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Impact of Harvest Year on Amino Acids and Sugars in Potatoes and Effect on Acrylamide Formation during Frying

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Acrylamide is formed via the Maillard reaction between reducing sugars and asparagine in a number of carbohydrate-rich foods during heat treatment. High acrylamide levels have been found in potato products processed at high temperatures. To examine the impact of harvest year, information on weather conditions during growth, that is, temperature, precipitation, and light, was collected, together with analytical data on the concentrations of free amino acids and sugars in five potato clones and acrylamide contents in potato chips (commonly known as crisps in Europe). The study was conducted for 3 years (2004−2006). The contents of acrylamide precursors differed between the clones and the three harvest years; the levels of glucose were up to 4.2 times higher in 2006 than in 2004 and 2005, and the levels of fructose were 5.6 times higher, whereas the levels of asparagine varied to different extents. The high levels of sugars in 2006 were probably due to the extreme weather conditions during the growing season, and this was also reflected in acrylamide content that was approximately twice as high as in preceding years. The results indicate that acrylamide formation is dependent not only on the content and relative amounts of sugars and amino acids but also on other factors, for example, the food matrix, which may influence the availability of the reactants to participate in the Maillard reaction.

KEYWORDS: Acrylamide; potato crisps; asparagines; glucose; fructose; sucrose; harvest year; weather conditions

INTRODUCTION

The potato is one of the world's major staple food crops (*1*). Today, the consumption of boiled potato has decreased in several countries but, conversely, the consumption of fried and roasted potatoes products has increased. In 2002, Swedish researchers reported that heat-treated carbohydrate-rich food products, such as potato chips (commonly known as crisps in Europe), contained acrylamide (*2*), a compound that is neurotoxic to humans (*3*) and classified as a probable human carcinogen (*4*). Some of the first reports on acrylamide in foods showed levels of up to 5800 μg kg^{-1} in industrially prepared potato chips (*2, 5–8*), but in recent reports, the levels are considerably lower (*9, 10*). Potatoes contain relatively high amounts of glucose, fructose, sucrose, and asparagine (*11–16*), which have been shown to produce acrylamide via the Maillard reaction (*17, 18*). Much research has been devoted to mitigation strategies, and the formation of acrylamide in fried potato products has been demonstrated to depend on several factors, for example, potato variety, precursor content, product shape and size, pretreatment, frying time and temperature, and moisture content in the final product (*19, 20*).

Processing conditions can be optimized and controlled to minimize acrylamide formation in contrast to the initial content of acrylamide precursors in the potatoes that depend on variety, growing conditions, and location. The genotype of the potato influences the pool of free amino acids as well as the concentration of sugars (*12, 14, 21*). In addition, the nutritional status of the soil, fertilization, location, and weather conditions during growth influence the initial precursor composition (*11, 14, 16, 22–24*). Most reports on the content of free amino acids and sugars in potatoes and acrylamide in chips concern one occasion during one growing season (*25–29*), and it is questionable if the results are applicable also for coming years as weather conditions can vary considerably (*14*).

We report the contents of free amino acids and sugars in different potato clones grown at the same location in southern Sweden for three consecutive years, 2004−2006, combined with data on temperature, precipitation, and radiation conditions during the growing seasons, and the acrylamide content in chips.

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MATERIALS AND METHODS

Potato Clones. Five potato clones were studied; Saturna and Lady Rosetta are two varieties commonly used for chip production, and Bintje is a well-established variety normally used for the production of French fries, whereas Hulda and SW 91 102 (hereafter denoted SW) derive from the breeding program of Svalöf Weibull AB, Sweden. Hulda and SW are resistant to low-temperature sweetening but are not available on the market.

The potato clones were grown at the same location in southern Sweden in 2004, 2005, and 2006. Fertilizers (88 kg of N ha^{-1}, 40 kg of P ha^{-1}, and 144 kg of K ha^{-1}), herbicides, and fungicides against late blight were applied according to local practices. The nitrogen status of the soil was 14.0, 17.9, and 12.7 kg of N ha^{-1} at the end of April in 2004, 2005, and 2006, respectively. The potatoes were harvested in September, and after wound healing for 2 weeks at 15 °C, the tubers were stored for 5 weeks at 6 °C and 98% relative air humidity before the experiments were conducted in November each year. In 2004, all five clones were analyzed, but for the next two years, Bintje was excluded because of its tendency to form very high levels of acrylamide; besides, Bintje is not a common chip variety. In 2005, Lady Rosetta was not analyzed because of lack of material.

Weather Data. Information on the average temperature, total precipitation measured as millimeters of rain, and global radiation for each month of the three growing seasons was recorded at the growing location. Air temperature was measured in degrees Celsius at 1.8 m above soil level. Global radiation was registered as mWh cm^{-2} at 1.8 m above soil level. The "normal values" are based on measurements done during 30 years between 1960 and 1990 (average of 30 years). The registrations followed the regulations set up by the Swedish Meteorological and Hydrological Institute (SMHI).

Sample Preparation. Between 12 and 20 potatoes of different sizes (tuber length = 6−10 cm) were selected during each sampling occasion. Chips were made in a laboratory-scale equipment as described by Viklund et al. (*30*). Briefly, potatoes were divided lengthwise, and half of each potato was homogenized and used for precursor analyses. The other halves were cut into 1.5 mm slices, which were deep fried in rapeseed oil for 4 min. The oil temperature was monitored with thermocouples (type K, 0.1 mm). The initial oil temperature was 180 °C, and the final temperature was 160 °C. All experiments were performed in duplicate.

Analyses. The dry matter contents of the tubers and potato chips were determined as described previously (*30*). The amino acid concentrations were determined using HPLC and a fluorescence detector, and the glucose, fructose, and sucrose concentrations were determined using gas chromatography and a flame ionization detector as previously described (*14*). The analytical methods have been evaluated in interlaboratory trials within the NORDACRYL project (http://www.nordicinnovation.net/_img/04005_nordacryl_final_report_web.pdf). The mean differences between duplicate precursor determinations were 4% for asparagine and 5% for sugars.

In 2004, the acrylamide content in the potato chips was analyzed using HPLC and mass spectrometry (LC-MS/MS) as described by Viklund et al. (*30*). In 2005 and 2006, the following changes were made; a longer column, a Genesis AQ column (150 × 4.6 mm i.d., 4 μm) equipped with a guard column (10 × 4.6 mm i.d., 4 μm) (Grace Vydac, Jones Chromatography) was used, and the flow rate was 0.5 mL min^{-1}. This column reduced some interfering peaks and increased the sensitivity. Before extraction, the samples were spiked with deuterium-labeled acrylamide (Polymer Source, Inc., Dorval, QC, Canada). Sheath and auxiliary gas flows were set to 80 and 20 units, respectively, and the heated capillary temperature was 175 °C. Single injections were made from each sample in 2004, whereas duplicate injections were made in 2005 and 2006. The mean difference between double injections in acrylamide analysis was 4%.

Statistical Analyses. Statistical analyses were performed using Minitab Statistical Software v. 13 (Minitab Inc., State College, PA). Significant differences were evaluated with the general linear model followed by Tukey's multiple-comparisons test. A value of $p \leq 0.05$ was considered to be significant.

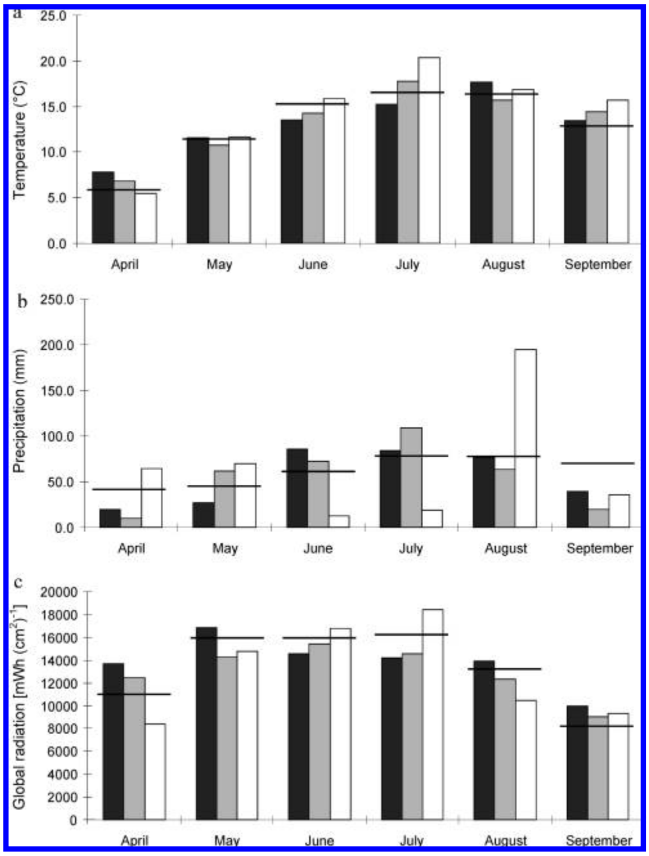

Figure 1. (**a**) Mean temperature, (**b**) total precipitation, and (**c**) total global radiation in 2004 (black bars), 2005 (gray bars), and 2006 (white bars). The vertical lines indicate normal values for the growing location.

RESULTS AND DISCUSSION

Weather conditions during growth had a great impact on the free amino acid and sugar contents in the different potato clones which, in turn, affected the formation of acrylamide in chips. Data on mean temperature, total precipitation, and global radiation for each month during the three growing seasons are summarized in **Figure 1**; the normal values are indicated with black lines. The mean temperature tended to be higher in 2006 than in 2004 and 2005 (**Figure 1a**). In 2006, the precipitation differed from the other years, being extremely low in June and July and extremely high in August (**Figure 1b**). The light conditions varied somewhat, with a tendency toward more global radiation in June and July of 2006 (**Figure 1c**).

Within each year, the contents of sugars (**Table 1**) and free amino acids (**Table 2**) differed between the potato clones. This is in agreement with results from other studies (*12, 14, 15, 28, 29*). For the three years studied, there were also major differences between the contents of amino acids and sugars within each clone. This is probably related to weather conditions during growth. For example, SW, which was a very low asparagine clone in 2004 and 2005, was in the same range as the others in 2006. Also, Lady Rosetta had a much higher content of asparagine in 2006. Other studies have shown that sometimes the effect of the year may obscure the effect of the variety (*14*). The asparagine content in potatoes may be affected by the nitrogen status and the fertilization of the soil; that is, the higher the nitrogen level, the higher the asparagine content (*23, 31*). The level of total nitrogen in the soil (measured in the latter half of April each year) was lowest in 2006, and the fields had been supplied

Table 1. Dry Matter, Sugars, and Asparagine (Milligrams per Gram of Dry Weight), the Ratio between Asparagine and Total Amino Acids in Tubers from Different Clones during Three Consecutive Years, and the Content of Acrylamide in Chips (Milligrams per Kilogram of Chips)[a]

			mg g $^{-1}$					
clone	year	dry matter (%)	glucose	fructose	sucrose	asparagine	molar ratio Asn/Total amino acids[b]	acrylamide (μg kg^{-1})
Saturna	2004	26	4.3 a	2.3 a	9.4 a	8.1 ab	0.53	3100 ab
	2005	27	1.8 b	1.1 b	9.3 a	11.2 cd	0.48	2800 b
	2006	20	7.5 c	6.2 c	6.1 b	9.6 ae	0.43	6300 cde
Hulda	2004	23	3.4 d	2.0 ad	6.0 b	12.5 cf	0.44	3200 ab
	2005	27	3.8 ad	1.6 bd	6.9 bc	10.1 de	0.37	3400 ab
	2006	23	5.6 e	3.7 e	6.5 b	10.5 de	0.37	7600 c
SW	2004	29	4.0 ad	1.8 ad	10.5 a	5.5 g	0.35	1800 b
	2005	28	4.8 f	2.1 ad	7.1 bc	5.6 g	0.33	2900 b
	2006	26	7.1 c	5.2 f	12.9 d	10.3 de	0.47	5200 ade
Lady Rosetta	2004	27	2.9 g	1.7 bd	8.7 ac	7.3 b	0.45	2400 b
	2005							
	2006	24	5.5 e	4.6 fg	9.1 a	13.1 f	0.58	5000 ae
Bintje	2004	26	9.8 h	4.2 eg	9.2 a	8.3 ab	0.46	7600 cd
	2005							
	2006							

[a] No significant differences between figures sharing the same letters within each column, $p < 0.05$. [b] Cysteine, methionine, and tryptophan were not analyzed.

Table 2. Free Amino Acids (Milligrams per Gram of Dry Weight) in Tubers from Different Clones during Three Consecutive Years[a]

		amino acids (mg g^{-1})															
clone	year	Asn	Gln	His	Arg	Ser	Asp	Glu	Thr	Gly	Ala	Tyr	Pro	Val	Phe	Ile+Leu	Lys
Saturna	2004	8.1	1.3	0.1	0.8	0.3	1.8	1.1	0.2	0.1	0.2	0.4	0.3	0.3	0.2	0.1	0.4
	2005	11.2	2.4	0.3	1.4	0.3	2.5	2.3	0.4	0.1	0.2	0.5	0.5	0.4	0.4	0.2	0.5
	2006	9.6	2.6	0.5	0.2	0.4	2.6	2.7	0.4	0.2	0.2	0.2	0.9	0.4	0.6	0.5	0.9
Hulda	2004	12.5	5.9	0.2	1.9	0.4	2.7	1.4	1.3	0.1	0.2	0.4	0.7	0.4	0.4	0.2	0.8
	2005	10.1	4.9	0.5	1.7	0.5	3.6	1.8	1.1	0.1	0.1	0.4	0.9	0.5	0.8	0.4	0.9
	2006	10.5	6.3	0.5	0.3	0.5	3.1	2.3	1.1	0.1	0.1	0.2	0.9	0.6	0.8	0.4	1.0
SW	2004	5.5	2.0	0.3	2.2	0.4	1.7	1.8	0.4	0.1	0.1	0.3	0.3	0.3	0.2	0.2	0.6
	2005	5.6	1.6	0.3	1.5	0.3	2.3	2.7	0.7	0.2	0.2	0.3	0.4	0.3	0.3	0.2	0.6
	2006	10.3	2.5	0.4	0.4	0.4	2.5	3.1	0.3	0.1	0.2	0.1	0.4	0.4	0.3	0.2	0.6
Lady Rosetta	2004	7.3	2.3	0.2	2.3	0.3	1.4	1.2	0.1	0.1	0.1	0.2	0.4	0.2	0.2	0.1	0.5
	2005																
	2006	13.1	2.6	0.3	0.4	0.3	2.0	2.2	0.3	0.1	0.1	NA	0.4	0.3	0.3	0.2	0.5
Bintje	2004	8.3	2.6	0.2	0.9	0.4	1.9	1.5	0.3	0.1	0.2	0.4	0.6	0.3	0.3	0.2	0.6
	2005																
	2006																

[a] Values are means of duplicate determinations.

with the same amount of nitrogen each year. However, the extremely high precipitation in August 2006 (**Figure 1b**) may have induced nitrogen leakage from the soil, thus making nitrogen more available for the tubers. Saturna had, however, a lower content of asparagine than in 2005. Notably, the content of aspartic acid and glutamic acid increased from 2004 to 2006, whereas the content of arginine in 2006 was <25% of that in 2004. These data indicate that the clones have different abilities to cope with the varying growing conditions.

For all clones tested, the glucose and fructose contents were significantly higher in 2006 than in the preceding years. In 2006, the levels of glucose were between 1.5 and 4.2 times higher than in 2004 and 2005 and the levels of fructose were between 1.9 and 5.6 higher. With regard to the levels of sucrose, the levels were significantly lower in Saturna in 2006 than in 2004 and significantly higher in SW, whereas no significant differences were found for Hulda and Lady Rosetta (**Table 1**). These differences may be explained by the precipitation and temperature during growth (*22, 23*). In 2004 and 2005, the total precipitation in August was more or less normal for the growth location in southern Sweden (**Figure 1b**). On the contrary, June and July of 2006 were very dry and warm (**Figure 1a**) followed by heavy rain in August. Second growth occurred in 2006 in all of the clones in the present study, and this is typical for drought periods followed by rainy periods (*23*). Such stressful conditions may increase the sugar content (*22, 23*). However, there are divergent opinions about whether soil moisture causes increased or decreased levels of reducing sugars, as reviewed by Kumar and Sing (*22*). Low temperature will negatively influence tuber growth and development. The temperature during growth is second only to the cultivar with regard to sugar levels (*22*). Light has been shown to affect the plant growth and tuber initiation (*23*). In our study, the light conditions varied somewhat between the seasons, but no extreme deviations from normal values were observed.

Within each year there were no significant differences in acrylamide content between the clones (**Table 1**) except for Bintje, which was studied in only 2004. The acrylamide content in Bintje chips was between 2.4 and 4.2 times higher than in the other chips. This high acrylamide level was expected because Bintje is a variety known to have high sugar content. Bintje is not usually used for chip production. In 2006, the chips had around twice as high acrylamide content as in 2004 and 2005, and this difference was significant. For example, the acrylamide content in Hulda chips was similar to the high levels in Bintje chips in 2004. These results clearly show that the harvest year can have a high impact on acrylamide formation in potato chips and probably also in other fried potato products, for example, French fries.

The differences in acrylamide content between the years can be related to the different levels of precursors, especially sugars as reported in other studies (*11, 24, 28, 29*). In the present study, we did not find any significant correlation between acrylamide and sugars for each of the different years. However, when data from all years were inspected, there was a weak correlation between acrylamide and glucose ($r = 0.74$, $p = 0.009$) and fructose ($r = 0.78$, $p = 0.004$). Such a poor correlation was also observed in similar experiments performed by Elmore et al. (*16*). In this study we did not find any significant correlation between acrylamide and asparagine, probably because of the limited number of samples, but in a previous study, we have shown that chips from tubers with low levels of asparagine contain the lowest amounts of acrylamide at similar sugar concentrations (*15*). Furthermore, competition between asparagine and other amino acids for participation in the Maillard reaction has emerged as a determinant of acrylamide formation in model systems. Heating potato cakes at 160 °C for 20 min has shown good correlation between the concentration of asparagine in potato tubers, expressed as the proportion of the total amino acid pool, and acrylamide formation (*16*). In the present study, we determined the molar ratio between asparagine and the total amount of amino acids (**Table 1**). The ratio varied between 33 and 58%, but no correlation was found with the acrylamide content. The data in **Table 2** show that asparagine is the dominant free amino acid in potato, which has been shown in other studies (*13, 16, 32, 33*). The lack of correlation between precursors and acrylamide formation indicates that the formation of acrylamide is dependent not only on the content and the relative amounts of sugars and amino acids but also on other factors, for example, the food matrix, which may influence the availability of the reactants to participate in the Maillard reaction.

To summarize, the results of this study clearly show that the contents of free amino acids and sugars can vary significantly both between clones and also between years for the same potato clone. This variation consequently influences the acrylamide content in the chips. The high levels of acrylamide precursors in 2006 were probably due to a long period of drought followed by heavy rain, which induced high sugar concentrations in the tubers. Our data show that the relationship between acrylamide formation and amounts of precursors is complex, and probably the reaction activity or capacity of the precursors in the food matrix is of great importance.

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