Semi-preparative isolation of Fn-type inulin from hydrolyzed globe artichoke inulin

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In this study, Fn-type oligofructose ranging from 2 to 4 fructose units were purified from hydrolyzed globe artichoke inulin on a semi-preparative scale and then characterized by High Pressure Anion Exchange Chromatography – Pulse Amperometric Detection (HPAEC-PAD) and ElectroSpray Ionization Mass Spectrometry (ESI-MS). GFn-type inulin with an average degree of polymerization above 80 were extracted from globe artichoke by hot water, purified and freeze-dried. This high DP fructan was then hydrolyzed by a commercial endo-inulinase which led to an enriched Fn-type product, as the enzyme broke the chains into 2-4 fructose units. These hydrolysis-products were individually isolated and purified by High Pressure Size Exclusion Chromatography (HPSEC). The purity of each Fn molecule was checked by HPAEC-PAD, while the molecular weight was determined by ESI-MS experiments in the infused mode which confirmed the degree of polymerization of the purified inulo-oligosaccharides.

Isolation and characterization of Fn-type inulin from globe artichoke inulin hydrolysis

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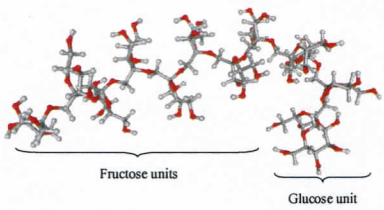
Introduction

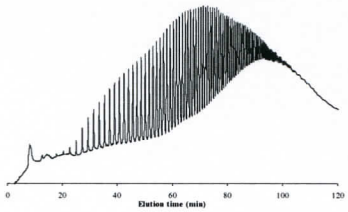
Inulin is a natural storage carbohydrate mainly found in plants from the *Asteraceae* family. This oligofructose is used as a nondigestible dietary fiber for its bifidogenic properties, but also for techno-functional properties in many foodstuff preparations. It is not only a simple molecule, but a mixture of oligo- and/or polysaccharides composed of fructose unit chains (linked by β-(2 →1) D-fructosyl-fructose bonds) of various length, terminated generally by a single glucose unit (linked by an α(1→2)-D-glucopyranosoyl bond). So, the general formula may be depicted as GFn or Fn, with G as glucose and F fructose, and n characterizing the number of total units.
Inulin can be a source of production of oligosaccharide, when a strong endo-inulinase action is exerted on it. This hydrolysis leads to an enriched Fn-type product, as the enzyme breaks only each 2-4 fructose units.
Globe artichoke inulin was extracted, purified and hydrolyzed by endo-inulinase. Inulin Fn-molecules were produce and purification was achieved using a semi-preparative HPSEC separation of each specific degree of polymerization. Purity of each fractionated Fn was ascribed by HPAEC-PAD and ESI-MS.



Experimentation and results

Extraction of globe artichoke inulin

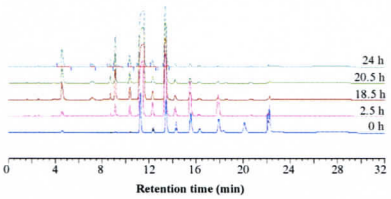
Inulin was extracted from 12 kg globe artichoke by 50 l distilled water (at 80°C, pH > 6 by NaOH). Extracted juice was filtered on 1 mm and 5 µm filters. Inulin was then precipitated by cooling, and the precipitate was centrifuged at 3000 g for 20 min.



The DP profile of extracted globe artichoke inulin showed a relatively high DP content.
DPn was determined as the number of fructose units per number of glucose units plus one glucose unit. An average degree of polymerisation number (DPn) of about 80 was found for globe artichoke inulin.

Hydrolysis of globe artichoke inulin

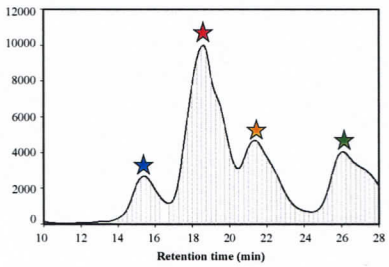
2.5 g globe artichoke inulin was mixed with 50 ml of distilled water and two drops of NaOH 0.1M, and heated to boiling. The pH was adjusted to 4.52 by AcNa (0.5M) and HCl (0.5M) in order to be in the optimal working range of the enzyme. A volume of 0.5 ml of endo-inulinase AOEI 400UI/ml (Beldem, Belgium) was added. The solution was then placed at 50°C for 24 h. The final product of the enzymatic reaction was lyophilized.
During the course of globe artichoke inulin hydrolysis by endo-inulinase, aliquots of the reaction mixture were periodically withdrawn and analyzed by HPAEC-PAD.



Fn were initially less important in the sample than GFn, and the endo-inulinase hydrolysed linear β-2,1 linked fructose polymers to F, F_2, F_3 and F_4. After 24 h hydrolysis, F_3 was the major product, followed by F4. No diminution of the F_3 and F_4 content was observed for a hydrolysis time higher than 24 h.

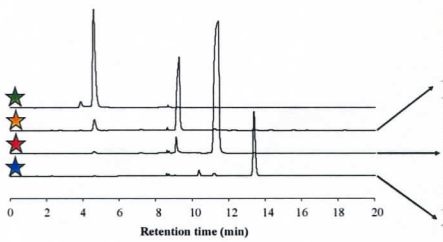
Purification of F2, F3 and F4

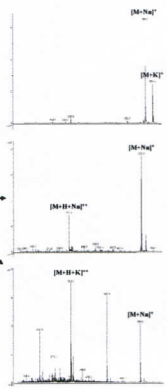
High Performance Size Exclusion Chromatography (HPSEC) was utilized to obtain a pure Fn fraction on a semi-preparative scale. F_{2-4} were purified by injecting 180 µl of a 23.6° brix solution of hydrolyzed globe artichoke inulin on a MCI Gel CH04S column (200x10mm) using a HPLC Waters coupled to a Refractive Index (RI) detector. Elution was performed by Milli-Q water (85°C) at 0.4 ml.min^{-1}.



HPSEC chromatograms of hydrolyzed artichoke inulin revealed four peaks. HPAEC-PAD characterization of these peaks permitted the identification of fructose, F_2, F_3 and F_4. HPSEC provided sufficiently good separation of extracted Fn as some relatively pure fractions were obtained for mass spectrometry characterization.
The HPSEC fractionation used in this study yielded enough pure products for the NMR experiments (interpretation in progress).

Fractions were collected from the inlet of the refractometer each 0.25 min and analyzed by HPAEC-PAD each 0.75 min by diluting 10 µl of the collect in 1 ml milli-Q water, in order to demonstrate the purity of the sample. Then, the purest fractions were put together and freeze dried.
ElectroSpray ionization with a quadrupole ion trap mass analyzer has been utilized to ascertain structural detail of purified F_{2-4}. In this approach, the neutral samples were ionized in ElectroSpray, which provides a profile of intact components. The samples were directly infused at 100 µl.h^{-1} from a 2% solution (w/w) of F_{2-4} in Milli-Q water.





The positive-ion mode mass spectra of $Fpy(F_{fu})_1$, $Fpy(F_{fu})_2$ and $Fpy(F_{fu})_3$ shows their sodiated molecule $[M+Na]^+$ at m/z 365.1, 527.1 and 689.2 respectively. The potassiated ion molecular $[M+K]^+$ was also detected for $Fpy(F_{fu})_1$. The mass spectrum of $Fpy(F_{fu})_2$ presents the double charged $[M+H+Na]^{++}$ at m/z = 272.1, while $[M+H+K]^{++}$ is observed for $Fpy(F_{fu})_3$ at m/z = 353.1. Spectrum of $Fpy(F_{fu})_3$ presents peaks at m/z = 527.1 and m/z = 203.0, probably resulting from the fragmentation of $Fpy(F_{fu})_3$ into fructose and the associated tri-fructose, in the sodiated form.

Conclusions

This work allowed the extraction of globe artichoke inulin, which had a relatively high degree of polymerization content (DP~ 80) and was well adapted to produce a high Fn / GFn ratio from endo-inulinase hydrolysis.
F_{2-4} were efficiently purified by HPSEC from the hydrolysis by endo-inulinase of the extracted globe artichoke inulin. ElectroSpray Ionization – Mass Spectrometry permitted to ascertain the degree of polymerization of purified molecules.

Acknowledgments

Financial support was provided by the Walloon Region of Belgium and Warcoing Research sprl. The authors are grateful to Mrs Lynn Doran for technical assistance.

Poster presented at the 3rd International Symposium on the Separation and Characterization of Natural Synthetic Macromolecules, Amsterdam, Netherlands, 31 January - 2 February 2007