

Acrylamide Formation from Asparagine under Low Moisture Maillard Reaction Conditions. 2. Crystalline vs Amorphous Model Systems

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The formation of acrylamide was investigated in model systems based on asparagine and glucose under low moisture Maillard reaction conditions as a function of reaction temperature, time, physical state, water activity, and glass transition temperature. Equimolar amorphous glucose/asparagine systems with different water activities were prepared by freeze drying and were shown to quickly move to the rubbery state already at room temperature and a water activity of above 0.15. The acrylamide amounts were correlated with physical changes occurring during the reaction. Pyrolysis and kinetics of acrylamide release in amorphous and crystalline glucose/asparagine models indicated the importance of the physical state in acrylamide formation. In amorphous systems, acrylamide was generated in higher concentrations and at lower temperatures as compared to the crystalline samples. Time and temperature are covariant parameters in both systems affecting the acrylamide formation by thermal processes. On the other side, the water activity and glass transition temperature do not seem to be critical parameters for acrylamide formation in the systems studied.

KEYWORDS: Acrylamide; Maillard reaction; low moisture; crystalline; amorphous; water activity; glass transition temperature

INTRODUCTION

Numerous studies have recently been performed to understand the formation of acrylamide in carbohydrate-rich foods obtained by thermal processing (1, 2). Work addressing the question of precursors suggested the naturally occurring amino acid L-asparagine (Asn) as the major direct precursor of acrylamide when heated under low moisture conditions in the presence of reducing sugars or carbonyl compounds (3–8). At elevated temperatures and under low moisture conditions, the formation of acrylamide was attributed to Maillard type reactions employing intermediates of Asn, such as the corresponding Schiff base (7–10), decarboxylated Amadori compound (8–10), Strecker aldehyde (3), and deamination product (7, 8), the latter (3-aminopropionamide) also generating acrylamide under aqueous conditions (11).

Various parameters affect the reaction yields of acrylamide from Asn, such as the concentration and type of carbonyl compounds as well as the reaction conditions. The interplay between reaction temperature and time has been suggested as an important factor (12, 13). Lowering the pH leads to reduced acrylamide amounts by decreasing the nucleophilicity of the α -amino group of Asn (14, 15). Furthermore, α -hydroxy ketones have been shown to be more reactive than α -hydroxy aldehydes in transforming Asn into acrylamide under pyrolytic conditions

(4, 7, 9, 10, 13, 16). We found, for example, that fructose is by a factor of 3–6 more efficient as compared to glucose (Glc) under the same pyrolytic conditions (9, 16), which, from a chemical point of view, is a rather surprising finding.

Apart from reaction time and temperature, the major difference between thermal procedures leading to high or low amounts of acrylamide from Asn is the water content of the reaction system, which directly influences its physical state. The water activity has been shown to be one key factor to consider in the Maillard reaction (17, 18). In particular, it is critical for the shelf life quality of industrially processed dry food products (19, 20).

In our recent paper (13), we discussed the role of physical properties of crystalline model systems on the formation of acrylamide and suggested the melting point of sugars as an important parameter. As most food systems occur in the amorphous state, the aim of this study was to study model mixtures of Asn and Glc in both crystalline and amorphous states under low moisture Maillard reaction conditions to better understand how acrylamide formation is affected by the physical state of the reaction system.

EXPERIMENTAL PROCEDURES

Materials. Asn, Glc, and acrylamide were from Fluka/Aldrich (Buchs, Switzerland). Formic acid, lithium chloride (LiCl), potassium acetate (CH₃COOK), phosphorus pentoxide (P₂O₅), and pure water were

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purchased from Merck (Darmstadt, Germany). Acetonitrile was obtained from Baker (Deventer, The Netherlands). All other reagents were of analytical grade and were used without further purification.

Caution. Acrylamide (CAS 79-06-1) is classified as toxic and may cause cancer. Wear suitable protective clothing, gloves, and eye/face protection when handling this chemical.

Proton Transfer Reaction Mass Spectrometry (PTR-MS). The PTR-MS from IONICON Analytik GmbH (Innsbruck, Austria) was used to follow on-line generation and release of acrylamide in model systems as recently described (13, 16). The full mass spectrum from m/z 21 to m/z 220 was monitored with a 0.2 s dwell time per mass to measure all compounds released from the model reaction mixture. In addition, the oven and the sample temperatures were also monitored as well as the specific ion at m/z 72 as a characteristic signal of the protonated acrylamide with a 2 s dwell time. Equimolar Glc/Asn mixtures were heated (25–190 °C, 5 °C/min) in an oven, and the headspace was analyzed on-line by PTR-MS.

Differential Scanning Calorimetry (DSC). This was performed with a DSC 220C (Seiko Instruments, Chiba, Japan), which is based on the heat flow principle and cooled with liquid nitrogen, as recently described (13). Samples of Glc/Asn mixtures (0.1–0.2 mmol) were hermetically sealed in the DSC crucibles for the closed system or left open for the open system and scanned with the heating scan rate of 5 °C/min using an empty crucible as the reference. All thermal analysis experiments were performed in duplicate. The onset and the endset temperatures of the phenomena observed were determined as the intersection points of the tangents to the heat flow curve step and to the pre- and poststep baselines using the DSC software from Seiko.

High-Performance Liquid Chromatography (HPLC). Acrylamide was quantified by liquid chromatography using a HPLC Series 1100 (Agilent, Palo Alto, CA) equipped with a DAD detector (UV/vis wavelength 200–900 nm, G1315A), as recently described (13). The mobile phase used for the HPLC separation of hydrophilic compounds was a mixture composed of water and acetonitrile (93/7, v+v) with 264 μ L of formic acid added. The compounds were separated on a DIONEX ion pack ICE-AS1 column (Olten, Switzerland): 9 mm \times 250 mm; bead diameter, 75 μ m; bead type, microporous; cross-linking, 8% divinylbenzene; ion exchange group, sulfonic acid, with an isocratic flow rate of 1 mL/min. Quantification was based on external calibration curves (seven points) established in the concentration range of 0.5 – 7.0×10^{-5} mol/L. The linearity was for all standard curves $r^2 > 0.999$ with a detection threshold of 10^{-6} mg acrylamide injected (13).

Pyrolysis Procedures. Open Reaction Systems. Asn (0.33 g, 2.5 mmol) was dry-mixed with Glc (0.45 g, 2.5 mmol) and placed in a reaction vessel that was held at a defined temperature in an oven. After the addition of the reactants, the formation of volatile compounds was monitored by PTR-MS. Reaction kinetics was monitored from room temperature up to 190 °C with a 5 °C/min temperature program.

Closed Reaction Systems. The chemicals of interest were heated in a temperature-controlled heating module (Brouwer, Luzern, Switzerland) in tightly closed vacuum hydrolysis tubes that were immersed in silicone oil. After a defined heating period (e.g., 5 min), the tubes were cooled on ice. The residue was extracted from the tube with a mixture (2–25 mL) of water/acetonitrile (93/7, v+v) and sonicated for 15 min. The sample was then passed through a filter (0.2 μ m) and injected (20–100 μ L) as such on the column and analyzed according to the conditions described above.

Preparation of Equimolar Amorphous Systems. Samples at $a_w = 0.07$. Glc (900 g) and Asn (660 g) were mixed with water (4.7 L) in a flask (10 L) corresponding to 25% total solid (TS) content. The solution was heated at 60 °C in a water bath during 150 min. The monophasic solution was then quickly transferred into a liquid nitrogen bath cooled at -170 °C to avoid Glc crystallization. The solid system obtained after evaporation of the liquid nitrogen was then stored at -55 °C in a freezer. Water was removed using a FCV 600 freeze dryer (Secfruid, Lausanne, Switzerland). The product was slowly lyophilized at -40 °C for 3 days and then from -40 to 20 °C in 36 h (1.7 °C/h, 0.001 mbar) to avoid collapse phenomena.

Samples at $a_w = 0.11$ and $a_w = 0.22$. The amorphous system with a_w at 0.07 was placed into desiccators for 3 weeks with LiCl and CH₃-

COOK for obtaining Glc/Asn amorphous systems with water activities at 0.11 and 0.22, respectively.

RESULTS AND DISCUSSION

In the course of our studies on the formation of acrylamide from binary mixtures of Asn and reducing sugars under low moisture conditions, we have obtained results that could not fully be explained by the chemical reactivity of the precursors. We have recently suggested the physical state of the precursors to be an important factor in acrylamide formation in crystalline sugar/Asn systems (13). To evaluate this phenomenon in more realistic models, we investigated amorphous equimolar Glc/Asn mixtures with respect to acrylamide formation. In addition, we studied the influence of water activity and glass transition temperature in these model systems.

Preparation of Amorphous Glc/Asn Powders. The preparation of amorphous powders based on Glc and Asn turned out to be a first challenge, as crystallization and collapse phenomena can easily occur during sample processing, especially if the phase diagram is not known. We succeeded to obtain an amorphous Glc/Asn powder by heating at 60 °C a solution containing equimolar Glc/Asn with a 25% TS content allowing solubilization of Asn, followed by flash-freezing with liquid nitrogen to avoid Glc crystallization. The glass transition temperature of this amorphous aqueous sample was determined by DSC to be $T_g' = -53$ °C (data not shown), indicating that the sample should be stored under this temperature to avoid crystallization. The solid was then carefully lyophilized during 5 days to obtain a powder with a water content of 1.65% (measured by gravimetry using the following conditions: in the presence of P₂O₅, 20 mbar, 2 h, 70 °C) and a water activity of 0.07. The powder showed a low bulk density without collapse. A few traces of crystallization were observed by polarized microscopy, but the specific wavelengths at 2280 and 2236 nm characteristic for Glc (21) and Asn crystals, respectively, were not observed by near-infrared spectroscopy. Therefore, this system was considered as amorphous.

Figure 1 visualizes the DSC of this amorphous system with a heat treatment of 5 °C/min. The glass transition, i.e., the phase change from the amorphous to the rubbery state, was the first physical change observed at the glass transition onset temperature of 43 °C that was characterized by a slight endothermic effect. The first onset of an exothermic signal occurred in the rubbery state ($T > T_g$) at 82 °C followed by a second, more pronounced exothermic phenomenon starting at about 110 °C.

Two other amorphous systems with different water activities were obtained from the amorphous sample with $a_w = 0.07$ by conditioning the water activity to 0.11 and 0.22 using salts such as LiCl and CH₃COOK, respectively. Using these samples, the relationship between glass transition temperature (T_g) and water

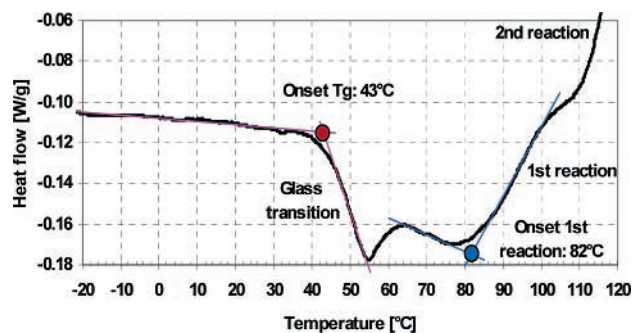


Figure 1. DSC of an amorphous equimolar mixture of Glc and Asn. The heating rate was 5 °C/min.

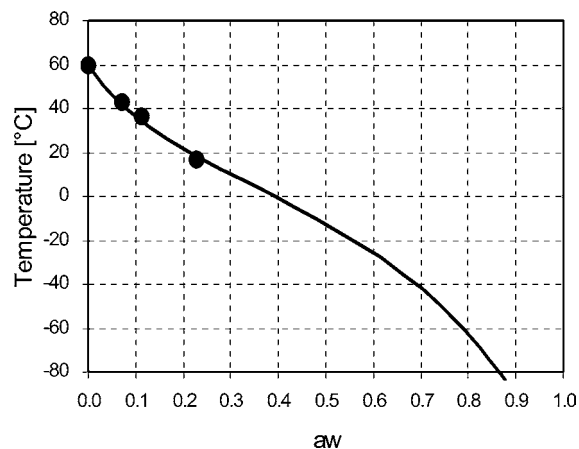


Figure 2. Glass transition temperature vs water activity of an equimolar amorphous Glc/Asn system.

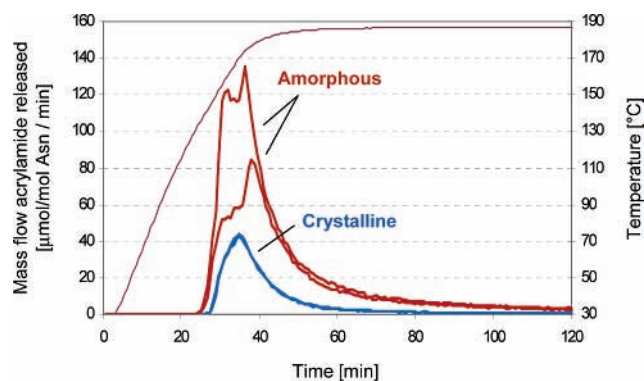


Figure 3. Kinetics of acrylamide release into the headspace during pyrolysis of equimolar amorphous or crystalline Asn/Glc monitored on-line by PTR-MS showing the entire experiment with a heating rate of 5 °C/min from 30 to 190 °C. Individual release curves of duplicates are shown.

activity (**Figure 2**) was determined (22) by combination of the Gordon–Taylor equation (T_g as function of water content) and the BET equation for the sorption isotherm of water (water content as function of water activity at 25 °C). As shown in **Figure 2**, an equimolar amorphous Glc/Asn system will quickly move to the rubbery state already at room temperature if the water activity is above 0.15.

Role of Physical State, Water Activity, and Glass Transition in Acrylamide Formation. The thermally induced formation of acrylamide from equimolar mixtures of crystalline and amorphous Asn/Glc was monitored in the headspace by on-line PTR-MS analysis measuring its characteristic ion at m/z 72 (13, 16). The general profile of acrylamide release is shown in **Figure 3**, indicating higher amounts of acrylamide produced from the amorphous sample (red curve). A better repeatability of acrylamide release was obtained in the crystalline system (blue curves), most likely due to the fact that the amorphous powder was less homogeneous and highly hygroscopic.

Moreover, the initial phase of the release curves in **Figure 3** shows that acrylamide was generated earlier in the amorphous mixture than in the crystalline sample. This means that acrylamide was formed at a lower temperature, i.e., starting at about 135 °C under these heating conditions. On the contrary, about 145 °C was required for the crystalline sample to observe acrylamide release under the same conditions. Interestingly, these findings are in concordance with the respective glass transition temperature and melting point of the two systems,

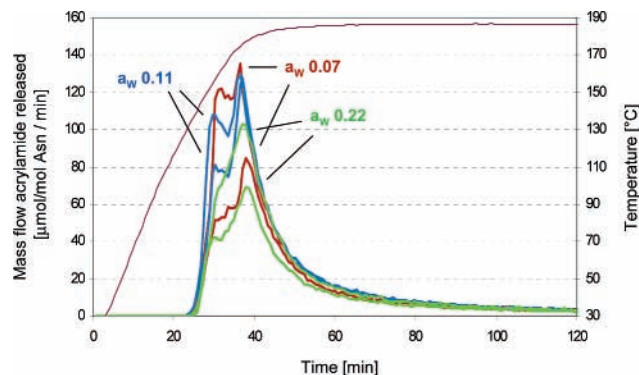


Figure 4. Kinetics of acrylamide release into the headspace during pyrolysis of equimolar amorphous Asn/Glc at different water activities monitored in real time by PTR-MS. Individual release curves of duplicates are shown. For the samples with $a_w = 0.07$ (red) and $a_w = 0.22$ (green), the lines are pointing to the maximum of the individual release curves.

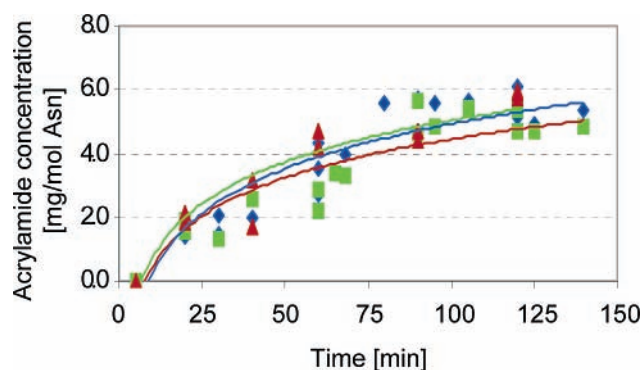


Figure 5. Formation of acrylamide upon time by heating at 110 °C amorphous Asn/Glc with a defined water activity of 0.07(▲), 0.11(◆), and 0.22 (■).

indicating that acrylamide is not released in detectable amounts as long as the reaction system is in the crystalline or amorphous state.

As shown in **Figure 4**, similar acrylamide release curves were obtained in amorphous systems independently of the water activity. These data indicate that in these model systems with water activities in the range of 0.07–0.22, there is no significant difference in acrylamide formation. Therefore, the formation of acrylamide cannot be correlated with water activity. This may partially be due to the limited reproducibility of the results obtained, in particular with the sample at $a_w = 0.07$, which was inhomogeneous and highly hygroscopic. Small differences in moisture content and crystal traces may trigger upon heat treatment modifications in structure and surface of the solid matrix that may affect the release of volatile compounds such as acrylamide. This effect may lead to high variations, as the released acrylamide amounts only represent a part of the acrylamide formed at a given time.

In addition, acrylamide was quantified in amorphous Glc/Asn samples heated under pyrolytic conditions at 110 °C as a function of time. As shown in **Figure 5**, the data obtained with the various amorphous systems indicate that reaction time influences much more acrylamide formation than water activity does. It should be mentioned, however, that the data obtained by PTR-MS (**Figure 4**) that show only acrylamide in the headspace can hardly be directly compared with HPLC data (**Figure 5**) representing total acrylamide amounts. Nevertheless, both experiments suggest a rather limited effect of water activity on acrylamide formation.

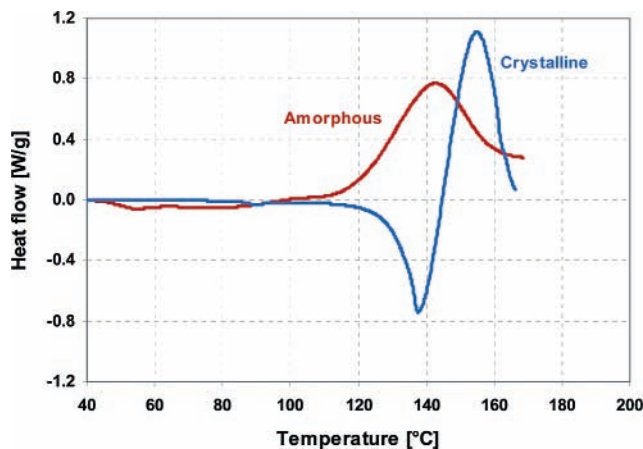


Figure 6. DSC of equimolar mixtures of Asn and Glc in the amorphous ($a_w = 0.07$) and crystalline states.

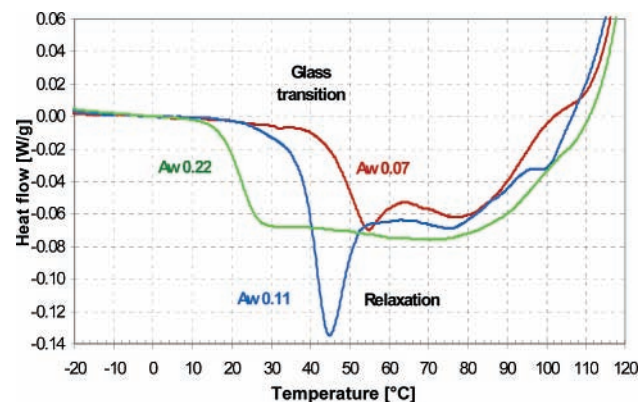


Figure 7. DSC of equimolar amorphous Asn/Glc at different water activities. The heating rate was 5 °C/min.

Physical State Changes Upon Pyrolysis. To better understand the meaning of the physical changes observed, the thermal behavior of model reaction systems containing crystalline or amorphous Glc/Asn was characterized by DSC. As shown in Figure 6, the first thermal event for the amorphous system (red curve) occurred at about 43 °C corresponding to the glass transition (see also Figure 1). A positive slope was observed from 110 to 145 °C exhibiting the well-known exothermic phenomenon, which is linked to the chemical (Maillard) reaction. For the crystalline Glc/Asn system, the melting of the sugar occurred at about 140 °C (endothermic

signal), followed by the exothermic Maillard reaction signal. It indicates that the amorphous sample is less stable and consequently more reactive than the crystalline sample, probably due to the increased molecular mobility in amorphous systems. Thus, the browning (Maillard) reaction is well-correlated with the melting behavior of the precursor system, which is in line with the kinetics of acrylamide formation as indicated by PTR-MS (Figure 3).

When looking at the DSC of the amorphous samples with different water activities (Figure 7), the different glass transition points for each amorphous powder can clearly be seen as follows: the higher the water activity the lower the glass transition temperature. However, after this thermal event, all curves overlap indicating that the exothermic signal of the reaction is not dependent on the T_g : The chemical reaction needs at least 80 °C to be initiated, which applies even to the most dehydrated amorphous matrix. These observations are fully consistent with the results described previously for acrylamide formed in these different models (13).

Effect of Reaction Time and Temperature. Pyrolysis of closed reaction systems performed as a function of temperature and time resulted in data summarized in Figure 8. These results confirm that the amorphous systems A and B generate acrylamide at lower temperatures than crystalline samples C and D. This difference is more pronounced at the 5 min heat period with higher heat load (samples A and C). From a quantitative point of view, amorphous systems generate more acrylamide when the heating period is short (sample A). Increasing the heating period to 60 min resulted in total acrylamide amounts, which were similar for both the crystalline and the amorphous system (B and D). Furthermore, the total acrylamide amounts generated were lower as compared to the shorter heat treatment (A and C). As already reported for crystalline systems (10, 13), these results confirm the importance of the covariant nature of time and temperature, which is applicable to amorphous systems as well.

In conclusion, this work has shown that acrylamide formation is, indeed, affected by the physical state of the reaction system. Acrylamide is preferably generated in amorphous systems at lower temperatures than in crystalline samples. Reaction time and temperature markedly influence acrylamide formation and degradation in the thermal process. On the other side, we have demonstrated in equimolar Glc/Asn model systems that acrylamide formation was not much influenced by water activity and glass transition temperature, thus suggesting these parameters

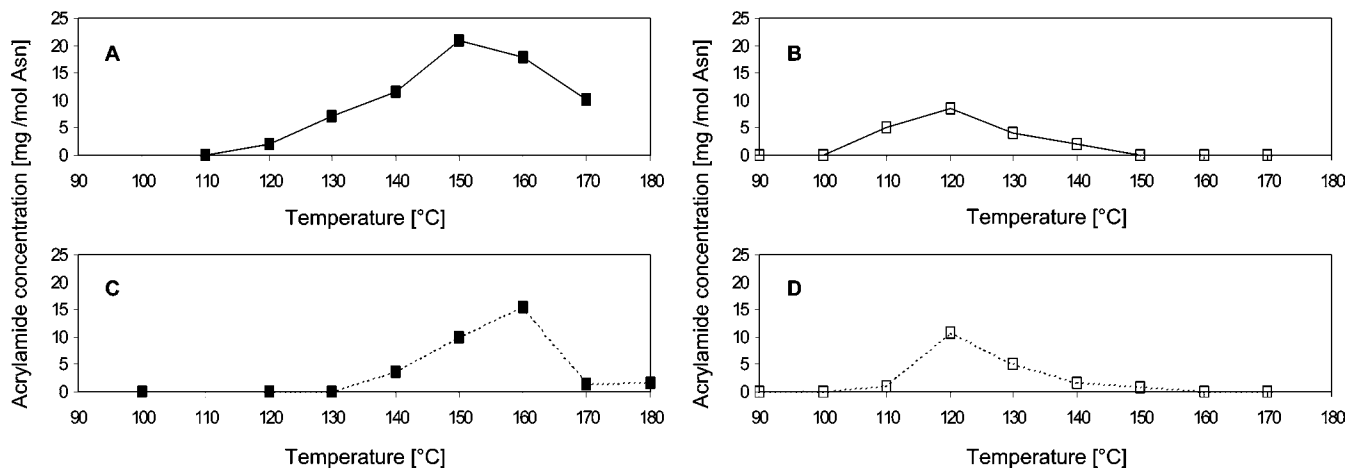


Figure 8. Formation of acrylamide by heating equimolar mixtures of Asn and Glc in the amorphous or crystalline state for 5 and 60 min, i.e., (A) amorphous, 5 min; (B) amorphous, 60 min; (C) crystalline, 5 min; and (D) crystalline, 60 min.

to be of limited relevance in this context. In general, a more detailed characterization of the solid matrix, e.g., interface, surface, porosity, and crystal water, may result in valuable information to better understand the phenomena of solid state Maillard reaction and the kinetics of acrylamide formation and its release.

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