DOI:10.1021/jf102697b

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

The Effect of High Pressure−High Temperature Processing Conditions on Acrylamide Formation and Other Maillard Reaction Compounds

Kristel De Vleeschouwer, Iesel Van der Plancken, Ann Van Loey, and Marc E. Hendrickx*

Laboratory of Food Technology, Leuven Food Science and Nutrition Research Center, Center for Microbial and Food Technology, Faculty of Bioscience Engineering, Katholieke Universiteit Leuven, Kasteelpark Arenberg 22, B-3001 Heverlee, Belgium

The effect of high pressure−high temperature (HPHT) processing on the formation of acrylamide and other Maillard-type reaction compounds was investigated in order to elucidate the impact of HPHT conditions on the different stages of the Maillard reaction. This study was performed in equimolar asparagine−glucose model systems that were treated at various HP/HT conditions (100−115 °C, 400−700 MPa, 0−60 min), and, for comparison, the model system was also heat-treated at ambient pressure. On the treated samples, the concentration of acrylamide, reactants, hydroxymethylfurfural, organic acids, and melanoidins was determined and the pH prior to and after treatment was measured. Based on the measured responses, the retarding effect of high pressure on the overall Maillard reaction was demonstrated; no or little differences were observed between 400 and 700 MPa. The study was conducted in two types of buffer, i.e. phosphate and MES buffer. In case of acrylamide, aspartic acid and browning, a higher concentration was generated in the MES buffer system, but these differences with the phosphate buffer system could be ascribed to pH changes resulting from the application of combined high pressure and high temperature. Based on the results, acrylamide formation is not expected to pose a major hazard to HPHT-treated products.

KEYWORDS: Acrylamide; high pressure−high temperature processing; Maillard reaction

INTRODUCTION

In April 2002 considerable amounts of acrylamide, a potential human carcinogen (*1*), were detected in carbohydrate-rich foods, such as potato and cereal products, that were heated at temperatures above 100 °C (*2*). The Maillard reaction between the amino acid asparagine and a reducing sugar was shown to be the predominant formation pathway for acrylamide (*3*,*4*). Besides toxicological implications (such as acrylamide generation), the Maillard reaction is responsible for the formation of desired as well as undesired color and flavor compounds and is therefore considered to be one of the most important chemical reactions in determining the quality of heated foods. Consequently, control of this reaction is an important issue to food processors.

High pressure−high temperature processing (HPHT) is a novel processing technology in food processing. This technology allows the use of shorter treatment times and reduced maximal processing temperatures as compared to conventional heat sterilization processes, and results in many cases in a better preservation of color, flavor, texture, and nutrients for a preset microbial safety (*5*,*6*). High pressure pasteurization (400−600 MPa, 10−40 °C) has already been implemented industrially for a number of applications, such as fruit juices (*7*,*8*); high pressure sterilization (600−900 MPa, 80−120 °C) has the potential to be implemented industrially on short to medium term. In the context of its industrial implementation it is, however, unclear to what extent and how the HPHT process influences Maillard-related reactions and the associated formation process-induced contaminants and product quality characteristics (flavor, color). HPHT has been shown to affect chemical reactions e.g. pectin demethoxylation and β-elimination, folate destruction, anthocyanin degradation, and so on (*9*−*11*). So far, detailed studies concerning the effect of high pressure sterilization on the Maillard reaction and related potential carcinogens (such as acrylamide) are, to the best of our knowledge, not available. The few studies that are available on the effect of high pressure sterilization conditions on the Maillard reaction are mostly restricted to a single HPHT combination (400 or 650 MPa, 100 °C) and a specific group of Maillard reaction products (such as flavor compounds or browning products) (*12*, *13*). In general, formation of both flavor and browning products is strongly influenced by the application of high pressure; in the case of browning products, the formation was strongly suppressed at 400 MPa compared to atmospheric pressure (*12*), whereas the study on flavor compounds revealed a modified odor profile for glucose−proline systems treated at 650 MPa in comparison to conventionally (0.1 MPa) heated samples (*13*). In addition, increased formation of α-dicarbonyl compounds and arginine modifications was reported in model systems with increasing pressure up to 600 MPa (*14*). Besides, limited information is available on the effect of combined high pressure and

*Corresponding author. Tel: +32-16-32.15.85. Fax: +32-16-32.19.60. E-mail: marc.hendrickx@biw.kuleuven.be.

 Published on Web 10/25/2010

mild temperature (40−70 °C) on the Maillard reaction. Studies focusing on a single group of Maillard reaction products (such as flavor compounds or browning products) reported an overall retarding effect of pressure (400 MPa) (*15*, *16*). Other research groups investigated the effect of pressure on the different stages of the Maillard reaction. Some studies reported the acceleration of the initial stage of the Maillard reaction with pressure (up to 800 MPa) and the retardation of advanced Maillard reaction pathways (*17*), while others observed only the suppression of the advanced Maillard reaction stages with pressure (up to 600 MPa) and no (considerable) effect on the initial stage (*18*, *19*). Similar results were obtained by Moreno et al. (*20*), however, only in unbuffered amino acid−sugar systems at an initial pH ≤ 8.0 at 60 °C and 400 MPa. In buffered systems at an initial pH ≤ 8.0, on the other hand, pressure slowed down the Maillard reaction in the initial stages. These effects were attributed to the pressure-induced pH changes of the buffer system. In unbuffered and buffered systems at an initial pH of 10.2, high pressure accelerated both the initial and advanced stages of the Maillard reaction. Similar results on pH effects have been reported by Hill et al. (*21*) at 40−60 °C and 600 MPa. In spite of the fact that not all results reported are unambiguous, the results point out that the overall Maillard reaction is strongly affected by high pressure and effects might differ between different stages. In addition, the pH of a system and the buffer applied can have a considerable impact on the observed pressure effects; therefore, a well-considered buffer selection is required (*22*).

Hitherto, the effect of combined high pressure and high temperature conditions on the formation of acrylamide has not been investigated. Its possible formation under conditions of HPHT is, however, relevant with respect to shelf-stable low-acid foods that require sterilization conditions in order to ensure their microbial safety. A possible food category to which this relates are the vegetable-based puree baby foods, which contain relevant precursors for acrylamide formation and in which even the lowest concentration of this process-induced contaminant can have toxicological consequences for the target group. Therefore, in this paper the formation of acrylamide was investigated under conditions of combined high pressure and high temperature (HPHT) in asparagine−glucose model systems at an initial pH of 6.0. In addition, the reaction precursors and several products/intermediates of the Maillard reaction were included in the study in order to evaluate the impact of HPHT on the different stages of the Maillard reaction.

MATERIALS AND METHODS

Sample Preparation. The preliminary study was conducted by using two types of equimolar asparagine−glucose model systems, one with high moisture content and one with low moisture content. The high-moisture model systems were prepared by mixing an equimolar concentration (0.1 M) of L-asparagine (≥99.5%, Sigma-Aldrich) and D-glucose (99.5%, Sigma-Aldrich) in buffer at an initial pH of 6.0. For the thermal treatment at atmospheric pressure, citrate buffer (0.05 M) was used, whereas for the HPHT treatment MES buffer (0.05 M) was applied. Low-moisture model systems consisted of an equimolar mixture of L-asparagine and D-glucose. In order to obtain a homogeneous mixture, both reactants were thoroughly mixed in the presence of water, subsequently frozen with liquid nitrogen and finally freeze-dried (0.01 mbar vacuum pressure, Alpha 2-4, Christ, Osterode, Germany) until a powder was obtained. The resulting powder was mixed with water to obtain a final moisture content of 16.7% (w/w).

The kinetic experiments were performed with asparagine−glucose model systems prepared by mixing 20% of an equimolar concentration L-asparagine and D-glucose with 80% of buffer (0.1 M) at an initial pH of 6.0. In order to include temperature and/or pressure effects on the pH of the buffer, the study was performed in two different types of buffer, i.e. the "temperature-stable" phosphate buffer and the "pressure-stable" MES buffer.

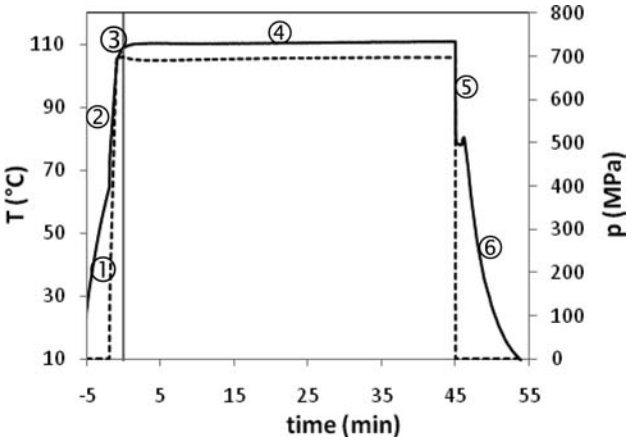

Figure 1. Illustration of the temperature−pressure profile monitored in an equimolar low-moisture asparagine−glucose model system treated in the POM sample holder at 110 °C and 700 MPa: temperature (full line), pressure (dashed line). ① heating to initial temperature at ambient pressure, ② adiabatic heating during pressure buildup, ③ 1 min equilibration period, ④ isothermal−isobaric conditions, ⑤ adiabatic cooling during pressure release, ⑥ cooling in ice bath.

Sample Processing. *Heat Treatment at Ambient Pressure*. Samples were heated in hermetically closed reactor tubes (inox, 8 mm × 100 mm, custom-made) in order to avoid as much as possible side phenomena during heating, such as fluctuations in water activity due to water evaporation and absorption of oil (heating medium), which could affect acrylamide formation. Heat treatment was performed in a thermostated oil bath (UH2D, Grant Instruments Ltd., Cambridge, England) at temperatures between 100 and 120 °C for different heating times. After thermal treatment, samples were immediately cooled in ice water to stop any further reaction. During the reaction and subsequent cooling phase, temperature of the samples was registered within the closed reactor tubes at regular time intervals (4 s) using thermocouples (type T, Thermo Electric Benelux, Balen, Belgium) connected to a data logger (TM 9616, Ellab, Roedovre, Denmark).

High Pressure Thermal Processing. High pressure experiments were performed in a laboratory scale multivessel high-pressure equipment (Resato, Roden, The Netherlands), consisting of six individual vessels surrounded by an isolated heating coil, connected to a cryostat. This equipment allows computer controlled pressure buildup, temperature control (up to 120 °C) and data logging of both pressure and temperature.

A protocol was developed to treat samples in a reproducible way at semi-constant HPHT. By means of illustration, the temperature and pressure history of a low-moisture asparagine−glucose model system is shown in **Figure 1**. Cylindrical, polyoxymethylene acetale (POM) sample holders (85 mm long, 12 mm internal diameter, and 3 mm thickness) were filled with sample and closed with a movable stopper sealed with an O-ring. These sample holders were custom-made to optimally fill the vessels, so the ratio of sample volume to pressure medium volume is as large as possible and constant. In addition, the sample holders were wrapped in plastic bags that were hermetically sealed in order to prevent contamination of the high pressure liquid in case of sample leakage. In case leakage was observed, samples were excluded from further analysis. Prior to the HPHT treatment the sample holders (containing the samples) were cooled to 10 °C in a cryostat. Subsequently, the sample holders were transferred to the pressure vessels already equilibrated at the desired process temperature. The vessels were closed and the temperature in the sample holder was allowed to rise to an initial temperature, which was dependent on the desired process temperature after pressure buildup. This preheating was the result of heat transfer from the pressure medium to the samples. This initial temperature was experimentally determined beforehand for each pressure/temperature combination under study. Sample holders with a 1.6 mm hole were used to allow temperature measurement inside the sample holder using a 36.8 mm type J thermocouple attached to the pressure vessel stopper. Subsequently, pressure was built up very fast, increasing in 5 s from 0.1 to 150 MPa and then from 150 MPa to the set pressure at a rate of 10 MPa/s. This was accompanied by a temperature rise due to compression heating. After

attaining the desired pressure, the individual vessels were isolated. During pressure holding the temperature rise of the sample continued due to the higher temperature of the pressure medium. A constant temperature was reached within 1 min. After preset time intervals the individual vessels were decompressed. Exactly 1 min after pressure release, the samples were removed from the vessel and immediately cooled in ice water to stop any further reaction.

Postprocessing Sample Handling. After the heat and HPHT treatments, the pH and browning of the samples were determined immediately at room temperature, after which the samples were frozen and stored at −40 °C prior to further analysis.

Analytical Techniques. *Acrylamide Analysis*. The analysis of acrylamide was performed by gas chromatography coupled to mass spectrometry with chemical ionization based on the method described by Biedermann et al. (*23*) without prior derivatization of acrylamide. After acrylamide extraction and further cleanup as described previously (*24*), the sample was analyzed using a 5973 inert GC−MS system (Agilent Technologies, Diegem, Belgium). One microliter was injected at low temperature, i.e. 60 °C, on a HP-InnoWax column (30 m × 250 μm i.d., 0.25 μm film thickness, Agilent Technologies) with a 0.5 m × 530 μm i.d. precolumn of deactivated fused silica. The chromatographic separation was carried out with a constant flow rate of 2 mL/min of helium as carrier gas using the following temperature program: the initial oven temperature was set to 60 °C (0.2 min), from which the temperature was increased to 100 °C at a rate of 35 °C/min and further augmented at a rate of 12 °C/min to 230 °C (3 min). The detection was performed with a quadrupole mass spectrometer operating in positive chemical ionization mode with 20% methane as ionization gas in "selected ion monitoring" (SIM) at *m*/*z* 72 (acrylamide), *m*/*z* 86 (methacrylamide) and *m*/*z* 88 (butyramide). Acrylamide concentration was quantified using methacrylamide (≥99%, Merck) as an internalstandard, which was added at the start of the sample preparation step. By comparing this internal standard with a second internal standard, i.e. butyramide (≥98%, Fluka), added to the samples prior to injection, losses during sample preparation and analysis could be accounted for.

Sugar Analysis. Samples were diluted (1:5000 or less, depending on the sugar concentration) and analyzed by high-performance anion-exchange chromatography (HPAEC; Dionex Bio-LC system, Sunnyvale, CA) using a CarboPac PA1 column (4 × 250 mm) with corresponding guard column at 30 °C. An isocratic gradient of 150 mM sodium hydroxide was used as the eluent for the sample analysis with a flow rate of 1 mL/min. After 10 min the eluent concentration was increased to 200 mM NaOH in order to regenerate the column. Sugars, i.e. glucose and fructose, were detected using an ED50 electrochemical detector in the pulsed amperometric detection mode (PAD; Dionex, Sunnyvale, CA) and quantified by use of an internal standard, lactose.

Amino Acid Analysis. The analysis of amino acids was performed using the EZ:faast amino acid analysis kit (Phenomenex, Torrance, CA). Thermally treated and HPHT-treated samples were diluted so that the total amino acid concentration was maximally 0.01 M. The procedure for sample preparation consists of a solid phase extraction step, followed by a derivatization step and liquid/liquid extraction. Derivatized samples were analyzed by gas chromatography coupled to mass spectrometry. Samples were injected (2.0 μL) at 250 °C in split mode (1:15) onto a Zebron ZB-AAA column (10 m × 0.25 mm i.d., Phenomenex). The oven temperature was initially set at 110 °C (1 min) and further increased to 320 °C at a rate of 30 °C/min. Helium was used as carrier gas at a constant flow rate of 1.1 mL/min. The detection was carried out in electron ionization mode in a scan range from *m*/*z* 45 to *m*/*z* 450. Quantification for asparagine was performed based on the ratio of the peak area of the *m*/*z* 155 ion to the peak area of the *m*/*z* 158 ion for the internal standard, norvaline. Aspartic acid was quantified based on the ratio of the peak area of the *m*/*z* 216 ion to the peak area of the internal standard.

Analysis of 5-Hydroxymethylfurfural (*HMF*). HMF was analyzed on the undiluted samples, filtered (0.45 μm) prior to injection, by means of HPLC equipped with a 1200 series UV-DAD detector (Agilent Technologies, Diegem, Belgium), using an analytical Prevail RP C_{18} column (250 × 4.6 mm, 5 μm particle size, Grace, Deerfield, IL) with corresponding guard cartridge (7.5 × 4.6 mm) at 25 °C. The eluent was a 7.5% methanol solution in reagent-grade water, at a flow rate of 0.6 mL/min. Detection was performed at 280 nm; quantification was performed using external calibration curves based on peak area (Chemstation software).

Analysis of Organic Acids. Prior to injection (10 μL), the undiluted samples were filtered (0.45 μm). Subsequently, organic acids were analyzed by means of HPLC equipped with a 1200 series UV-DAD detector (Agilent Technologies, Diegem, Belgium), using an analytical Prevail Organic Acid column (250 × 4.6 mm, 5 μm particle size, Grace, Deerfield, IL) with corresponding guard cartridge (7.5 × 4.6 mm) at 25 °C. An isocratic gradient of 25 mM potassium phosphate buffer (pH 2.5) was used as the eluent for the sample analysis with a flow rate of 1 mL/min. Organic acids were detected by their absorbance at 210 nm; quantification (for formic and acetic acid) was performed using external calibration curves based on peak area (Chemstation software).

Determination of Brown Color Formation. Browning was determined spectrophotometrically by measuring the absorbance at 470 nm. When necessary, samples were diluted with reagent-grade water. The corresponding melanoidin concentration, formed from asparagine and glucose, could be calculated from the absorbance measured by using an extinction coefficient of 282 L/mol·cm, as described by Knol et al. (*25*).

RESULTS

Selection of the Model System. An equimolar asparagine−glucose model system was used to study the kinetics of acrylamide formation and other related Maillard-type reaction compounds under HPHT conditions. This type of model system has frequently been used in previous studies concerning the effect of temperature on acrylamide generation (*25*−*29*). The moisture content to be used, which is known to have a decisive impact on acrylamide generation (*30*, *31*), is still to be determined with relation to the range of HPHT conditions used in this study. Therefore, initially a high-moisture asparagine−glucose (0.1M) model system in MES buffer (pH 6.0) was treated for a limited number of times at 700 MPa combined with temperatures in the range of 100 to 120 °C. For comparison, the same type of model system, however, dissolved in citrate buffer (pH 6.0), was treated in the same temperature range at atmospheric pressure. Buffer selection was performed based on their p*K* dependence on pressure and/or temperature to ensure only minimal pH changes due to processing conditions applied (*22*). On the treated samples, the concentration of acrylamide was determined (data not shown). After an initial lag phase, the concentration generated at atmospheric pressure rose with increasing time and temperature, as was already described before (*24*, *27*, *29*−*31*). In the case of high pressure (i.e., 700 MPa), the effects are less clear, as the acrylamide concentration of most of the HPHT-treated samples is below the limit of detection (i.e., 20 ppb). Based on these results, however, it can be stated that high pressure strongly retards the generation of acrylamide under high-moisture conditions. Because of the limited formation of acrylamide under conditions of high moisture, the occurrence of this reaction was verified under conditions of low moisture, i.e. at an initial moisture content of 16.7% (w/w). Irrespective of the lower amount of water in the system, the temperature rise during pressure buildup (due to adiabatic heating) was comparable to that observed for the corresponding HPHT conditions under high-moisture conditions. The absolute acrylamide concentration generated under low-moisture conditions (data not shown) is higher (factor ∼4) compared to the concentration generated under high-moisture conditions and is no longer below the detection limit. The data for acrylamide concentration reveal an increasing trend as a function of time and temperature, as was also observed in the literature for other low-moisture systems (*30*,*31*) and for the treatments at atmospheric pressure under high-moisture conditions. Also under low-moisture conditions, the concentration of acrylamide generated by heating at atmospheric pressure is much higher compared to the concentrations generated at elevated pressure, again implying the retarding effect of pressure on acrylamide generation. Besides a pressure of 700 MPa, the impact of another pressure level (i.e., 500 MPa) on acrylamide generation was checked

Table 1. Overview of the Selected HT/HP Treatments Performed in Phosphate Buffer (×) and MES Buffer (+)

	100 °C	105 °C	110 °C	115 °C
0.1 MPa	×	×	×	×
400 MPa			×+	
500 MPa			×+	
600 MPa	×+	×+	×+	×+
700 MPa			×+	

at 110 °C. Comparison of the acrylamide concentrations obtained at these two pressure levels confirms this retarding effect with increasing pressure.

Based on these results, it can be concluded that for the kinetic study a low-moisture model system is required in order to make sure that quantifiable acrylamide concentrations are formed and that the Maillard reaction takes place up to a certain degree in the HPHT range (400−700 MPa, 100−115 °C, 0−60 min) under consideration.

Kinetics of Acrylamide and Other Maillard-Type Reaction Related Compounds. For the kinetic study an asparagine−glucose model system containing 20% of an equimolar mixture of the reactants and 80% of buffer (at an initial pH of 6.0) was selected. This corresponds to a higher moisture content than applied for the low-moisture systems used in the preliminary study in order to better control entrapping of air in the sample holders. This model system was treated under different HT/HP-t (high temperature−high pressure−time) combinations using two types of buffer. For comparison, the model system was also treated in the same temperature range, at ambient pressure. In order to minimize possible temperature and/or pressure effects on the pH of the buffer, the study was performed in two different types of buffer, i.e. the "temperature insensitive" phosphate buffer (0.1 M) and the "pressure insensitive" MES buffer (0.1 M) (*22*). An overview of the selected HT/HP treatments used is shown in **Table 1**. In the treated samples, different compounds/characteristics related to the Maillard reaction were quantified in order to elucidate the effect of combined high pressure high temperature on the different pathways of the Maillard reaction and the related formation of acrylamide, as in the literature (cf. Introduction) the effect of HPHT on these different pathways is not unambiguous or not known.

Acrylamide. The concentration of acrylamide generated as a function of time and temperature or combined high pressure−high temperature in the 20% equimolar asparagine−glucose model systems in phosphate buffer at pH 6.0 is shown in **Figure 2A−E**. As discussed in the preliminary study, generally the concentration of acrylamide increases with time and temperature up to a maximum (attained after about 40 min at the highest reaction temperature tested), and decreases again when higher temperatures are applied, such as at 115 °C (**Figure 2A**,**C**,**D**). The observed decrease can be ascribed to reaction of acrylamide with other reactive groups present in the system under consideration (*24*). The absolute acrylamide concentrations generated under HPHT conditions in MES buffer (**Figure 2D**,**E**) are much higher (factor 15 to 20) compared to the concentration formed in the phosphate buffer system (**Figure 2A**,**B**). Furthermore, the acrylamide concentration generated in both buffer systems is significantly lower than the concentration formed under atmospheric pressure (**Figure 2C**), confirming the retarding effect of high pressure on acrylamide generation, as expected based on the preliminary results. This is in line with the observations reported in the literature that high pressure retards the overall Maillard reaction (*12*, *15*, *16*). When comparing the different levels of high pressure applied, limited differences were observed between the various levels of high pressure tested in the MES buffer system as well as in the phosphate buffer system.

The kinetics of acrylamide concentrations can be assessed by means of single-response modeling including a series of two consecutive reactions, with acrylamide as the final product. The corresponding kinetic parameters (i.e., rate constant and activation energy) can, however, only be estimated with a large degree of uncertainty and therefore were not reported in this manuscript. This large uncertainty is presumably related to the uncertainty concerning the mechanism under HPHT and the lack of data on undefined compounds from the initial phase of the Maillard reaction.

pH and Organic Acids. The final pH of the treated samples was measured at room temperature. The pH can be used as an indication for the degree to which the Maillard reaction has occurred, as organic acids are formed along with the reaction and as their concentration increases, the buffer concentration becomes insufficient to buffer the system, resulting in a pH drop. The time course of the net pH change is represented in **Figure 3A−C**, showing the results for only the phosphate buffer system because of very limited differences between both buffer systems. The net pH decreases with time, and this pH drop is clearly dependent on the treatment temperature, as can be seen from **Figure 3A**,**C**. Comparison of the pH change in the HPHT- and HT-treated samples indicates a retarding effect of high pressure on the overall Maillard reaction, as the pH drop at atmospheric pressure (**Figure 3C**) is more pronounced than under conditions of high pressure (600 MPa) (**Figure 3A**). Contrarily to temperature, the effect of high pressure on the net pH change as a function of time is negligible within the range tested (400 to 700 MPa) (**Figure 3B**); this was also observed for the formation of acrylamide. Based on the time course of the pH, the effect of temperature on the overall Maillard reaction is confirmed as well as the retarding effect of high pressure on this reaction. The level of high pressure applied is, however, not determinant in the range of 400 to 700 MPa.

Besides the pH, organic acids were measured. Formic and acetic acid are the predominant organic acids that are expected to be formed in systems containing glucose and/or fructose (*32*−*34*). However, none of the samples contained a detectable concentration of one of these acids. This implies that other unidentified acids, of which the presence is confirmed by the detected profiles (data not shown), are presumably responsible for the observed pH drop.

Reactants: Amino Acids and Sugars. The concentrations of the reactants, i.e. glucose and asparagine, remained mainly unchanged as a function of treatment time under conditions of high pressure contrarily to the treatments at ambient pressure that resulted in a decrease as a function of time and temperature (results not shown). This again confirms the retarding effect of high pressure on the Maillard reaction as already assumed based on the changes in acrylamide concentration and pH (as discussed above).

Besides glucose, no fructose (a possible isomerization product) was detected during sugar analysis. This implies that the isomerization reaction between glucose and fructose is suppressed under conditions of high pressure. A decreased isomerization under high pressure conditions (400 MPa) was also reported for the saccharide lactose (*35*).

The analysis of amino acids, on the other hand, revealed the formation of aspartic acid (**Figure 4A−E**), which is also observed when heating asparagine−glucose model systems at temperatures between 120 and 200 °C at atmospheric pressure (*29*, *31*). The concentration of aspartic acid (expressed per mole initial asparagine concentration) increases with time and temperature, as can be seen from **Figure 4A**,**C**,**D**, and is comparable for the phosphate buffer system heated at atmospheric pressure (**Figure 4C**) and treated under combined HPHT conditions (**Figure 4A**). The formation of aspartic acid as a function of time and temperature in the MES buffer system (**Figure 4D**) is,

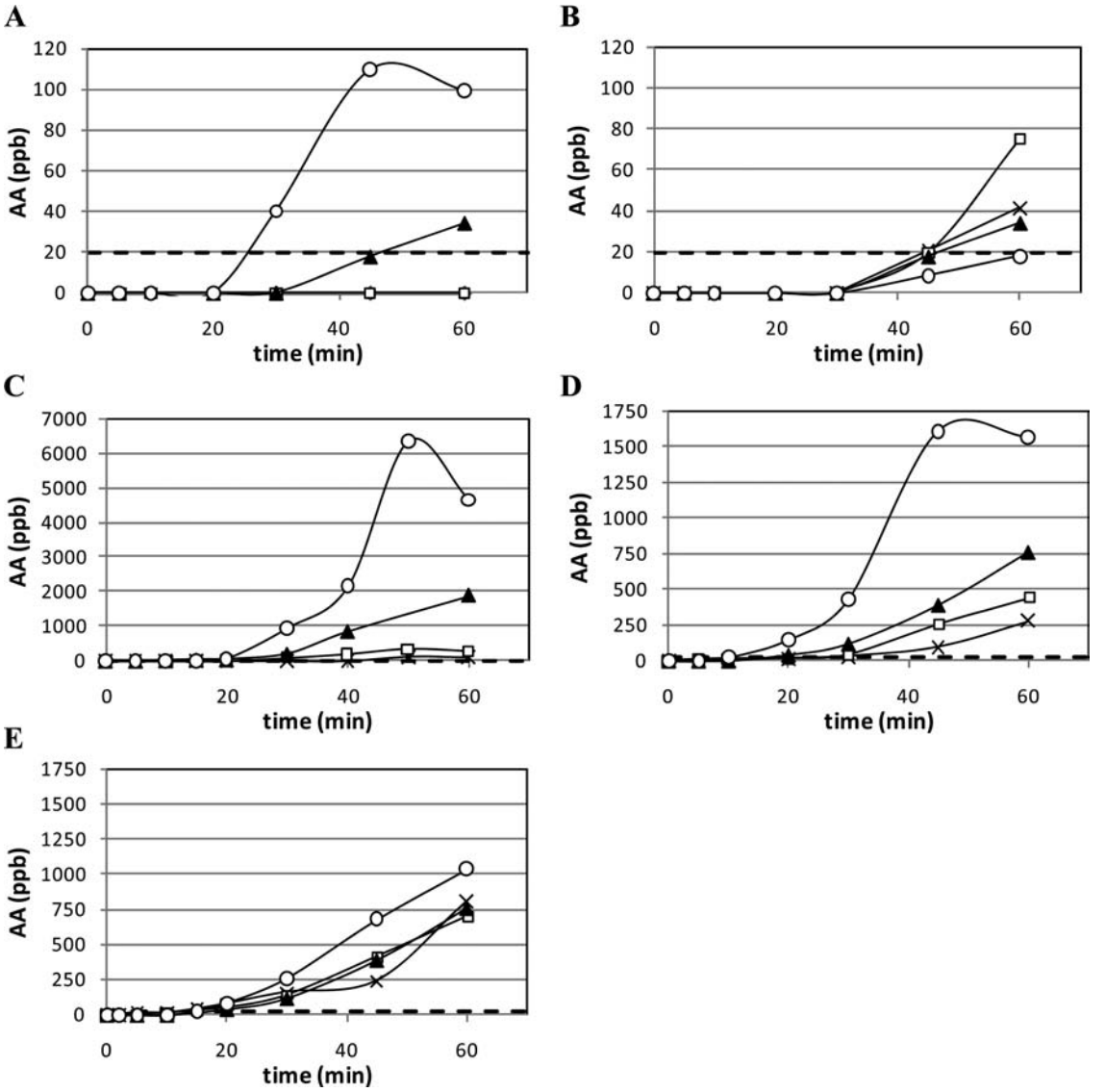

Figure 2. Time course of acrylamide in a 20% asparagine−glucose model system in sodium phosphate (**A**, **B**, **C**) or MES buffer (**D**, **E**) (0.1 M, pH 6.0). Effect of temperature: 100 (×), 105 (□), 110 (▲), and 115 °C (○) at 600 MPa (**A**, **D**) or ambient pressure (0.1 MPa) (**C**). Effect of pressure: 400 (×), 500 (□), 600 (▲), and 700 MPa (○) at 110 °C (**B**, **E**). The dashed line indicates the limit of detection (i.e., 20 ppb) of the analysis.

however, in all cases higher (factor ∼1.5) compared to the phosphate buffer system (**Figure 4A**). Besides on temperature, the changes in aspartic acid concentrations as a function of time are also dependent on changes in pressure. These changes are, however, much less pronounced within the pressure range tested compared to the temperature range tested. Moreover, aspartic acid formation seems to be most pronounced at the highest pressure tested, i.e. 700 MPa (especially clear for the MES buffer system (**Figure 4E**)). As was observed in **Figure 4A**,**D** for the effect of temperature, the aspartic acid concentration per mole initial asparagine concentration for the effect of pressure in the MES buffer system (**Figure 4E**) is also higher than the concentration formed in the phosphate buffer system (**Figure 4B**). In thermal studies (in the range of 120 to 200 °C), aspartic acid was proposed to be formed from asparagine through chemical conversion due to the high heating temperatures applied and the related decrease in pH during the reaction; this was confirmed by overall changes in pH (measured after the reaction), as a result of ammonium release, in high-moisture systems, however, mainly at temperatures equal to or higher than 160 °C (*29*). When comparing the yield of aspartic acid per mole initial asparagine in the 20% equimolar asparagine−glucose systems used in this study after a 60 min treatment at 115 °C (and atmospheric or high pressure) with the one observed after 60 min at 120 °C in high-moisture (0.1 M) and low-moisture equimolar asparagine−glucose model systems, it seems to fit in between. This trend seems realistic, since the yield per mole initial asparagine concentration generally increases with decreasing moisture content (*29*, *31*).

Hydroxymethylfurfural (*HMF*). HMF was not detected in the thermal nor the HPHT-treated samples. The absence of HMF in the model system heated at atmospheric pressure is not unexpected since only very low HMF formation was reported under more favorable reaction conditions (i.e., higher pH and temperature) in buffered glucose−glycine model systems by Martins (*32*).

Browning. Brown color formation as observed in amino acid−sugar systems is the result of melanoidin formation and possible caramelization reaction(s). Melanoidins are brown-colored polymers that are end products of the Maillard reaction. Browning can be quantified spectrophotometrically, and if an extinction coefficient is available or can be determined for the specific amino acid−sugar mixture under investigation, one can calculate the corresponding concentration of melanoidins formed. In the case of asparagine−glucose mixtures the extinction coefficient is reported

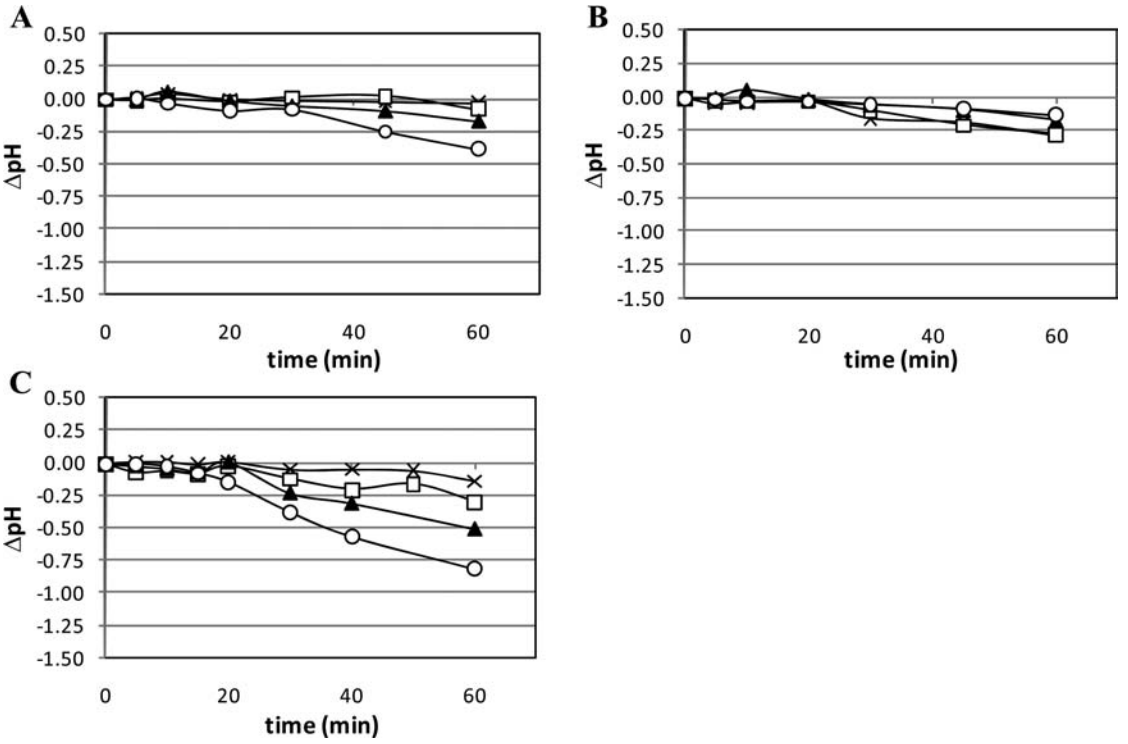

Figure 3. Time course of pH (measured at room temperature) in a 20% asparagine−glucose model system in sodium phosphate buffer (0.1 M, pH 6.0). Effect of temperature: 100 (×), 105 (□), 110 (▲), and 115 °C (○) at 600 MPa (**A**) or ambient pressure (0.1 MPa) (**C**). Effect of pressure: 400 (×), 500 (□), 600 (▲), and 700 MPa (○) at 110 °C (**B**).

to be 282 L/mol·cm (*25*). The apparent concentration of melanoidins formed is shown in **Figure 5A−E**. From **Figure 5**, anincrease of browning with reaction time and temperature can be observed. Browning in the samples heated at atmospheric pressure (**Figure 5C**) is, however, much more pronounced as compared to browning under conditions of high pressure (**Figure 5A**,**B**). This again confirms the retarding effect of high pressure on the overall Maillard reaction. Moreover, the higher the pressure applied, the lower the level of browning, which corresponds to the trend observed for acrylamide formation. The same trend was observed also for the MES buffer systems (**Figure 5D**,**E**), but the absolute concentration of melanoidins formed is for all reaction conditions higher for the MES buffer system compared to the phosphate buffer system.

DISCUSSION

Based on the course of acrylamide concentration and other Maillard reaction related products/intermediates determined as a function of treatment time, temperature and pressure in an equimolar asparagine−glucose system in phosphate buffer on the one hand and in MES buffer on the other hand, it is clear that acrylamide generation and the overall Maillard reaction are retarded under high pressure. Furthermore, these reactions also seem to occur at a higher rate in MES buffer compared to phosphate buffer. The discrepancies for the different products/intermediates between the phosphate and the MES buffer system can in all cases be explained by changes in pH due to temperature and/or pressure. These changes are determined by the temperature and pressure dependence of the pK_a of the buffer. The temperature dependence of K_a has been reported in the literature for several types of buffers (*22*, *36*). The pressure dependence can be calculated by means of Planck's equation (eq 1),

$$\left(\frac{\mathrm{d}\ln(K_a)}{\mathrm{d}p}\right)_T = \frac{\Delta V}{RT} \qquad (1)$$

where p is the pressure (MPa), T the absolute temperature (K), ΔV the reaction volume at atmospheric pressure ($cm^3 \cdot mol^{-1}$) and R the gas constant 8.3145 $cm^3 \cdot MPa \cdot K^{-1} \cdot mol^{-1}$ (*22*, *37*−*40*). Based on these values the influence of combined temperature and pressure on the pH of both buffer systems tested was calculated; for the HPHT conditions applied in this study, the theoretical changes in pH are listed in **Table 2** for both buffer systems under consideration. The reaction volumes used to calculate these pH changes were the values reported by Bruins et al. (*22*) for MES buffer and by Byrne and Laurie (*40*) for phosphate buffer. Based on these values listed in **Table 2**, it can be seen that phosphate buffer is little temperature dependent, but strongly dependent on pressure, resulting in a pH drop of 1.2 to 1.6 units for the HPHT conditions under consideration. Contrarily, for MES buffer, which is mildly temperature dependent, but little pressure dependent, the selected HPHT conditions result in a pH drop of maximally 0.79 unit. Based on these calculated pH changes, the pH drop for the phosphate buffer system is two times as high as for the MES buffer system. This difference in pH change explains the difference in the concentration of acrylamide and of other Maillard-type reaction products/intermediates between the phosphate and MES buffer system. However, based on the much larger pH drop in phosphate buffer, a more pronounced retardation of the Maillard reaction would be expected than is seen in our results. This latter observation could be explained by the reported enhancing effect of phosphate ions on the Maillard reaction (*41*, *42*).

Based on the time course of different responses characterizing different stages of the Maillard reaction and the course of the pH (measured prior to and after treatment), the retarding effect of high pressure on the overall Maillard reaction was demonstrated; this was most pronounced at the highest pressure tested (i.e., 700 MPa) in the case of acrylamide and browning, and was mainly pressure independent (within the tested range of 400−700 MPa) for aspartic acid and the pH change. Due to the lack of

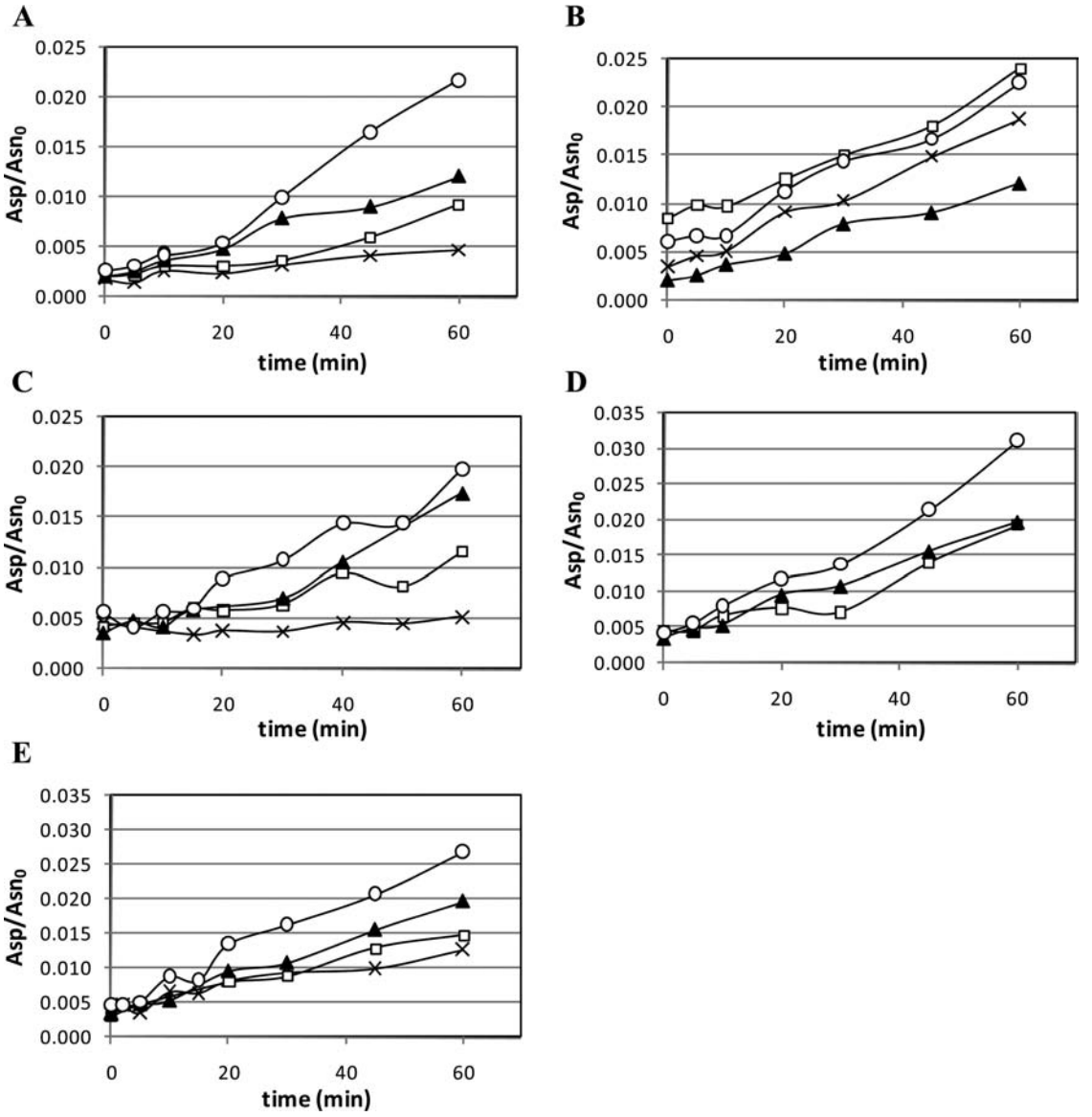

Figure 4. Time course of aspartic acid (expressed per mole initial asparagine concentration) in a 20% asparagine−glucose model system in sodium phosphate (**A**, **B**, **C**) or MES buffer (**D**, **E**) (0.1 M, pH 6.0). Effect of temperature: 100 (×), 105 (□), 110 (▲), and 115 °C (○) at 600 MPa (**A**, **D**) or ambient pressure (0.1 MPa) (**C**). Effect of pressure: 400 (×), 500 (□), 600 (▲), and 700 MPa (○) at 110 °C (**B**, **E**).

formation of HMF as an intermediate Maillard reaction product, the effect of high pressure on the Maillard reaction in this paper could only be evaluated based on its effects on browning and acrylamide. Since browning products are the end products of the Maillard reaction, a decrease of these products with increasing pressure allows us to state that pressure has a net retarding effect on the overall Maillard reaction. However, as the Maillard reaction is a complex reaction network consisting of different stages and pathways, the effect of high pressure on each of the individual reactions within the Maillard reaction might therefore be different; this has already been demonstrated by other research groups for specific compounds (*12*−*14*). The effect of high pressure on each reaction separately will depend on the type of reaction; for example, reactions involving the formation of hydrogen bonds are known to be favored by high pressure because bonding results in a decrease in volume of the molecules (*43*). It is, however, difficult to build a full picture of the mechanism of the Maillard reaction under high pressure, and available information has been summarized in the Introduction. The study described in this paper was conducted in two types of buffers (i.e., phosphate and MES buffer, respectively a "temperature insensitive" and a "pressure insensitive" buffer), and it revealed small differences in the concentration of the different compounds between the two buffer systems. For the MES buffer system a consistently higher concentration of acrylamide, aspartic acid and melanoidins was generated. The differences between phosphate and MES buffer system could principally be ascribed to differences in pH changes that are induced by application of pressure and/or temperature; a more pronounced pH drop (up to 1.6) was observed in the phosphate buffer system, whereas only half of this pH drop was estimated to occur in the MES buffer system.

As pointed out before, the novelty of this study resides in the investigation of the formation of acrylamide under high pressure−high temperature conditions. Based on the results obtained in model systems, it could be demonstrated that maximal acrylamide concentrations generated during a 60 min HPHT treatment at 115 °C and 600 MPa (∼1700 ppb) are considerably lower than during a conventional heat treatment (∼6500 ppb). Moreover, the time frame that is relevant for high pressure sterilization applications is much shorter (3−5 min holding time at 121 °C)

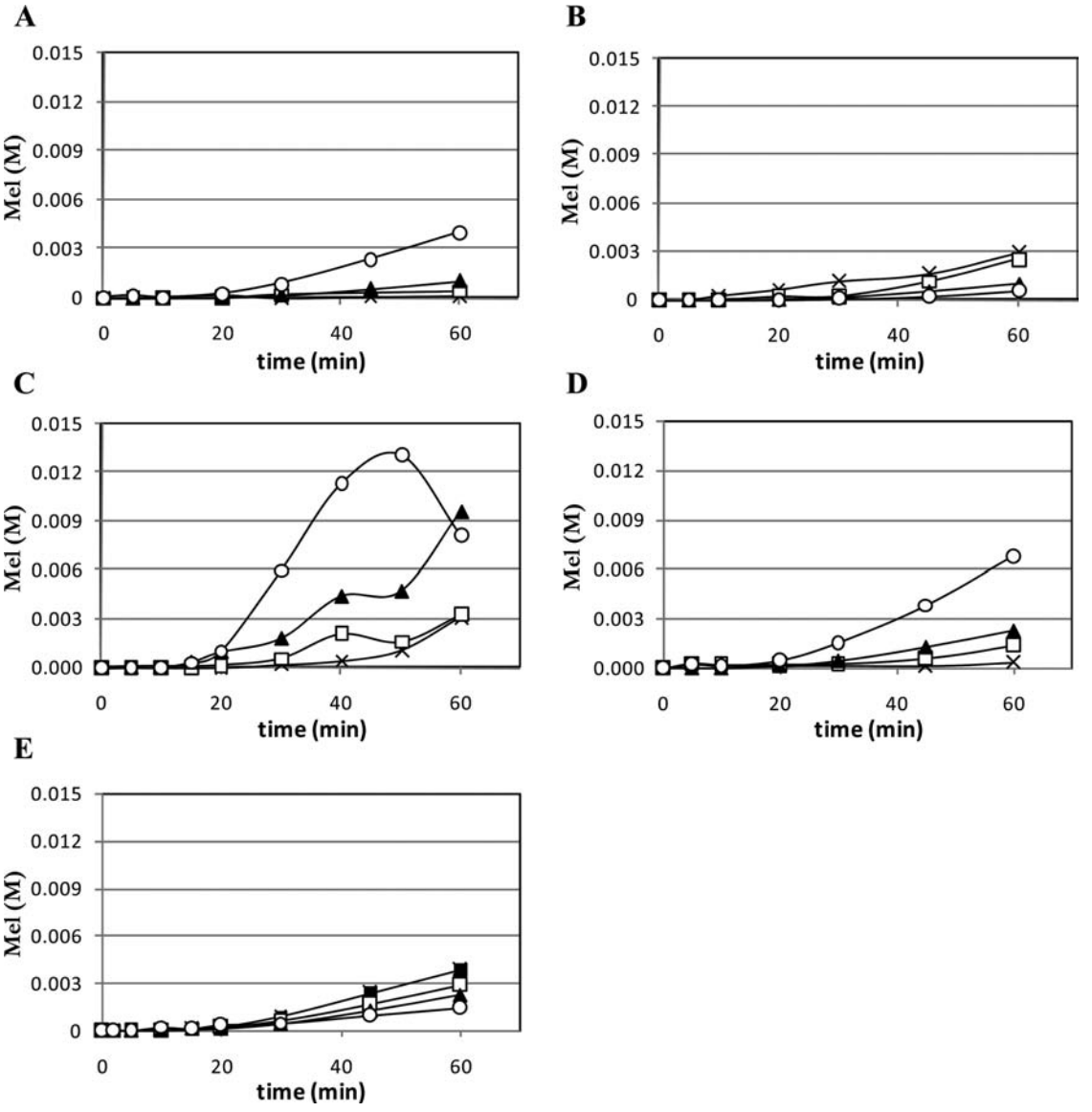

Figure 5. Time course of melanoidins in a 20% asparagine−glucose model system in sodium phosphate (**A**, **B**, **C**) or MES buffer (**D**, **E**) (0.1 M, pH 6.0). Effect of temperature: 100 (×), 105 (□), 110 (▲), and 115 °C (○) at 600 MPa (**A**, **D**) or ambient pressure (0.1 MPa) (**C**). Effect of pressure: 400 (×), 500 (□), 600 (▲), and 700 MPa (○) at 110 °C (**B**, **E**).

Table 2. Calculated Change in pH That Occurs during Thermal or Combined HPHT Treatment

		pH change	
P (MPa)	*T* (°C)	phosphate buffer	MES buffer
0.1	100	−0.22	
	105	−0.24	
	110	−0.25	
	115	−0.27	
400	110	−1.21	−0.78
500	110	−1.37	−0.75
600	100	−1.52	−0.61
	105	−1.52	−0.67
	110	−1.52	−0.73
	115	−1.52	−0.79
700	110	−1.65	−0.71

than the frame selected in this study for kinetic purposes. Based on these considerations and the fact that only high water content products are relevant for high pressure processing applications, acrylamide formation is not expected to pose a major hazard to this type of product. However, validation in real food products is required.

ABBREVIATIONS USED

AA, acrylamide; Asp, aspartic acid; Asn, asparagine; HMF, hydroxymethylfurfural; HPAEC high-performance anion-exchange chromatography; HPHT, high pressure−high temperature; Mel, melanoidins; PAD, pulsed amperometric detector; POM, polyoxymethylene or polyacetale.

LITERATURE CITED

(1) IARC (International Agency for Research on Cancer). Acrylamide. *IARC Monogr. Eval. Carcinog. Risks Hum.* **1994**, *60*, 389−433.
(2) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Analysis of Acrylamide, a Carcinogen Formed in Heated Foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998−5006.
(3) Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448−449.
(4) Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P. A.; Robert, M.-C.; Riediker, S. Acrylamide from Maillard reaction products. *Nature* **2002**, *419*, 449−450.

(5) Matser, A. M.; Krebbers, B.; van den Berg, R. W.; Bartels, P. V. Advantages of high pressure sterilisation on quality of food products. *Trends Food Sci. Technol.* **2004**, *15*, 79−85.
(6) De Roeck, A.; Mols, J.; Duvetter, T.; Van Loey, A.; Hendrickx, M. Carrot texture degradation kinetics and pectin changes during thermal versus high-pressure/high-temperature processing: A comparative study. *Food Chem.* **2010**, *120*, 1104−1112.
(7) Deliza, R.; Rosenthal, A.; Abadio, F. B. D.; Silva, C. H. O.; Castillo, C. Application of high pressure technology in the fruit juice processing: benefits perceived by consumers. *J. Food Eng.* **2005**, *67*, 241−246.
(8) Barbosa-Cánovas, G. V.; Juliano, P. Food Engineering: Integrated Approaches. In *Food Engineering—Integrated approaches*; Gutiérrez-López, G. F., Ed.; Springer: New York, 2008; pp 9−46.
(9) Verlinde, P.; Oey, I.; Hendrickx, M.; Van Loey, A. High-pressure treatments induce folate polyglutamate changes in intact broccoli (*Brassica oleraceae* L. *cv. Italica*) tissue. *Food Chem.* **2008**, *111*, 220−229.
(10) De Roeck, A.; Duvetter, T.; Fraeye, I.; Van der Plancken, I.; Sila, D. A.; Van Loey, A.; Hendrickx, M. Effect of high-pressure/high-temperature processing on chemical pectin conversions in relation to fruit and vegetable texture. *Food Chem.* **2009**, *115*, 207−213.
(11) Verbeyst, L.; Oey, I.; Van der Plancken, I.; Hendrickx, M.; Van Loey, A. Kinetic study on the thermal and pressure degradation of anthocyanins in strawberries. *Food Chem.* **2010**, *123*, 269−274.
(12) Heberle, I.; Schieberle, P.; Hofmann, T. Influence of High Hydrostatic Pressure on the Formation of Non-enzymatic Browning Products Formed in Maillard-type Reactions. In *Advances in High Pressure Bioscience and Technology II*; Winter, R., Ed.; Springer: Berlin, 2003; pp 341−345.
(13) Deters, F.; Hofmann, T.; Schieberle, P. Influence of High Hydrostatic Pressure on the Formation of Key Maillard-type Flavour Compounds from D-glucose and L-proline. In *Advances in High Pressure Bioscience and Technology II*; Winter, R., Ed.; Springer: Berlin, 2003; pp 347−350.
(14) Alt, N.; Schieberle, P. Model Studies on the Influence of High Hydrostatic Pressure on the Formation of Glycated Arginine Modifications at Elevated Temperatures. *J. Agric. Food Chem.* **2005**, *53*, 5789−5797.
(15) Bristow, M.; Isaacs, N. S. The effect of high pressure on the formation of volatile products in a model Maillard reaction. *J. Chem. Soc., Perkin Trans. 2* **1999**, 2213−2218.
(16) Frank, O.; Heberle, I.; Schieberle, P.; Hofmann, T. Influence of high hydrostatic pressure on the formation of intense chromophores formed from pentoses and primary amino acids. *Int. Congr. Ser.* **2002**, *1245*, 387−388.
(17) Isaacs, N. S.; Coulson, M. Effect of pressure on processes modelling the Maillard reaction. *J. Phys. Org. Chem.* **1996**, *9*, 639−644.
(18) Tamaoka, T.; Itoh, N.; Hayashi, R. High Pressure Effect on Maillard Reaction. *Agric. Biol. Chem.* **1991**, *55*, 2071−2074.
(19) Schwarzenbolz, U.; Klostermeyer, H.; Henle, T. Maillard-type reactions under high hydrostatic pressure: Formation of pentosidine. *Eur. Food Res. Technol.* **2000**, *211*, 208−210.
(20) Moreno, F. J.; Molina, E.; Olano, A.; López-Fandiño, R. High-Pressure Effects on Maillard Reaction between Glucose and Lysine. *J. Agric. Food Chem.* **2003**, *51*, 394−400.
(21) Hill, V. M.; Ledward, D. A.; Ames, J. M. Influence of High Hydrostatic Pressure and pH on the Rate of Maillard Browning in a Glucose-Lysine System. *J. Agric. Food Chem.* **1996**, *44*, 594−598.
(22) Bruins, M. E.; Matser, A. M.; Janssen, A. E. M.; Boom, R. M. Buffer selection for HP treatment of biomaterials and its consequences for enzyme inactivation. *High Pressure Res.* **2007**, *27*, 101−107.
(23) Biedermann, M.; Biedermann-Brem, S.; Noti, A.; Grob, K. Two GC-MS Methods for the Analysis of Acrylamide in Foodstuffs. *Mitt. Lebensmittelunters. Hyg.* **2002**, *93*, 638−652.
(24) De Vleeschouwer, K.; Van der Plancken, I.; Van Loey, A.; Hendrickx, M. Impact of pH on the Kinetics of Acrylamide Formation/Elimination Reactions in Model Systems. *J. Agric. Food Chem.* **2006**, *54*, 7847−7855.
(25) Knol, J. J.; van Loon, W. A. M.; Linssen, J. P. H.; Ruck, A.-L.; van Boekel, M. A. J. S.; Voragen, A. G. J. Toward a Kinetic Model for Acrylamide Formation in a Glucose-Asparagine Reaction System. *J. Agric. Food Chem.* **2005**, *53*, 6133−6139.
(26) Becalski, A.; Lau, B.P.-Y.; Lewis, D.; Seaman, S. W. Acrylamide in Foods: Occurrence, Sources, and Modeling. *J. Agric. Food Chem.* **2003**, *51*, 802−808.
(27) Claeys, W. L.; De Vleeschouwer, K.; Hendrickx, M. E. Kinetics of Acrylamide Formation and Elimination during Heating of an Asparagine-Sugar Model System. *J. Agric. Food Chem.* **2005**, *53*, 9999−10005.
(28) Hedegaard, R. V.; Frandsen, H.; Granby, K.; Apostolopoulou, A.; Skibsted, L. H. Model Studies on Acrylamide Generation from Glucose/Asparagine in Aqueous Glycerol. *J. Agric. Food Chem.* **2007**, *55*, 486−492.
(29) De Vleeschouwer, K.; Van der Plancken, I.; Van Loey, A.; Hendrickx, M. The kinetics of acrylamide formation/elimination in asparagine-glucose systems at different initial reactant concentrations and ratios. *Food Chem.* **2008**, *111*, 719−729.
(30) De Vleeschouwer, K.; Van der Plancken, I.; Van Loey, A.; Hendrickx, M. Kinetics of Acrylamide Formation/Elimination Reactions as Affected by Water Activity. *Biotechnol. Prog.* **2007**, *23*, 722−728.
(31) De Vleeschouwer, K.; Van der Plancken, I.; Van Loey, A.; Hendrickx, M. Investigation of the Influence of Different Moisture Levels on Acrylamide Formation/Elimination Reactions using Multiresponse Analysis. *J. Agric. Food Chem.* **2008**, *56*, 6460−6470.
(32) Martins, S. I. F. S. (Ed.), *Unravelling the Maillard reaction network by multiresponse kinetic modelling*. Doctoral dissertation, University of Wageningen, Wageningen, 2003.
(33) Brands, C. M. J.; van Boekel, M. A. J. S. Reactions of Monosaccharides during Heating of Sugar-Casein Systems: Building a Reaction Network Model. *J. Agric. Food Chem.* **2001**, *49*, 4667−4675.
(34) Davídek, T.; Devaud, S.; Robert, F.; Blank, I. Sugar Fragmentation in the Maillard Reaction Cascade: Isotope Labeling Studies on the Formation of Acetic acid by a Hydrolytic β-Dicarbonyl Cleavage Mechanism. *J. Agric. Food Chem.* **2006**, *54*, 6667−6676.
(35) Moreno, F. J.; Villamiel, M.; Olano, A. Effect of High Pressure on Isomerization and Degradation of Lactose in Alkaline Media. *J. Agric. Food Chem.* **2003**, *51*, 1894−1896.
(36) McKenzie, H. A.; Dawson, R. M. C. pH and Buffers. In *Data for Biochemical Research*, 2nd ed.; Dawson, R. M. C., Elliott, D. C., Elliott, W. H., Jones, K. M., Eds.; Oxford University Press: London, 1969; pp 475−508.
(37) Neuman, R. C.; Kauzmann, W.; Zipp, A. The Dependence of Weak Acid Ionization in Aqueous Buffers. *J. Phys. Chem.* **1973**, *77*, 2687−2691.
(38) Kitamura, Y.; Itoh, T. Reaction Volume of Protonic Ionization for Buffering Agents. Prediction of Pressure Dependence of pH and pOH. *J. Solution Chem.* **1987**, *16*, 715−725.
(39) Hitchens, T. K.; Bryant, R. G. Pressure Dependence of Weak Acid Ionization in Deuterium Oxide Solutions. *J. Phys. Chem. B* **1998**, *102*, 1002−1004.
(40) Byrne, R. H.; Laurie, S. H. Influence of pressure on chemical equilibria in aqueous systems—with particular reference to seawater. *Pure Appl. Chem.* **1999**, *71*, 871−890.
(41) Rizzi, G. P. Role of Phosphate and Carboxylate Ions in Maillard Browning. *J. Agric. Food Chem.* **2004**, *52*, 953−957.
(42) Bell, L. N. Maillard reaction as influenced by buffer type and concentration. *Food Chem.* **1997**, *59*, 143−147.
(43) Yaldagard, M.; Mortazavi, S. A.; Tabatabaie, F. The principles of ultra high pressure technology and its application in food processing/preservation: A review of microbiological and quality aspects. *Afr. J. Biotechnol.* **2008**, *7* (16), 2739−2767.

Received for review July 12, 2010. Revised manuscript received October 4, 2010. Accepted October 9, 2010. K.D.V. and I.V.d.P. are postdoctoral researchers funded by the Research Fund K. U. Leuven. This work was financially supported by the Commission of the European Communities, Framework 6, Priority 5 "Food Quality and Safety", Integrated Project NovelQ FP6-CT-2006-015710 and the Katholieke Universiteit Leuven.