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# Influence of Storage Practices on Acrylamide Formation during Potato Frying

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A number of parameters linked to storage of potatoes were evaluated with regard to their potential to influence the acrylamide formation in French fries. Acrylamide, which is a potential human carcinogen, is reported to be formed during the frying of potatoes as a result of the reactions between asparagine and reducing sugars. This study was conducted using three potato varieties (Bintje, Ramos, and Saturna) typically used in Belgium, The Netherlands, and the northern part of France for French fry and crisp production. Saturna, mainly used in crisp production, appeared to be the least susceptible for acrylamide formation during frying. Especially storage at low temperatures (4 °C) compared to storage at 8 °C seemed to enhance acrylamide formation due to a strong increase in reducing sugars caused by low-temperature storage. Because of the reversible nature of this physiological reaction, it was possible to achieve a significant reduction of the reducing sugars after a reconditioning of the cold-stored potatoes for 3 weeks at 15 °C. All changes in acrylamide concentrations could mainly be explained by the reducing sugar content of the potato ($R^2 = 0,84$, $n = 160$). This means that, by ensuring a low reducing sugar content of the potato tuber, the risk for acrylamide formation will largely be reduced. Finally the use of a sprout inhibitor did not influence the composition of the potato, and thus acrylamide formation was not susceptible to this treatment.

**KEYWORDS: acrylamide; potato; storage temperature; storage time; reconditioning; frying**

## INTRODUCTION

The potato (*Solanum tuberosum* L.) is one of the world's major staple food crops. In 2003, $310 \times 10^{12}$ ton potatoes were produced (*1*). This tuber is an important element in the daily diet for a majority of people. Potatoes are an important source of highly nutritional proteins, fibers, potassium, and vitamins. They can be processed in different ways: boiled, crisped, baked, roasted, fried, etc.

In 2002 the Swedish National Food Administration detected high concentrations of acrylamide in common heated starch-rich foods such as French fries (www.slv.se). This attained public concern, because acrylamide is genotoxic and a potential carcinogen to humans, classified in Group 2A by the International Agency for Research on Cancer (*2*).

It has been stated that acrylamide is generated during a side reaction of the Maillard reaction. Crucial participants in this reaction are an amino acid (asparagine) and reducing sugars (fructose and glucose) (*3*−*6*). Asparagine provides the backbone of the acrylamide molecule, while reducing sugars are essential co-reactants in the formation of N-glycoside intermediates, which lead to the formation of acrylamide. Fried products, especially French fries and crisps, belong to the food category with probably the highest concentrations of acrylamide recorded so far (*7*). The reason for this strong susceptibility to acrylamide formation is the abundance of free asparagine present in the potato (*8*). The acrylamide formation only takes place at temperatures above 100 °C (*7*), which makes the frying process an ideal condition. The Maillard reaction, on the other hand, is essential for its contribution to the color and flavor of fried potatoes.

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Acrylamide formation during frying of potatoes can be reduced by adjustments in the food preparation stage itself as illustrated by several authors (*9*−*11*). Moreover, acrylamide formation during frying can be influenced by selection of the appropriate raw material (*12*). Obviously, acrylamide formation will largely be influenced by the potato composition, particularly with regard to its sugar and amino acid content. Several papers on the influence of storage temperature on the reducing sugars of potatoes were published (*13*, *14*). Obviously the consequences with regard to acrylamide formation during frying have been documented (*15*−*18*). However, apart from the storage temperature, also other storage factors may have an effect on acrylamide formation during frying of potatoes, factors such as reconditioning and the use of a sprout inhibitor. These aspects have hardly been studied before. Therefore this paper considers these and other aspects related to the storage of potatoes (typically grown in Belgium, The Netherlands, and in the northern part of France) and the influence on acrylamide formation during deep-fat frying.

Bintje, Ramos, and Saturna were the varieties used in this study. Bintje is a very important variety in Belgium since it has a share of over 60% in the total potato production. Its high market share can be explained by its wide applicability, apart from frying. Ramos is an interesting variety, because this variety can be stored at 6 °C, which is lower than for the other varieties (8 °C). This means they can be stored longer without sprouting. Saturna has a share of 6% in the Belgian potato production and is mainly used in crisp production.

## MATERIALS AND METHODS

**Materials.** Potatoes (*Solanum tuberosum* L. varieties: Bintje, Ramos, Saturna) stored at respectively 4 and 8 °C in the specially adapted storage facility at the Interprovincial Research Institute for Potato production (Rumbeke-Beitem, Belgium) were sampled starting from mid-October (week 42) of the year 2003 until mid-May (week 20) of the year 2004. To suppress sprouting potatoes were coated with powder, CIPC (Chlorpropham, isopropyl-*N*-(3-chlorophenyl)carbamate) was used in the storage house at 8 °C at 2 kg Luxan-Grostop (1.34% active substance) $ton^{-1}$ potatoes. Samples were taken every 2 or 4 weeks. Potatoes stored at 4 °C, not treated with CIPC, were reconditioned in weeks 8, 16, and 24 of storage. Reconditioning was done by storing potatoes at 15 °C, in two batches, one treated with CIPC and one without. The CIPC treatment during reconditioning was carried out using fumigation with Luxan-Grostop HN. The reconditioning process was carried out for 3 (R3) and 5 weeks (R5).

**Reagents and Chemicals.** For amino acid analysis, an injection buffer with the following composition was applied: 16.3 g of lithium acetate, 100 $\mu$L of caprylic acid (purum, ≥98%), 7.5 mL of formic acid (puriss. p.a., ∼98%) (supplied by Fluka Chemie GmbH Buchs, Switzerland), and 25 mL of high-performance liquid chromatography (HPLC) methanol (Labscan Ltd, Ireland) in 900 mL of demineralized water brought to pH 2.2 with trifluoroacetic acid and adjusted to 1 L with demineralized water (Fluka Chemie GmbH Buchs, Switzerland). For the gas chromatographic determination of the sugars, the following aqueous solutions were prepared: Carrez I (14% $K_4Fe(CN)_6$, Merck, Germany) and Carrez II (30% $ZnSO_4$, Chem-lab, Belgium). Furthermore, hexamethyldisilazane, trifluoroacetic acid from Chem-lab (Belgium), an internal standard solution (6 mg $mL^{-1}$ phenyl-$\beta$-D-glucopyranoside (Sigma-Aldrich, Belgium)), and an oximation reagent (2.5 g of hydroxylaminehydrochloride (UCB, Belgium) in 100 mL of dry pyridine (Merck, Germany)) were applied for the determination of sugars. For acrylamide determination, 2,3,3-[$D_3$]-acrylamide (Polymer Source Company, Canada) and acrylamide (Sigma Chemical Company, St.Louis, MO) were used as standards. HPLC-grade methanol of Merck (Germany), HPLC-grade water (Milli-Q, Waters Corporation, Milford, MA), and glacial acetic acid 99% (Vel, Belgium) were the solvents applied in the acrylamide determination. For the crude protein and total free amino acid content, sulfuric acid (Chem-lab, Belgium), trichloroacetic acid (Acros Organics, Belgium), 0.05 N titrisol solution HCl (Merck, Germany) and Kjeltab CX (Thompson & Capper Ltd., UK) were used. Determination of the starch content was assessed with Carrez I, Carrez II, and a solution of 25 mL of hydrochloric acid (37%, VWR, Belgium) in 1 L of demineralized water. All reagents were of analytical grade or better unless otherwise mentioned.

**Frying.** For frying experiments, sample batches of 20 potatoes (caliber = >5 cm) were taken. From every potato one French fry was cut out in length of the center of the potato. This French fry had a cross-section of 1 × 1 cm and a length of 5−10 cm. In total, 20 French fries were rinsed and fried as described below. The remainder of the potatoes were cut in small cubes and kept at −18 °C in order to analyze the composition of the raw material. Before frying, potatoes (batch of 20 French fries) were rinsed two times with 400 mL of water and put in a cup of water (400 mL) for approximately 15 min. They were subsequently superficially dried with a paper towel. Par-frying was performed in a Frifri FFE 41 (Switzerland) of 15 kW and a capacity of 20 L of oil for exactly 3 min at a temperature of 180 °C (±5 °C). French fries were cooled to room temperature between par-frying and finish frying. Finish frying was carried out with a Fritel Frying (Belgium) machine of 3.2 kW and a capacity of 5 L of oil, for exactly 2 min at 180 °C (±5 °C). The frying temperature was carefully monitored with a digital thermometer (Testo 925 with waterproof needle probe for measurements between −60 and 250 °C, Belgium). After frying and cooling, potatoes were homogenized with a Braun-multiquick mixer (Braun, GmbH, Spain) and frozen at −18 °C until analyzed for their acrylamide content.

**Chemical Characterization of the Potato.** *Dry Matter (DM) Content.* The determination of the DM content was based on the AOAC Official Method (930.15) (*19*). Briefly, 5 g of homogenized potatoes were 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 (*20*). Crude potatoes were homogenized; 1.5−2 g of this potato mix was transferred into a Kjeldahl tube to which 10 mL of $H_2SO_4$ and 1 Kjeltab CX (catalyst compound) were added. The decomposition was done in a destruction block (420 °C) until a clear solution was obtained. Distillation was carried out with a 2200 Kjeltec Auto (FOSS Tecator, Sweden). The obtained distillate was titrated with 0.05 M HCl. For the calculation of the total 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 (paper filtrate), the filtrate was used in the Biotronik LC3000 amino acid analyzer. The separation of the amino acids is based on a different partition of the amino acid cations between a cation-exchange resin and five consecutively used lithium acetate buffer solutions of an increasing pH (A, 2.85; B, 3.30; C, 4.25; D, 8.00; E, 10.30) and ionic strength (A, 0.16 N; B, 0.18 N; C, 0.20 N; D, 0.20 N; E, 0.40 N). The detection of the separated amino acids is based on a color reaction using a buffered ninhydrin solution and a continuous measurement of the absorbance at 570 nm (for the alpha amino acids, giving rise to a purple blue color) and at 440 nm (for the imino acids, giving rise to a yellow brown complex) (*21*).

*pH*. For the determination of the pH, 90 mL of distilled carbon dioxide free 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 (*22*) 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. 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.

*Sugars*. Mono- and disaccharides were assessed by gas chromatographic analysis after an aqueous extraction from homogenized potatoes. Before extraction in water, phenyl-$\beta$-D-glucopyranoside was added as internal standard at a concentration of 6 mg $mL^{-1}$. After incubation for 30 min at 60 °C, a cleanup step was carried out with Carrez I and

II (5 mL each). This obtained solution was filtrated, and 1 mL of the obtained solution was dried under nitrogen. The residue was derivatized in two steps, first an oximation with 100 $\mu$L of oximation reagents (30 min at 60 °C), second to trimethylsilylesters with 100 $\mu$L of hexamethyldisilizane and 10 $\mu$L of trifluoraceticacid (10 min at ambient temperature). Analyses were carried out using a Varian 3380 gas chromatograph equipped with a flame-ionization detector (Varian Instrument Group, Walnut Creek, CA). The chromatographic parameters were: stationary phase (5%-phenyl)-methylpolysiloxane, film thickness 0.25 $\mu$m, 30 m × 0.32 mm inside diameter (i.d.) (Agilent Technologies, Palo Alto, CA, USA); mobile phase: He at 1 mL $min^{-1}$, split 1/40, injector temperature = 250 °C; detector temperature = 340 °C; injection volume = 1 $\mu$L; temperature program = 180 °C for 1min, ramp at 15 °C $min^{-1}$ to 290 °C. The flame ionization detector was operated with hydrogen and air at, respectively, 30 and 300 mL $min^{-1}$ and helium at 20 mL $min^{-1}$ as makeup gas.

*Acrylamide*. The determination of acrylamide was carried out using an accreditated method based on the ISO 17025 standards. In the experimental procedure, acrylamide was extracted from food with water before a cleanup of the extract on solid-phase extraction combining Oasis HLB and Bond Elut-Accucat cartridges as described in the US FDA methodology (http://www.cfsan.fda.gov/~dms/acrylami.html). A further concentration step of three times by evaporation was introduced before analysis using the LC-MS/MS technique. The chromatographic separation was performed on a $\mu$-Bondapak $C_{18}$ 300 × 3.9 mm, 10 $\mu$m analytical column (Waters Corporation, Milford, MA) with 0.1% acetic acid in water as the mobile phase at a flow rate of 0.6 mL $min^{-1}$. A split of the flow rate (1:1) with a Valco piece was applied before the entrance into the MS/MS detector consisting of a Quattro *micro* triple quadrupole system from Micromass (Manchester, UK). Protonated acrylamides, produced by positive electrospray ionization in source, were chosen as precursor ions. Identification of acrylamide was based on the relative retention time and on two diagnostic ions (precursor (72 > 71.99) and one daughter ion (72 > 55) obtained by collision with Argon) selected for the multiple reaction monitoring (MRM). Determination of acrylamide in samples was made by a linear calibration curve set on standard solutions over the concentration range from 0 to 1000 $\mu$g/kg using 2,3,3-[$D_3$]-acrylamide (daughter ion: 75 > 58) as internal standard for the recovery correction. The limits of the method are, respectively, 10 and 20 $\mu$g acrylamide $kg^{-1}$ foodstuff for detection and quantification.

**Statistical Analysis.** Statistical analysis of the data was performed using SPSS version 12.0 (SPSS Inc., Chicago, IL). General linear model, univariate analysis was performed to determine significant influences ($p \leq 0.05$) of treatments (i.e., variety, storage time and temperature, etc.) on intrinsic factors (i.e., DM content, reducing sugar content, etc.). Post hoc comparison of means (Duncan) was carried out to determine significant differences between the different levels within each treatment. The chosen level of significance was 0.05. The term significant is used to indicate differences for which $p \leq 0.05$.

## RESULTS AND DISCUSSION

In practice, potatoes are stored at 8 °C. However, due to climatic conditions, lower storage temperatures may be unavoidable. On the other hand, at lower preservation temperatures, sprouting can be inhibited without the use of chemicals and the potatoes are less susceptible to diseases. Thus, low-temperature preservation may be interesting for organic potato production. The sweetening, which is associated with this low-temperature storage, could be restored by temperature reconditioning of potatoes (*13*, *23*−*25*). Therefore in this study two storage temperatures were considered together with a reconditioning process of the cold stored potatoes.

**Influence of Storage Time and Temperature on the Composition of the Potato.** *Reducing Sugars.* The results obtained for the fructose and glucose content are shown in, respectively, **Figures 1 and 2** for the three varieties, which were stored during 24 weeks at 4 and 8 °C. The reducing sugar concentration in potatoes stored at 8 °C did not change significantly over the whole storage period for all varieties studied (**Figures 1 and 2**). In addition, no significant differences between the three varieties were detected at this storage temperature.

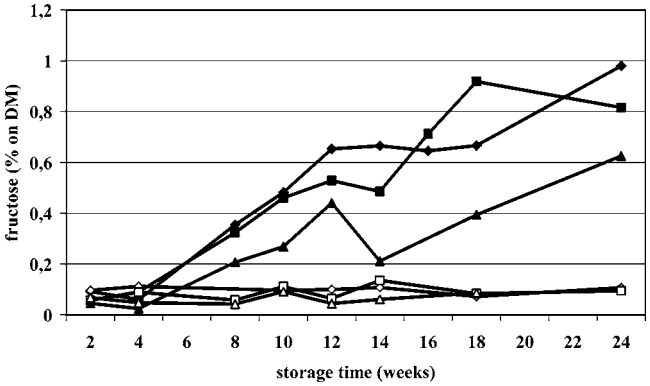


**Figure 1.** Influence of storage time and temperature on the fructose concentration of Bintje, Ramos, and Saturna, expressed in % on DM. (◆ = Bintje, 4 °C; ■ = Ramos, 4 °C; ▲ = Saturna, 4 °C; ◇ = Bintje, 8 °C; □ = Ramos, 8 °C; Δ = Saturna, 8 °C.)

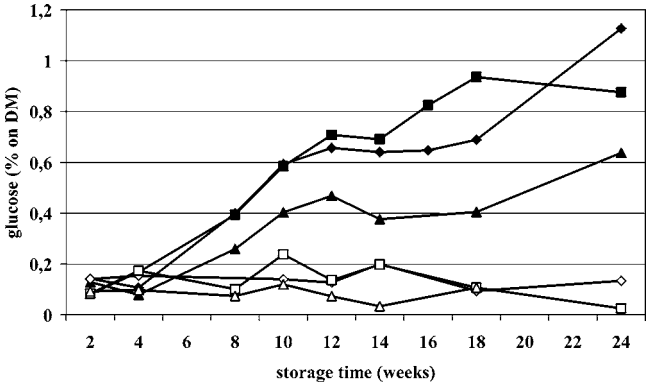


**Figure 2.** Influence of storage time and temperature on the glucose concentration of Bintje, Ramos and Saturna, expressed in % on DM. (◆ = Bintje, 4 °C; ■ = Ramos, 4 °C; ▲ = Saturna, 4 °C; ◇ = Bintje, 8 °C; □ = Ramos, 8 °C; Δ = Saturna, 8 °C.)

At 4 °C, a significant difference in fructose and glucose content between potatoes stored during the first 8 weeks and those stored for a longer period was observed. Before week 8 of storage, the average fructose and glucose content for variety Bintje was, respectively, 0.08 and 0.12% on DM. From storage week 8 until week 24, the fructose content increased from 0.36 to 0.98% on DM (**Figure 1**). The glucose content increased similarly, from 0.40% on DM in week 8 of storage up to 1.13% on DM in week 24 of storage (**Figure 2**). Similar trends were observed for the two other varieties studied. Olsson et al. (*15*) have found a decrease at the end of storage. These observations were not present because of the shorter storage period. The increase in the reducing sugar concentration on a dry matter basis can be attributed to the fact that potatoes stored at low temperatures accumulate sugars in the tuber (*14*, *26*−*29*). It should be noted that a gradual cooling of 8 weeks of the potatoes was applied before reaching the final storage temperature of 4 °C; this elucidates the lower amount of reducing sugars present during the first 8 weeks of storage.

When comparing the different varieties in their behavior to cold storage, it could be observed that especially the varieties Bintje and Ramos showed the highest concentration in fructose and glucose levels, compared to Saturna (**Figures 1 and 2**).

**Table 1.** Influence of Reconditioning after Initial Storage at 4 °C and Influence of Storage at 8 °C during Weeks 8 and 24 on the Fructose, Glucose, and Sucrose Concentrations, Expressed as % on DM (R0, 0 Weeks of Reconditioning; R3, 3 Weeks of Reconditioning; R5, 5 Weeks of Reconditioning; ND, Not Determined)[a]

| | | Bintje | | Ramos | | Saturna | |
|---|---|---|---|---|---|---|---|
| | | 8 weeks cold storage | 24 weeks cold storage | 8 weeks cold storage | 24 weeks cold storage | 8 weeks cold storage | 24 weeks cold storage |
| fructose (% on DM) | R0 (4 °C) | 0.35b | 0.98c | 0.32b | 0.82c | 0.21b | 0.63c |
| | R3 | 0.08a | 0.13a | 0.13a | 0.22a | 0.07a | 0.12a |
| | R5 | 0.08a | ND | 0.09a | ND | 0.05a | ND |
| | 8 °C | ND | 0.11 | 0.06 | 0.09 | 0.04 | ND |
| glucose (% on DM) | R0 (4 °C) | 0.40b | 1.13c | 0.39b | 0.88c | 0.26b | 0.64c |
| | R3 | 0.10a | 0.11a | 0.23a | 0.25a | 0.09a | 0.12a |
| | R5 | 0.16a | ND | 0.12a | ND | 0.07a | ND |
| | 8 °C | ND | 0.13 | 0.1 | 0.03 | 0.07 | ND |
| sucrose (% on DM) | R0 (4 °C) | 0.93b | 0.69b | 1.19b | 0.89b | 0.90b | 0.86b |
| | R3 | 0.47a | 0.30a | 0.5a | 0.31a | 0.49a | 0.38a |
| | R5 | 0.63ab | ND | 0.38a | ND | 0.6ab | ND |
| | 8 °C | ND | 0.48 | 0.51 | 0.24 | 0.69 | 0.59 |

[a] Different letters in the same column and for one sugar indicate significant differences ($p \leq 0.05$) by Duncan test.

Saturna contained a significantly lower amount of reducing sugars. Several authors reported widely varying contents of reducing sugars within a given cultivar as well as between potato cultivars. Mean contents of reducing sugars which have been reported for Bintje and Saturna are, respectively, 0.5 and 0.9% on DM (*8*, *30*). For variety Ramos, no reported concentrations were available. Differences in the reducing sugar content can among others be clarified by seasonal variability (*31*). In agreement with list references (*15*, *32*), the influence of cold storage in respect to sugar development is variety dependent. Furthermore it can be stated that variety Ramos, which has a possible storage temperature of 6 °C, does not respond better to cold storage compared to Bintje.

When varieties were compared in their ability to suppress sprouting during cold storage, Ramos and Saturna appeared to be least susceptible for sprouting. After 32 weeks of cold storage, a sprout of 1 cm was visible. This is the limit of acceptance for industrial processing. Bintje on the other hand already started sprouting after 18 weeks of cold storage.

Apart from unintended occurring cold storage of potatoes due to climatic conditions, cold storage of potatoes can be beneficial due to sprout suppression during long-term storage, minimization of physiological weight loss (i.e., $H_2O$ and DM) due to decreased respiration, and a reduction in losses associated with bacterial and fungal pathogens (*33*). Despite the many advantages of low-temperature storage, the undesirable Maillard browning during potato chip frying operations is a major drawback, as this results in the production of dark-colored chips that are unacceptable to the consumer due to their appearance and bitter taste (*33*, *34*). To circumvent these disadvantages however, reconditioning of the potatoes can be applied.

Therefore cold-stored potatoes were reconditioned at 15 °C for 3 or 5 weeks after 8, 16, and 24 weeks of cold storage. After 3 weeks of reconditioning, sprouts of around 10 cm occurred on the tuber, but the tuber tissue remained firm. After 5 weeks of reconditioning, a small root was formed. The reducing sugar concentrations are presented in **Table 1** before and after reconditioning. For variety Bintje, the reducing sugar content present after 8 weeks of cold storage was 0.75% on DM; after reconditioning it dropped to 0.18% on DM. Thus, despite the fact that during the first 8 weeks of storage the temperature was only gradually decreased and therefore the reducing sugar content was relatively low, the reducing sugar content could significantly be reduced by reconditioning. After 24 weeks of cold storage, the fructose concentration increased up to 0.98%, the glucose concentration up to 1.13% on DM for Bintje, while after reconditioning, the concentrations decreased up to levels comparable to those observed in potatoes stored at 8 °C (**Table 1**). For Bintje, the fructose and glucose concentrations after 3 weeks reconditioning were, respectively, 0.13 and 0.11% on DM, while for the potatoes stored at 8 °C for 24 weeks the concentrations of fructose and glucose were, respectively, 0.11 and 0.13% on DM. These results were similar for variety Saturna. This is in contrast to variety Ramos, where the reducing sugar content after reconditioning appeared to be higher than in potatoes stored at 8 °C. From the results presented in **Table 1**, it could also be concluded that, by extending the reconditioning period up to 5 weeks, a further decrease of the reducing sugar content could not be achieved. This is in accordance with the results of Isherwood (*23*) and Iritani and Weller (*24*). They postulated that only the sugars formed during cold storage could be reduced; thus a minimum amount of sugar remained to be present. Moreover sugars formed during senescent sweetening could neither be reduced. For variety Ramos, storage at 4 °C might lead to a partially irreversible reconversion of reducing sugars to starch such that reconditioning could not reduce the amount of reducing sugars any further (*13*). Hence reconditioning proved to be able to eliminate most of the disadvantages of cold storage. The decrease in reducing sugars during reconditioning is caused by an increase in the respiration rate due to the higher temperatures. Hereby a part of the reducing sugar content is lost in respiration or converted into starch (*25*). This could lead to a considerable improvement of the baking color. Furthermore, it could be stated that other parameters such as pH, dry matter, and total protein, free amino acid, and starch content were not significantly influenced by the reconditioning process (results not shown).

*Sucrose.* The influence of storage temperature and period on the sucrose concentration is presented in **Figure 3** for the three varieties. Tissue concentrations of sucrose were higher and showed greater fluctuations relative to levels of reducing sugars; this was also reported by Blenkinsop et al. (*31*). It can be noted that, at 4 °C, the sucrose concentration stayed fairly constant. Furthermore, the sucrose content was slightly but not significantly higher in potatoes stored at 4 °C than those stored at 8 °C. This is in accordance with several authors (*13*, *35*−*38*). The sucrose concentration in potatoes stored at 8 °C however showed a decrease, although not a significant trend. Nonetheless the decrease in sucrose concentration for variety Bintje amounts up to 50%; the decrease was not significant probably due to

**Table 2.** Difference between Varieties, Storage Temperature, and Time in Contents of DM, pH, Crude Protein, Total Free Amino Acid, and Asparagine of Potatoes Stored for 0 and 24 Weeks

| | Bintje | | | Ramos | | | Saturna | | |
|---|---|---|---|---|---|---|---|---|---|
| storage temperature | | 4 °C | 8 °C | | 4 °C | 8 °C | | 4 °C | 8 °C |
| week in storage | 0 | 24 | 24 | 0 | 24 | 24 | 0 | 24 | 24 |
| DM (%) | 22.14 | 22.20 | 21.63 | 20.64 | 22.12 | 23.01 | 25.84 | 23.66 | 24.21 |
| pH | 6.28 | 6.23 | 6.29 | 6.39 | 6.27 | 6.39 | 6.29 | 6.14 | 6.30 |
| crude protein (% on DM) | 10.74 | 11.66 | 12.25 | 10.96 | 10.24 | 9.40 | 9.39 | 12.06 | 11.44 |
| total free amino acid content (% on DM) | 4.18 | 4.60 | 4.58 | 4.44 | 3.86 | 4.27 | 3.41 | 4.11 | 3.52 |
| free asparagine (% on DM) | 1.67 | 1.63 | 1.73 | 1.89 | 1.54 | 1.84 | 1.67 | 1.93 | 1.60 |

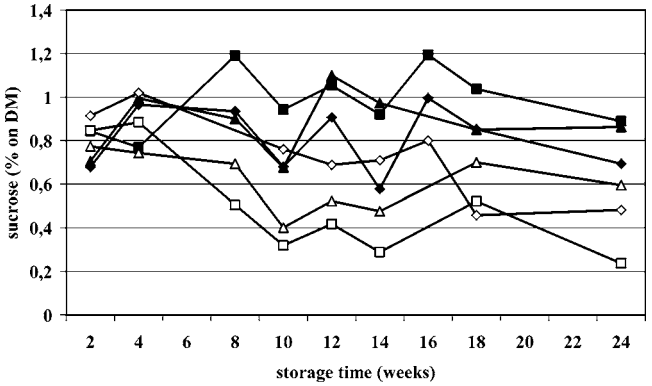


**Figure 3.** Influence of storage time and temperature on the sucrose concentration of Bintje, Ramos and Saturna, expressed in % on DM. (◆ = Bintje, 4 °C; ■ = Ramos, 4 °C; ▲ = Saturna, 4 °C; ◇ = Bintje, 8 °C; □ = Ramos, 8 °C; Δ = Saturna, 8 °C.)

the large variability present in the sucrose concentrations. During the reconditioning process, a significant decrease in the sucrose content was noticeable (**Table 1**). A reconditioning period of 5 weeks did not cause any extra decrease.

*DM Content and pH.* The dry matter content and the pH are presented in **Table 2** for the three varieties, stored at 4 and 8 °C at the beginning and end of storage. It can be observed that storage temperature and time did not noticeably influence the dry matter content and pH. With concern for the variety, it can be noticed that Saturna had a slightly higher amount of dry matter, which is positive for crisp production. Differences in pH of the tuber between the 3 varieties were very small. Statistical analysis confirmed these conclusions. No significant influence of storage temperature and time could be found. These results are in accordance with Blenkinsop et al. (*17*), who found that the DM content remained relatively constant upon storage. This is an indication that the storage conditions were adequate in limiting weight loss due to transpiration.

*Crude Protein and Total Free Amino Acid Content.* The crude protein and the total content of free amino acids are shown in **Table 2**. No significant differences were found between the two storage temperatures, and the crude protein content was fairly constant in time. Blenkinsop et al. (*39*) found similar results. Fitzpatrick and Porter (*40*) found a breakdown of proteins to free amino acids during prolonged storage of potato tubers. This protein degradation has been associated with the end of tuber dormancy and the mobilization of nitrogen reserves for sprout formation (*41*−*43*). Again it may be concluded that the storage facilities were optimal; so sprouting was not initiated yet, and consequently the protein degradation could not be observed.

No differences were found in the total free amino acid content in potatoes stored at 4 or 8 °C. This is in accordance to the results obtained by Brierley et al. (*44*). Furthermore, storage time did not influence the total free amino acid content of the three varieties.

Asparagine is by far the most abundant free amino acid in potato tubers (*44*−*46*). In this study the asparagine content of the potato did not seem susceptible to different storage temperatures and long time storage. No significant differences could be found between storage at 8 °C and storage at 4 °C. Moreover, no alteration could be found in asparagine concentration when potatoes were stored for a longer period. The asparagine content may vary from variety to variety, but from **Table 2** there appeared to be very little difference between the three varieties. In addition however, the asparagine concentration may also be influenced by other factors such as soil type, fertilizer application, and climate (*47*).

*Starch.* The observed results did not show any significant influence of storage time and temperature on the starch content of the potato (results not shown). In literature, the storage temperature has been suggested to influence the starch content. Especially during cold-induced sweetening in stored potatoes, starch degradation occurs (*14*, *25*, *48*). It should be noted however that the loss of starch required to provide the observed increases in the reducing sugar levels is not always measurable, since on average 70% of tuber dry matter is starch but less than 1−2% is soluble sugar (*49*).

**Influence of Variety, Storage Time, and Temperature on Acrylamide Formation.** In the previous section, the influence of three potato varieties, storage time, and temperature on the composition of the tubers was studied in detail. From these tubers, French fries were prepared by par-frying for exactly 3 min at 180 °C (±5 °C) followed by finish frying at 180 °C (±5 °C) for 2 min. After homogenization, acrylamide content was determined and evaluated in order to correlate these data with the previous observations on the tuber composition. The repeatability of this frying process was determined to verify the reliability of the method. The frying procedure described above was repeated 10 times on potatoes from the same variety and storage conditions, subsequently acrylamide concentrations were determined on each batch of finished fries. Average acrylamide concentration amounted 364 ± 99 $\mu$g kg$^{-1}$.

As shown in **Figure 4**, the acrylamide concentration in the French fries from the three potato varieties studied is given as a function of storage time for two storage temperatures studied. Acrylamide formation after frying in potatoes stored at 8 °C was not significantly influenced by prolonged storage, and this for the three varieties studied. This is in accordance to Noti et al. (*17*), where the acrylamide potential remained low. At 4 °C however, an increase in the acrylamide content of the French fries was observed for the three varieties, starting after about 8 weeks of storage of the potatoes. After about 14 weeks, the slope of the curve became less steep (**Figure 4**). This continued at least until week 30 of storage. In consideration of the average acrylamide concentration in the French fries for the last 10 weeks of tuber preservation, it can be observed that the cold stored Saturna variety ($\bar{X}$ = 1731$\mu$g kg$^{-1}$) seemed significantly less prone to acrylamide formation during frying compared to

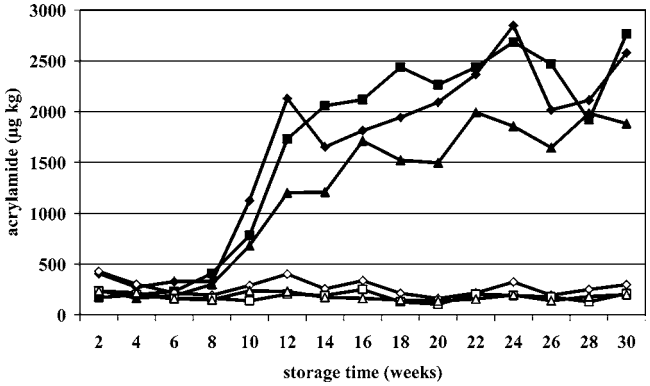


**Figure 4.** Influence of storage time and temperature on acrylamide formation during frying of three varieties (Bintje, Ramos, Saturna) stored at 4 °C and 8 °C over 24 weeks, expressed in $\mu$g kg$^{-1}$. (◆ = Bintje, 4 °C; ■ = Ramos, 4 °C; ▲ = Saturna, 4 °C; ◇ = Bintje, 8 °C; □ = Ramos, 8 °C; Δ = Saturna, 8 °C.)

**Table 3.** Influence of Reconditioning after Initial Storage at 4 °C during 8, 16, and 24 Weeks and the Influence of CIPC Treatment during Reconditioning on the Acrylamide Concentration in French Fries, Expressed in $\mu$g kg$^{-1}$ (R0, 0 Weeks of Reconditioning; R3, 3 Weeks of Reconditioning; R5, 5 Weeks of Reconditioning)[a]

| | | acrylamide ($\mu$g kg$^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | weeks in cold storage | R0 | R3 | | R5 | |
| | | | CIPC | no CIPC | CIPC | no CIPC |
| Bintje | 8 | 329a | 387a | 375a | 226a | 296a |
| | 16 | 1811c | 820b | 760b | 725b | 491b |
| | 24 | 2874d | 714b | 518b | 422b | 515b |
| Ramos | 8 | 408a | 211a | 287a | 279a | 147a |
| | 16 | 2118c | 1169b | 812b | 722b | 432b |
| | 24 | 2686d | 906b | 484b | 1150b | 695b |
| Saturna | 8 | 300a | 170a | 267a | 213a | 118a |
| | 16 | 1712b | 413a | 378a | 316a | 348a |
| | 24 | 1854b | 242a | 415a | 495a | 440a |

[a] Different letters per variety indicate significant differences ($p \leq 0.05$) by Duncan test.

the cold stored Bintje ($\bar{X}$ = 2279$\mu$g kg$^{-1}$) and Ramos ($\bar{X}$ = 2382 $\mu$g kg$^{-1}$) varieties. These observations are in agreement with the trends observed with regard to the reducing sugar content of the tubers upon storage. At 8 °C, no significant changes in the reducing sugar concentration could be observed. At 4 °C however, a strong increase in both the fructose and glucose content was observed upon 8 weeks of storage, corresponding to the time at which the acrylamide formation in potatoes increases as well.

From the above-mentioned results, it is obvious that cold storage strongly enhances acrylamide formation during frying. The increase in acrylamide was clearly associated with the increase in the reducing sugar content. Reconditioning however proved to be very effective in decreasing the reducing sugar content after cold storage (**Table 1**). Therefore the effects of this reconditioning process on the acrylamide formation during frying were studied as well. In **Table 3**, acrylamide levels of the French fries prepared from the respective tubers are summarized.

As can be observed, acrylamide concentrations in fries from tubers stored for 8 weeks at 4 °C did decrease slightly but not significantly after reconditioning of the tuber at 15 °C. Previously however, a further significant decrease in the reducing sugars was observed (**Table 1**). After 16 and 24 weeks of cold storage, however, reconditioning for 3 weeks already caused a

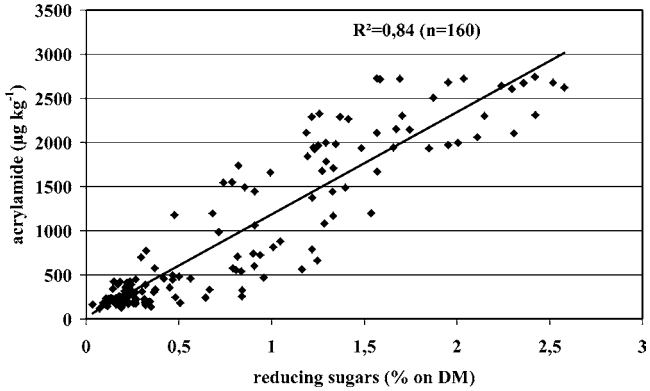


**Figure 5.** Acrylamide as a function of the reducing sugars in potato tubers.

significant and drastic reduction in the acrylamide produced during frying. Reconditioning of cold-stored potatoes for 5 weeks did not cause a significant decrease in the acrylamide formation during frying. This is in agreement with the results of the reducing sugar content of the tubers reported in **Table 1**. It should be stated, however, that the acrylamide formation in reconditioned potatoes does not decrease to the level that is observed in potatoes stored at 8 °C. Thus reconditioning eliminates most but not all of the negative effects of cold storage.

From all the previous observations, it can be confirmed that, upon storage, especially the reducing sugars of the potato enhance the acrylamide formation during frying. To verify this conclusion, a correlation was made, for all the data available, between the observed acrylamide content of the French fries available and the reducing sugar concentration of the corresponding tuber. These results are shown in **Figure 5**. A high correlation ($R^2$ = 0.84, $n$ = 160) was obtained between the reducing sugar concentration of the tuber and the acrylamide content after frying. The correlation between acrylamide formation and the asparagine concentration in the tuber was very low ($R^2$ = 0.02, $n$ = 160). Consequently, by control of the amount of reducing sugars present in the tuber, the formation of acrylamide during frying can be reduced for a big extent. These results are in accordance with Amrein et al. (*50*, *51*), Becalski et al. (*52*), and Williams (*53*). Nonetheless this does not mean that there are no other precursors available acrylamide formation (*6*, *54*, *55*)

**Influence of a Sprout Inhibitor on Acrylamide Formation and on the Composition of the Potato.** Finally, the influence of the use of a sprout inhibitor on acrylamide formation was studied on potatoes that were cold stored and reconditioned. During reconditioning, potatoes start to sprout and thus a chemical sprout suppressor needs to be applied. In this study, a comparison was made between tubers in reconditioning at 15 °C treated with and without CIPC. With application of CIPC during reconditioning, no sprout nor root formation occurred, in contrast to the untreated tuber where sprouts were already forming after 3 weeks of reconditioning. The sprout inhibitor did not have a significant influence on the composition of the potato (Results not shown). These findings are similar to those of Blenkinsop et al. (*56*), who found no effect of CIPC on tuber protein or solids content. In particular, CIPC treatment had no effect on tuber concentrations of glucose, fructose, and sucrose for all investigated varieties. In line of the previous results, acrylamide concentration in the finished fry showed no consistent trend as a function of CIPC treatment during reconditioning (**Table 3**). Therefore it was concluded that acrylamide formation during frying was not significantly influenced by a CIPC treatment during reconditioning.

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