Influence of D-glucose polymers on acrylamide elimination during heating – a model study

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Summary

Acrylamide was applied on naturally occurring D-glucose polymers – potato, rice, wheat and maize starch as well as cellulose and heated at temperatures between 100 °C and 180 °C to study catalytic effects of their crystalline structures on acrylamide elimination at given conditions. At chosen time intervals, samples were analysed for acrylamide contents by GC-MS using negative chemical ionization procedure. The same experiment was carried out with Teflon as the blank matrix. All polymers were found to bring about a decrease in acrylamide contents due to polymerization reactions stimulated by their crystalline structures to be confirmed by X-ray powder diffraction technique, when residual acrylamide contents were lower for all D-glucose polymers in comparison to Teflon. The greatest decrease in acrylamide content was observed for the cellulose matrix able to decrease it by 25%. The kinetic analysis of these processes showed that the influence of cellulose on the rate of acrylamide elimination was highest for temperatures slightly above 100 °C. In general, importance of cellulose to AA elimination is stressed by the fact that its crystalline structure is stabile during heating in presence of water in real food systems while starches loosing their catalytic effects due to disappearance of their crystalline structures forming amorphous swollen matrix.

Keywords

cellulose; starch; acrylamide; GC-MS; elimination; polymerization; kinetics; X-ray powder diffraction technique

Acrylamide (AA) is a toxic compound formed during thermal processes of food production as one of numerous products of Maillard reactions taking place between reducing saccharides and aminoacids, 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]. Because AA is formed from precursors frequently occurring in raw materials, reliable procedures preventing totally its formation seem to be unrealistic. On the other hand, several papers have been published dealing with possibilities of reducing its contents in final food products [4–6] such as asparagine removal by enzymatic splitting [7], addition of antioxidant agents [8] or application of divalent ions to prevent formation of a Schiff base, which is an essential intermediate of AA formation [9]. A principally different way of AA elimination is its elimination in the presence of NaCl and its additives [10–12]. These compounds were found to be able to stimulate polymerization reactions of AA leading to formation of biologically inactive polyacrylamide [13, 14].

D-glucose polymers are major components of foods formed frequently in vegetable materials. Among them, starch and cellulose are the dominant ones, affecting considerably also technological, sensorial and nutritional properties of food products. Starch is, in general, a polymer composed of two different types of macromolecules, amylose and amylopectin. Amylose is a linear polymer containing 1000–2000 D-glucose unit cells linked by α-(1→4) bonds [15]. Amylopectin is a branched-chain polysaccharide with a much larger molecule. The number of glucose unit cells varies widely but may be as high as 10^6. In addition to α-(1→4) linkages, amylopectin has branches at about every 24–30 D-glucose unit cells through

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α-(1→6) linkages. These branches continue with α-(1→4) linkages, but then they may have subsidiary branching giving a tree-like structure. Starch is biosynthesized as granules with dimensions in the range of 1 to 100 μm and its properties strongly depend on their crystalline ultrastructure [16]. In general, starch chains crystallize in two polymorphic forms, the A- and B-type structures based on left-handed double-stranded helices parallel-packed into monoclinic and hexagonal unit cells, respectively [17]. Cellulose is the major polysaccharide in the primary and secondary cell walls composed of linear polymer chains of β-(1→4) linked glucose unit cells. In nature, cellulose never occurs as a single chain, but from the moment of its biosynthesis it occurs as a crystalline array of microfibrils which contains 36 parallel polysaccharide chains stiffened by intra- and inter-molecular hydrogen bridge bonds [18]. Due to the fact that the role of inorganic crystalline structures on elimination of AA by its polymerization reactions has already been found [13, 14], the aim of this work was to study effects of D-glucose crystalline structures on AA behaviour during heating.

MATERIALS AND METHODS

Chemicals

Acrylamide of p.a. purity was purchased from Fisher Scientific (Loughborough, United Kingdom) and 2,3,3-D3 AA (98%) was purchased from Cambridge Isotope Laboratories (Andover, Massachusetts, USA). Acetone SupraSolv was obtained from Merck (Darmstadt, Germany), acetonitrile ChromaSolv and Methanol ChromaSolv from Sigma-Aldrich (Steinheim, Germany). Starches and acid-washed cellulose were purchased from Fluka (Steinheim, Germany).

Instruments

For AA determination, a 6890N gas chromatograph equipped with a 5973 inert mass selective spectrometer (both from Agilent Technologies, Palo Alto, California, USA) was used.

Metal Block Thermostat was purchased from Liebisch, Bielefeld, Germany, and EcoScan Temp JKT Temperature Meter equipped with Probe 3T520C was obtained from Eutech Instruments Europe (Nijkerk, Netherlands). Nylon filters (pore size, 0.45 μm) were purchased from Supelco (Bellefonte, Pennsylvania, USA).

Powder X-ray diffraction patterns were measured with a powder diffractometer 1730/10 (Philips, DA Best, Netherlands) connected to PC for data collection. Radiation was generated by a CuK α source (0.154128 nm). Exciting voltage was 40 kV, anode current was 35 mA. The instrument was operated over the 2 theta range of 3–60° and a step size of 0,02°. Sample was surface plain, placed in a nickel sample holder, measured and stored at room temperature. Samples were crushed using an agate pestle and mortar prior to measurements and presented as a lightly compressed powder disk.

Experiment

An amount of 1 g of D-glucose polymer and 100 μg of AA dissolved in acetone were placed in a 40 ml glass tube equipped with a PTFE/silicone septum, and acetone was removed using a stream of nitrogen. Then, the tubes were heated in the thermostat from 100 °C to 180 °C at a heating rate of 2 °C·min^{-1}. The temperature of reactants inside the tubes was monitored by a thermometer. At chosen temperatures, the contents of four vessels were cooled, treated and analysed. For comparison purposes, Teflon instead of D-glucose polymer was used as a blank.

AA determination

After cooling, D3-AA as an internal standard and 5 ml of a mixture of acetonitrile – methanol (8 : 2, v/v) were added, the vessel content was sonicated for 5 min, filtered and analysed by GC-MS. A volume of 1 μl of the extract was applied into a splitless injector (purge time 0.5 min at 250 °C) and separation was carried out using an Agilent 122-3232 30 m × 0.25 mm × 0.25 μm fused silica capillary column coated with a DB-FFAP phase. The column was held at 50 °C for 1 min, then heated to 250 °C at a rate of 10 °C·min^{-1}. The carrier gas (helium) flow was maintained at 0.8 ml·min^{-1} by an electronic control of pressure. Under these conditions, AA and D3-AA eluted at 13.2 min. The data accumulation was not initiated until 12 min to avoid detection of the large peaks of acetonitrile and methanol. AA amount was determined from the ratio of the peak area of AA to the peak area of the known amount of spiked D3-AA. The detection was carried out by the mass detector working in a selected ion monitoring mode; the ions were obtained by the negative chemical ionization procedure using methane as the reagent gas. The mass of the most intense fragments was 70.15 and 73.15 m/z, respectively.

RESULTS AND DISCUSSION

First, the presence of crystal structures in starches and cellulose was studied using X-ray

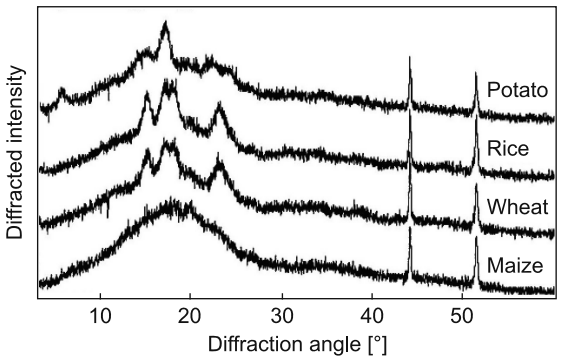

Fig. 1. Diffraction patterns of starches.
Two sharp diffraction lines at ~ 44° and ~ 51.5° 2Θ originate from sample holder due to sample transparency.

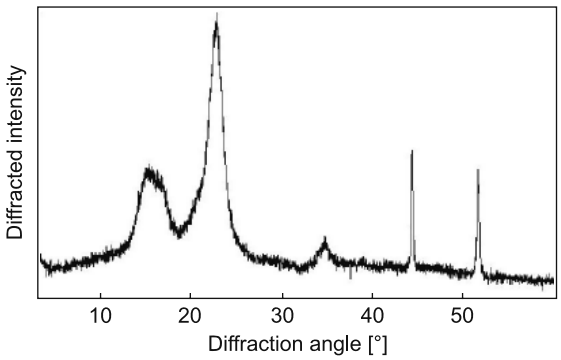

Fig. 2. Diffraction pattern of cellulose.
Two sharp diffraction lines at ~ 44° and ~ 51.5° 2Θ originate from sample holder due to sample transparency.

powder diffraction (XRPD) technique. Crystalline nature of starches and cellulose was confirmed by XRPD measurements, as shown in Fig. 1 and Fig. 2. It is necessary to note that the XRPD patterns of all samples are composed of the broad diffraction peaks superposed on characteristic amorphous halo pattern that indicates the lack of three-dimensional long range ordered structure and small crystal size, which is typical for paracrystalline phases.

The changes in AA contents were followed under non-isothermal conditions because of impossibility to reach immediate isothermal conditions in the vessels. In particular, at temperatiures above 100 °C, the isothermal conditions are ill-defined [14]. Therefore, we decided to use the well-defined linear heating with the heating rate of 2 °C·min^{-1}. AA contents decreased with the incerease in the temperature (Fig. 3).

For comparison, the same experiment as for starches and cellulose was carried out using Teflon powder as the blank polymer matrix. As can be seen, the rate of AA elimination was the slowest in the Teflon matrix, while AA elimination occured most rapidly in the cellulose matrix where the AA content was reduced by 25%. For the starches, the decrease in AA content was between 19 and 22%.

The kinetic data of AA elimination were treated using the procedure described in [14]. The procedure is based on the use of non-Arrhenian temperature function

$$k(T) = AT^m \quad (1)$$

where A and m are parameters. Since the tempera-

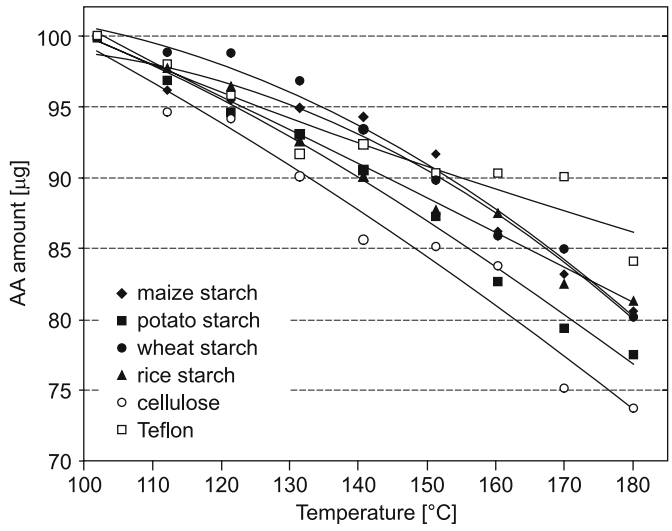

Fig. 3. Decrease in acrylamide amounts heated in the presence of D-glucose polymers and Teflon.

Tab. 1. Kinetic parameters describing the kinetics of AA elimination in various matrices and the values of temperature functions and half-times for temperatures of 120 °C and 180 °C.

Polymer	a [min^{-1}]	m	k (120 °C) [min^{-1}]	τ (120 °C) [min]	k (180 °C) [min^{-1}]	τ (180 °C) [min]
maize	3.49×10^{-3}	9.357	2.97×10^{-3}	233	1.12×10^{-2}	61.7
potato	5.68×10^{-3}	4.146	5.29×10^{-3}	131	9.52×10^{-3}	72.8
rice	4.42×10^{-3}	4.803	4.07×10^{-3}	170	8.05×10^{-3}	86.1
wheat	4.25×10^{-3}	7.404	3.74×10^{-3}	185	1.07×10^{-2}	64.7
cellulose	6.63×10^{-3}	3.374	6.25×10^{-3}	111	1.01×10^{-2}	68.7
Teflon	2.37×10^{-3}	8.415	2.05×10^{-3}	339	6.77×10^{-3}	102

ture of the samples increases linearly, the dependence of temperature on time can be expressed as

$$T = T_0 + \beta t \tag{2}$$

where T_0 is the starting temperature and β stands for the heating rate. Then, for the first-order kinetics of AA elimination, one can get [14]:

$$g = g_0 \exp\left[-\frac{aT_r\vartheta^{m+1}}{\beta(m+1)}\right] \tag{3}$$

where g_0 and g are the amounts of AA in the reaction vessel at the temperatures T_0 and T, respectively, ϑ is the reduced temperature defined by the equation

$$\vartheta = \frac{T}{T_r} \tag{4}$$

where T_r is a reference temperature. In this paper, the reference temperature 126.85 °C (400 K) is chosen. The parameter a is given as

$$a = AT_r^m \tag{5}$$

Eq. (3) 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). The resulting values of the parameters are listed in Tab. 1, the experimental points and fitted curves are shown in Fig. 3.

The kinetic parameters a and m, obtained from the non-linear regression, enable modelling the kinetics of AA elimination without a deeper insight into its mechanism [14]. It is possible to calculate the AA amount at a chosen time for any time-temperature regime. For a chosen constant temperature, the value of temperature function can be obtained by the relationship [14]

$$k = a\vartheta^m \tag{6}$$

and the related half-time of the reaction (τ) can be obtained by the equation

$$\tau = \frac{\ln 2}{k} \tag{7}$$

The calculated values of temperature functions and half-times for the temperatures of 120 °C and 180 °C are also shown in Tab. 1. It can be seen from the values of half-times that the elimination of AA was the slowest on the Teflon matrix. All D-glucose polymer matrices increased the rate of AA elimination in comparison to Teflon. Unambiguously, the lowest half-time of AA elimination at 120 °C was seen for the cellulose matrix while the half-times for starch matrices were higher by 30–120%. The situation was quite different at 180 °C where cellulose and starches (except for the rice starch) had very similar values of half-times. However, also at this temperature, elimination of AA on Teflon had the lowest rate.

CONCLUSIONS

On the basis of experimental results, these remarks might be postulated as follows:

- Due to their crystalline structures, D-glucose polymers are able to bring about a decrease in AA content by stimulation of its polymerization reactions during heating.
- The most pronounced effect has cellulose, which decreased the AA amount by 25%.
- Catalytic effects of cellulose are significant due to the fact that the cellulose does not lose its crystal structure during thermal processes in a real food matrix in contrast to starches, which are forming an amorphous swollen matrix due to interaction with water during heating, what is typical for real food production procedures.

– The kinetic analysis of the process showed that the influence of cellulose on the rate of AA elimination was highest for temperatures slightly above 100 °C.

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