Short communication

Determination of Acrylamide in Malt with GC/MS

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Abstract

In food, acrylamide is produced in the course of Maillard reaction and its precursors are reducing saccharides and amino acid asparagine. Acrylamide formation in food depends on food composition and processing conditions. Significant quantities are formed during heat treatment above 120 °C, mostly at 150–180 °C, while at still higher temperatures the extent of formation decreases.

Barley is the raw material for malt production. It is a crop with high content of nitrogen compounds and a high content of starch. During malting, enzymatic activity leads to an increase in the content of reducing saccharides in malt, and during kilning, biochemical changes lead to melanoidin production and these conditions are favourable for acrylamide formation. These processes were studied in several malts using gas chromatography-mass spectrometry. Acrylamide was determined in significant amounts from several μg kg^{-1} – mg kg^{-1}.

Keywords: Acrylamide, gas chromatography, mass spectrometry, malt

1. Introduction

In 2002 the detection of acrylamide (Figure 1), neurotoxic and potentially carcinogenic substance, in food processed at temperatures >120 °C was reported.[1–3] High acrylamide content was found especially in food with a high starch content, such as food from potatoes and cereals.[4]

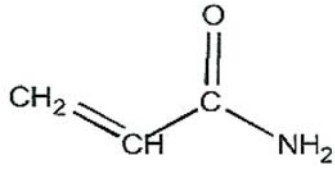

Figure 1. Structural formula of acrylamide (C_3H_5NO, CAS 79-06-1).

Acrylamide originates in the Maillard reaction and its precursors are reducing sugars and asparagine. The mechanisms leading to acrylamide formation in food depend on food composition and processing conditions.[5,6] There are three reaction phases:[7,8]

– production of glycosylamine, followed by Amadori rearrangement,
– dehydration and fragmentation of saccharides accompanying Strecker degradation of amino acids occur,
– Strecker aldehyde undergoes further reduction and dehydration reactions and acrylamide is created.

Besides this, studies of model systems have shown that under certain conditions acrolein and acrylic acid also participate in acrylamide formation after reaction with asparagine in foods rich in lipids.[8,9] Further research in model systems of glucose and asparagine showed the effect of temperature and time of heating. Acrylamide formation was low within the temperature range of 120–140 °C; if increased to 160–180 °C, the content increased dramatically. After reaching 180 °C, acrylamide production declines. The decline in acrylamide formation with higher temperatures can be explained by the fact that acrylamide as an intermediate product of Maillard reaction decomposes into other products.[9,10] Certain processes during production of malt are also favourable for acrylamide formation.[11]

The most frequently used methods for acrylamide determination are[12] HPLC-MS and GC-MS. In the latter case, acrylamide is derivatised by bromation.[13,14]

The advantage of acrylamide bromation is that the product is more volatile and less polar. The resulting derivate is readily extracted from aqueous solutions and it can be more easily detected with GC-MS. Conversion of acrylamide to 2,3-dibromopropionamide is usually performed by an addition of anhydrous potassium bromide, hydro-

bromic acid and a saturated solution of bromine in water. By adding trietylamine, non-stable 2,3-dibromopropionamide is converted to more the stable derivate 2-bromopropenamid.[14]

2. Materials and Methods

2.1. Chemicals

The following substances were used as standards: acrylamide (1 mg mL^{-1}) in methanol (Absolute Standards, USA), ($^{13}C_3$) acrylamide (1 mg mL^{-1}) in methanol (Cambridge Isotope Laboratories, USA), 2,3-dibromopropionamide (1 mg mL^{-1}) in methanol (Absolute Standards, USA). Bromine, potassium bromide, hydrobromic acid, sodium thiosulphate and trietylamine were all obtained from Merck (Germany). Methanol, ethylacetate (for HPLC, Chromservis, Czech Republic) were used.

2.2. Malt Samples

Samples of barley malt for the determination of acrylamide were taken in the course of malting (kilning). The first sample was taken at 60 °C and the last one at 210 °C, sampling was done in intervals of 10 °C. In addition, samples of special and coloured malts were taken (Table 1).

Table 1. The samples of special and coloured malts used in this study.

Pale malt	Pilsener Munich
Special malt	Smoked Melanoidin
Caramel Malt	CARAPILS® CARAHELL® CARARED® CARAAMBER® CARAMUNICH® CARAAROMA®
Roasted malt	CARAFA® CAAFA®SPECIAL
Wheat malt	Wheat – pale Wheat – dark CARA – WHEAT Wheat – roasted
Rye malt	Rye – pale Rye – caramel Rye – roasted

2.3. Sample preparation

10 µl of internal standard ($^{13}C_3$ acrylamide) and 50 mL of distilled water (60 °C) were added to the ground sample (5 g). After sonication (20 min) the homogenate was transferred quantitatively into centrifuge tubes and centrifuged at 8000 rpm^{-1} for 30 min. 2 g of KBr and HBr acid (pH 0–1) were added to 5 mL of supernatant. After cooling, 2 mL of bromine water was added. Contents in the flask were stirred and the flask was placed in a container with crushed ice for 10 h into the refrigerator. After bromination the excess bromine was titrated with $Na_2S_2O_3$ (1 mol L^{-1} solution) until discoloration occurred. The content of the flasks was transferred to teflon centrifuge tubes, 5 mL of ethylacetate was added to each tube and the content was shaken for 3 min and then centrifuged at 5000 rpm^{-1} for 5 min. After centrifugation, 1 mL of the organic phase was delivered with a pipette to a plastic microtube and triethylamine (0.2 mL) was added. Microtubes were shaken and after 15 min, they were centrifuged at 5000 rpm^{-1} for 5 min. After centrifugation, the tube content was delivered to glass vials and analyzed using GC/MSD.

2.4. Instrumentation

Gas chromatograph Trace GC Ultra Finnigan with mass detector Trace DSQ Thermo Finnigan was used with the DB-WAX capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; J&W Scientific, USA). The column was held at 50 °C (1 min), then programmed at 15 °C min^{-1} to 150 °C (5 min). The temperature of the PTV injector was 200 °C in splitless mode (1 min). Transfer line temperature was 200 °C. The mass selective detector operated in selected ion monitoring (SIM) mode with positive electron impact (EI) ionization. The carrier gas was helium with a flow 1.5 mL min^{-1}. Under these conditions, the retention time of acrylamide and ($^{13}C_3$) acrylamide derivatives was 16.34 min.

Identification of acrylamide was conducted on the basis of retention time and for 2-bromopropenamide of specific ions *m/z* 149 and 151, quantification was performed using a calibration curve. To attain reliable results and maximum selectivity, the isotopically marked ($^{13}C_3$) acrylamide (*m/z* 152.154 for 2-bromo($^{13}C_3$)propenamide) was used as an internal standard.

3. Results and Discussion

The calibration curve was linear in the range from 30 to 620 µg kg^{-1} (acrylamide content in a real sample) with the correlation coefficient of 0.9985, LOQ was 25 µg kg^{-1}. Recovery was confirmed by samples spiked with isotopically marked ($^{13}C_3$) acrylamide and it ranged from 72%–86%.

Figure 2 shows the results of the dependence of acrylamide formation on kilning temperature. Maillard reactions occur at two main stages in the malting process; during wort boiling and in the production of speciality malts. Therefore, there is a strong possibility that acrylamide could be found in beer.

During kilning, acrylamide is already created from 60 °C. Formation of acrylamide at temperatures from 60–100 °C can be explained by long heating at temperatures above 60 °C. Malt is pre-dried for even 10 h at temperatures of 55–65 °C, after which kilning at temperatures follows, which is specific for the given type of malt (to 225 °C), held from 90 min to 2.5 h. Maximal acrylamide formation was found in the temperature interval 150–170 °C. Then, a decrease in acrylamide formation follows. The decrease may be explained by the fact that acrylamide as an intermediate product of Maillard reaction.[7,8]

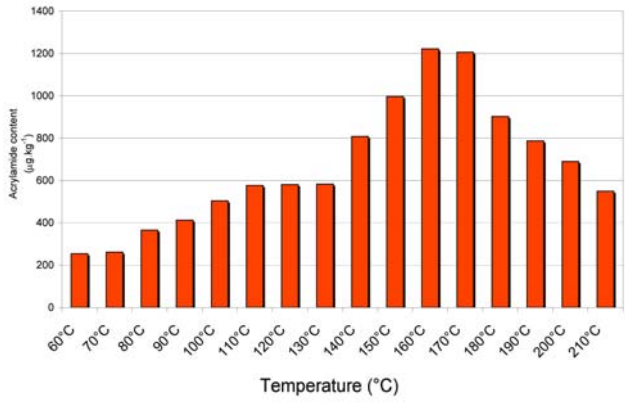

Figure 2. Dependence of acrylamide formation on kilning temperature.

Figure 3 shows acrylamide content in special and coloured malts Coloured and special malts are typically prepared by roasting kilned malt, a process comparable to coffee roasting.[15] In pale malts, acrylamide content was in the range of 630–660 μg kg^{-1}. In special melanoidin malt its content was 2210 μg kg^{-1}. Melanoidin malt has the same acrylamide content as the malt sampled in the course of kilning at 130 °C. This high value of acrylamide corresponds to the melanoidin malt preparation conditions, at which the Maillard reaction is promoted. Rye malts exhibit lower acrylamide contents in caramel and roasted malts than barley malts prepared at the same temperatures. Lower acrylamide content is caused by different contents of asparagines and reducing sugars in modified rye malt. In a similar way, the lower acrylamide content in wheat malts can be explained.

In roasted barley malts, the highest acrylamide content is in the malt CARAFA® SPECIAL. The highest acrylamide content of all the malts analyzed was determined in malts of the type CARAMÜNICH (3084 μg kg^{-1}). This corresponds to the method of caramel malt production (kilning temperature 150–170 °C) and conforms with the described dependence of acrylamide formation on temperature during kilning.

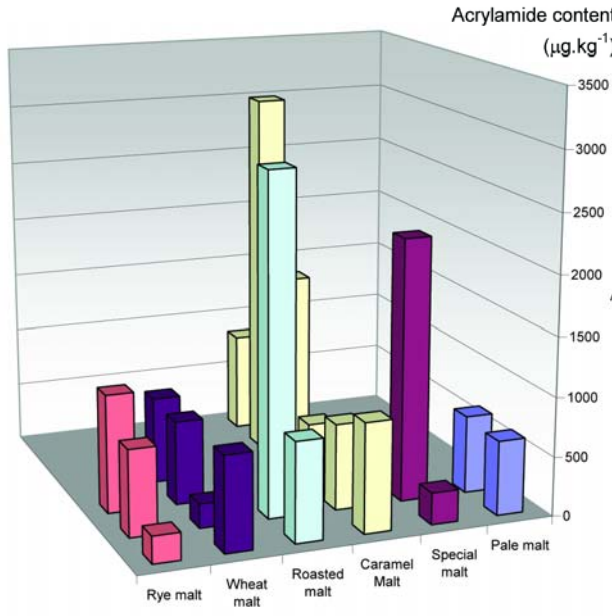

Figure 3 Acrylamide content in special malts.

4. Conclusion

Malt analyses proved the dependence of acrylamide content on kilning temperature. With the increasing temperature, acrylamide content in malt increased until 180 °C, after which it starts to decrease, due to further reactions of non-enzymatic browning. Higher acrylamide contents were found in special and coloured malts. This fact is connected with higher temperatures during kilning of these malts.

5. Acknowledgement

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6. References

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Povzetek

Tvorba akrilamida v hrani je posledica Maillardove reakcije. Prekurzorji so reducirajoči sladkorji in aminokislina asparagin. Na tvorbo akrilamida vpliva tudi temperatura, pospešena je pri temperaturah nad 120 °C, zlasti pa pri 150–180 °C, medtem ko pri še večjih temperaturah akrilamid sodeluje v nadaljnjih reakcijah in se njegova vsebnost zmanjša. Ječmen je surovina, iz katere pridelujemo slad. Vsebuje veliko dušikovih spojin in škroba. Med pridelavo sladu se vsebnost reducirajočih sladkorjev povečuje zaradi encimskih reakcij. Biokemijske reakcije med nadaljnjimi procesi vodijo to tvorbe melanoidina. Nadaljnji nastanek akrilamida smo preučevali v več vzorcih sladu z uporabo plinske kromatografije z masnospektrometrično detekcijo. Določili smo ga v intervalu med μg kg^{-1} in mg kg^{-1}.