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# REVEALING TRANSFORMATION PATHWAYS AND DEGRADATES OF INULINE BY NEW ELSD-METHOD

**Abstract:** Within current experimental report a newly developed high performance liquid chromatography (HPLC) method for the measurement of pure inulin and its thermaly degraded derivatives using evaporative light scattering (ELS) detection is proposed. The fructan composition has been characterised by a chromatographic method developed after several optimizations of sample preparation procedure and apparatus. The procedure contains a direct detection of native and thermally treated inulin/fructooligosaccharide components after dissolving samples in water at room temperature and by detecting their chain-distribution with the use of HPLC coupled with ELS detection. The thermal treatment procedures were selected from 150°C up to 230°C (10 °C increments each sample) in order to determine the ideal heating temperature and treating period for preserving inulin's oligomeric or/and polymeric structure. The method is accurate, simple and without interferences from the detectable signals of mono- and disaccharides as thermal decomposition products of inulin. The analytical procedure makes it unnecessary to use artificial hydrolysis of the macromolecule.

Key words: inulin, HPLC, evaporative light scattering detection, degradates, thermal treatment, carbohydrate polimers

## Introduction

Inulin-type fructans can be found in more than 36.000 plant species and they might be regarded as the most abundantly occurring carbohydrates the in nature following starch.[1] Inulin is a polydisperse substance with linear chains containing fructose monomers having a terminal glucose moiety. The number of fructose monomers is typical for the plant produicing inulin. The chain length of inulin varies from 3 to 65 monomer molecules in chicory root with an average degree of polymerisation (DP) of 10.[2]

Inulins with a terminal glucose are known as alpha-D-gluco-pyranosyl-[beta-D-fructo-furanosyl](n-1)-D-fructo-furanosides (or fructo-oligosaccharides), which are abbreviated $GpyF_n$. Inulins without glucose are beta-D-fructo-pyranosyl-[D-

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fructofuranosyl](n-1)-D-fructo-furanosides (or inulo-oligosaccharides), abbreviated as $FpyF_n$. Letter n refers to the number of fructose residues in inulin; py is the abbreviation for pyranosyl, the simplest structural formulas are shown in figure 1.[3, 4] In contrast to the previously applied sample preparation methods (solution in hot water and filtering), comparison of distinctive protocols has been performed. Throughout this study inulin powder-samples of *Chicorium intybus L.* (chicory), Dahlia species (dahlia), *Helianthus tuberosus* (Jerusalem artichoke) were treated in parallel at 8 different temperature values for 9 distinctive time periods. Thermal treatments were carried out from 150 °C up to 230 °C (10 °C increments each sample) in order to characterise the thermal degradation of inulin and determine all the produced oligo- and polymers.[5, 6, 7]

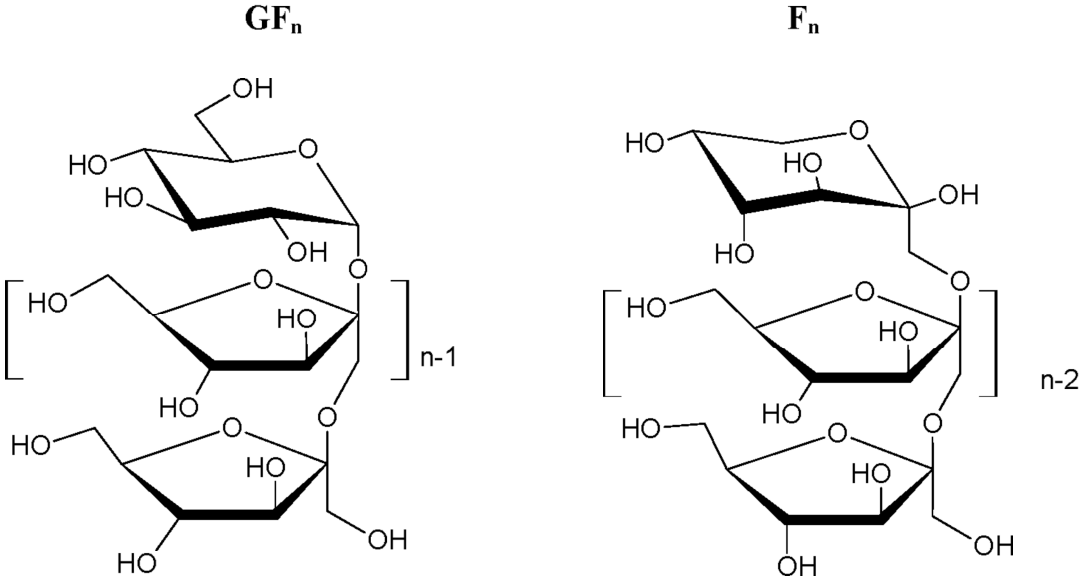


***Figure 1.*** *The structure of inulin, n or m equal to the number of fructose units, G= glucose, F=fructose.*

Relevance of the studies is enhanced by the fact that major prebiotic effect might be attributed to fructans depending substantially on their degree of polymerization.[8, 9] Up to now by using GC, HPLC (High Performance Liquid Chromatography) with RI or PAD techniques average polymerisation degree of inulin was found to be 20. Isolation and identification of fructan oligomers has been accomplished between DP2-DP4[2] so far. The aim of the work was to obtain interpretable chromatographic data of non-heated inulin and its decomposition products, and to reveal the degradation pathways by altering the heating temperature and heating period of inulin. This was accomplished by an accurate, new and simple method achieved by detecting the after-treatment arising degradates

and measuring their quantity using HPLC and ELS (evaporative light scattering) detection. On the other hand structural comparison of the degradates led to clear comprehension of the thermal transformation process.

**Experimental procedure**

High purity nutritional inulin powder was obtained from Sensus (Roosendaal, The Netherlands). Chicory inulins for chemical use were from Sigma Aldrich (Steinheim, Germany) and Fluka, (Buchs, Switzerland), medium chain nutrition grade chicory and artichoke inulins were obtained from Sensus (Roosendaal, The Netherlands), Orafti (Belgium) and from Ökoszervíz Kft. (Szentendre, Hungary). Chemical reagents used for chromatography were from Merck (Darmstadt, Germany). High purity 1-kestose, 1,1-kestotetraose, 1,1,1-kestopentaose were obtained from Noack Group of Companies (Vienna, Austria) for the identification of low-molecular weight oligomers. The sample preparation procedure was carried out without previous enzymatic or chemical hydrolysis of powder samples. The chromatographic detection was carried out by using ultrahigh-purity water and acetonitrile as gradient mobile phases. The liquid chromatography was carried out using a HPLC system consisting of a SIL-20A autosampler, an LC-20AB binary pump, a DGU-20$A_3$ degasser, a CBM20A communication module, a CTO-20A column chamber all from Shimadzu Manufacturing Inc. and a Prevail Carbohydrate column (250mm × 4,6 mm, 5μm) from Altech Association Inc. (Deerfield, Ireland). All inulin samples were treated at all the nine heating temperatures and expositional times. Inulin samples (0.8 grams) were placed in opened glass vials and have been heated in an oven at temperatures shown in table 1. Sample preparation resumed only to water dissolution and filtering the samples. 10 ml of hot (85°C) ultra-high purity water was added to the inulin samples, the solutions were sonicated for 60 minutes and syringe filtered (0.45 μm, nylon) into a glass autosampler vial for injection, standard solutions were prepared using low-molecular weight oligomers. Detection and identification of inulin thermal degradates of standard oligomers were performed by the above mentioned method using a PL-ELS-2100 evaporative light scattering liquid chromatographic detector. Injection volume was 8 μl of samples containing 80 mg carbohydrate per 1 ml ultra-high pure water. The flow rate of the eluent was 0.4 ml/min. 112 minutes were needed for complete separation for control (non-heated) inulin samples and 77 minutes for the heated samples with a gradient mobile phase of acetonitrile-water (initial rate: 88/12).

***Table 1.*** *Temperatures of the thermal treating and exposition times.*

| | Heating temperature, °C | Time of exposition, min |
|---|---|---|
| Inulin samples | 150 | 22,5 |
| | 160 | 20,0 |
| | 170 | 17,5 |
| | 180 | 15,0 |
| | 190 | 12,5 |
| | 200 | 10,0 |
| | 210 | 7,5 |
| | 220 | 5,0 |
| | 230 | 2,5 |

## Results and discussion

In order to investigate the pathways of thermal degradation and its impact on initial structure of inulins, chromatographic separation of control (non-heated) inulin samples were also performed. By applying high-performance liquid-chromatography coupled with evaporative light scattering detection of the non-heated sample (Figure 2.) oligo-, and polymer derivatives were separated efficiently ranging from DP3 up to DP31. Fructooligosaccharide standards are only available from 3 to 5 DPs, while the upper fructan oligomers appeared in the complex polymeric chromatograms were also identified and isolated. From alteration of retention times and molecular weights exact number of fructose units might be concluded. The applied method resulted in a chromatogram with solid evidences on the DPs of specific components. The principal fructo- oligosaccharides are well known and relevant peak IDs were confirmed by using the standards mentioned above (Figure 3.). The homologue peaks can be assigned as being the chromatographic signals of carbohydrate oligomers increased by one fructose unit (Figure 4.).

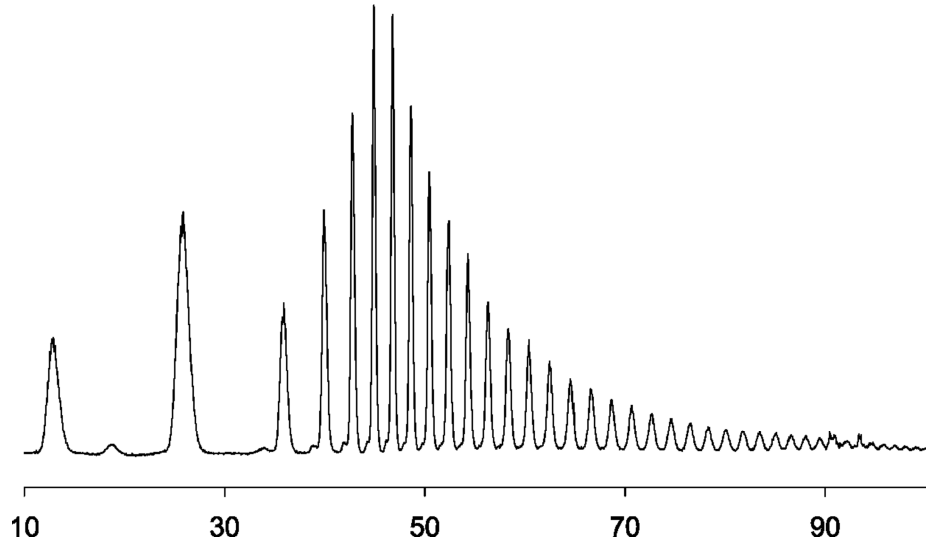


***Figure 2.*** *ELS detected HPLC chromatogram of non-heated, control inulin sample.*

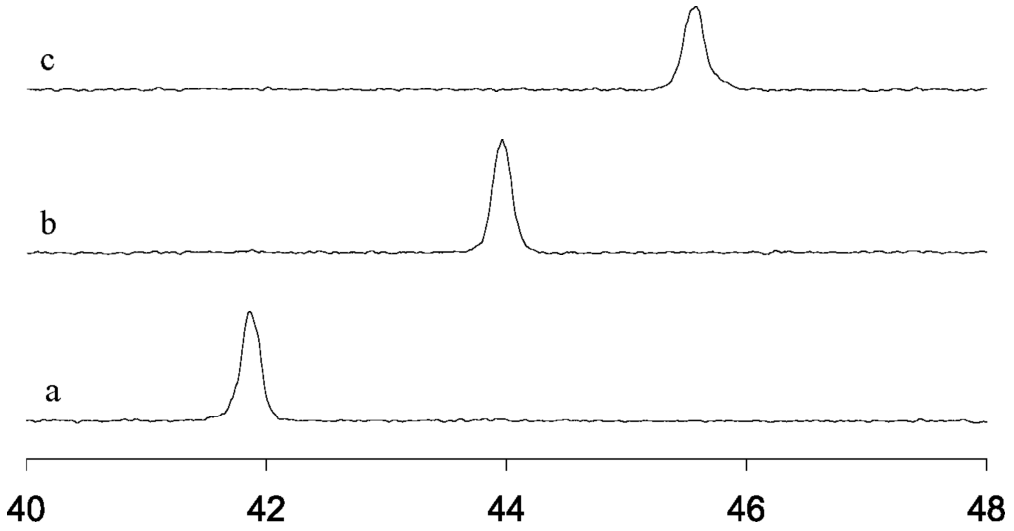


***Figure 3.*** *HPLC signals of the three known standards (a: trimer, RT: 41.86 min.; b: tetramer, RT: 43.97 min.; c: pentamer, RT: 45.58 min.).*

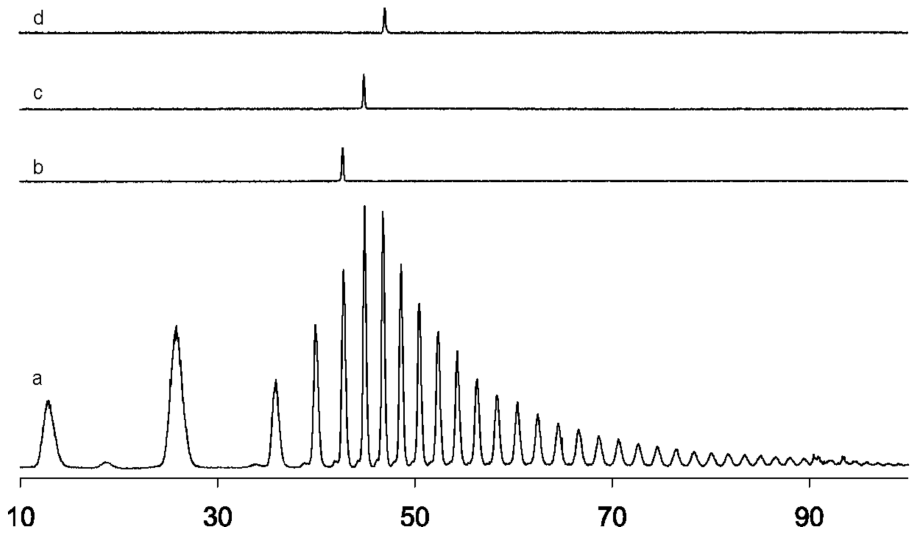


***Figure 4.*** *HPLC Data Comparison of the three known standards and heated inulin at 180 °C (a: heated inulin, b: trimer, c: tetramer, d: pentamer)*

The chromatograms of heated samples (Figure 5.) provided with relevant information on the degradation of the oligo-derivatives. In cases of the heated samples the increased occurrence of thermally degraded derivatives is proportionally correlated with the temperature. It was clearly observed that the chromatographic signals of products having lower polymerisation degree were enhanced with increased extent of thermal treatment, meanwhile higher polymers completely disappeared (Figure 5.). During heating experiments fructose and glucose content remained unchanged. Only saccharose level was slightly raised with the increase of time-interval and temperature of the applied thermal treatment.

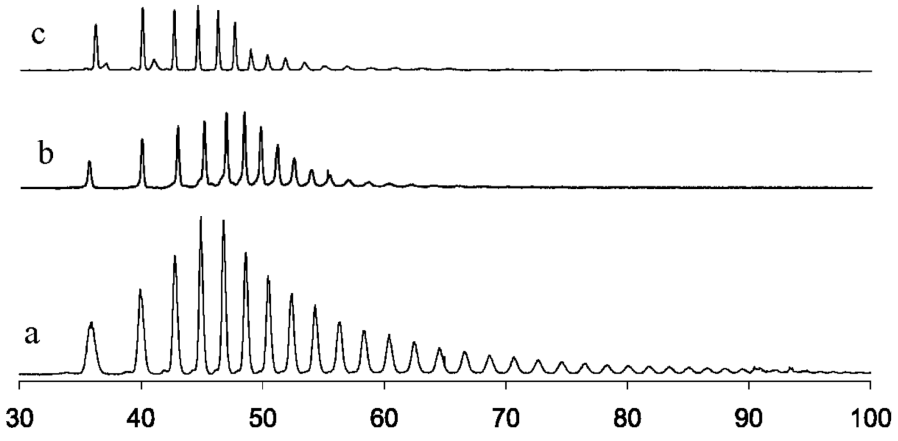


***Figure 5.*** *Data comparison of „Intact" and heated inulin (a: control; b: 180 °C; c: 210 °C).*

## Conclusions

It can be concluded that intense thermal treatment of inulin leads to decrease of the amount of „intact" inulin from *Cichorium intybus L.* Degradation process could be well observed in the chromatograms of the heated samples. Fructan oligomers appeared in the chromatograms were isolated and identified.

Accurate structural information of oligo-and polymer derivatives (from DP3 up to DP31) were obtained by applying high-performance liquid-chromatography coupled with evaporative light scattering detection. From alteration of retention times and molecular weights exact number of fructose units might be concluded.

One of the most important conclusions of the experiments is that fructans with or without thermal treatment may be directly determined without previous enzymatic or chemical hydrolysis with the application of a simple sample preparation procedure avoiding usage of harmful chemicals and complex extraction processes. Thus, HPLC-ELS might be regarded as a very powerful tool of fructan detection as its resolution power is able to separate each degree of polymerisation within macromolecule of inulin.

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