Elimination of acrylamide by polymerization catalysed by inorganic food components

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Summary

This paper summarizes results of the experiments aimed at the behaviour of acrylamide (AA) in contact with NaCl and additives of table salt at elevated temperatures. In the first experiment, samples were heat-treated at 171.1 °C for 10 min. Presence of NaCl in the matrix brougth about a decrease in AA content of up to 40%, when the highest decrement in the AA content was observed in the range of 0–1 % (w/w) of NaCl added. Than, differential scanning calorimetry measurements indicated that the elimination of AA was caused by its polymerization at elevated temperatures and stimulated by NaCl. Far more intensive support of polymerization showed potassium ferrocyanide and potassium iodate (as table salt additives), which decreased AA content by 61 % and 88 %, respectively, in comparison to pure NaCl. Comparing infrared spectra of AA and the product formed during the experiments, it was found that all inorganic compounds brought about polymerization of AA molecules through new C-C linkages among vinyl groups, while carbonyl as well as amino groups of AA molecules remained in the original state. Based on the treatment of experimental results, parameters characterizing the kinetics of AA polymerization at non-isothermal conditions were established.

Acrylamide (AA) is a toxic compound formed during thermal processes of food production as one of numerous products of Maillard reaction taking place between reducing saccharides and amino acids, when key roles play such compounds as reducing saccharides and asparagine [1, 2]. Formation and occurrence of AA in various foods is considered a risk factor due to its ability to increase probability of postmenopausal endometrial and ovarian cancer [3]. Since AA is formed from precursors frequently occurring in raw materials, procedures preventing totally its formation seem to be unrealistic. On the other hand, several papers have dealt extensively with procedures of AA elimination in final food products [4–6]. Up to date, a lot of procedures have been developed to minimize AA content in food matrices, such as amino acids addition [7, 8], usage of additional or special technological procedures [9, 10], removal of asparagine by enzymatic degradation using the enzyme asparaginase [11], addition of antioxidant agents [12], application of divalent ions to prevent formation of the Schiff base, which is an essential intermediate of AA formation [13], or optimization of formulations and production procedures in the bakery process itself [14]. A principally different way of AA removal is its elimination in the presence of NaCl and additives of table salt. This paper summarizes our achievements in the study of AA behaviour in contact with inorganic salts, which are able to initiate polymerization reactions leading to the formation of biologically inactive polyacrylamide.

Effect of NaCl on acrylamide during heating [15]

Equimolar mixture of asparagine and D-glucose was placed into a glass tube and heated at 171.1 °C for 10 min. After that, the sample was cooled and analysed for AA content. The same conditions

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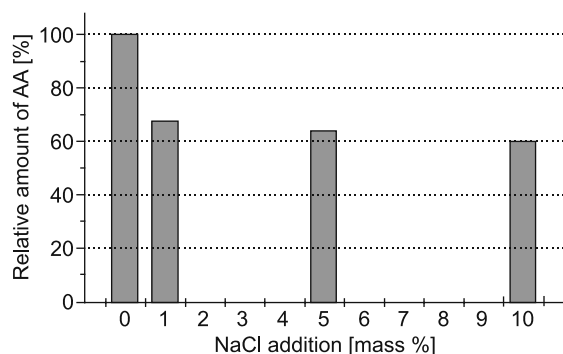Peter Šimko, Tel.: 00 421 2 5557 4622, Fax: 00 421 2 5557 1417, e-mail: peter.simko@vup.sk

Fig. 1. Relative amount of acrylamide in an equimolar mixture of asparagine and glucose after heat treatment at 171.1 °C for 10 min, as a function of NaCl addition.

were maintained, when NaCl was added to the mixture at 1, 5 and 10%. Every heating experiment and determination was repeated for 27 times to obtain sufficient amount of data for calculation of standard deviation as well as relative standard deviation. The decrease in AA content resulting from the heat treatment is shown in Fig. 1. As can be seen, addition of NaCl had considerable inhibitory effects on AA formation in the studied systems. However, the decrease was not linear with the addition of NaCl and the dependence was hyperbolic-like with the tendency to reach a limit value for NaCl addition above 10%. The high decrement in the AA content (by 32%) was already observed for the addition of 1% of NaCl, which is important for practical applications in real technological procedures during thermal food processing. On the other side, the increase in the added amount from 1 to 10% had less effect, when AA content decreased only by 8%.

Study of AA behaviour in contact with NaCl as followed by differential scanning calorimetry [16]

The effect of NaCl on AA behaviour during heating was studied by differential scanning calorimetry (DSC). Pure NaCl, pure AA and the mixture AA and NaCl (1 : 1, w/w) were heated at a heating rate of 10 K·min⁻¹ and recorded. In Fig. 2, the record of NaCl is a horizontal line, which indicates that no process occurs in NaCl when heated up to 180 °C. The DSC record of pure AA shows an endothermic peak with the onset at a temperature of 82 °C corresponding to melting of AA. At 156 °C, an exothermic peak starts, which is obviously connected with polymerization of AA. In the DSC record of the mixture of AA and NaCl,

one can see that melting occurs at the same temperature as for pure AA. However, the polymerization peak is shifted by 12 °C to lower temperatures, so indicating a strong acceleration of the AA polymerization by NaCl. The elimination of AA may occur not only by AA polymerization to biologically inactive polyacrylamide but, in a real food matrix, also by the reaction of AA with other compounds. The DSC measurements have shown that the AA elimination by polymerization is strongly accelerated by the presence of NaCl, which is apparently the reason of the lower AA content in the model food matrix.

Confirmation of AA polymerization in the presence of NaCl and additives of table salt [17]

Individual inorganic compounds (NaCl, $K_4[Fe(CN)_6]$, KIO_3) and AA dissolved in methanol were placed into a 40 ml glass vessel and methanol was removed using a stream of nitrogen. Then, the vessels were sealed tightly with PTFE/silicone septa and heated in a thermostat from 102 °C to 180 °C at a heating rate of 2 °C·min⁻¹. The temperature of reactants inside the vessels was monitored by a thermometer. Systems were sampled periodically by carrying out three parallel measurements. After reaching the selected temperature, the vessels were cooled and their contents were dissolved in methanol. Then, deuterium-labeled acrylamide (D3-AA) dissolved in methanol was added, sonicated for 5 min, filtered and analysed by GC-MS. For Fourier transform infrared (FTIR) spectrometry analysis, 0.5 g of the inorganic compound and AA were mixed and homogenized thoroughly in a mortar dish, then the mixture
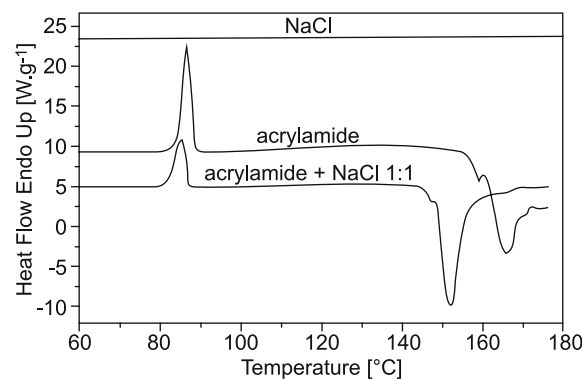

Fig. 2. DSC records of NaCl, pure acrylamide and the mixture of acrylamide and NaCl (1 : 1, w/w) at a heating rate of 10 K·min⁻¹.
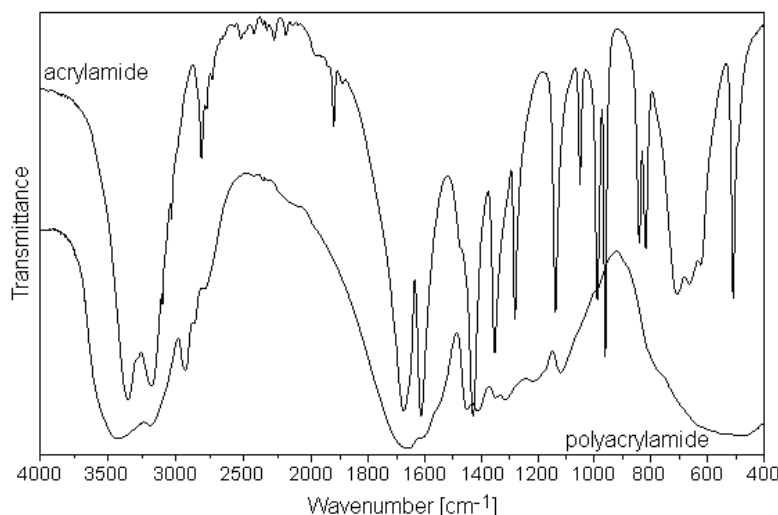
Fig. 3. Infrared spectrum of acrylamide (top) and the isolated product, polyacrylamide (bottom) in a KBr pellet at room temperature.

was placed into a glass tube, sealed tightly with PTFE/silicone septa, placed in a thermostat and heated to 180 °C for 20 min. After cooling, 10 ml of water was mixed with the tube content and the formed gel was centrifuged. To remove completely the inorganic component, the gel washing was repeated ten times, and then it was dried in a laboratory drier at 80 °C. Finally, KBr pellets containing the product and AA were prepared for the FTIR analysis.

FTIR spectra of AA and the product in the mid-infrared region of 4000–400 cm^{-1} are shown in Fig. 3. The AA bands at 3454 cm^{-1} and 3184 cm^{-1} assigned as NH_2 stretching antisymmetric and symmetric modes, respectively, remained practically at the same wave numbers in the polymer spectrum and kept similar shapes and intensities. On the other hand, AA bands corresponding to the C–H stretching modes were above 3000 cm^{-1}, which is typical for olefinic hydrogens and normally indicates the presence of sp^2-hybridized carbon in CH bonds. In the product, these bands disappeared and the new ones appeared at 2952, 2932 and 2867 cm^{-1}, respectively. The area below 3000 cm^{-1} is typical for C–H stretching modes of saturated hydrocarbons with secondary and tertiary bonded carbon in methylene –CH_2– and methine CH groups, respectively. Thus, NH_2 group can be identified from this part of the spectrum of the product and, in comparison with AA, disappearance of the vinyl group and the presence of a saturated carbon chain is notable. IR spectrum of the amide group is characteristic for several modes described in the terms of amide vibrations as amide I (C=O stretching), amide II (NH_2 deformation or scissor-

ing), amide III (C–N stretching), amide IV (C=O deformation or rocking), amide V (NH_2 wagging) and amide VI (C=O wagging). In the condensed-state spectra, the amide I and II modes appear as strong bands in the regions (1680 ± 40) cm^{-1} and (1610 ± 30) cm^{-1}, respectively [22]. Hence, the assignment of AA bands at 1674 cm^{-1} and 1614 cm^{-1} as C=O stretching and NH_2 deformation modes is obvious. C=C stretching mode occurring in this region at 1648 cm^{-1} is very weak in the IR spectrum and, consequently, not useful for diagnostic purposes. The spectrum of the product in this region is very similar to the AA spectrum indicating that both amide modes are present. It means that the amide group remains unchanged in the product. Frequency upshift of C-N stretching mode (amide III) at 1412 cm^{-1} and >CH_2 deformation mode at 1450 cm^{-1} were used as a proof of AA polymerization under the influence of γ-irradiation [23]. The changes regarding the vinyl group can be monitored through its very characteristic three wagging modes including all three hydrogens in –CH=CH_2 group. In two of them, hydrogen atoms in *trans* and *cis* positions participate. They appear in a narrow region (975±35) cm^{-1} and in a wide one (565±165) cm^{-1} [22], and they are also known as CH=CH *trans* and *cis* wagging. The former is usually present as a strong band and often is marked as =CH wagging. Both ending hydrogen atoms mainly participate in the third wagging mode appearing in the region (895±85) cm^{-1}. This mode is marked as =CH_2 wagging. It is usually present as a strong band and additionally accompanied by the very characteristic medium-intensive overtone around 1900 cm^{-1}. This mode

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is sensitive to α-bonded atom and appears in the region (950 ± 30) cm^{-1} in compounds with C=O bond [22]. Therefore, the strong absorptions at ≈975 cm^{-1} and ≈950 cm^{-1} with the overtone at ≈1900 cm^{-1} are the most useful infrared bands to elucidate the –CH=CH$_2$ structure. In the IR spectrum of AA, there is =CH wagging mode at 990 cm^{-1} and =CH$_2$ wagging mode at 962 cm^{-1} with the overtone at 1921 cm^{-1}. Absence of these three bands in the IR spectrum of the product indicates the transformation of the vinyl group into a saturated carbon chain similarly as the above-mentioned results for N-H and C-H stretching regions.

Non-isothermal kinetics of AA elimination and its acceleration by NaCl and additives of table salt [18]

The changes in AA content were followed under non-isothermal conditions. The reason for use of these experimental conditions was the impossibility to establish immediately the isothermal conditions in reaction vessels, in particular at temperatures above 100 °C. The results of measurements showed that the sample reached the target temperature after approximately 5 min, when the approach to the target temperature followed an exponential course. It means that the measurement under "isothermal" conditions would be non-isothermal and the "isothermal" conditions would be ill-defined. Therefore, it was decided to use the well-defined linear heating conditions with the heating rate of 2 °C·min^{-1}. The measured dependence of AA content on temperature is shown in Fig. 4. As follows from the experimental results, NaCl strongly accelerated the decrease in AA content.

Mechanisms of the processes in condensed phase are very often unknown or too complicated to be characterized by a simple kinetic model. They tend to occur in multiple steps that have different rates. To describe their kinetics, methods based on the single-step approximation are frequently used. Within the approximation, the rate of the complex multi-step condensed-state process can be formally described as

$$\frac{d\alpha}{dt} = k(T)f(\alpha) \qquad (1)$$

where t is time, T is temperature, α is the conversion of the process under study, $k(T)$ and $f(\alpha)$ are temperature and conversion functions, respectively. Eq. (1) is mostly called the general rate equation.

The temperature function in Eq. (1) is almost exclusively expressed by the Arrhenius equation. For non-isothermal processes with linear heating, the temperature integral cannot be expressed in a closed form when the Arrhenius equation is used. It has been discussed that, for the single-step approximation, there is no need to be confined to the Arrhenius equation. Other functions leading to the closed form of the temperature integral were suggested. We chose the function

$$k(T) = AT^{m} \qquad (2)$$

where A and m are parameters. Considering the conversion function, for the non-isothermal kinetic data it is recognized that almost any $f(\alpha)$ can satisfactorily fit the experimental data. We chose a first-order conversion function in the form:

$$f(\alpha) = 1 - \alpha \qquad (3)$$

Combination of Eq. (1) – Eq. (3) gives after some manipulations:

$$\int_{0}^{\alpha}\frac{d\alpha}{1-\alpha} = \int_{0}^{t}AT^{m}dt \qquad (4)$$

For linear heating, the dependence of temperature on time can be expressed as

$$T = T_0 + \beta t \qquad (5)$$

where T_0 is the starting temperature and β stands for the heating rate. Combining Eq. (4) and Eq. (5), one can get after integration and some rearrangement:

$$\alpha = 1 - \exp\left[-\frac{AT^{m+1}}{\beta(m+1)}\right] \qquad (6)$$

In Eq. (6), it is assumed that no process occurs at the beginning of the experiment so that the lower temperature integration limit was set $T_0 = 0$ K. The conversion of AA polymerization is defined as

$$\alpha = \frac{g_0 - g}{g_0} \qquad (7)$$

where g_0 and g are the amounts of AA in the reaction vessel at the temperatures T_0 and T, respectively.

In order to avoid the manipulation with extremely small values of the parameter A, for the treatment of experimental results we defined the reduced temperature, ϑ:

$$\vartheta = \frac{T}{T_r} \qquad (8)$$

where T_r is the reference temperature. In this paper, the reference temperature of 126.85 °C

(400 K) was chosen. Then, Eq. (2) can be re-written as

$$k = a \vartheta^m \qquad (9)$$

where

$$a = A T_r^m \qquad (10)$$

Finally, Eq. (6) takes the form

$$\alpha = 1 - \exp\left[-\frac{a T_r \vartheta^{m+1}}{\beta(m+1)} \right] \qquad (11)$$

Combining Eqs. (7) and (11), one can get:

$$g = g_0 \exp\left[-\frac{a T_r \vartheta^{m+1}}{\beta(m+1)} \right] \qquad (12)$$

Eq. (12) was used for the treatment of experimental data. The adjustable parameters were g_0, a and m. For the minimization of the sum of squares between experimental and fitted values of the AA amount, non-linear curve fit was applied using Origin 5 (OriginLab, Northampton, Massachusetts, USA) software. The experimental points and fitted curves are shown in Fig. 4. This single-step approximation is a mathematical tool for describing the kinetics in a simple way represented by Eq. (1), irrespective of the complexity of the overall process. For a complex process, the adjustable parameters in the temperature function have no mechanistic significance or physical meaning, so there is no reason to be confined to the Arrhenius relationship and the use of Eq. (2) is fully justified. The kinetic parameters obtained thus do not have any mechanistic significance. Knowing their values, however, one can model the kinetics of the process without a deeper insight into its mechanism. It is possible to calculate α at a chosen time

for any time-temperature regime. For a constant temperature, taking into account Eq. (9), the time for a first-order reaction can be expressed as

$$t_\alpha = -\frac{\ln(1-\alpha)}{k} = -\frac{\ln(1-\alpha)}{a \vartheta^m} \qquad (13)$$

CONCLUSIONS

1. AA elimination can be accelerated by the addition of NaCl, which is able to initiate polymerization reactions of this food contaminant.
2. Inorganic salts $K_4[Fe(CN)_6]$ and KIO_3, used as table salt additives, exhibit much stronger effects on AA elimination during thermal processing than pure NaCl.
3. Formation of polyacrylamide has been proven by FTIR spectroscopy, which confirmed formation of C-C backbone polymer, while the carbonyl and amino groups remained unchanged.
4. With regard to high AA reactivity and complexity of a real food matrix, polymerization may represent just one of conceivable pathways of acrylamide elimination taking place during thermal processes.
5. For description of AA elimination, the equation and parameters characterizing the kinetics of AA polymerization, e.g. the rate of its elimination at non-isothermal conditions, can be obtained.
6. The kinetic parameters obtained facilitate the calculation of rate constant values for any temperature in an isothermal regime.
7. This approach simplified considerably the complexity of the overall processes taking place in food matrix during heating and makes possible modelling the kinetics of AA elimination without a deeper insight into its mechanism.
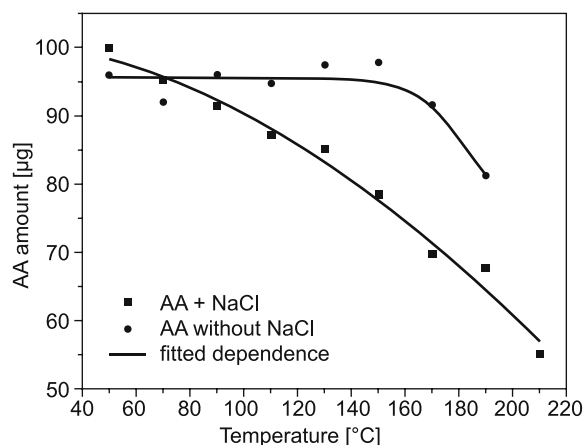
Fig. 4. Changes in acrylamide amount in non-isothermal conditions as a function of temperature.

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