Heat-Induced Degradation of Inulin

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Abstract: Heat treatment of inulin at 135 to 190°C leads to a decrease in the measurable amount of the fructan, when quantified as fructose after enzymatic hydrolysis. Using high-performance anion-exchange chromatography with pulsed amperometric detection, degradation of the fructan chains and concomitant formation of low-molecular products was observed, most likely representing di-D-fructose dianhydrides. Heat-induced degradation of inulin during thermal processing of foods like bakery products must be taken into account within the discussion about possible prebiotic properties of the fructan.

Keywords: inulin; fructooligosaccharide; HPAEC-PAD; prebiotic; di-fructose dianhydrides

INTRODUCTION

Inulin, a mixture of linear fructans consisting of 1,2-β-linked fructose bound to a terminal sucrose residue, is used as prebiotic food ingredient in milk or bakery products. Doses of 4 to 5 g inulin per day shall be efficient in stimulating the growth of gastrointestinal bifidobacteria. The degree of polymerisation (dp) of inulin varies from 2 to 70 [1, 2]. Oligofructose is a partial enzymatic hydrolysate of native inulin with a dp ranging from 2 to 10 [3]. Several reports in the literature indicate that such low-molecular inulin or oligofructose shows superior prebiotic and bifidogenic effects compared to long-chain inulin [4–6]. Up to now, only very limited information exists on reactions of inulin during food processing. It was shown that the total content of fructan was reduced during baking of bread to 33% [1, 3], but structural consequences resulting from this reactions are not known. Furthermore, knowledge about heat-induced degradation of the carbohydrate is important with respect to food labelling concerning the functionality of inulin. The purpose of our study was to quantify the total amount of inulin after dry heating as well as to monitor structural changes using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

EXPERIMENTAL

Materials and methods. Inulin from chicory was from Sigma-Aldrich (Deisenhofen, Germany). For quantitative determination of the inulin content, a commercial test kit from Megazyme International (Bray, Ireland) was applied. Chemicals used were of highest purity available.

Determination of inulin. The instructions by the manufacturer were strictly followed. The assay from Megazyme is based on enzymatic hydrolysis of sucrose, starch and maltooligosaccharides present in the sample by the combined action of an enzyme mix (sucrase, pullulanase, maltase and β-amylase), followed by reduction of the sugars to the corresponding sugar alcohols by treatment with alkaline borohydride. Fructan and inulin are then hydrolysed to fructose and glucose with fructanase (exo-inulinase). The released sugars are measured photometrically after derivatisation with *p*-hydroxybenzoic acid hydrazide (PAHBAH).

HPAEC-PAD. A dedicated chromatography system was used, consisting of a pump 709, detector 817, valve unit 812 and filtration sample processor 788, controlled via Metrodata IC Net 2.3 software (all from Metrohm GmbH & Co. KG, Filderstadt, Germany). The column was a Metrosep A Supp 1 (250 × 4.6 mm; Metrohm, Filderstadt, Germany). Injection volume was 20 µl of samples containing

between 1 to 2 mg carbohydrate per ml distilled water. Elution was performed at a column temperature of 35°C and a flow rate of 1 ml/min, using a solution of 10 mM sodium acetate in 100mM sodium hydroxide as buffer A and 200 mM sodium acetate in 200 mM sodium hydroxide as buffer B. The elution programme started with an isocratic elution at 13% B for 10 min, followed by a linear gradient to 100% B within 55 min, isocratic elution at 100% B for 50 min and a final linear gradient from 100% B to 13% B within 20 min. Detection was achieved by triple-pulsed amperometry with a gold electrode. Pulse programme of the detector electrode potential was +0.05 V (0 to 0.4 s), +0.075 V (0.41 to 0.6 s) and –0.15 V (0.61 to 1.0 s) with a sample time of 100 ms. The flow cell was operated at 35°C.

Heating experiments. Inulin samples (0.5–1 g) placed in open glass vials, were heated in an oven up to 60 min at temperatures between in 100 to 195°C.

RESULTS AND DISCUSSION

Dry heating leads to a decrease of the amount of "intact" inulin from chicory when measured as fructose after enzymatic hydrolysis (Figure 1). Significant degradation is observed for temperatures between 165 and 195°C, which corresponds to conditions used for baking. For this temperatures, between 50 to 90% of inulin degradation was found already after 30 min of heating, increasing to nearly complete degradation after 60 min.

This results indicate that due to thermal treatment of inulin, reaction products must be formed in which glycosidic bonds are no longer accessible for β-fructosidase. Our data are in agreement with Fretzdorff and Welge [7] as well as Praznik *et al*. [1], who reported that between 10 to 30% of inulin is degraded during conventional bread baking. Only few studies have been published concerning degradation of inulin during heating. For mapping changes in the inulin profiles occurring during thermal treatment, high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) was applied.

As can be seen from Figure 2, fructose oligomers starting from a dp of 5 up to 40 can be separated within a run time of 135 minutes. Range of dp was estimated using commercial 1,1,1-kestopentaose. Based on this signal, the dp values increase step by step with increasing retention time [8]. Separation of short-chain oligofructoses with dp values below 4 was not possible. The dp values of the commercial inulin sample from chicory roots, which was used for the heating experiments, were mainly between 13 and 30 (Figure 2a). With increasing temperature and heating time, the intensity of signals characteristic for long fructose chains decreased and an increase of signals due to low-molecular compounds could be detected (Figures 2b,c). After heating 15 min at 195°C, signals of fructose oligomers have nearly disappeared, concomitant with the formation of one dominating peak eluting immediately at the start of the chromatogram (Figure 2d). Separation of this compounds with an apparent dp below 5 was not possible. In this context it seems noteworthy, that Christian and Manley-Harris [9] were able to characterise di-fructose dianhydrides (DFDAs) as products formed during heating of inulin in the presence of citric acid. It remains open, whether DFDAs are formed also during food processing, being responsible for the observed inulin degradation. Furthermore, consequences for the prebiotic properties of inulin will have to be clarified. Quantification of DFDAs in processed

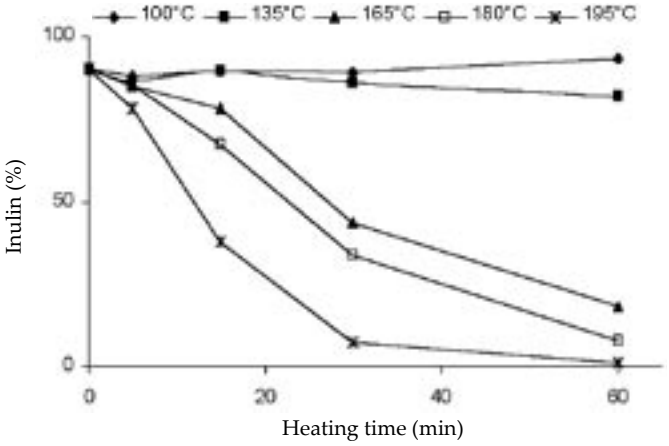

Figure 1. Degradation of inulin from chicory during dry heating. Inulin was quantified as fructose after enzymatic hydrolysis

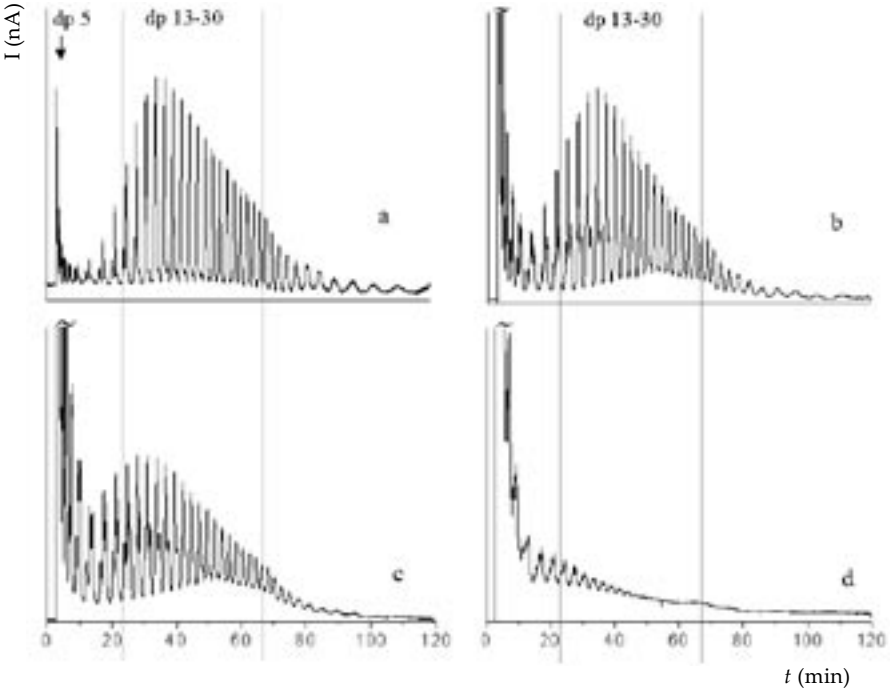

Figure 2. HPAEC-PAD of inulin from chicory. a – unheated sample, b – 15 min at 165°C, c – 15 min at 180°C, d – 15 min at 195°C

foods might be a suitable tool in order to control heat-induced changes of inulin.

CONCLUSIONS

Dry heating of inulin under the conditions used for baking leads to a significant decrease of the fructan and the formation of low-molecular degradation products, most likely di-fructose dianhydrides, which cannot be hydrolysed by fructanase. The consequences of this heat-induced degradation reactions on the prebiotic properties of inulin are not yet studied.

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