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DETERMINATION OF INULIN AND FRUCTOOLIGOSACCHARIDES COMPOSITION IN SELECTED PLANT

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ABSTRACT

Inulin and Fructooligosaccharides (FOS) are naturally occurring sugars with potentially beneficial nutritional effect. High-Performance Liquid Chromatogram (HPLC) was developed to measure Inulin and FOS in selected vegetables and crops. HPLC separation showed that inulin content ranged between 1.35 -33.8% ;whereby the lowest value was found in barley, var. Giza 126 and wheat, the highest content was found in Chicory and Garlic. Total FOS content ranged between 0.22-27%, the lowest value was found in wheat bran, the highest was found in onion.

Keyword: Inulin- Fructooligosaccharides- High-Performance Liquid Chromatogram

INTRODUCTION

Inulin and Fructooligosaccharides (FOS) are indigestible carbohydrates composed of fructose units attached by β (2→1) bond and finished or not with a molecule of glucose, which is linked to fructose through an α (1→2) bond *(Roberfroid , 1999).* FOS were defined as a mixture of Kestose (GF_2), Nystose (GF_3) and Fructofuranosylnystose (GF_4). *(Lewis,1993).*

Inulin and FOS have been shown to exhibit beneficial health effects by stimulating the growth of bifidobacteria in the human colon, by suppression of harmful bacteria and reduction of serum cholesterol concentration *(Gibson and Roberfroid, 1995).*In animal experiments it was shown that inulin and FOS increase mineral absorption *(Schlz-Ahrens et al., 2001; Raschka & Daniel, 2005).*

Inulin and FOS are naturally occurring in many plants including banana, onion, wheat, barley, asparagus, and Jerusalem artichokes *(Spiegal et al.,1994).* Due to their physiochemical properties , sweetening power and low caloric value FOS have been added to pastry , confectionery , and dairy products . Their energy value is theoretically lower than that of sucrose. *(Roberfroid et al, 1993).*

The objective of the present work is to optimize the conditions for the extraction and determination of inulin and fructooligosaccharides (FOS) in selected plants.

MATERIALS AND METHODS

Materials:

Jerusalem artichoke tubers and barley were purchased from the Agriculture Research Center, Giza. The chicory roots, wheat, whole grain and wheat bran were purchased from a local store for medicinal herbs. Onions,

var. Giza 6, var. Tantawi and garlic were obtained from local retailer. The inedible portion was removed and discarded while the remaining edible portion was cut into small parts, lyophilized and ground to a fine powder.

Sample Preparation:

Methanolic extraction of FOS:

Approximately two grams of finely ground samples were placed in a round bottom flask followed by the addition of 30 ml of aqueous methanol (70%) and CaCO3 (0.1g) . The flasks were quick fit and connected to a condenser. Extraction of the FOS was completed at 70-75ºC for 30 min, with occasional shaking every 5 minutes. Upon cooling, the content of the flask was transferred quantitatively using successive methanol washing to a centrifuge tube. The clear supernatant obtained after centrifugation for 5 min ;1000 rpm was aspirated. The sediment was washed with aqueous methanol (2 ml) and centrifugation was repeated . The combined supernatants were concentrated in Buchi rotary evaporator to approximately 3 ml . Prior to the HPLC separation, the concentrate was diluted with equal volume of acetonitrile (v/v).

Extraction of inulin with boiling water:

One grams of finely ground samples were placed in a round bottom flask followed by the addition of 25 ml of boiling distilled water and CaCO3 (0.1g) . The flasks were heated at 100^{0}C under reflux for 60 min with occasional shaking every 5 minutes. Upon cooling, the content of the flask were filtered using filter paper. The filtrate was desalted by passing on ion-exchange column containing a bed mixture of cation (one part) and anion (five parts) exchange resins. The column was washed with deionized water. The first 3ml eluted were discarded. The following three milliliters containing the water extract were collected in a vial for subsequent HPLC separation.

HPLC separation:

The High Performance Liquid Chromatography (HPLC) instrument (Knauer Co., Germany) was equipped with a (250 X 4 mm) column packed with NH_2P (amino propyl polymer) with particle size 5 μ. The gradient for the mobile phase was acetonitrile: water at a ratio of 70: 30. Injection volume was 20 µl; the flow rate 0.8 ml / min; Temperature was 20◦C. The eluted FOS units were detected using A Refractometer detector (Knauer Co, Germany). Signals from the detector were recorded simultaneously by the data system Euro Chrom 2000.

A column packed with Aminex HPX-87C was also used for the separation of inulin. The gradient for the mobile phase was water. Injection volume was 0.5-1 ml; the flow rate was 0.5 ml / min; temperature was 70◦ C. The eluted FOS units were detected using refractometer detector. Signals from the detector were recorded simultaneously by the data system Euro Chrom 2000.

Components (GF_n units) were quantitated by measuring peak areas and comparing them to a standard curve generated by plotting area counts against concentration of, FOS Wako pure (Japan) standards, and Raftiline (Orafti Company)

RESULTS AND DISCUSSION

Figure (1) illustrates a typical HPLC chromatogram for the separation of the methanolic extract of the authentic FOS solution standard (20.45 mg/ml). Retention times of 11.71; 14.83 and 19.02 min for kestose, nystose and fructofuranosylnystose, respectively, were obtained under the experimental conditions, when the NH_2P was used in the packing of the column. The methanolic extract of onion var.Giza 6 behaved similar to the authentic standard, with regard to the respective retention times (Figure2).

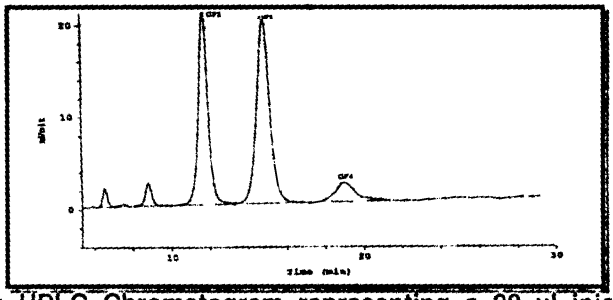

Figure (1): HPLC Chromatogram representing a 20 µl injection of a standard Fructooligosaccharides solution 20.45mg/ml containing Kestose (GF2; 11.718 min), Nystose (GF3; 14.836 min), Fructofuranosylnystose (GF4; 19.020min).

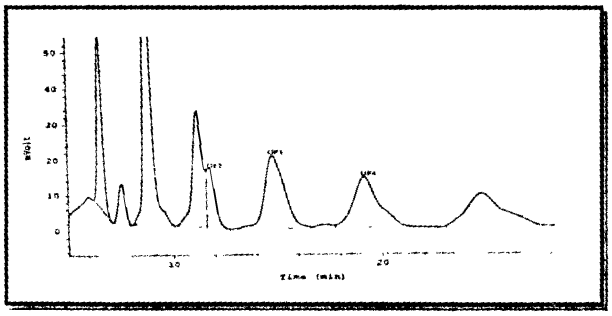

Figure (2): Fructooligosaccharides profile of onion , var. Giza 6 (20 µl injection) after methanolic extraction : Kestose (GF2; 11.733 min), Nystose (GF3; 14.8 min), Fructofuranosylnystose (GF4; 19.200 min) it was similarly to standard, with regard to the respective retention times.

A typical HPLC chromatogram for the separation of the water extract of the authentic Raftiline solution standard 20mg/ml appeared in Figure (3). Retention times of 8.23 min for inulin were obtained under the experimental conditions, when the Aminex HPX-87C was used in the packing of the column. The water extract of onion var. Giza 6 showed similar behavior to the authentic Raftiline, with regard to the respective retention times (Figure 4).

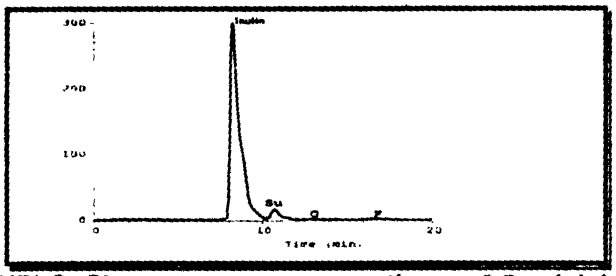

Figure (3): HPLC Chromatogram representing a 0.5 ml injection of Raftiline solution 20mg/ml :(inulin; 8.231 min) sucrose (Su; 10.739 min), Glucose (G; 12.991min)and fructose(F;16.693min).

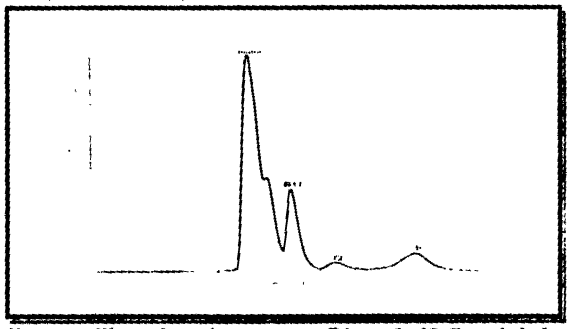

Figure (4): Inulin profile of onion, var. Giza 6 (0.5 ml injection) after water extraction (inulin ; 8.321min), sucrose (Su; 10.606 min), Glucose (G;12.907 min)and fructose(F; 17.059min) it was similarly to standard, with regard to the respective retention times.

The FOS composition of selected vegetables and crops are presented in Table (1), results are presented on dry matter (DM) basis. The onion was a rich source of total FOS with mean levels of 26.7 % DM, while wheat bran was the lowest. Inter and intra variation were found in the concentrations of Kestose ; nystose and fructofuranosylnystose.

Table (1): Fructooligosaccharides composition of selected vegetables and crops.

Ingredient	Kestose g/100g	Nystose g/100g	Fructofuranosylnystose g/100g	Total FOS g/100g
Vegetables				
Jerusalem Artichoke	7.09	4.52	4.13	15.75
Onion (var.Tantawi)	4.64	8.26	6.25	19.15
Onion,(var. Giza 6)	4.95	11.57	10.26	26.77
Garlic	0.65	1.13	0.80	2.58
Chicory	0.29	0.17	1.58	2.04
Crops				
Barley, (var. Giza 126)	2.14	0.30	0.21	2.65
Barley,(var. Giza 131)	1.78	T	T	1.78
Wheat , whole grain	1.40	0.52	T	1.91
Wheat, Bran	0.19	0.03	T	0.22

T = traces

The GF_3 unit represented the largest percentage of total onion FOS, followed by GF_4 unit.

Inulin content of chicory was the highest compared to other plant sources, while that of barley, var. Giza 126, was the lowest (Table 2).

Table (2): Inulin content of selected vegetables and crops.

Ingredient	Inulin g/100g
Vegetables	
Jerusalem Artichoke	21.804
Onions, var. Giza 6	15.492
Garlic	29.135
Chicory	33.840
Crops	
Barley, whole grain, var. Giza 126	1.368
Barley, whole grain, var. Giza 131	3.086
Wheat , whole grain	1.552
wheat bran	2.037

The current HPLC method was able to accurately separate and quantitatively measure individual GF_n units compared to the HPLC method of *Sims et al. (1991)*. In comparison with other studies, the present study showed higher values than those reported by *Campbell et al,(1997)* with the exception of Jerusalem artichoke and wheat bran that show lower values in total FOS in our study. This may be attributable to sampling technique, sample origin, and extraction.

In conclusion, inulin and FOS are present in significant amounts in a wide variety of common foods and food ingredients. HPLC method is a viable means for quantifying inulin and FOS contents of food and feed. The method provides excellent separation and detection of inulin and FOS.

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تقدير مركبات الانيولين و سكرات الاليجو فركتوز في بعض من النباتات المختارة

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تنتشرمركبات الانيولين و سكريات الأوليجو فركتوز فى الطبيعة حيث توجد بحوالى ٣٦٠٠٠ صنف بالمملكة النباتية ، نالت هذه المركبات أهمية خاصة بعد أن أعلن عن آثارها الايجابية على الصحة العامة من خلال تحسينيا لوظائف عمل القولون ، الحد من تكاثر البكتريا الممرضة والى رفع كفاءة الأستفادة من العناصر المعدنية .

تم تقدير تركيز الانيولين و مركبات سكريات الأوليجو بواسطة الفصل الكروماتوجرافى عالى الأداء في بعض من الخضروات و المحاصيل بعد استخلاصيا مائيا او كحوليا.

و قد كان المحتوي من مركب الانيولين في النباتات المختارة يتراوح ما بين ١,٣٥-٣٣,٨% حيث كان اعلي محتوي من مركب الانيولين في نبات الشيكوريا و الثوم ،أقل محتوي كان الشعيرالمغطاة صنف جيزة ١٢٦.

يترواح المحتوي من سكرات الاليجو فركتوز ما بين ٠,٢٢-٢٧ % حيث كان اعلي محتوي في نبات البصل و أقل محتوي في ردة القمح.