The dataset was subdivided according to product type; something that had not been attempted in the previous study (Powers et al. 2013). This was based on three broad design descriptors: standard, thick

Table 3. Number of observations (*n*), mean (ng g^{-1}), standard error of mean (SE), 90% and 95% quantile (Q90 and Q95) values for acrylamide in samples of potato crisps from Europe for each month using data from 2002 to 2016. The Q90 and Q95 values are also given for the period from 2011 to 2016. Values other than *n* are shown in bold where they are greater than 1000 ng g^{-1}.

Month	*n*	Mean	SE	Q90	Q90 2011–2016	Q95	Q95 2011–2016
January	5566	484.6	4.796	894.9	820.0	**1140**	**1050**
February	5646	498.5	4.868	950.0	875.0	**1200**	**1120**
March	6775	511.1	4.984	949.0	867.0	**1210**	**1090**
April	6690	489.5	4.537	910.5	850.0	**1170**	**1090**
May	7153	519.4	5.564	996.2	935.0	**1300**	**1200**
June	5888	449.0	4.793	860.0	790.0	**1140**	**1020**
July	5730	314.9	3.646	590.0	560.0	790	740.0
August	5729	305.0	3.418	550.0	500.0	740	630.8
September	6307	317.0	3.303	580.8	530.0	777	680.0
October	5899	387.1	3.570	710.0	650.0	900	800.0
November	6804	428.3	3.877	799.1	701.4	**1030**	912.4
December	5676	439.1	4.144	818.9	719.7	**1010**	891.6

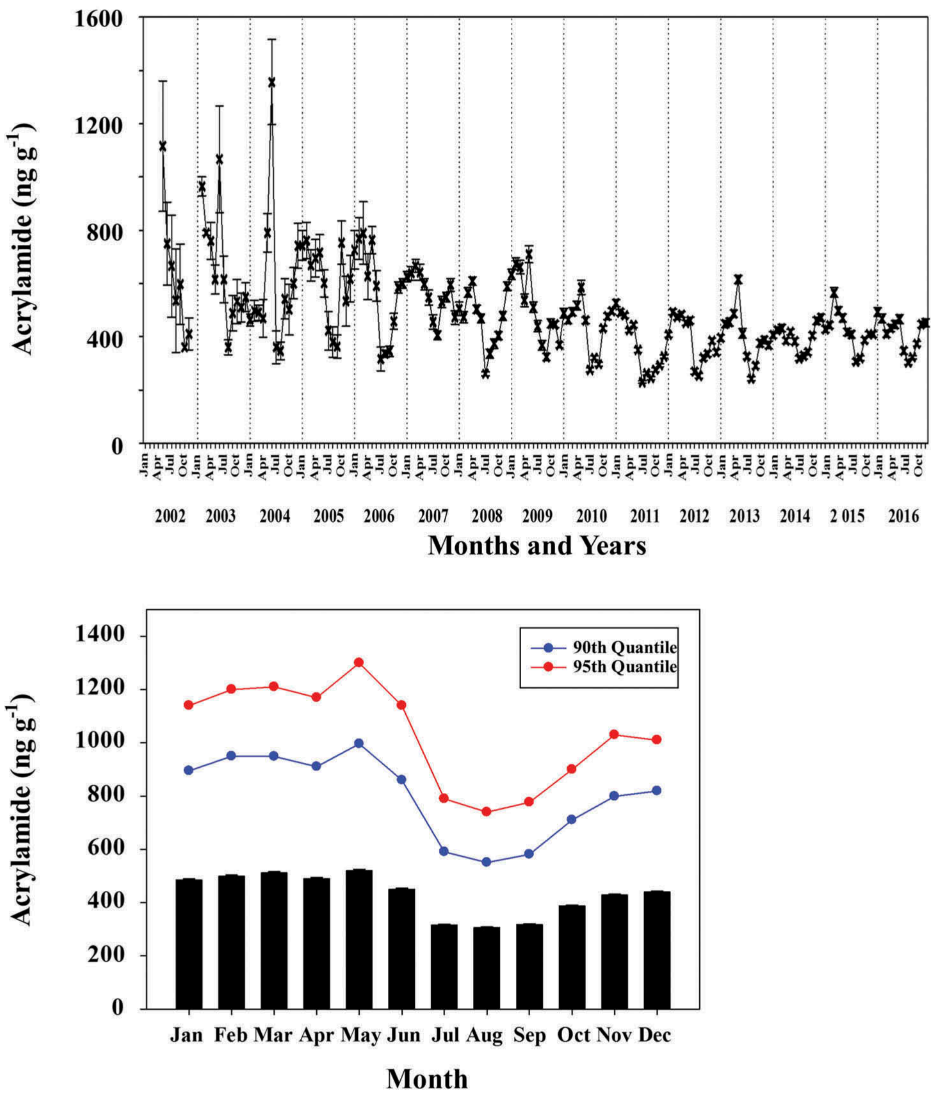

Figure 3. Seasonality in acrylamide levels in samples of potato crisps from 2002–2016.

Top panel: mean acrylamide levels over time (2002–2016) with standard errors, plotted monthly. Bottom panel: mean acrylamide levels per month over all years with standard errors and with trend in 90% and 95% quantiles.

cut, and ridge/wave. The standard type had been produced from a slice of potato with a flat or straight surface on both sides (the two largest surface areas) of the slice. Slice thickness was typically greater than 0.127 cm and no more than 0.165 cm across the potato web. The thick cut type was the same except that the slice thickness was equal to or greater than 0.165 cm and typically no more than 0.216 cm across the potato web. The ridge/wave type had been produced from a slice of potato with a ridge/wave or crinkle-cut design on one or both sides (the two largest surface areas) of the slice. Slices were typically greater than 0.165 cm and typically no more than 0.216 cm thick across the potato web, i.e. the same thickness as the thick cut type (while it is possible to produce thinner ridge/wave potato crisps, such products are relatively uncommon as they tend to be more fragile and subject to increased breakage after frying).

There were 58,794 samples of standard crisps, 3574 of the thick cut type and 11,495 of the ridge/wave type, and the means, standard errors, numbers of values, means on the $\log_e$ scale, Q90, Q95 and maximum values of acrylamide by type and year are given in Table 4. ANOVA revealed a significant interaction between type and years ($p < 0.001$, F-test), indicating that the fluctuation in acrylamide over time was not consistent for the three types. The main effects of types and years were also highly significant ($p < 0.001$, F-test). The means on the $\log_e$ and raw scales, with average LSD (5%) for comparisons and standard error, are shown graphically in Figure 4.

Notably, the mean acrylamide level for the thick cut type was 1044 ng g^{-1} in 2006, the first year for which data for that type were available, compared with 555 ng g^{-1} for the standard type, a difference of 88%. It was even higher in 2007 at 1322 ng g^{-1}, more than twice the mean for the standard type of 558 ng g^{-1}. By 2008 it was still approximately 50% higher than for the standard type, but by 2009 it had fallen to the point where there was no significant ($p < 0.05$, LSD) difference between the two types. The mean acrylamide level for the ridge/wave type, on the other hand, was higher than the standard type in 2002 but lower from 2003 to 2005, albeit with very low sample numbers in those years. In 2006 it was again higher than in the standard type, but as with the thick cut type the difference with the standard type declined in subsequent years. Since 2009 the levels of acrylamide for all three types have come together.

Regions

The dataset was also sub-divided according to the region of Europe (north, south, east or west) from which the samples originated (Table 5). Statistical analysis of the full set of data on the $\log_e$ scale using ANOVA to test the main effects and interactions between regions and years revealed a significant ($p < 0.001$, F-test) interaction between these factors. The means on the $\log_e$ scale and the average LSD (5%) on 73,813 degrees of freedom are given in Table 6 and plotted in Figure 5, as are the raw means with standard errors. The values of acrylamide in the north and east were generally higher than in the south and west. However, from 2006 onwards in the east and 2013 onwards in the north, acrylamide levels moved progressively towards those in the south and west, although levels in the north remained higher than in the other regions right up to 2016.

The north not only had higher means than the other regions but also much higher proportions of samples that exceeded the 1000 ng g^{-1} mark (Table 6). Even as late as 2012, the proportion of samples exceeding this level was 39.15%, or almost two in five, and in 2016 it was still 12.41%, or approximately one in eight. This was exacerbated by seasonality, and the proportion of samples exceeding 1000 ng g^{-1} in each month for the period 2011 to 2016 (the plateau period) is shown graphically in Figure 6. The problem for the north region in the first half of the year is clearly evident from the graph, with, for example, almost a third (32.67%) of samples for May exceeding 1000 ng g^{-1} during that period.

Comparisons with EFSA data

Data from EFSA's acrylamide monitoring programme for the years 2011 to 2015 were obtained through a public access to documents request and are given in Supplementary File S2. There were 1506 entries in the dataset that were not already included in the manufacturers' dataset. Of these 1506 entries, 181 had no analytical method recorded, 109 had been obtained using a

Table 4. Mean (ng g^{-1}), standard error of mean (SE), number of values (n), mean of log$_e$ data, Q90, Q95 and maximum values of acrylamide by type and year in samples of potato crisps from Europe 2002 to 2016. Means are shown in bold where they are greater than 1000 ng g^{-1}.

	Standard							Thick cut							Ridge/wave						
Year	Raw Mean	SE	n	Mean (log$_e$)	Q90	Q95	Max	Raw Mean	SE	n	Mean (log$_e$)	Q90	Q95	Max	Raw Mean	SE	n	Mean (log$_e$)	Q90	Q95	Max
2002	751	90.9	40	6.346	1530	2100	2500	*	*	*	*	*	*	*	**1010**	790	2	6.445	2274	2432	1800
2003	580	27.8	131	6.232	988	1128	2080	*	*	*	*	*	*	*	394	130.0	5	5.819	910	1063	910
2004	656	29.5	283	6.300	1234	1594	4450	*	*	*	*	*	*	*	385	24.8	38	5.876	554	688	840
2005	629	22.1	208	6.303	1087	1201	1780	*	*	*	*	*	*	*	544	74.4	22	6.121	1125	1300	1300
2006	555	12.5	993	6.118	1000	1299	2830	**1044**	159.4	21	6.701	2280	2745	2800	663	29.9	137	6.361	1190	1301	1960
2007	558	7.3	2739	6.123	996	1246	4710	**1322**	298.0	19	6.850	2560	4415	5900	611	17.3	448	6.269	1087	1364	2500
2008	468	5.3	5206	5.893	900	1170	4300	701	75.4	47	6.287	1500	1815	1900	493	14.3	439	6.044	919	1100	2400
2009	506	6.5	5420	5.888	1080	1440	6000	473	23.5	406	5.900	889	1300	4000	471	11.9	667	5.982	850	1033	2800
2010	439	4.8	8838	5.824	798	1060	12,000	383	15.7	514	5.710	656	856	3000	434	6.8	1619	5.918	720	918	3900
2011	344	2.8	9084	5.609	660	840	3090	483	10.6	939	5.963	939	1136	2400	364	5.6	2190	5.706	656	847	2700
2012	401	3.6	6850	5.791	746	960	4500	511	15.1	653	6.008	1062	1417	2150	443	9.5	1153	5.877	881	1169	2520
2013	429	4.7	4646	5.829	840	1100	2635	508	17.8	289	6.063	944	1118	2100	311	14.1	436	5.497	538	701	2550
2014	395	3.9	4122	5.798	740	910	1800	387	13.2	410	5.789	685	829	2049	407	7.9	1178	5.811	787	980	1600
2015	417	4.3	4929	5.835	759	990	2600	401	30.7	91	5.817	675	1220	1510	441	8.2	1387	5.884	849	1050	2100
2016	394	4.3	5305	5.758	736	980	3200	450	20.8	185	5.943	800	928	1680	461	8.7	1774	5.894	870	1200	3400

* No data present.
LSD (5%) for comparing means on the log$_e$ scale = 0.1893.

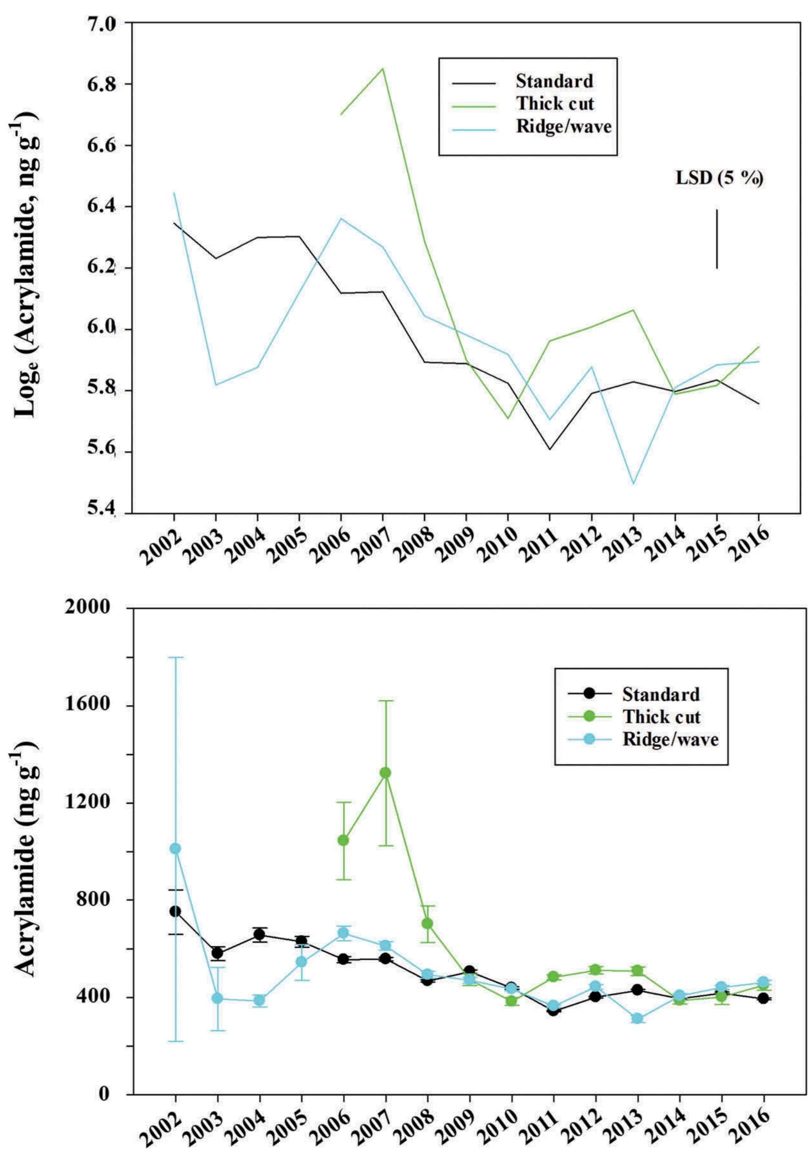

Figure 4. Overall mean acrylamide levels (ng g^{-1}) in samples of potato crisps of different types (standard, thick cut and ridge/wave) shown over years from 2002–2016, on the log$_e$ scale showing the average LSD (5%) on 73,822 degrees of freedom (top) and on the raw scale with standard errors (bottom).

Table 5. Grouping of 25 European countries into geographic regions (north, south, east and west) that formed the basis of the regional analysis of acrylamide levels. Note that there were no observations from Estonia, Luxembourg, Malta or Slovenia in the dataset.

North	South	East	West
Denmark	Cyprus	Bulgaria	Austria
Finland	Greece	Croatia	Belgium
Lithuania	Italy	Czech Republic	France
Latvia	Portugal	Hungary	Germany
Norway	Spain	Poland	Ireland
Sweden		Romania	Netherlands
			Switzerland
			United Kingdom

non-standard analytical method, 45 appeared to be duplicated and three were apparently from unprocessed material (the 'Data Removed' tab of Supplementary File S2 details those observations that were excluded). This left 1168 observations for analysis (the 'Data Analysed' tab of Supplementary File S2), including 44 cases where the acrylamide value was missing due to it being below the limit of quantification (LOQ) or limit of detection (LOD) values. These

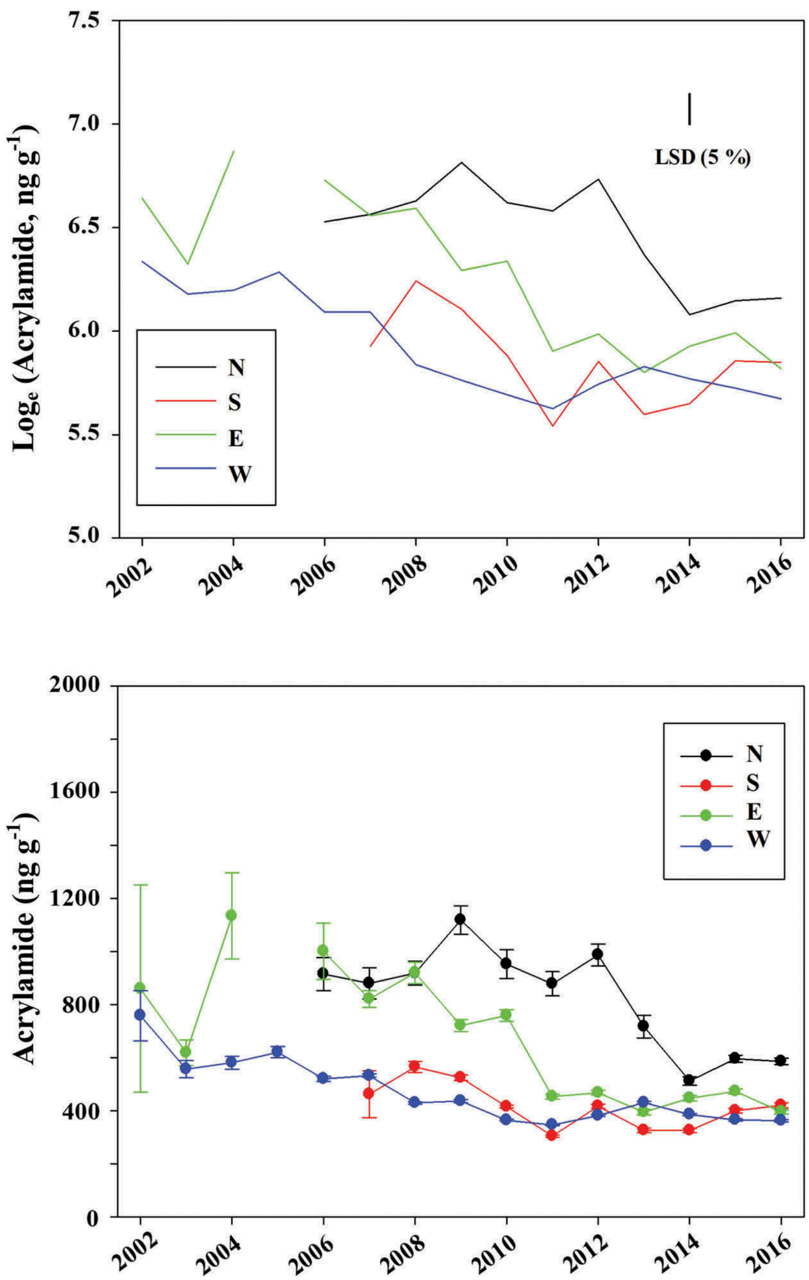

Figure 5. Overall mean acrylamide levels (ng g^{-1}) in samples of potato crisps from different regions of Europe (north, south, east and west: Table 5), shown over years from 2002–2016, on the log$_e$ scale showing the LSD (5%) on 73,813 degrees of freedom (top) and on the raw scale with standard errors (bottom).

Table 6. Mean (ng g^{-1}), standard error of mean (SE), number of values (*n*), proportion (%) of samples with more than 1000 ng g^{-1} and mean of $\log_e$ data for acrylamide by region and year in samples of potato crisps from Europe, 2002 to 2016. Mean values are shown in bold where they are greater than 1000 ng g^{-1}.

		Year														
Region		2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
North	**Mean**	*	*	*	*	915	880	918	**1118**	953	878	987	718	512	596	586
	SE	*	*	*	*	61.9	59.5	44.9	53.8	54.0	45.6	41.6	43.0	15.7	13.7	12.0
	n	0	0	0	0	122	129	159	173	153	148	235	133	375	1021	1241
	% > 1000	*	*	*	*	31.97	26.36	34.59	45.66	35.95	29.73	39.15	20.30	7.47	16.16	12.41
	Mean ($\log_e$)					6.528	6.565	6.630	6.816	6.621	6.581	6.734	6.370	6.080	6.147	6.160
South	**Mean**	*	*	*	*	*	462	565	525	416	305	417	326	326	400	421
	SE	*	*	*	*	*	88.0	21.1	10.3	4.6	4.0	7.0	9.4	8.3	8.9	10.4
	n	0	0	0	0	0	17	129	861	2585	2351	1578	560	472	645	725
	% > 1000	*	*	*	*	*	5.88	3.88	8.59	2.44	1.11	3.68	1.61	0.85	3.26	4.97
	Mean ($\log_e$)						5.928	6.243	6.106	5.883	5.541	5.854	5.599	5.650	5.857	5.849
East	**Mean**	860	619	**1134**	*	**1001**	821	920	721	759	454	468	395	448	473	396
	SE	390.0	48.5	162.6	*	105.5	32.1	40.4	23.1	22.6	9.5	8.7	10.8	10.1	9.3	7.8
	n	2	35	25	0	36	262	291	758	1395	1430	989	569	706	966	1112
	% > 1000	50.00	11.43	40.00	*	33.33	24.05	33.68	19.92	17.06	6.85	4.55	3.34	4.11	6.11	2.97
	Mean ($\log_e$)	6.642	6.324	6.868		6.729	6.558	6.594	6.293	6.338	5.904	5.986	5.801	5.928	5.992	5.818
West	**Mean**	759	557	581	621	520	532	430	437	365	347	382	431	386	366	363
	SE	94.6	32.7	23.9	21.3	9.8	6.6	4.5	6.0	3.5	2.8	3.6	5.1	3.9	3.9	4.6
	n	40	101	296	230	993	2798	5113	4701	6838	8284	5854	4109	4157	3775	4186
	% > 1000	22.50	6.93	10.47	13.48	7.96	7.93	5.30	8.55	3.07	2.63	4.36	6.72	3.61	2.30	3.68
	Mean ($\log_e$)	6.336	6.179	6.197	6.286	6.092	6.091	5.838	5.763	5.693	5.626	5.745	5.828	5.770	5.724	5.673

* No data present. Average LSD (5%) for comparing means on the $\log_e$ scale = 0.1460.

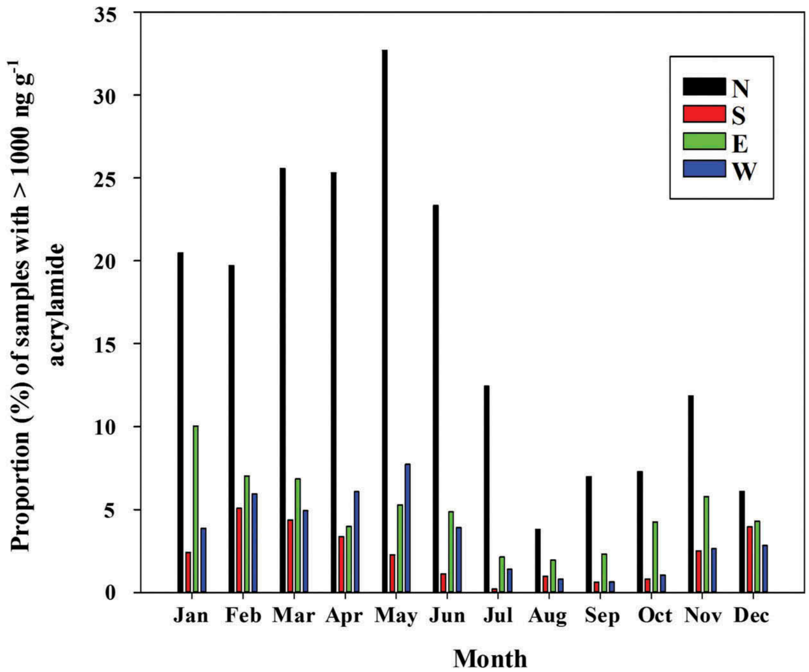

Figure 6. Proportion (%) of samples with more than 1000 ng g^{-1} acrylamide for each month over the period 2011–2016 for geographic regions.

were appropriately set at the LOQ or LOD values to avoid exclusion of such important data.

The dataset was a fraction of the size of the manufacturers' dataset used for the major part of the study. In addition, while the dataset included samples from every month, it was heavily weighted towards March and November because Member States are encouraged to sample in those months, with only 179 of the 1168 samples (15%) coming from the three months of July, August and September, when levels would be expected to be at their lowest. It also contained observations from Estonia, Luxembourg, Malta and Slovenia, which were not represented in the manufacturers' dataset, but no observations from Bulgaria, Switzerland, Czech Republic, Latvia, the Netherlands, Portugal or Romania.

The means (ng g^{-1}) of the EFSA data were 621 ± 49 for 2011, 600 ± 39 for 2012, 521 ± 37 for 2013, 575 ± 31 for 2014, and 743 ± 47 for 2015, while the proportion (%) of samples exceeding the current Indicative Value of 1000 ng g^{-1} was 17.5 in 2011, 14.0 in 2012, 14.5 in 2013, 15.9 in 2014 and 22.4 in 2015. Thus, as with the manufacturers' data, there was little difference between the years. Seasonality (not shown) broadly followed the same trend as shown in Figure 3 for the manufacturers' data, although there were too few samples in some months to make the analysis meaningful (there were only 11 observations for December in total over all five years, for example). Notably the values were considerably higher than those for the manufacturers' data: the mean for 2015, for example, was 76% higher than that for the manufacturers' data for that year (743 compared with 422 ng g^{-1}), with more than four times the proportion of samples exceeding the 1000 ng g^{-1} level (22.4% compared with 5.2%).

Discussion

We have previously shown (Powers et al. 2013) a clear downward trend in manufacturers' data on acrylamide in potato crisps in Europe between 2002, the year in which the presence of acrylamide in food was first reported (Tareke et al. 2002), and 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%. In the present study, we have shown that acrylamide levels levelled off in subsequent years, with the 2016 figure of 412 ng g^{-1} actually being higher than that for 2011, although it still represented a 46% reduction from 2002. This suggests that the most effective acrylamide reduction measures had been developed and implemented by 2011, and that 2011 was a particularly good year with respect to low acrylamide-forming potential of the crop. The measures that were implemented included improved control of cooking temperature and duration, monitoring of moisture levels in the finished product, post-frying quality control based on colour, switching to very low-sugar potato varieties, used only within their optimum storage window, and the careful control of storage temperature and conditions. Manufacturers have also introduced checks on potato sugar concentration at time of harvest, during storage and at the factory gate. Selection of potato lots has been prioritised based upon quality of the raw materials and the intended finished product design. It is important that manufacturers continue to monitor latest research and trial new tools as they become available. Possible additional tools that are already available but not widely applied include blanching, vacuum frying or par vacuum frying, and the application of pulsed electric field technology, all of which may be effective in some product types but also impact product cost and quality. Even with additional processing measures, further substantial reductions may not be achievable without a step change in the acrylamide-forming potential of the raw material; in other words, the breeding of potato varieties with much lower concentrations of reducing sugars and/or free asparagine, together with improved stability during cold storage.

Potato varieties with greatly reduced acrylamide-forming potential have been developed and marketed in the U.S.A. by the Simplot Company, using RNA interference (RNAi) to reduce the expression of a tuber-specific asparagine synthetase gene, *ASN1* (Rommens et al. 2008; Chawla et al. 2012). The varieties, called Innate® and Innate® Gen. 2, also have reduced activity of two genes encoding enzymes of starch breakdown, phosphorylase L (*PhL*) and starch-associated R1 (*R1*), as well as a gene (*PPO5*) encoding polyphenol oxidase, an enzyme involved in bruising and, in the case of Innate® Gen. 2, resistance to late blight. However, there is currently no prospect of these or any other genetically modified varieties being grown in Europe. It is possible that similar improvements could be made using a non-GM, genome editing technique such as CRISPR (Song et al. 2016). However, the application of that technology to potato is still in development, and there is continued uncertainty over how it will be regulated in Europe. In the meantime, European manufacturers and consumers will have to wait for progress to be made by conventional breeding techniques, which is likely to be relatively slow and limited.

The fact that this study shows acrylamide levels in potato crisps in Europe to have levelled off in recent years is particularly important given that the European Commission has recently opened the public consultation process for its revised risk management measures for acrylamide in food (European Commission 2017). The proposals include renaming Indicative Values as Benchmark Levels and reducing the Benchmark Level for potato crisps to 750 ng g^{-1} (note that the Q90 in our dataset exceeded 750 ng g^{-1} for half the year, from January to June, even in the plateau period from 2011 to 2016). The proposals also state that the setting of Maximum Levels (MLs) for acrylamide in certain foods should be considered in the future. Indeed, the possibility of MLs being imposed on sectors of the food industry that do not show sufficient progress in reducing acrylamide levels has already been discussed by the European Parliament's Environment, Public Health and Food Safety Committee (31 January 2017:

http://www.emeeting.europarl.europa.eu/committees/agenda/201701/ENVI/ENVI(2017)0130_1/sitt-3826045). The results of this study suggest that while impressive reductions in acrylamide levels were achieved in potato crisps in the first decade after acrylamide was identified as a problem, further progress, including compensating for differences across the seasons and between regions, may be difficult to achieve.

One factor that might be expected to affect acrylamide in crisps is the design or type of crisp, and that was investigated in this study, with the dataset subdivided according to three broad design descriptors: standard, thick cut, and ridge/wave. Both the thick cut and ridge/wave types were produced from thicker potato slices than the standard type, meaning that the surface area to volume ratio would be lower. However, the study provided no evidence that a thicker cut could reduce acrylamide formation on a per weight basis, suggesting that acrylamide forms throughout the crisp. Indeed, acrylamide levels were higher in the thicker types of crisp in the early years of the study, although this difference had disappeared by the later years, suggesting that there may be more potential for acrylamide to form in thicker crisps but that manufacturers devised and applied measures to counterbalance this. These measures may include the selection of batches of potatoes with the lowest reducing sugar content for products in which the risk of acrylamide formation is high, and/or adaptations to production and design processes.

Another factor that was shown to have an effect on acrylamide levels was the region from which the sample originated, with samples from the north (comprising Denmark, Finland, Lithuania, Latvia, Norway and Sweden), in particular, having higher levels of acrylamide than samples from the other regions, and a much higher proportion of samples exceeding the 1000 ng g^{-1} mark, particularly in the first six months of the year. There is anecdotal evidence that this is due to environmental rather than varietal effects, with potatoes of the same variety having higher acrylamide-forming potential when grown in the north than they do when grown in other regions. This needs to be confirmed in a scientific study, and research carried out to find an explanation. Lower temperature is one possible cause, but low temperatures would also be experienced by plants growing further south but at higher elevations. Differences in day length may therefore be a more likely explanation: the origins of European potatoes appear to be in equatorial Colombia, with adaptation to the changing day length of higher latitudes a feature of potato's adoption as a European crop. The striking and enduring seasonality of the proportion of samples with more than 1000 ng g^{-1} acrylamide in the north also raises questions about the stability of sugar concentrations in potatoes in storage in that region.

The reductions in acrylamide levels in potato crisps that have been achieved in Europe since 2002 represent a clear vindication of the approach taken by the European Commission and industry. Nevertheless, future decisions must be informed by reliable data. The previous study of acrylamide levels in crisps from 2002 to 2011 (Powers et al. 2013) discussed the fact that the levels of acrylamide reported by EFSA were consistently higher than those reported by manufacturers (EFSA reported a mean of 675 ng g^{-1} for 2010, for example, while the mean for the much larger manufacturers' dataset for 2010 was 435 ng g^{-1}). Comparing the manufacturers' and EFSA data for 2011 to 2015 (the most recent data provided to us by EFSA) in the present study showed the same trend, with the EFSA data consistently showing higher means and proportions of samples above the current Indicative Value of 1000 ng g^{-1}.

The manufacturers' data were supplied entirely by companies that are members of the European Snacks Association (ESA), representing approximately 40–50% of potato crisp volume sold across Europe. Awareness of the FoodDrinkEurope Toolbox measures among those companies is high, and the lower acrylamide levels in that dataset could reflect the fact that their products have been subjected to a higher level of acrylamide mitigation measures than perhaps is the case for products produced by non-ESA members. On the other hand, the EFSA dataset may have shown statistical bias because it was comparatively very small and samples were not analysed evenly throughout the year, with relatively few samples coming from July to September, when acrylamide levels would be expected to be at their lowest. Furthermore, many of the acrylamide measurements supplied to EFSA by Member States had to be excluded from our analysis because the analytical method used was not reported, or the values were not quantitated using mass spectrometry, or they were apparent duplicates. This illustrates the need to compile and analyse large and representative datasets, acquired using standardised methods, to produce an accurate picture of the current situation and trends across time and regions that can inform decision-making.

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Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Nigel G. Halford http://orcid.org/0000-0001-6488-2530

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