

Influence of Fertilization on Acrylamide Formation during Frying of Potatoes Harvested in 2003

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The quality of the potato has been found to vary, when grown under different agricultural and environmental conditions, such as the level of fertilization. Consequently these factors may influence the acrylamide formation during the preparation of French fries. These assumptions were studied on three varieties: Bintje, Ramos, and Saturna from the harvest of 2003. Decreasing N fertilization caused increases in the reducing sugar concentration from 60% up to 100% on DM for all varieties studied. Due to a high correlation between the reducing sugar content and the generation of acrylamide during frying, this resulted in a parallel increase in the acrylamide concentration of the French fries. Thus by lowering the amount of N fertilizer, an increase of 30–65% of the acrylamide generation during frying could be observed. It seems of extreme importance to find an appropriate balance between the level of N fertilizer in order to diminish acrylamide formation but on the other hand to obtain an acceptable tuber and to consider the environmental impact. All results reported should be seen in the perspective of the warm growing season of 2003.

KEYWORDS: Acrylamide; potato; fertilization; frying

INTRODUCTION

Shortly after the discovery of the possible carcinogen acrylamide in foods in 2002, French fries emerged as a product with one of the highest levels with an average value of 334 $\mu\text{g kg}^{-1}$ and a maximum value of 5312 $\mu\text{g kg}^{-1}$ (1). This raised public concern because potato products are staple food for a big part of the world's population. Since then a lot of research has been conducted on the formation, reduction, and prevention of acrylamide in fried potatoes.

Asparagine is considered as the main amino acid responsible for acrylamide formation, whereas the presence of reducing sugars is necessary to affect the conversion of this amino acid into acrylamide (2, 3). Bearing in mind that in potatoes free asparagine is present in considerable excess compared to the amount of reducing sugars, the reducing sugars will be the limiting factor in the acrylamide formation in French fries, and

thus they will particularly determine the formation of acrylamide (4). A strong correlation between acrylamide formation and the reducing sugar levels available in the potato was previously elucidated by several authors (4–6).

Any modifications performed on the raw material constituents will inevitably influence the Maillard reaction and its products and, concomitantly, the organoleptic properties of the food. Thus changes in the chemical composition can be useful in reducing acrylamide formation during frying; however, these measures must be placed in the perspective of consumer acceptance. However, by selecting the appropriate tubers, acrylamide can be minimized to levels below 100 $\mu\text{g kg}^{-1}$ (7). Therefore, a detailed knowledge of the influence of agricultural and storage conditions on the composition of the potato tubers is of fundamental importance.

The aim of this study was to investigate three potato varieties from the 2003 harvest, which were submitted to different levels of N fertilization. Heavy fertilization is routinely applied since the potato is a crop with high nutrient requirements (8). Due to environmentally related problems in various countries however there is a growing social and even legal pressure to decrease the amount of nitrogen fertilization applied in general agricul-

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ture. As a consequence, in some countries potato growers are also bound to restricted fertilization levels. Therefore it is important to test the effect of a decrease in the nitrogen fertilization on the chemical composition and subsequently on the acrylamide formation after frying. Because changes in the chemical composition of the potato caused by different N fertilization levels are also depending on the potato variety, three different varieties were studied. The varieties used were Bintje, Ramos, and Saturna.

MATERIALS AND METHODS

Materials. Potatoes (*Solanum tuberosum* L. varieties: Bintje, Ramos, Saturna) obtained from the Interprovincial Research Institute for Potato Production (Rumbeke-Beitem, Belgium) were sampled every 2 or 4 weeks starting from mid-October (week 42) of 2003 until mid-May (week 24) of 2004. These potatoes were grown under three different N fertilization levels. The three different levels of N fertilization were advice N, half of the advice N, and no N. The advice nitrogen was determined based on soil analysis. The initial nitrogen level in the soil was as follows: nitrite nitrogen, 20.8 kg N ha⁻¹ for a depth of 0–30 cm, 15.2 kg N ha⁻¹ for a depth of 30–60 cm, and 10.4 kg N ha⁻¹ for a depth of 60–90 cm; ammonium nitrogen, 4.6 kg N ha⁻¹ for a depth of 0–30 cm, 1.0 kg N ha⁻¹ for a depth of 30–60 cm, and 0.1 kg N ha⁻¹ for a depth of 60–90 cm. On the basis of these values, the advice N applied was fractioned and applied as follows: 160 kg N ha⁻¹ was added before planting for all varieties; the second fraction was added at tuber initiation around 6 weeks after planting. The amount applied depended on the cultivar: Bintje, 65 kg N ha⁻¹; Ramos, 35 kg N ha⁻¹; Saturna, 55 kg N ha⁻¹. A decrease in fertilization was chosen due to the fact that there are restrictions on the rates of N applications for environmental reasons; furthermore, restrictions are necessary to avoid yield decreases due to a higher salinity of the soil and finally to avoid a too low dry matter content of the tuber (9).

Potatoes were harvested in week 39 (end of September) and subsequently stored at 8 °C. To suppress sprouting, CIPC (chlorpropham, isopropyl-*N*-(3-chlorophenyl)carbamate) was used at the beginning of storage in the storage house at 8 °C at 2 kg Luxan-Grostop (1.34% active substance) ton⁻¹ potatoes.

Sample Preparation and Frying. Potatoes were rinsed, cut, and fried for acrylamide determination as described in De Wilde et al. (6). The remainder of the potatoes was cut in small cubes and kept at -18 °C in order to analyze the raw material composition. The repeatability test of the frying procedure was also described in De Wilde et al. (6) and showed that the frying process was repeatable with a relative standard deviation of 27%.

Reagents and Chemicals. All reagents and chemicals used in the following chemical analysis were described in De Wilde et al. (6).

Chemical Characterization of the Potato. Dry Matter (DM) Content. The determination of the DM content was based on the AOAC Official Method (930.15) (10). Briefly, 5 g of homogenized potatoes was mixed with calcined sea sand and placed in the oven at 105 °C until constant weight was reached.

Crude Protein Content. The total Kjeldahl protein content was determined according to Egan et al. (11). Crude potatoes were homogenized with a household mixer (Braun MR 5550 MCA, Spain). An amount of 1.5–2 g of this potato mix was transferred into a Kjeldahl tube to which 10 mL of H₂SO₄ and 1 Kjeltab CX (catalyst compound) were added. The digestion was done in a destruction block (420 °C) until a clear solution was obtained. Distillation was carried out with a 2200 Kjeltac Auto (FOSS Tecator, Sweden). The obtained distillate was titrated with 0.05 M HCl. For the calculation of the crude protein content a conversion factor of 6.25 was used.

Free Amino Acid Content. Mixed crude potatoes (15 g) were transferred into a quantitative flask of 100 mL and diluted to 100 mL with 15% trichloroacetic acid (TCA, v/v). After incubation (10 min at ambient temperature) and filtration, the filtrate was diluted in injection buffer and used in the Biotronik LC3000 amino acid analyzer as described in De Wilde et al. (6).

pH. For the determination of the pH, 90 mL of distilled water was added to 10 g of homogenized potato. After filtration, the pH of the solution was measured with a pH electrode (Schott, Germany).

Starch. The procedure of Browne and Zerban (12) was used with minor modifications. After homogenization of the crude potatoes, 2.5 g of potatoes was treated with 50 mL of the HCl solution. This solution was boiled for 15 min. During this step, starch was converted to sugars, mainly glucose. After a cleanup step with Carrez I and II, an adjustment to 100 mL was carried out with the HCl solution. After filtration the hydrolysate was measured in a polarimeter (Hilger and Watts, England). The extent of polarization is related to the concentration of the optically active molecules in the solution by the equation $\alpha = [\alpha]lc$, where α is the measured angle of rotation, $[\alpha]$ is the optical activity (which for acid hydrolyzed potatoes amounts 184), l is the path length, and c is the concentration.

Sugars. Mono- and disaccharides were assessed by gas chromatographic analysis as described by De Wilde et al. (6). Briefly, after aqueous extraction, addition of an internal standard (phenyl- β -D-glucopyranoside) and cleanup, the filtrate was derivatized and injected in a Varian 3380 gas chromatograph equipped with a flame ionization detector (Varian Instrument Group, Walnut Creek, CA) with a stationary phase (5% phenyl)methylpolysiloxane, film thickness 0.25 μ m, 30 m \times 0.32 mm inside diameter (i.d.) (Agilent Technologies, Palo Alto, CA).

Acrylamide. The determination of acrylamide was carried out using an accredited method based on the ISO 17025 standards with minor modifications described in (10). Acrylamide was extracted from the French fries with water before the cleanup step. A further concentration step by evaporation was introduced before analysis using the LC-MS/MS technique. Determination of acrylamide in samples was made by a linear calibration curve set on standard solutions over the concentration range from 0 to 1000 μ g kg⁻¹ using 2,3,3-acrylamide-*d*₃ (daughter ion: 75 > 58) as internal standard for the recovery correction. The limits of the method are, respectively, 10 and 20 μ g acrylamide kg⁻¹ foodstuff for detection and quantification (13).

Statistical Analysis. Statistical analysis of the data was performed using SPSS version 12.0 (SPSS Inc., Chicago, IL). Due to the large extent of the experimental setup no repetitions of each single sample point were present. A general linear model was used to process the data statistically. Univariate analysis was performed to determine significant influences ($p \leq 0.05$) of factors (i.e., variety, fertilization level, etc.) on intrinsic factors (i.e., DM content, reducing sugar content, etc.). When no interaction was present between the parameters, e.g., between storage time and dry matter content, but when there is a significant interaction, e.g., between variety and dry matter content, post hoc comparison of the average values over storage time could be carried out to determine significant differences between the varieties in their dry matter content. The chosen level of significance was 0.05.

RESULTS AND DISCUSSION

Influence of Fertilization on the Chemical Composition of the Potato. After statistical analysis it could be concluded that the pH, dry matter, crude protein, total free amino acid, starch, and sugar content were not significantly influenced by the storage time for the three varieties studied. This is in line with the results obtained in previous experiments concerning the influence of storage time on the acrylamide formation of varieties Bintje, Ramos, and Saturna (6). Therefore, the average values were used to determine the influence of different fertilization levels on the chemical composition.

Dry Matter Content and pH. Fertilization had a nonsignificant influence on the average dry matter content of the three potato varieties studied (Table 1). Except for Bintje however, if no fertilization was applied, a significantly higher dry matter content was observed. Previously, several authors found that the dry matter content increased with decreasing N application (14). Low N fertilizer application causes the haulm weight to become smaller, thus the dry matter content of the tubers increases. In

Table 1. Influence of Decreasing N Fertilization on the Selected Characteristics of Three Potato Varieties^a

	Bintje			Ramos			Saturna		
	advice N	advice N/2	no N	advice N	advice N/2	no N	advice N	advice N/2	no N
DM (%)	22.45 a ^b	22.86 a	24.66 b	23.46 a	23.66 a	23.09 a	24.16 a	24.75 a	23.65 a
pH	6.29 a	6.37 a	6.40 a	6.33 a	6.39 a	6.39 a	6.25 a	6.29 a	6.25 a
crude protein (% on DM)	11.03 c	9.80 b	8.01 a	10.52 b	8.51 b	7.33 a	11.59 c	9.44 b	8.13 a
net protein (% on DM)	5.38 b	5.44 b	3.39 a	5.16 c	4.10 b	3.59 a	6.74 c	5.23 b	4.21 a
total free amino acids (% on DM)	4.42 b	2.80 a	2.74 a	3.98 b	3.08 ab	2.48 a	3.19 b	2.78 ab	2.44 a
nonprotein compounds (% on DM)	5.65 b	4.36 a	4.62 a	5.36 b	4.41 b	3.74 a	4.85 b	4.21 a	3.92 a
other nitrogen compounds (% on DM)	1.23 a	1.56 a	1.88 a	1.38 a	1.33 a	1.26 a	1.66 a	1.43 a	1.48 a
starch (% on DM)	72.04 a	74.62 a	73.46 a	75.45 a	76.21 a	74.68 a	76.35 a	77.13 a	76.48 a
fructose (% on DM)	0.10 a	0.14 b	0.18 c	0.087 a	0.094 a	0.17 b	0.063 a	0.070 a	0.097 b
glucose (% on DM)	0.14 a	0.19 a	0.28 b	0.13 a	0.14 a	0.27 b	0.084 a	0.067 a	0.14 b
sucrose (% on DM)	0.73 a	0.59 a	0.74 a	0.50 a	0.41 a	0.51 a	0.61 a	0.45 a	0.73 a
asparagine (% on DM)	1.78 b	1.06 a	1.11 a	1.69 c	1.23 b	0.90 a	1.56 c	1.18 b	0.97 a
acrylamide ($\mu\text{g kg}^{-1}$)	272 a	417 ab	444 b	179 a	207 a	295 b	181 a	190 a	235 b

^a Stored at 8 °C over 24 weeks. Results are the average over a 24 week period as explained in the Results and Discussion. ^b Different letters in the same row and per variety indicate significant differences ($P \leq 0.05$) by the Duncan test.

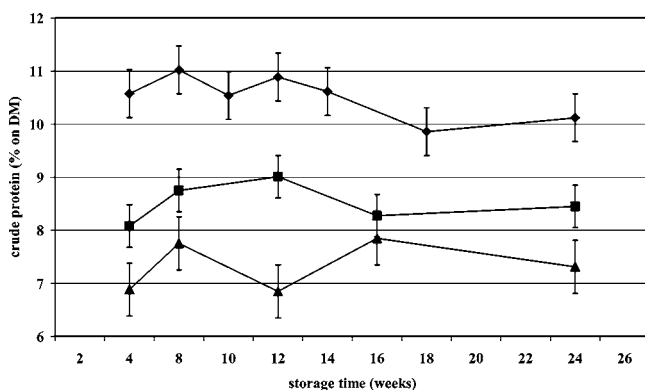


Figure 1. Influence of three N fertilization levels on the crude protein content of variety Ramos stored at 8 °C over 24 weeks of storage, expressed as % of DM (◆ = advice N, ■ = advice N/2, ▲ = no N) (error bars are \pm standard deviation ($P \leq 0.05$), $n = 5$, except for advice N, $n = 7$).

this study however, the difference in dry matter content between the three fertilization levels was smaller and not significant in the tubers of varieties Ramos and Saturna. Concerning pH, no significant differences were found between the fertilization levels for the three varieties (**Table 1**).

Crude Protein and Total Free Amino Acid Content. As can be observed from **Table 1** and **Figure 1**, a decrease in the dose of N fertilizer resulted in significant decreases in the crude protein content and subsequently in decreases in the net protein content. This is confirmed by several authors (15). The crude protein content remained moreover stable during storage for all varieties (**Figure 1**) (results for Bintje and Saturna are not shown). The decrease in the crude protein content with decreasing nitrogen fertilization could be attributed to a decrease of both the nonprotein compounds and the total free amino acid concentration while both showed to be significantly dependent

Table 2. Influence of N Fertilization Levels on the Average Absolute Free Amino Acid Values ($n = 5$) of Variety Bintje Stored over 24 Weeks

amino acid	absolute (% on dry matter)		
	advice N	advice N/2	no N
ornithine	0.01 a ^a	0.00 a	0.01 a
lysine	0.11 b	0.08 a	0.09 ab
arginine	0.25 b	0.14 a	0.12 a
histidine	0.05 b	0.03 a	0.04 a
γ -aminobutyrate	0.24 a	0.23 a	0.22 a
phenylalanine	0.07 b	0.04 a	0.06 ab
tyrosine	0.04 a	0.02 a	0.04 a
leucine	0.03 b	0.02 a	0.02 a
isoleucine	0.07 a	0.04 a	0.05 a
methionine	0.04 a	0.02 a	0.02 a
valine	0.14 b	0.09 a	0.09 a
alanine	0.05 b	0.03 a	0.04 ab
proline	0.24 b	0.06 a	0.13 a
glutamine	0.43 c	0.31 b	0.19 a
glutamic acid	0.41 c	0.20 b	0.11 a
asparagine	1.78 b	1.06 a	1.11 a
serine	0.09 b	0.05 a	0.05 a
threonine	0.06 a	0.04 a	0.05 a
aspartic acid	0.30 a	0.33 a	0.29 a

^a Different letters in the same row indicate significant differences ($P \leq 0.05$) by the Duncan test.

upon the fertilization. Only the other nitrogen compounds (i.e., purine derivatives, pyrimidine derivatives, nitrogenous glycol derivatives, etc. (16)) were not significantly influenced by the increasing fertilization level (**Table 1**).

On individual free amino acid basis, a similar trend could be observed, although not all free amino acids are affected to the same extent (17) (**Table 2**). The asparagine concentration decreased in an analogous way for the three varieties with decreasing N fertilization with values of 37.6%, 43%, and 37.7%

for varieties Bintje, Ramos, and Saturna, respectively (results not shown for varieties Ramos and Saturna). More variability between the varieties could be observed in the trend of the other amino acids with decreasing N fertilization. For example, glutamine decreases with values from 8% to 56% for Saturna and Bintje, respectively, and valine decreased from 23% to 37% for Saturna and Bintje, respectively. Consequently, it could be observed that the influence on the main acrylamide precursor, asparagine, was less variety dependent than the other amino acids.

Starch and Sugars. The observed results did not show any significant influence of fertilization on the starch content of the tuber (**Table 1**). Other studies have observed that intensive N fertilization decreases the content of starch in potato tubers, which is for potato frying an undesirable affect (18). In this study, the applied fertilization corresponds to the one which is commonly used in practice, so no excessive N was applied. This explains why the effect on the starch content was not significant.

In **Table 1** a negative relationship between N fertilization and the reducing sugar content (fructose and glucose) in the tuber is observed for the three varieties studied. The reducing sugar content in the tubers increased significantly with around 92% on DM for variety Bintje, 100% on DM for variety Ramos, and 60% on DM for variety Saturna. Thus, this increase due to a decrease in the nitrogen fertilization is variety specific. The reducing sugar concentration in variety Saturna appeared to be less significant to different N fertilization levels. The increase in the reducing sugar content was not significant when half of the advice N was applied, but appeared to be significantly different when no fertilization was applied to the soil.

In contrast to the observed results for the reducing sugar content, no significant relationship between the fertilization level and the sucrose content of the tubers was observed (**Table 1**). Previous findings confirm the statement that N fertilization influences the reducing sugar accumulation (19–24). The higher intake of N leads to a reduction in available mono- and disaccharides in the plant metabolism, because these are used for the biosynthesis of amino acids and especially with higher N fertilization for amides (glutamine and asparagine) (25). Although the study investigates the behavior of tubers grown in an exceptionally hot and dry summer, the outcome of our study confirms the results found in the literature. Therefore, we could say that the obtained results can be useful to investigate the influence on the acrylamide formation although a confirmation of the obtained results will be necessary.

Influence of Nitrogen Fertilization on the Amount of Acrylamide. After chemical characterization of the tubers, French fries of the same tubers were cut and fried as described above. Acrylamide content was determined and evaluated in order to correlate these data with the previous observations on the tuber composition.

Results as a function of storage time and nitrogen fertilization level for Bintje are shown in **Figure 2**. As can be observed, again no characteristic changes occur in the generation of acrylamide upon frying throughout the whole storage period for all nitrogen fertilization levels. It can be concluded that fries from tubers grown under advice N have a significantly lower acrylamide concentration than fries from tubers grown without added N fertilization. The acrylamide concentration in fries from tubers grown under advice N/2 appeared to be not significantly different from the highest and lowest amount of N fertilizer. This means that the N fertilization can be reduced until a certain threshold level. When the fertilization level becomes too low or zero the acrylamide content increases significantly. The

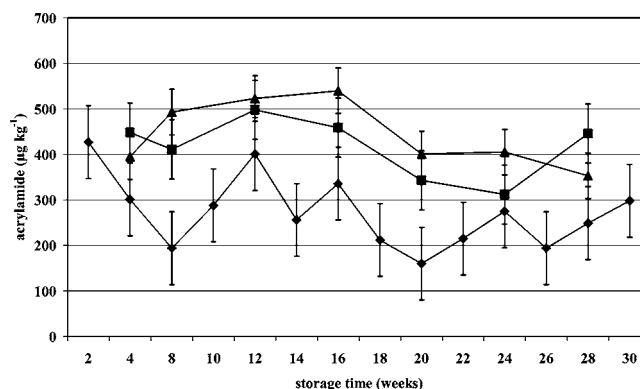


Figure 2. Influence of three N fertilization levels on acrylamide formation during frying of variety Bintje stored for 30 weeks at 8 °C, expressed as $\mu\text{g kg}^{-1}$ (\blacklozenge = advice N, \blacksquare = advice N/2, \blacktriangle = no N) (error bars are \pm standard deviation ($P \leq 0.05$), $n = 7$, except for advice N, $n = 14$).

amount of acrylamide was up to $241 \mu\text{g kg}^{-1}$ higher (week 20, variety Bintje) when decreasing the amount of N fertilizer. This is more than twice the acrylamide concentration in fries from potatoes cultivated under a normal level of N fertilization (**Figure 2**).

These results are in agreement with those observed for the other varieties investigated (results not shown). Average acrylamide levels in fries as a function of the N fertilization level are included in **Table 1**. It can be observed as well that a significant difference in the acrylamide content can be observed between the treatment without fertilization and the treatments with fertilization (advice N and advice N/2).

As stated before, the main precursors for acrylamide are the reducing sugars and asparagine (2, 4, 26, 27). The limiting factor for the formation of acrylamide in potatoes is the content of reducing sugars, because potatoes contain a high amount of free asparagine (4, 28). These findings are confirmed in this study. While asparagine decreases with decreasing N fertilization, the reducing sugar content increases, and subsequently acrylamide formation increases for all varieties. Thus, it is clear that the observed changes in acrylamide formation are similar to those occurring in the reducing sugar concentrations. This is also clear from the decreases observed in the reducing sugar content when the N fertilization increased. The lowest decrease was present in the variety Saturna. This is also the case for the acrylamide concentration after frying, where the acrylamide concentration decreased for 60%, while for varieties Bintje and Ramos this decrease amounted 92% and 100%, respectively, with increasing nitrogen fertilization. Therefore it is important to adjust the nitrogen fertilization to the potato variety used.

When the asparagine content was correlated with the acrylamide concentration in the fries, the correlation was only $R^2 = 0.024$ ($n = 160$). This in contrast to the reducing sugar content which showed a strong correlation with the acrylamide formation, $R^2 = 0.84$ ($n = 160$). Because 16% of the acrylamide formation could not be explained by the reducing sugar concentration, it could be possible that other compounds are also involved. A possible hypothesis is that due to the decrease in the total free amino acid concentration some amino acids, which could have a beneficial effect due to competition with asparagine, decreased with decreasing N fertilization. When correlating the molar amount of the free amino acids and acrylamide of variety Bintje, a negative correlation could be observed for the amino acids histidine ($R^2 = 0.55$, $n = 16$) and arginine ($R^2 = 0.64$, $n = 16$). Some results clearly indicate that amino acids can reduce the content of acrylamide. Several

authors (29, 30) stated that the addition of glycine to dough and potato products reduced the acrylamide content. Levine and Smith (31) found that the addition of methionine or cysteine decreased acrylamide formation in a cracker model probably due to competition with asparagine in reactions that would produce acrylamide and that cysteine can react directly with acrylamide, causing its elimination.

It can be concluded that finding an optimum N fertilization level will be very difficult. The influence of fertilization and the content of the reducing sugars depend strongly on the climatic conditions; thus, the obtained conclusions cannot be generalized, but because they are very similar to what has been found in the literature they can give a good indication. Cultivating potatoes under a high level of N fertilization can be positive with regard to acrylamide formation; on the other hand it can be very detrimental for the tuber, potato yield, and the environment. Thus a balance between both phenomena should be achieved.

ABBREVIATIONS USED

DM, dry matter; N, nitrogen; TCA, trichloroacetic acid.

LITERATURE CITED

- (1) International Agency for Research on Cancer. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*; Lyon, France, 1994; Vol. 60, pp 389–433.
- (2) Becalski, A.; Lau, B. P. Y.; Lewis, D.; Seaman, S. W. Acrylamide in foods: occurrence, sources, and modeling. *J. Agric. Food Chem.* **2003**, *51*, 802–808.
- (3) Zyzak, D. V.; Sanders, R. A.; Stojanovic, M.; Tallmadge, D. H.; Eberhart, B. L.; Ewald, D. K.; Gruber, D. C.; Morsch, T. R.; Strothers, M. A.; Rizzi, G. P.; Villagran, M. D. Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem.* **2003**, *51*, 4782–4787.
- (4) Becalski, A.; Lau, B. P. Y.; Lewis, D.; Seaman, S. W.; Hayward, S.; Sahagian, M.; Ramesh, M.; Leclerc, Y. Acrylamide in french fries: Influence of free amino acids and sugars. *J. Agric. Food Chem.* **2004**, *52*, 3801–3806.
- (5) Amrein, T. M.; Bachmann, S.; Noti, A.; Biedermann, M.; Barbosa, M. F.; Biedermann-Brem, S.; Grob, K.; Keiser, A.; Realini, P.; Escher, F.; Amado, R. Potential of acrylamide formation, sugars, and free asparagine in potatoes: A comparison of cultivars and farming systems. *J. Agric. Food Chem.* **2003**, *51*, 5556–5560.
- (6) De Wilde, T.; De Meulenaer, B.; Mestdagh, F.; Govaert, Y.; Vandeburie, S.; Ooghe, W.; Frassel, S.; Demeulemeester, K.; Van Peteghem, C.; Calus, A.; Degroot, J.; Verhé, R. The influence of storage practices on acrylamide formation during frying. *J. Agric. Food Chem.* **2005**, *53*, 6550–6557.
- (7) Grob, K.; Biedermann, M.; Biedermann-Brem, S.; Noti, A.; Imhof, D.; Amrein, T.; Pfefferle, A.; Bazzocco, D. French fries with less than 100 µg/kg acrylamide. A collaboration between cooks and analysts. *Eur. Food Res. Technol.* **2003**, *217*, 185–194.
- (8) Mondy, N. I.; Koch, R. L. Influence of nitrogen-fertilization on potato discoloration in relation to chemical composition 0.1. Lipid, potassium, and dry-matter content. *J. Agric. Food Chem.* **1978**, *26*, 667–669.
- (9) Crozier, C. R.; Creamer, N. G.; Cubeta, M. A. Fertilizer management impacts on stand establishment, disease, and yield of Irish potato. *Potato Res.* **2000**, *43*, 49–59.
- (10) Association of Official Analytical Chemists. In *Official Methods of Analysis*, 15th ed.; Method 930.15; Helrich, K., Ed.; AOAC: Arlington, IL, 1990.
- (11) Egan, H.; Kirk, R.; Sawyer, R. In *Pearson's Chemical Analysis of Foods*, 8th ed.; Egan, H., Kirk, R., Sawyer, R., Eds.; Churchill Livingstone: London, 1981; Chapter Nitrates and Nitrites, pp 66–71.
- (12) Browne, C. A.; Zerban, F. N. *Physical and Chemical Methods of Sugar Analysis*; John Wiley & Sons: New York, 1941.
- (13) Govaert, Y.; Pavesi, A.; Scheers, E.; Frassel, S.; Weverbergh, E.; Van Loco, J.; Degroot, J. M.; Goeyens, L. Optimisation of a method for the determination of acrylamide in foods. *Anal. Chim. Acta*, submitted for publication, 2005.
- (14) Kolbe, H.; Muller, K.; Olteanu, G.; Gorea, T. Effects of nitrogen, phosphorus and potassium fertilizer treatments on weight-loss and changes in chemical-composition of potato-tubers stored at 4-degrees-C. *Potato Res.* **1995**, *38*, 97–107.
- (15) Eppendorfer, W. H.; Bille, S. W. Free and total amino acid composition of edible parts of beans, kale, spinach, cauliflower and potatoes as influenced by nitrogen fertilization and phosphorus and potassium deficiency. *J. Sci. Food Agric.* **1996**, *71*, 449–458.
- (16) Burton, W. G. *The Potato*, 3 ed.; Longman Scientific and Technical: Essex, 1989.
- (17) Rexen, B. Studies of protein of potatoes. *Potato Res.* **1976**, *19*, 189–202.
- (18) White, R. P.; Sanderson, J. B. Effect of planting date, nitrogen rate, and plant spacing on potatoes grown for processing in Prince-Edward-Island. *Am. Potato J.* **1983**, *60*, 115–126.
- (19) Kang, J. G.; Yang, S. Y.; Kim, S. Y. Effects of nitrogen levels on the plant growth, tuberization and quality of potatoes grown in aeroponics. *J. Korean Soc. Hortic. Sci.* **1996**, *37*, 761–766.
- (20) Kolbe, H.; Mueller, K.; Olteanu, G.; Gorea, T. Effects of nitrogen, phosphorus and potassium fertilizer treatments on weight loss and changes in chemical composition of potato tubers stored at 4 °C. *Potato Res.* **1995**, *38*, 97–107.
- (21) Roe, M. A.; Faulks, R. M.; Belsten, J. L. Role of reducing sugars and amino-acids in fry color of chips from potatoes grown under different nitrogen regimes. *J. Sci. Food Agric.* **1990**, *52*, 207–214.
- (22) Iritani, W. M.; Weller, L. Influence of low fertility and vine killing on sugar development in apical and basal portions of russet Burbank potatoes. *Am. Potato J.* **1978**, *55*, 239–246.
- (23) Hughes, J. C. The effects of storage temperature, variety and mineral nutrition on sugar accumulation. *Aspects Appl. Biol.* **1986**, *13*, 28–33.
- (24) Westermann, D. T.; James, D. W.; Tindall, T. A.; Hurst, R. L. Nitrogen and potassium fertilization of potatoes—sugars and starch. *Am. Potato J.* **1994**, *71*, 433–453.
- (25) Kolbe, H. Kartoffeldüngung unter differenzierten ökologischen Bedingungen. Ph.D. Dissertation, Georg-August-Universität, Göttingen, Germany, 1990.
- (26) Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P. A.; Robert, M. C.; Riediker, S. Acrylamide from Maillard reaction products. *Nature* **2002**, *419*, 449–450.
- (27) Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448–449.
- (28) Amrein, T. M.; Schonbachler, B.; Rohner, F.; Lukac, H.; Schneider, H.; Keiser, A.; Escher, F.; Amado, R. Potential for acrylamide formation in potatoes: data from the 2003 harvest. *Eur. Food Res. Technol.* **2004**, *219*, 572–578.
- (29) Amrein, T. M.; Schonbachler, B.; Escher, F.; Amado, R. Acrylamide in gingerbread: Critical factors for formation and possible ways for reduction. *J. Agric. Food Chem.* **2004**, *52*, 4282–4288.
- (30) Brathen, E. B.; Kita, A.; Knutsen, S. H.; Wicklund, T. Addition of glycine reduces the content of acrylamide in cereal and potato products. *J. Agric. Food Chem.* **2005**, *53*, 3259–3264.
- (31) Levine, A.; Smith, R. E. Sources of variability of acrylamide levels in a cracker model. *J. Agric. Food Chem.* **2005**, *53*, 4410–4416.

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