# Isolation of Reaction Products Resulting from Heat-Induced Degradation of Inulin

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**Abstract**: From inulin which had been heated for 30 min at 200°C, four di-D-fructose dianhydrides (DFDAs) were isolated using flash chromatography and final purification by semipreparative HPLC, followed by identification via NMR spectroscopy. The DFDAs α-D-Fru*f*-1,2':2,3'-β-D-Fru*f* (DFA III), α-D-Fru*f*-1,2':2,1'-β-D-Fru*f* (DFA I), α-D-Fru*f*-1,2':2,1'-α-D-Fru*f* (DFA VII) and β-D-Fru*f*-1,2´:2,1´-β-D-Fru*f* were identified. The yield of the isolated DFDAs varied depending on the DP of the used inulin. Using the isolated DFDAs as reference compounds, quantification of the disaccharides in commercial bakery products via high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was possible.

**Keywords**: inulin; di-D-fructose dianhydrides; DFDA; NMR; bakery products

## INTRODUCTION

Inulin is an energy source of various plants like chicory and Jerusalem artichoke and contains fructan chains of different degree of polymerization (DP). It is used to increase the amount of dietary fibre or as prebiotic ingredient in particular in dairy and bakery products (Roberfroid 2007). Previous studies in our laboratory showed that dry heating of inulin under conditions resembling baking (up to 1 h heating between 135°C and 195°C) induced a significant degradation of the fructan ranging from 20 to 100%, which significantly influences quantification of inulin especially in heat-treated samples. Furthermore, we had observed the formation of low-molecular degradation products which were cleavable by acid to fructose (Boehm *et al*. 2005). Inspired by studies of Christian and Manley-Harris (2000), the aim of the present study was to identify degradation products formed during a dry heating of inulin.

## MATERIALS AND METHODS

Inulin (Raftiline® HP) was from Orafti (Belgium). All chemicals used were of highest purity available. Amounts of 1 g of Raftiline® HP were heated at 200°C for 30 min. Analytical characterization was performed by HPAEC with pulsed amperometric detection as described by Boehm *et al*. (2005). Degradation products were isolated from the heated inulin, dissolved in water (500 mg in 5 ml), by flash chromatography, using a column (450 × 30 mm) filled with cellulose (Merck, Darmstadt, Germany), which was eluted with a mixture of ethylacetate/ethanol/glacial acetic acid/water (3:2:2:2). Fractions of 5 ml were collected and those containing individual compounds were pooled. The solvent was removed and the dried residues were dissolved in acetonitrile/water (85:15). Further purification was performed via semipreparative HPLC with RI-detection, using an amino-modified column (250 × 8 mm; Knauer, Berlin, Germany), eluted isocratically with acetontrile/water 85:15. After removing the solvent, the isolated compounds were identified by nuclear magnetic resonance spectroscopy (NMR). The $^{1}$H-, $^{13}$C-, HSQC-, HMBC- and COSY-spectra were recorded. Further details are described in Trabs *et al*. (unpublish).

## RESULTS AND DISCUSSION

Raftiline® HP, an inulin with a degree of polymerization of 24, was heated for 30 min at a tempera-

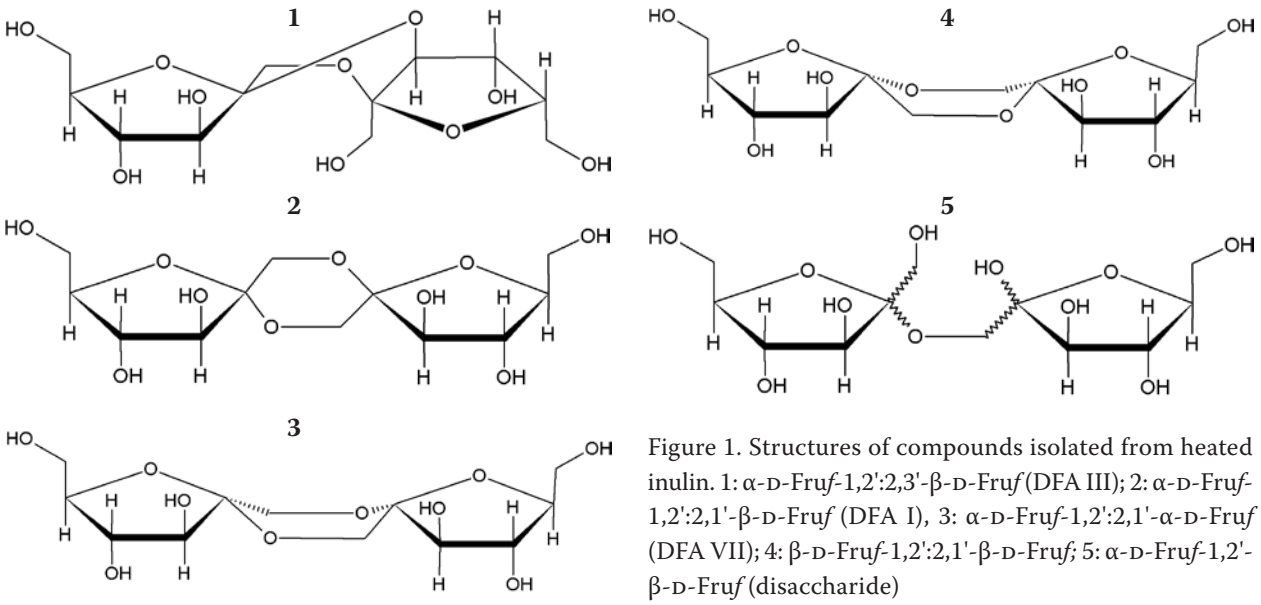


Figure 1. Structures of compounds isolated from heated inulin. 1: α-D-Fru*f*-1,2':2,3'-β-D-Fru*f* (DFA III); 2: α-D-Fru*f*-1,2':2,1'-β-D-Fru*f* (DFA I), 3: α-D-Fru*f*-1,2':2,1'-α-D-Fru*f* (DFA VII); 4: β-D-Fru*f*-1,2':2,1'-β-D-Fru*f*; 5: α-D-Fru*f*-1,2'-β-D-Fru*f* (disaccharide)

ture of 200°C. The resulting caramel contained a wide range of degradation products, like fructose, glucose and possible di-D-fructose dianhydrides (DFDAs) as demonstrated by thin layer chromatography (Boehm *et al*. 2006). DFDAs were isolated from this mixture using flash-chromatography and semipreparative HPLC on an amino phase. Identification was achieved by nuclear magnetic resonance spectroscopy ($^{1}$H-spectrum, $^{13}$C-spectrum, HSQC-, HMBC-, COSY-spectrum). Among the five chromatographically pure isolated substances, four different DFDAs could be identified, namely α-D-Fru*f*-1,2':2,3'-β-D-Fru*f* (DFA III), α-D-Fru*f*-1,2':2,1'-β-D-Fru*f* (DFA I), α-D-Fru*f*-1,2':2,1'-α-D-Fru*f* (DFA VII) and β-D-Fru*f*-1,2':2,1'-β-D-Fru*f* (Figure 1). The yield of the isolated DFDA varied depending on the DP of the used inulin. In addition to the DFDAs, a disaccharide (compound 5 in Figure 1) was isolated. Further studies concerning its origin are necessary.

Using the isolated DFDAs as reference compounds, quantification of the disaccharides in

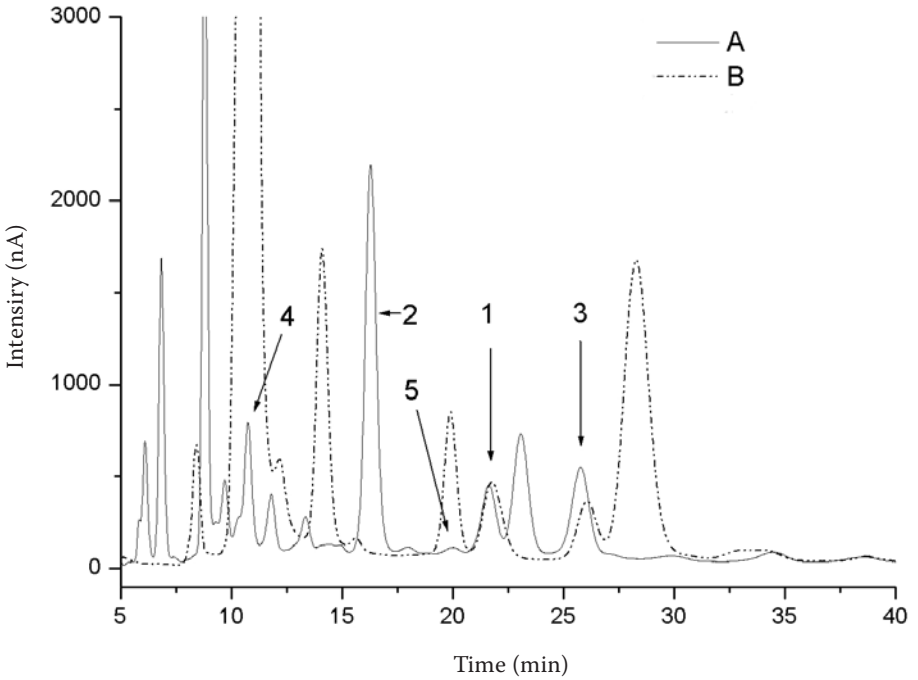


Figure 2. HPAEC-PAD chromatogram of (A) an inulin caramel syrup after heating for 30 min at 200°C and (B) a biscuit containing inulin caramel syrup. Numbers refer to compounds shown in Figure 1

inulin caramel syrups (Figure 2A) and food samples such as commercial bakery products was possible using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). For further studies, biscuits containing different amounts of inulin caramel syrup (5–7%) as an exchange for sucrose were used. After aqueous extraction, different DFDAs could be detected. In the analysed bakery products, mainly DFA III, DFA I and DFA VII could be measured (Figure 2B). Due to coelution, the other isolated compounds (compound 4 and 5 in Figure 1) could not be detected in more complex food matrices. For DFA III and DFA VII, amounts ranging around 120 mg/100 g for each of the DFDAs and for DFA I around 10 mg/100 g were found in the analysed biscuits, indicating that DFDAs are formed during conventional baking processes. As DFDAs are discussed as possible bioactive compounds (Hara & Kondo 2005), further studies on the formation of DFDAs in complex foods or certain food constituents such as caramels or sugar couleurs are promising.

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