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ARTICLE

Implementation of Acrylamide Mitigation Strategies on Industrial Production of French Fries: Challenges and Pitfalls

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This study evaluated various additives or process aids on the industrial production of French fries, based on their acrylamide mitigation potential and other quality parameters. The application of acetic and citric acid, calcium lactate and asparaginase was investigated on the production of frozen par-fried French fries at the beginning and end of the 2008 and 2009 potato storage season. Despite the fact that some of these treatments significantly reduced acrylamide content of the final product in preliminary laboratory experiments, their application on the industrial production of French fries did not result in additional acrylamide reductions compared to the standard product. Asparaginase was additionally tested in a production line of chilled French fries (not par-fried). Since for this product a longer enzyme−substrate contact time is allowed, a total asparagine depletion was observed for the enzyme treated fries after four days of cold storage. French fries upon final frying presented acrylamide contents below the limit of detection (12.5 μg kg^{-1}) with no effects on the sensorial properties of the final product.

KEYWORDS: Acrylamide; mitigation strategies; pH acidification; asparaginase; industrial trials; par-fried French fries; chilled French fries

INTRODUCTION

Acrylamide is classified by the IARC as "probably carcinogenic to humans" (*1*) and is formed during the Maillard reaction between asparagine and carbonyl compounds, such as reducing sugars (*2*, *3*). Fried potato products are important in the acrylamide issue since they contain the main precursors necessary for its formation and moreover they contribute on average for an important part of the dietary exposure to this process contaminant (*4*, *5*), apart from other food commodities, including cereals products, coffee, chocolate and potato snacks.

Several studies have been reported regarding the impact of additives on the formation of acrylamide. Organic acids are known for their mitigating effect due to the protonation of asparagine amino groups at low pH. This would block the nucleophilic addition of asparagine with a carbonyl compound, preventing the formation of the corresponding Schiff base, a key intermediate in the Maillard reaction and in the formation of acrylamide (*6*−*11*). Mono- and divalent cations (e.g., Na^+ and Ca^{2+}) were indicated to efficiently reduce acrylamide formation. It was postulated that these ions could interact with asparagine so that the Schiff base formation was again prevented (*9*, *11*−*15*). In addition, the effect of calcium ions on the pH decrease of the food matrices associated with acrylamide reductions was also reported (*11*, *16*). The pH decrease may be explained due to the competitive displacement of protons from ionizable functional groups, containing oxygen, nitrogen, or sulfur atoms that share electrons with hydrogen atoms (*17*). Asparaginase, an enzyme that hydrolyzes asparagine to aspartic acid and ammonia, can reduce acrylamide formation in foods by removal of the precursor asparagine (*18*−*21*). Thus, acrylamide formation is successfully reduced upon addition of the enzyme in dough based products, given that asparagine is readily available for the enzyme to be converted to aspartic acid.

Since the discovery of acrylamide in foods, stakeholders together with the scientific community have investigated possible strategies to mitigate acrylamide formation in potato products. The European Food and Drink Federation (CIAA) established a Technical Acrylamide Expert Group in 2003 and created the "Acrylamide Toolbox". The toolbox represents an updated and robust medium for the categorization and summarization of formation and mitigation of acrylamide in various foods (*22*). However, to date, no publicly available data exists on the evaluation of the various promising additives/processing aids on their acrylamide mitigation potential when applied on a real French fry industrial processing line. Therefore, the main purpose of this study was to confirm the acrylamide-lowering impact of several mitigation recipes successfully tested in the laboratory on an industrial level and their effect on the sensorial properties of the final product. Thus, the application of acetic and citric acid, calcium lactate and asparaginase was evaluated on an industrial

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production line throughout the 2008 and beginning of 2009 potato storage seasons. Furthermore, the application of asparaginase in chilled (not par-fried) French fries was also evaluated. These treatments were investigated regarding their potential to reduce acrylamide and their influence on sensorial properties of the final product and on fat uptake during final frying.

MATERIALS AND METHODS

Reagents and Chemicals. Chemicals used for pretreating French fries were of food grade quality. Sodium chloride was obtained in the retail market. Calcium chloride 36% (w/v) was supplied by Brenntag (Belgium) and L-lactic acid (50% w/v) was provided by Purac Biochem (The Netherlands). Glycine (98.5%), succinic acid (>99%), L-ascorbic acid (>99%), erythorbic acid (>99%) and adipic acid (99.6%) were purchased from Sigma Aldrich (Belgium). Magnesium chloride·$6H_2O$ was supplied by Merck (Belgium). Potassium chloride was supplied by Riedel-deHaen (Germany), and magnesium lactate dihydrate was supplied by Jost Chemicals (Belgium). Magnesium acetate anhydrous was supplied by Kemira ChemSolutions (The Netherlands). Acetic acid (19% v/v) was purchased from St. Martinus SA (Roosdal, Belgium). Calcium lactate (Puracal PP FCC) was donated by PURAC (Bingen, Germany), and the enzyme asparaginase (Acrylaway 3500 ASNU g^{-1}) was donated by Novozymes (Denmark). Citric acid monohydrate and sodium acid pyrophosphate (SAPP) were provided by the French fry producer where the industrial trials took place. Tetrasodium pyrophosphate (TSPP M221) was purchased from Caldic (Hemiksem, Belgium). HCl (25% w/w) and petroleum ether (bp 40−60 °C, Chem-Lab, Belgium) were used for the determination of the oil content. Ortho-phthaldialdehyde (OPA) and amino acid standards were purchased from Sigma Aldrich (Bornem, Belgium). HPLC grade acetonitrile and methanol were obtained from VWR (Leuven, Belgium). Trichloroacetic acid (TCA), tris(hydroxymethyl)-aminomethane (TRIS) were obtained from Acros Organics (Geel, Belgium). Ammonium test kit for asparaginase activity assay was purchased from Merck (Darmstadt, Germany). All reagents and chemicals used for the acrylamide and sugar analysis were described in Mestdagh et al. (*23*) and De Wilde et al. (*24*), respectively.

Raw Material. Potato (*Solanum tuberosum* L.), variety Bintje, was used for the study. A batch of potato tubers was supplied by a single producer for each trial. Fully refined palm oil (Cargill Refined Oils-Europe) was used for all the frying experiments.

Preliminary Lab Experiments. Chilled French fries (not par-fried) blanched at 70 °C for 20 min and treated with SAPP were supplied by a local company. Potato strips (200 g) submitted to the different chemical treatments were placed in a dip bath (containing 2 L of dip treatment) at 60 °C for 1 min. After drying on absorbent paper for 15 min, French fries were par-fried at 180 °C for 90 s and frozen at −18 °C after cooling. Final frying was performed at 175 °C for 150 s. French fries treated with asparaginase were dipped in a bath at 60 °C for 5 min. Potato strips were dried in an oven at 70 °C for 15 min (to see whether drying conditions would affect the enzymatic activity) and subsequently par-fried and fried as for the other treatments.

For each dipping treatment a control was done using distilled water.

Industrial Trials. *Frozen Par-Fried French Fry Industrial Trials.* Four industrial trials were performed on the production of frozen French fries, from December 2008 to November 2009. The trials took place in a line with a 5 ton h^{-1} capacity operating at 1 ton h^{-1} for trial purposes. Each concentration per treatment was tested for 45 min. The production process stages are described in **Figure 1A**. Production process steps were performed as in normal industrial practices unless indicated otherwise. Different treatments were added in the dip tank (**Figure 1A7**). Increments of each treatment were performed without empting the dip tank. After each additive the dip tank was emptied and cleaned. SAPP and dextrose were added in the dip tank (as in standard production) with an exception for the calcium lactate treatment or unless indicated otherwise. The pH in the dip tank was approximately 4.7 under standard conditions due to addition of SAPP. Drying conditions were as applied during standard production unless mentioned otherwise. Samples were stored at −18 °C according to packaging instructions until final frying.

Trial 1 (*December 2008*). (i) Control samples (control 1) were produced according to standard conditions at the start of the trial. (ii) Acetic acid (pH 3.6 and 3.3), (iii) citric acid (pH 4.0 and 3.5) and (iv) calcium lactate (0.03 and 0.05 M) were tested. Since the latter additive precipitates in the presence of SAPP, this was added in blancher 2 (**Figure 1A6**) and dextrose was added in the dip tank (**Figure 1A7**) together with the additive. (v) For the asparaginase treatments concentrations of 5000, 7500, and 10000 ASNU L^{-1} were tested; SAPP was added in blancher 1 (**Figure 1A5**). The temperature of blancher 2 was lowered to 58 °C and the temperature in the dip tank controlled in order to ensure not to exceed 55 °C for optimal enzymatic activity.

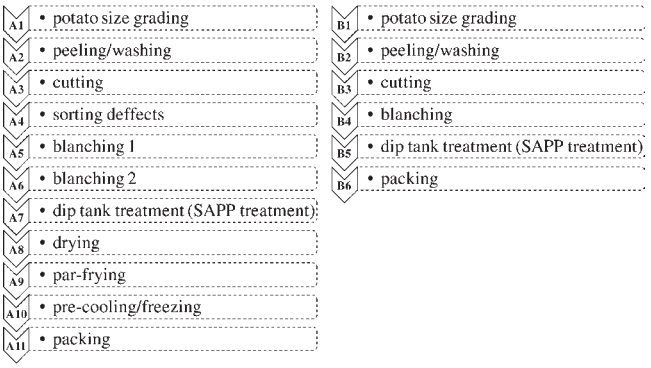

Figure 1. Production stages of frozen par-fried French fries (**A**) and chilled French fries (**B**).

Trial 2 (*May 2009*). (i) Control samples (control 1) were produced according to standard conditions at the start of the trial. (ii) Acetic acid (pH 3.6 and 3.3) and (iii) citric acid (pH 4.0 and 3.5) were tested. (iv) Calcium lactate (0.03, 0.05, and 0.08 M) was added in the dip tank (**Figure 1A7**) together with dextrose; SAPP was added in blancher 2 (**Figure 1A6**). (v) Asparaginase concentrations 5000, 7500, and 10000 ASNU L^{-1} were added in the dip tank (**Figure 1A7**) together with SAPP. The temperature of blancher 2 was lowered to 55 °C and the temperature in the dip tank controlled not to exceed 55 °C. Drying time was increased to 12 min for the enzyme treatment. (vi) The control sample (control 2) was produced under the same blanching and drying conditions as enzyme treated sample.

Trial 3 (*June 2009*). (i) Control samples (control 1) were produced according to standard conditions at the start of the trial and between each new treatment. (ii) Acetic acid (pH 3.6 and 3.3) and (iii) citric acid (pH 3.5, 3.0 and 2.0) were tested. (iv) Calcium lactate (0.05, 0.08, and 0.16 M) was added in the dip tank (**Figure 1A7**) together with dextrose, and SAPP was added in blancher 2 (**Figure 1A6**). (v) Asparaginase concentrations 7500, 10000, and 20000 ASNU L^{-1} were added in the dip tank (**Figure 1A7**) together with SAPP. The temperature of blancher 2 was lowered to 55 °C and the temperature in the dip tank controlled not to exceed 55 °C. The drying time was extended to 12 min. (vi) Control sample (control 2) was produced under the same blanching and drying conditions as enzyme treated sample.

Trial 4 (*November 2009*). Asparaginase (20000 ASNU L^{-1}) was added at different pH levels (6.0, 5.6, 5.2 and 4.7). In order to allow an appropriate blanching of the potatoes and to control the temperature of the dip for optimal enzyme activity, blanching conditions were modified from previous trials, blancher 1 (10 min, 84 °C) and blancher 2 (5 min, 60 °C) (**Figure 1A5** and **A6**, respectively. For pH levels of 6.0, 5.6, and 5.2 TSPP was used instead of SAPP. A typical pH of TSPP (0.5% w/w) in solution is approximately 9.0; therefore the pH was adjusted with addition of citric acid. A control sample without enzyme was produced for each pH level. Drying was performed according to standard conditions.

Chilled French Fry (*Not Par-Fried*) *Industrial Trial.* An industrial trial was performed in November 2009 with asparaginase on chilled French fries (not par-fried) in a line with 4 ton h^{-1} capacity. The production process stages are described in **Figure 1B**. Three concentrations of enzyme were tested, 625, 1250, and 2500 ASNU L^{-1}. Line operating conditions were standard. Due to temperature control requirements in the dip tank, French fries were produced in 100 kg batches. Samples were packed in 2.5 kg bags under modified atmosphere (with increased concentrations of CO_2) and stored at 4 °C according to packaging instructions. Standard product has a shelf life of 15 days (determined by the producer).

Final Frying of French Fries. *Frozen Par-Fried French Fries*. Three different bags were sampled per treatment. Each sample for chemical and color analysis consisted of 35 potato strips from each bag. Fryings were performed at 175 °C for 3 min in a semiprofessional thermostated deep-fryer (Fritel 2505, Belgium) containing 5 L of oil as previously described (*23*). The repeatability of acrylamide formation in French fries prepared according to this method presents a RSD of 15% (*23*). After color measurement, samples were homogenized and frozen for subsequent determination of dry matter, acrylamide and fat content.

Chilled (*Not Par-Fried*) *French Fries*. Samples were taken on days 5, 9, and 15 of product shelf life from three different bags and fried according to packaging instructions. Thirty-five potato strips from each bag were par-fried at 140 °C for 3.5 min and placed on a paper towel until reaching room temperature. The potato strips were then fried for 3 min at 160 °C. Samples were homogenized and frozen for subsequent acrylamide analysis. On a daily basis a sample was taken from 1 bag of each treatment for asparagine determination. Upon opening of the bag, the samples were immediately submitted to heated oil at 180 °C for 15 s for enzyme deactivation. Samples were homogenized and frozen for subsequent aspartate and asparagine analysis.

Sensorial Evaluation of French Fries. Sensorial evaluation of samples from the trials was performed under blind tests by four different groups of professional panelists (up to 24 panelists) from the French fry industry from Western Europe. The attributes considered for the sensorial tests were taste, odor and texture. Results report samples classified as in specification or not in specification.

Acrylamide Analysis. Acrylamide was determined in homogenized potato sample as described previously (*23*). After aqueous extraction, using [2,3,3-D_3]acrylamide as internal standard, the acrylamide extract was further cleaned up by solid-phase extraction. The extract was analyzed using LC−MS/MS with positive electrospray ionization.

Reducing Sugar Analysis. Mono- and disaccharides in homogenized potato sample were assessed as described earlier (*24*). Briefly, after aqueous extraction, addition of an internal standard (phenyl-β-D-glucopyranoside) and drying under nitrogen, the filtrate was derivatized and injected in a GC equipped with a flame-ionization detector.

Dry Matter Content Analysis. The dry matter content was determined, based on an official AOAC method (*25*). Briefly, 5 g of homogenized potatoes was mixed with calcinated sea sand and placed in the oven at 105 °C until a constant weight was obtained.

Color Evaluation of French Fries. The color of the French fries was measured using an Agtron process analyzer (model E15-FP, Nevada, USA), as described previously (*26*). Agtron values range from 0 (black) to 100 (white). The measurement was repeated three times after mixing the fries in between each measurement. The apparatus was calibrated using a white tile (Agtron value of 100), according to manufacturer's instructions.

Oil Content Analysis. Approximately 10 g of homogenized French fries was boiled for 15 min in a beaker containing 50 mL of HCl (25%) and covered with a watch glass. The solution was filtered over a wet filter paper. The filter was rinsed with hot water until the filtrate reached a neutral pH and dried. The oil was Soxhlet extracted with 150 to 200 mL of petroleum ether during 4 h. After solvent evaporation, the oil residue was dried until constant weight at 105 °C (*27*).

Asparagine Determination. Mixed potatoes (15 g) were transferred into a 100 mL quantitative flask and diluted to the mark with 15% trichloroacetic acid (TCA, v/v). Filtered samples were analyzed on a reversed-phase HPLC with an Agilent 1100 system equipped with an automated sample injector and a fluorescence detector (Agilent Technologies, Switzerland) (*28*). A precolumn derivatization of the samples was performed using OPA. The derivatized amino acids were separated on a Zorbax Eclipse AAA Rapid Resolution column (4.6 × 150 mm, Agilent Technologies) at a flow rate of 2 mL min^{-1}, using a gradient of solvent A (45% methanol, 45% acetonitrile and 10% water) and solvent B (45 mM $NaH_2PO_4 \cdot H_2O$, 0,02% NaN_3, pH 7.8). Quantification was done based on a four point calibration curve between 80 and 400 pmol μL^{-1}. By addition of the internal standard sarcosine, relative areas and relative retention times could be used for the identification and quantification.

Microbial Stability of Chilled French Fries (Not Par-Fried). Samples were analyzed for total aerobic count, yeast and mold, and lactic acid bacteria after 5, 9, and 15 days of storage for microbial stability. Chilled French fries have a shelf life of 15 days (determined by the producer).

Effect of pH on Asparaginase Activity in Solution (Lab Experiment). The effect of pH on enzyme activity was tested on phosphate buffered solutions (0.2 M) at pH 4.8, 5.2, 5.6, 6.0 and containing asparagine (30 μmol mL^{-1}), aspartate (47 μmol mL^{-1}), glutamine (57 μmol mL^{-1}), glutamate (57 μmol mL^{-1}) and SAPP (0.5%). Asparaginase (50 ASNU L^{-1}) was added to the solutions in a water bath at 55 °C. Samples were collected every 5 min (up to 15 min), and TCA 15% (v/v) was added in order to deactivate the enzyme prior to ammonia content determination by means of an ammonia test kit.

Statistical Analysis. Statistical analysis of the data was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL). Post hoc comparison of means (Duncan test) was carried out to determine significant differences ($p < 0.05$) between the different levels within each treatment.

RESULTS AND DISCUSSION

Preliminary experiments were performed in the lab with fifteen additives including acids, salts, amino acids and asparaginase, regarding their effect on sensorial attributes and their acrylamide reducing capabilities in French fries. Initially, screening experiments evaluated the highest concentration admissible for each additive without compromising the sensorial properties of the final product. In addition, the acrylamide lowering potential of these treatments was evaluated. The most successful treatments regarding acrylamide reduction in these lab experiments were acetic and citric acid, calcium chloride, and asparaginase (Supporting Information 1). These additives were considered for industrial trials, with an exception for calcium chloride, due to limitations related to the chloride content of the effluent. The latest was replaced by another calcium containing additive, calcium lactate. The selection of these treatments represents two possible mitigation recipes, pH decrease and asparagine elimination. Industrial trials were performed from December 2008 to November 2009 in order to evaluate the effect of these mitigation recipes on prefrozen French fries as a function of the potato storage season.

Industrial Trials on Frozen Par-Fried French Fries. The industrial production process of frozen French fries generally comprises the following stages. The potatoes entering the factory are first graded according to size (**Figure 1A1**). After peeling, washing and cutting (**Figure 1A2**,**A3**), the potato strips undergo a blanching treatment (**Figure 1A5**,**A6**). This is an important stage of the process since it influences final product specifications, such as color, fat content and texture (*29*). In addition, during blanching, acrylamide precursors are leached out leading to the reduction of acrylamide content in the final product (*30*). The potato strips are then immersed in a dip tank (**Figure 1A7**) containing sodium acid pyrophosphate (SAPP) and dextrose. SAPP reduces the darkening of the blanched potato cuts while dextrose contributes to a uniform and standardized color of the final product. The potato strips then proceed to a drying step (**Figure 1A8**), followed by par-frying (**Figure 1A9**). After a precooling stage, the product is frozen and packed (**Figure 1A10**,**A11**, respectively).

Table 1 presents the characterization of the raw material used in the trials and the effect of different treatments on the final product. Control 1 samples refer to French fries produced under standard operating conditions. Reducing sugar (RS) contents of the raw material used during the three trials increased throughout the potato storage season varying from 0.73 to 0.99 g (100 g DM)$^{-1}$. Acrylamide levels of control samples were similar (286 and 289 μg kg^{-1}) for the first and third trials while significantly lower ($p < 0.05$) for the second trial (111 μg kg^{-1}). Agtron color results equally demonstrated lighter colored French fries in the second trial compared to the first and third trials, despite that RS contents were higher in the second trial compared to the first. Control samples after final frying presented 9−10% of fat for the

Table 1. Characterization of the Raw Material Used in Industrial Trials of Frozen French Fries and Effect of Different Treatments on the Fat Content, Formation and Acrylamide Concentrations of the Final Product[a]

	Dec 2008	May 2009	Jun 2009
Raw Material			
RS (g (100 g DM)$^{-1}$)	0.73 ± 0.11	0.91 ± 0.00	0.99 ± 0.28
DM (%)	22 ± 0.0	20 ± 0.4	22 ± 0.9
Standard Process (Control 1)			
DM French fries (%)	43 ± 0.2	43 ± 0.4	45 ± 1.3
fat content (%)	10 ± 0.1	14 ± 0.4	9 ± 0.6
color Agtron	67 ± 1.2	73 ± 3.2	64 ± 2.0
acrylamide (μg kg^{-1})	286 ± 45	111 ± 33	289 ± 36
Acetic Acid			
DM French fries (%)[b]	43 ± 0.3	43 ± 1.0	46 ± 0.5
fat content (%)[b]	11 ± 0.5	14 ± 1.7	9 ± 0.1
color Agtron			
pH 3.6	72 ± 0.5 c_1	71 ± 1.8	63 ± 0.3
pH 3.3	69 ± 1.0 c_1	70 ± 0.7	64 ± 0.7
acrylamide (μg kg^{-1})			
pH 3.6	182 ± 41 c_1	109 ± 13	357 ± 37 c_1
pH 3.3	213 ± 14 c_1	149 ± 38	315 ± 13
Citric Acid			
DM French fries (%)[b]	43 ± 0.7	43 ± 0.1	45 ± 0.7
fat content (%)[b]	10 ± 0.3	15 ± 1.7	9 ± 0.1
color Agtron			
pH 4.0	70 ± 0.5 c_1	74 ± 0.7	N/A
pH 3.5	70 ± 1.8 c_1	66 ± 1.8 c_1	60 ± 0.5 c_1
pH 3.0	N/A	N/A	66 ± 0.8
pH 2.0	N/A	N/A	63 ± 1.4
acrylamide (μg kg^{-1})			
pH 4.0	216 ± 21	109 ± 13	N/A
pH 3.5	205 ± 30 c_1	165 ± 23	329 ± 25
pH 3.0	N/A	N/A	165 ± 24 c_1
pH 2.0	N/A	N/A	177 ± 30 c_1
Calcium Lactate			
DM French fries (%)[b]	43 ± 0.1	44 ± 1.0	45 ± 0.1
fat content (%)[b]	10 ± 0.8	14 ± 0.7	10 ± 0.3
color Agtron			
0.03 M	70 ± 0.4 c_1	61 ± 3.2 c_1	N/A
0.05 M	64 ± 1.0 c_1	66 ± 2.2 c_1	64 ± 0.4
0.08 M	N/A	65 ± 3.0 c_1	66 ± 0.5
0.16 M	N/A	N/A	63 ± 3.0
acrylamide (μg kg^{-1})			
0.03 M	230 ± 51	332 ± 54 c_1	N/A
0.05 M	302 ± 38	272 ± 35 c_1	315 ± 11
0.08 M	N/A	328 ± 44 c_1	152 ± 18 c_1
0.16 M	N/A	N/A	185 ± 47 c_1
Control 2			
DM French fries (%)	N/A	41 ± 0.3	44 ± 0.1
fat content (%)	N/A	12 ± 0.1	9 ± 1.6
color Agtron	N/A	64 ± 0.3 c_1	63 ± 2.2
acrylamide (μg kg^{-1})	N/A	298 ± 11 c_1	247 ± 40
Asparaginase			
DM French fries (%)[b]	43 ± 0.2	41 ± 0.8	45 ± 0.7
fat content (%)[b]	11 ± 0.3	12 ± 0.6	9 ± 0.2
color Agtron			
5000 ASNU L^{-1}	72 ± 1.4 c_1	63 ± 2.2 c_1	N/A
7500 ASNU L^{-1}	70 ± 2.3 c_1	62 ± 0.9 c_1	59 ± 0.5 c_1, c_2
10000 ASNU L^{-1}	71 ± 0.8 c_1	62 ± 1.0 c_1	59 ± 1.9 c_1, c_2
20000 ASNU L^{-1}	N/A	N/A	60 ± 0.5 c_1, c_2

Table 1. Continued

	Dec 2008	May 2009	Jun 2009
acrylamide (μg kg^{-1})			
5000 ASNU L^{-1}	309 ± 12	313 ± 23 c_1	N/A
7500 ASNU L^{-1}	282 ± 25	351 ± 20 c_1	411 ± 36 c_1, c_2
10000 ASNU L^{-1}	281 ± 48	289 ± 31 c_1	405 ± 20 c_1, c_2
20000 ASNU L^{-1}	N/A	N/A	297 ± 2

[a] N/A: not applicable. [b] Average of all concentrations tested (n = 3 per concentration). c_1: Significantly different from control 1. c_2: Significantly different from control 2, $p < 0.05$ by the Duncan test.

first and third trials and slightly higher for the second trial (14%). This is in agreement with lower dry matter contents of raw material used in the second trial (20%) compared to the first and third trials (22%). Lower dry matters may be linked to higher fat uptakes due to higher levels of water evaporation. A fair comparison of the raw material used in the three trials is hindered by several variables influencing raw material properties such as different growing and harvesting conditions, difference in concentrations of acrylamide precursors, texture, tissue aging, etc. This, however, represents the daily reality faced by the potato processing industry.

Effect of Acetic and Citric Acid and Calcium Lactate on Industrial Production of Frozen Par-Fried French Fries. The effect of two organic acids (acetic and citric acid) and calcium lactate on acrylamide formation, fat content and sensorial attributes of frozen French fries was investigated during three industrial trials (December 2008, May and June 2009). In order to ensure that the line was operating in a standard manner throughout the trials, the pH of the dip tank was monitored, and adjustments were made accordingly with addition of more acid to keep a stable pH.

Acetic acid (19% v/v) was added to the dip tank (**Figure 1A7**) at two pH levels, 3.6 and 3.3, during the three trials. Treated samples presented significantly lower ($p < 0.05$) acrylamide levels compared to the control sample in December 2008 (**Table 1**). This resulted in 36% and 26% of acrylamide reduction for pH 3.6 and 3.3 respectively. On the other hand, these fries were significantly lighter in color compared to the control. Consumers often fry French fries until desired color reached, irrespective of the frying instructions given on the packaging. Assuming this color is similar to the color of the control sample, a pretreatment which results in lighter colored French fries may be misleading regarding realistic acrylamide contents. Therefore, additional fryings were performed on these lighter in color samples until an Agtron color value of approximately 67 was reached (color of control sample). The acrylamide content for these French fries was 387 ± 31 μg kg^{-1}, being significantly higher ($p < 0.05$) compared to the control samples (286 ± 45 μg kg^{-1}). Thus, if consumers would fry these acetic acid pretreated French fries until the desired color is reached, the acrylamide contents would be significantly higher compared to nontreated French fries.

In the trial of May 2009, no significant differences were observed regarding acrylamide contents and Agtron color for the acetic acid treated French fries and control sample (**Table 1**). For this trial, the acrylamide content of the control sample was also lower compared to the other trials, as mentioned above. On the other hand, the trial of June 2009 resulted in acrylamide contents significantly higher ($p < 0.05$) for French fries pretreated at pH 3.6 compared to the control, while for the fries treated at pH 3.3 no significant difference was observed (**Table 1**).

Citric acid monohydrate was added in the dip tank (**Figure 1A7**) in a pH range between 4.0 and 3.5 in December 2008 and May 2009 trials, and pH levels ranging from 3.5 to 2.0 in June 2009. In the first trial acrylamide contents were significantly lower ($p < 0.05$)

Table 2. Reducing Sugar Contents throughout the Trials on Par-Fried Samples (December 2008 through June 2009)[a]

RS (g (100 g DM)$^{-1}$)	Dec 2008	May 2009	Jun 2009
control 1	0.33 ± 0.01	0.34 ± 0.01	0.47 ± 0.06
acetic acid pH 3.3	0.33 ± 0.04	0.36 ± 0.03	0.61 ± 0.01
citric acid pH 3.5	0.27 ± 0.00	0.18 ± 0.02	N/A
citric acid pH 2.0	ND	ND	0.64 ± 0.03
calcium lactate 0.05 M	0.39 ± 0.03	0.07 ± 0.01	N/A
calcium lactate 0.16 M	N/A	N/A	0.31 ± 0.00

[a] N/A: not applicable. ND: not determined.

and Agtron color significantly lighter ($p < 0.05$) for the pH 3.5 pretreated samples compared to the control (**Table 1**). These samples were fried as well until an Agtron value of approximately 67 was obtained, resulting in an acrylamide content of 400 μg kg^{-1}, significantly higher ($p < 0.05$) compared to the control. No significant differences were observed for acrylamide contents between citric acid treated samples and control samples in May 2009. Therefore in June 2009 further exaggerated lower pH levels (3.0 and 2.0) were tested. Samples dipped at pH 3.0 and 2.0 presented acrylamide levels significantly lower ($p < 0.05$) when compared to control, with acrylamide reductions of 43 and 39%, respectively. In this last trial, acrylamide reductions were not linked to color changes of the final product as observed in the first trial.

Calcium lactate was added in the dip tank (**Figure 1A7**) at concentrations ranging between 0.03 and 0.08 M in the December 2008 and May 2009 trials, and a higher concentration of 0.16 M was additionally tested in June 2009. Ca^{2+} has been described to have an acidifying effect on the potato surface, influencing therefore the acrylamide formation (*11*), although this additive would not influence the pH in the dip tank itself. Due to the incompatibility of this additive with SAPP, the latest was added in blancher 2 instead of the dip tank (standard industrial practices). Even with this extra precaution, precipitation occurred in the dip tank and in addition a white foam was observed on the surface of the potato strips. In December 2008 no significant differences ($p < 0.05$) were observed regarding acrylamide contents of the final product between calcium lactate treated fries and the control (**Table 1**). On the other hand, the color of the treated samples differed significantly from the color of the control. Samples treated with calcium lactate 0.03 M were significantly lighter in color, while samples treated with 0.05 M were significantly darker compared to the control. Such variability is presumed to be due to variations in the raw material. During the trial of May 2009 all calcium lactate treated samples contained significantly higher acrylamide contents and were significantly darker ($p < 0.05$) when compared with the control. When additional fryings were performed with these samples targeting the color of the control product, the French fry core was not properly cooked, thus acrylamide contents were not determined. The two highest concentrations (0.08 and 0.16 M) tested in June 2009 presented significantly lower ($p < 0.05$) acrylamide contents when compared to the control resulting in reductions of 47 and 36%, respectively. For these samples the color of the final product was not affected.

None of the pH modifying agents applied during the trials presented an effect on the fat content of the final product. Although the application of the above-mentioned treatments allowed acrylamide reductions in studies referred in the literature and previous preliminary lab experiments (Supporting Information 1), no consistent results of acrylamide reduction were obtained with the application of these treatments throughout the three trials. One possible explanation could be the variability of the raw material confirmed by the RS contents of par-fried samples determined throughout the trials (**Table 2**). In December 2008 the RS contents of the par-fried samples were fairly constant throughout the production day (0.27−0.39 g (100 g DM)$^{-1}$), while in May 2009 the RS levels varied from 0.07 to 0.36 g (100 g DM)$^{-1}$, although care was taken to use a single batch of potatoes per production day. The highest RS contents were observed in June 2009 (0.31−0.64 g (100 g DM)$^{-1}$). These results are an indication of raw material variability and could explain some inconsistent results obtained in the trials. A more plausible explanation however could be the low pH of the dip tank (4.7) used in industrial practices due to the addition of SAPP. Thus, control samples from the industrial trials would contain lower acrylamide contents when compared to control samples of the lab experiments (performed by dipping the potato strips in distilled water with a pH generally above 6.0). Therefore, higher acrylamide reduction yields were obtained in the lab experiments compared to the industrial trials. This hypothesis was confirmed in an additional experiment discussed further in this paper (**Table 3**).

Table 3. Effect of pH and Asparaginase on Acrylamide and Color of Frozen French Fries (November 2009)[a]

	pH values			
	4.7	5.2	5.6	6.0
	Acrylamide (μg kg^{-1})			
control	374 ± 15 c,d	363 ± 13 c	448 ± 18 e	566 ± 26 f
asparaginase	323 ± 19 b	291 ± 6 a	397 ± 19 d	346 ± 8 b,c
	Color (Agtron)			
control	61 ± 0.4 c,d	62 ± 1.1 c,d	56 ± 0.6 a,b	55 ± 0.9 a
asparaginase	60 ± 1.8 c	63 ± 0.4 e	57 ± 1.0 b	57 ± 1.1 b

[a] Different letters presented per row indicate significant differences ($p < 0.05$) by the Duncan test.

Effect of Asparaginase on Industrial Production of Frozen Par-Fried French Fries. Asparaginase, an enzyme which converts asparagine into aspartic acid and ammonia, has been successfully demonstrated to lower acrylamide formation in dough based products (*19*, *21*). French fries, on the other hand, consist of solid cut pieces. Therefore the contact between enzyme and substrate is less optimal, making the implementation of the enzyme treatment potentially more complex (*21*). Four industrial trials were performed regarding the application of asparaginase on frozen French fries. The first three trials investigated the effect of enzyme concentration on acrylamide reduction levels, fat content and sensorial attributes of frozen French fries. The fourth trial investigated the pH effect in the dip tank on the enzyme activity. General requirements for the enzyme application are the necessity of a blanching step prior to the treatment and temperature control of the dip. A blanching step is required for best enzyme performance since it produces micro structural changes in the potato tissue (e.g., starch gelatinization) facilitating the contact of asparaginase with asparagine (*20*). The optimum temperature of the asparaginase activity at pH 7 is 60 °C. Above this temperature the enzyme activity decreases rapidly (*21*). In industrial practices, the blanching process may include the use of up to three blanchers and in general blanching temperatures above 60 °C are applied. This may constitute a problem for controlling the temperature in the subsequent dip tank below 60 °C since potato strips entering the dip tank are at temperatures above optimal values for enzyme activity. Therefore, the application of the enzymatic treatment on the French fry process should consider the introduction of an extra cooling process step immediately after the blanching treatment. Since this was not feasible for the trials, the temperature of blancher 2 (**Figure 1A6**)

was lowered in order to obtain a temperature in the dip tank (**Figure 1A7**) of 50−60 °C for optimal enzymatic activity.

Van Hendriksen et al. (*21*) reported an acrylamide reduction of 59% in French fries when submitting the potato strips for 1 min in a dipping solution containing asparaginase 10500 ASNU L^{-1}. These results were attributed to the enzyme remaining active during drying rather than the duration of the dip treatment. Even though drying air temperature is higher than what can be tolerated by the enzyme, due to water evaporation from the surface of the potato strips, the product surface temperature will be lower than the applied drying temperature (*21*). For trial proposes, French fries were dipped for 1 min in the dip tank and drying time was extended compared to the standard process in order to allow enough time for enzyme activity. In **Table 1**, control 1 samples refer to French fries produced under normal operating conditions, while control 2 samples refer to French fries produced with adjusted blanching conditions (blancher 2 < 60 °C) without enzyme treatment. Asparaginase concentrations ranging from 5000 to 10000 ASNU L^{-1} were tested in December 2008. For this particular trial, SAPP was added in blancher 1 (**Figure 1A5**) since the adjusted temperature in the dip tank would no longer be optimal for SAPP action on (gray/blue) discoloration of French fries. Control 2 was not performed for this trial, therefore enzyme treated samples could only be compared to the control of the standard process (control 1). The application of the enzyme resulted in French fries significantly lighter ($p < 0.05$) in color compared to control 1, but no significant difference was observed for acrylamide content in the final product (**Table 1**). Enzyme treated samples presented blue spots after final frying. This may be explained due to the addition of SAPP in blancher 1 and being washed out in the second blancher and therefore not having an impact on gray/blue discoloration. In May 2009 the same enzyme concentrations were repeated and SAPP was added in the dip tank. An additional control was performed for the adjusted blanching conditions (control 2). Asparaginase treated fries and control 2 samples had significantly higher acrylamide contents and were significantly darker ($p < 0.05$) in color compared to control 1. This can possibly be explained by the lower temperatures applied in the second blanching step, leading to less removal of acrylamide precursors. No significant differences were observed between acrylamide contents of enzyme treated samples and control 2. Asparagine contents were determined on samples taken in different process stages including blanchers 1 and 2, dip tank, dryer and par-fryer. Samples were submitted to 180 °C for 15 s (oil immersion) for enzyme inactivation immediately after taken from the line. Asparagine concentrations decreased approximately 60% after the blanching step (from 0.66 ± 0.10 to 0.25 ± 0.04 g (100 g FW)$^{-1}$) and no further reductions were observed throughout the remaining process even upon application of increasing concentrations of asparaginase.

In June 2009, an enzyme concentration of 20000 ASNU L^{-1} was evaluated. No significant differences were observed regarding color and acrylamide contents between controls 1 and 2, even though blanching conditions were milder for control 2. All enzyme treated fries were significantly darker ($p < 0.05$) in color when compared to both controls. In addition, samples dipped in asparaginase 7500 and 10000 ASNU L^{-1} presented acrylamide contents significantly higher compared to controls 1 and 2 (**Table 1**). Asparagine contents were determined, and as for the previous trial no differences were observed between enzyme treated and control samples, demonstrating that the enzyme treatment had little or no effect on asparagine content of the potato strip.

Although preliminary lab tests (Supporting Information 1) and previous studies demonstrated the reducing impact of asparaginase on acrylamide formation in French fries, often these were carried out at pH levels above 4.7 (*21*) or the pH was not mentioned (*20*). This would result in control samples with higher levels of acrylamide and therefore could yield high acrylamide reductions. Moreover, pH values above 4.7 could have an influence on the enzymatic activity (contributing to higher acrylamide reductions). Therefore, the effect of pH on the enzymatic activity was investigated in the lab. Buffered solutions with pH ranging between 4.7 and 6.2 and containing asparagine, aspartate, glutamine, glutamate and SAPP (0.5%) were used for this experiment. Asparaginase (50 ASNU L^{-1}) was added to the solutions at 55 °C. Samples were taken every 5 min up to 15 min for ammonia content determination (with an ammonia test kit, which could potentially be used to control the enzyme activity in a production process). **Figure 2** shows that a limited ammonia concentration was detected at pH 4.7, suggesting that the pH used in industrial practices could be unfavorable for asparaginase application in French fry production. Increasing pH improved enzyme activity, and the highest activity was observed at pH 6.2. However, these tests were performed in solutions, and potatoes are known to have a buffering capacity. Consequently it was not yet clarified to what extent the pH in the dip tank would influence the enzyme activity in the potato strip itself. Therefore, a fourth industrial trial considering asparaginase application (20000 ASNU L^{-1}) at different pH levels (4.7−6.2) in the dip tank was performed. In order to obtain pH 6.0 in the dip tank a different type of pyrophosphate salt needed to be used, being tetrasodium pyrophosphate (TSPP). TSPP is used in the food industry as an emulsifier and a buffering agent. This agent presents a pH of approximately 9.0 at 0.5% (w/w) in solution. pH levels of 6.0, 5.6, and 5.2 in the trials resulted from the addition of citric acid to TSPP (0.5%). The pH of 4.7 was obtained with the use of SAPP without further adjustments (as used in current industrial practices). Acrylamide contents and Agtron color results of the final product are presented in **Table 3**. The raw potatoes used for this trial contained 0.49 ± 0.11 g (100 g DM)$^{-1}$ of RS and 19.2 ± 1.3% of dry matter. Results of control samples (non enzyme treated) indicate a strong pH effect on acrylamide formation. The lowest acrylamide contents in nontreated fries were obtained for pH 4.7 and 5.2. For these samples, acrylamide concentrations of 374 and 363 μg kg^{-1} were not significantly different ($p < 0.05$). Increasing the pH to 5.6 and 6.0 resulted in significantly higher acrylamide contents compared to the standard process fries (pH 4.7) corresponding to an increase of 20 and 51%, respectively (**Table 3**). Similar results have been reported by Jung et al. (*10*), when investigating the effect of pH on French fries. This study

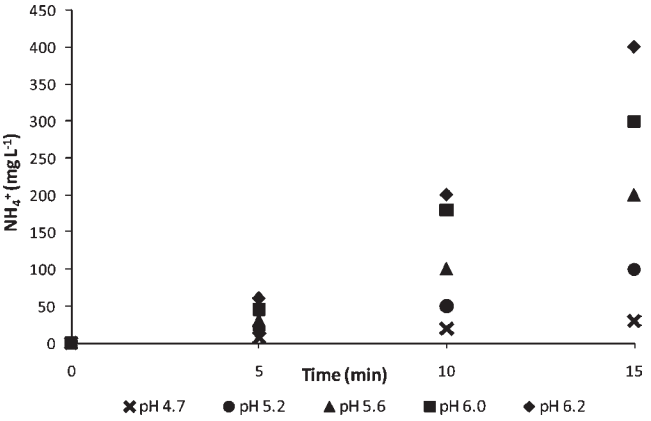

Figure 2. Effect of pH on asparaginase activity, expressed as NH_4^+ mg L^{-1} formation in a solution containing asparagine (30 μmol mL^{-1}), aspartate (47 μmol mL^{-1}), glutamine (57 μmol mL^{-1}) and glutamate (57 μmol mL^{-1}) and SAPP (0.5%) at 55 °C.

reported a control sample performed in distilled water with a pH of 6.2 and pH values of 5.2 and 4.9 for citric acid treated samples. Decreasing the pH from 6.2 to 5.2 resulted in a 67% reduction of acrylamide, however lowering the pH to 4.9 resulted in only 8% additional acrylamide reduction (*10*).

The pH effect on acrylamide formation was less evident upon addition of the enzyme. Moreover, acrylamide concentrations of the asparaginase samples treated at pH 4.7 and 6.0 were not significantly different. Differences between enzyme treated samples at pH 5.2 and 5.6 are probably due to variability of the raw material, e.g. RS contents. Overall, the pH effect on enzyme activity reported in the lab experiments (performed with solutions) was hindered when applying asparaginase to potato strips at pH 4.7 (14% of acrylamide reduction), probably due to the potato buffering capacity. Moreover, results from this trial indicate no advantage in increasing the pH in the dip tank to improve enzyme activity.

French fries treated at pH 6.0 and 5.6 (with and without enzyme) were darker in color compared to fries treated at pH 5.2 and 4.7 (**Table 3**). These differences in color may be explained by the pH effect on the Maillard reaction. Although the use of TSPP raised some concern regarding possible gray discoloration on the final product, such defect was not observed.

Effect of Acetic and Citric Acid, Calcium Lactate and Asparaginase on Sensorial Properties of Frozen Par-Fried French Fries. Samples from the above-described trials were tested for sensory attributes by different groups of professional panelists from the French fry industry throughout Western Europe (Supporting Information 2). Samples not in specification were considered by the professionals of the sector as nonsalable product, and that most probably would give rise to quality complaints. Control samples were evaluated together with the treated samples. Generally control samples were considered as in specification. Acetic acid treated fries were rejected among the panelists due to an acidic taste throughout the three trials. With an exception of the pH 3.6 sample in December 2008, more than 55% and up to 71% of the samples were not in specification according to the panelists. Also important to mention, during the trials, an intense vinegar odor was perceived in the production facility. This could possibly have negative consequences on line operators and other products being produced in the line's vicinity.

The addition of citric acid to the French fry process was also identified by the panelists, although to a lower extent when compared to the acetic acid treated samples. Even at pH levels lower than 3.3, the percentage of samples not in specification did not exceed 45%. In December 2008 and May 2009 a higher percentage of samples not in specification was identified at pH 4.0 compared to pH 3.5. The number of panelists were not the same for testing those two samples, and the reduced number of panelists evaluating the sample treated at pH 4.0 could account for the higher percentage of samples out of specification.

Calcium lactate treated samples were generally rejected in the sensorial tests at a concentration above 0.03 M. Different off tastes, such as, bitter, sour, chemical taste, nutty and soap, were described by the panelists. In addition a crunchier/harder texture was also reported.

The sensorial evaluation of French fries treated with pH lowering agents indicated that acetic acid was the pretreatment which resulted in a higher percentage of samples out of specification followed by calcium lactate and citric acid. Moreover, all acidifying agents had a negative impact on sensorial attributes of the final product.

Asparaginase treated samples of trials mentioned in **Table 1** that were classified as out of specification were justified due to an off taste described as slightly acid/green potatoes/earth taste/carton taste. Throughout these three trials, percentages of enzyme treated fries not in specification varied from 13 to 38%, indicating that asparaginase has an impact on the sensorial properties of French fries, although to a lower extent compared to the pH modifying treatments. French fries treated with asparaginase in November 2009 (**Table 3**) were considered together with samples treated with TSPP to be out of specification (17 to 50%) due to different off tastes.

Industrial Trials Regarding the Application of Asparaginase on Chilled French Fries (Not Par-Fried). Besides optimal temperature and pH, time for enzyme−substrate contact seems a crucial parameter for asparaginase to mitigate acrylamide formation in French fries. Since in the production of chilled French fries (**Figure 1B**) there is no par-frying or any other process step that could deactivate the enzyme, asparaginase could remain active throughout the product shelf life. This could result in total hydrolysis of the asparagine content into aspartic acid and ammonia and French fries free of acrylamide. This was tested on an industrial line in chilled French fries (not par-fried) in November 2009. The product was evaluated for sensorial attributes and microbial stability. Three asparaginase concentrations were added in the dip tank, 625, 1250, and 2500 ASNU L^{-1}. Line operating conditions were standard. For temperature control in the dip tank, French fries were produced in 100 kg batches. Samples were packed in 2.5 kg bags under modified atmosphere and stored at 4 °C according to packaging instructions. On a daily basis throughout product shelf life, a sample of each treatment was taken for asparagine analysis. Asparagine contents varied from 111 to 199 mg (100 g FW)$^{-1}$ in nontreated potato strips during the 15 days of product shelf life (**Figure 3**). Treated samples with an enzyme concentration of 625 ASNU L^{-1} were asparagine depleted after 6 days of chilled storage. Doubling the enzyme concentration allowed asparagine depletion after 5 days. At the highest enzyme concentration tested, asparagine was completely converted after 4 days. French fries were par-fried and fried on days 5, 9, and 15 of product shelf life for acrylamide content analysis and sensorial evaluation. Acrylamide concentrations of control samples varied from 90 ± 9.1 to 124 ± 21.5 μg kg^{-1} between days 5 to 15 and were not significantly different ($p < 0.05$). Enzyme treated samples presented acrylamide contents below limit of detection (LOD 12.5 μg kg^{-1}) after 5 days of storage. The final product was evaluated regarding sensorial aspects by the panelist group of the industrial partner where the trial was performed. No significant difference was detected between enzyme treated samples and control regarding sensorial aspects of the final product. Microbial stability tests performed at days 5, 9, and 15 of product shelf life demonstrated all products in specification, and no difference was observed between enzyme treated and control samples. Thus, product shelf life was not affected by the enzyme treatment.

As a conclusion, the trials performed on frozen French fries clearly demonstrated the important role of pH on acrylamide formation, which was greatly favored for pH levels above 5.2. No consistent results were obtained regarding acrylamide reduction on French fries when acidifying the dip treatment by addition of acetic and citric acid below pH 4.7. Although calcium lactate may have an acidifying impact on the potato tissue, no consistent acrylamide reduction results were obtained either upon its addition in the dip tank. The application of the enzyme in French fries which are par-fried is very limited in the current line set-ups due to the insufficient time allowed for enzyme activity from the dip tank until the par-fryer. In addition, other drawbacks such as temperature control in the dip tank and safety issues should be considered for asparaginase application. The time of enzyme−substrate contact allowed in chilled French fries (not par-fried) resulted in total asparagine depletion and therefore

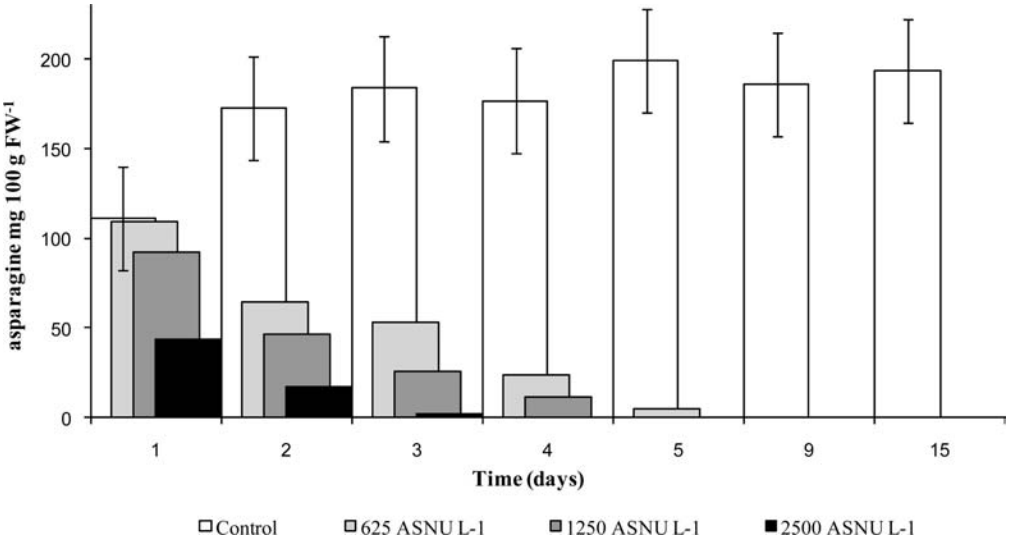

Figure 3. Effect of asparaginase on asparagine contents in chilled French fries (not par-fried) throughout the 15 days of product shelf life. Error bars represent standard deviation of controls.

limited acrylamide concentrations to be formed. Moreover, these results demonstrated that indeed the enzyme eliminates the acrylamide precursor asparagine without altering final product sensorial attributes and affecting the product shelf life, provided that sufficient enzyme−substrate contact time is allowed. The implementation of such treatment requires however modifications on the industrial lines and therefore additional investments besides the cost of the enzyme. Furthermore, chilled (not par-fried) French fries represent a specific segment market which is different and narrower compared to the frozen par-fried French fries. The application of the enzyme on chilled French fries implies the presence of residual enzyme activity on the product before frying. This is in disagreement with the definition of processing aid, and therefore the regulatory status regarding the use of the enzyme under these circumstances still needs to be clarified. Current industrial practices in the production of frozen French fries such as selection of potato varieties with low RS contents, potato storage temperature above 8 °C, selection of tubers larger in size, blanching of the potatoes and addition of SAPP (which acidifies the potato) already contribute for French fries with minimal acrylamide formation. Factors such as seasonal variability of the raw material, raw material characteristics (before and after blanching), the complexity of the blanching step (1, 2, or 3 blanchers), dip tank parameters (temperature, pH and duration) and other variables should be considered when implementing an acrylamide mitigation strategy on an industrial level while maintaining the expected product quality for the consumer. Other considerations such as feasibility, legislation, cost, effluent treatment, safety and comfort of the employees, ability to control dosage, etc. are equally relevant when considering the implementation of any change to an industrial process.

ABBREVIATIONS USED

SAPP, sodium acid pyrophosphate; TSPP, tetra sodium pyrophosphate; DM, dry matter; FW, fresh weight; RS, reducing sugars; OPA, ortho-phthaldialdehyde; TCA, trichloroacetic acid; TRIS, tris(hydroxymethyl)aminomethane.

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Supporting Information Available: (1) Effect of acids, salts, amino acids (dipping treatment 60 °C, 1 min) and asparaginase (dipping treatment 60 °C, 5 min), on acrylamide formation in French fries (lab experiment). (2) Effect of different treatments on sensorial attributes of frozen par-fried French fries produced on an industrial line. This material is available free of charge via the Internet at http://pubs.acs.org.

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