MICROWAVE-ASSISTED EXTRACTION OF INULIN FROM JERUSALEM ARTICHOKE AND PARTIAL ACID HYDROLYSES

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ABSTRACT
The Recent study aimed to identify the optimum conditions for inulin extraction from jerusalem artichoke tuber (JAT) powder using microwave assisted extraction method, and partially hydrolysis of purified inulin for producing fructooligosaccharide (FOS) using citric acid & microwave energy. The extraction conducted at different temperature (90 – 95 °C) at (700 W/5min, 450w/8 min and 350w/14 min). The extracted inulin was concentrated to (50 – 60) % of original volume using rotary evaporator, and purified by lime method. The purified inulin extract was concentrated to 32 brix and mixed with acetone (3:1 acetone : extract), kept for 24 h at 4 °C, then centrifuged at 10000g / 15 min., the precipitate dried at 55 °C. Acid hydrolysis of purified inulin carried out at different pH values (1.5, 2.5 & 3.0) at (90 ±2) °C. Aliquot of inulin hydrolysate were taken after (5, 10, 15, 20, 25 & 30) min and subjected to qualitative analysis by RP-HPLC and TLC. The obtained results indicated that the microwave assisted extraction at 700 W / 5 min. / 95 °C was superior as compared to the rest. The yield of extracted inulin was about 39.61 % and the extraction efficiency was 94.31 %, while the optimum condition for inulin acid hydrolysis appeared to be at 90 °C/ 15 min at pH 2.5 and 450 W. The qualitative analysis using (RP-HPLC & TLC) showed that the DP of inulin units ranged from (2 - 35) and for FOS ranged from (2 – 9) unit.

Keywords: hydrolysate, fructooligosaccharides, RP-HPLC, TLC, citric acid, microwave energy.
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INTRODUCTION

Inulin is a linear polymer (polydisperse) of fructose linked by $\beta$ (2 → 1) glycosidic bond and terminated with a D-glucose molecule linked to fructose by an $\alpha$ (1 → 2) glycosidic linkage, as in sucrose (22). Inulin serves as storage carbohydrate in many members of the Asteraceae family such as Helianthus tuberosus (Jerusalem artichoke), Cichorium intybus (chicory), Tarax-acum officinalis (dandelion) and Inula helenium (elecampane) (10). Although today, chicory roots is the main crop used for industrial production of inulin, the pursuit of improving the extraction quality of other crops does not bring to a stop since. Jerusalem artichoke tubers (JAT) is containing about 14 to 19% of inulin (from fresh weight) so it can be a valuable source of inulin (2). Inulin extracted from natural sources is characterized by a degree of polymerization (DP) ranging (2 - 60) units. The oligosaccharides of inulin with DP less than 10 units called of fructo-oligosaccharide (FOS) is one of the bestknown prebiotics (25). Inulin and FOS are considered as functional food that is beneficial to human health through decrease the risk of some diseases like colon cancer, intestinal infections, diabetes, constipation, obesity and increase intestinal absorption of some minerals such calcium and magnesium in small intestine (17, 30). Several methods for inulin extraction from fresh JAT have been adopted (13, 28). These methods are time consuming with low extraction efficiency. Therefore it is necessary to develop methods for inulin extraction which improve the extraction efficiency (19). Microwave assisted extraction [MAE] is a process of using microwave power to heat the solution through the extraction of the sample in order to separate analytes from the sample matrix into the solvent, it is promising alternative method to extracted bioactive compounds from plant sources (43). The highly localized pressure and temperature can accelerate the migration of target compounds from the sample to the extraction solution, thus increases the extraction efficiency compared to conventional extracts. The extraction efficiency by microwave increased because it's volumetric heating effect, leading a higher temperature of solvent result due to dipole rotation of the solvent in the microwave field. Thus increasing the solubility of biocompounds (42). This study was aimed to optimize the conditions for inulin extraction from JAT powder and for partially hydrolysis of purified inulin for producing fructo-oligosaccharides (FOS) using citric acid and microwave power.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents were of analytical reagent grade. These included potassium iodide, sodium hydroxide, Sodium acetate, tricalcium phosphate, sodium periodate, dinitrosalicylic acid, resorcinol and ascorbic acid (Merck, Germany). Citric acid, concentrated hydrochloric acid, maltodextrin and concentrated sulfuric acid (BDH - England), Glucose, Fructose, Sucrose, Lactose, cellulose, raffinose and 1-kestose were obtained from (Sigma-Aldrich, St. Louis, MO, USA) and (Merck Germany), Standard chicory inulin (Sigma, Berlin, Germany). Ethanol, butanol, aniline, diphenylamine, acetone, phosp-horic acid and glacial acetic acid were obtained from (Alpha Chemicals-India). Chromatographic silica gel 60 (20 cm×20 cm) precoated glass plates were obtained from (Merck, Germany).

Determination of free fructose, total fructose & inulin content in JAT

Free fructose, total fructose & Inulin content were determined according to the method described by Saengkanuk et al. (33) using following formula:

$$I = k \times (F_{tot} - F_t)$$

Where (I) is the inulin content, $(F_{tot})$ is total fructose content, $(F_t)$ is free fructose, and $(k)$ is a correction factor for the glucose part of the inulin and for the water loss during hydrolysis. In this study, $k = 0.995$ is adopted, this value is recommended for the unknown DP inulin.

Preparation of JAT powder

The JAT purchased from local market, washed with tap water to remove dust and other undesirable materials. The cleaned JAT was peeled and cut into slices 2 mm thick pieces. In order to avoid enzymatic browning, the slices were immersed in acidified boiled water (0.1% w/w ascorbic acid) and left for (2 – 3 min.) to obtain white inulin powder, then the JAT slices were dried at 60 °C for 10 h in an oven, milled and sieved through an (50 mesh)
sieve. The powdered JAT was kept in dry place, until extraction time (36).

**Microwave-assisted extraction of Inulin**

Inulin was extracted from JAT powder using a Microwave (silvercrest - German) at a frequency of 50 Hz's with six power settings according to Ruo-ling (32) method, with some minor modifications. Extraction of Inulin from JAT powder carried out as follows:

(a) Microwave power 700W, solid to liquid ratio (1 : 30) (w/v) and extraction time 5 min
(b) Microwave power 450W, same mixing ratio and extraction time 8 min
(c) Microwave power 350W, same mixing ratio and extraction time 14 min

The extraction process was conducted in duplicate. The obtained extracts were filtered through Muslin cloth and filter paper (Whitman No. 1), the residue of JAT was re-extracted under the same conditions, then all filtrate were concentrated to 50-60% of the original volume using rotary evaporator. The extract was turbid due to the presence of protein, pectin, and cell wall materials. These impurities were removed according to method described by Paseephol et al. (28). The Inulin extract was concentrated to 32°B and precipitated according to Ku et al. (20) method. Acetone was used at ratios of (3:1 v/v), then kept for 24 h in a refrigerator at 4°C, centrifuged at 10000g for 15 min, the precipitated inulin was taken and dried in an oven at a temperature of 55°C.

**Determination of inulin yield**

Inulin yield (%) was calculated according to the following eq.

\[ Y = \left( \frac{mL}{mJAT} \right) \times 100 \]  

(Temkov et al. 38)

Where, mL is the inulin mass obtained from the extraction and mJAT is the mass of artichoke tubers taken for the extraction.

**Extraction and determination of pectin**

Pectin was extracted from JAT powder following the method described by Liu et al. (21), and the yield of pectin was calculated using the following formula equation:

\[ \text{Pectin yield} \% = \left( \frac{m1}{m2} \right) \times 100 \]

Where \(m1\) (g) is the weight of dried pectin, \(m2\) (g) is the weight of dried material

**Inulin hydrolysis for producing FOS:** Partial hydrolysis of purified Inulin carried out due to method described by Fontana et al. (12).

Purified inulin solution (5%) was used to prepare the working solution with different pH values being (1.5, 2.5 and 3.0); citric acid solution 10% was used to adjust the pH of the above solutions. All solutions were heated to (90±2) °C using microwave power 450W for time periods (5, 10, 15, 20, 25 & 30) min. After the partial hydrolysis of inulin, the pH of the hydrolysate was adjusted to 7 using calcium hydroxide, the hydrolysate clarified (the salts precipitated) using the method described by Heding et al. (15). The supernatant was concentrated to 32 B°, FOS and inulin precipitated according to Ku et al. (20) method with some modifications. Acetone was used at ratios of (4:1 v/v), then kept for three days in a refrigerator at 4°C, centrifuged at 10000g for 15 min. The precipitate was washed with (60:40) (water: acetone) solution and centrifuged again at 10000 rpm for 10 min., then mixed with 10 ml distilled water, 1g of maltodextrin and 0.01g tri-calcium phosphate. The whole mixture placed in a drying oven at 110°C for 75 min to remove the remaining solvent then complete the drying at 55°C (35). The percentage of (FOS & inulin) remaining after inulin hydrolysis was calculated according to Ngampanya et al. (26) method, using the following equation:

**Determination of total carbohydrate**

Phenolsulphuric acid method was used to estimate total carbohydrate (9) using D-Fructose for standard curve preparation.

**Determination of reducing sugar**

Dinitrosalicylic acid (DNSA) method was used to estimate reducing sugar using D-Fructose for standard curve preparation (23).

**Determination of free fructose**

Free Fructose was determined by resorcinol reagent method using D-Fructose as standard (4).

**Determination of moisture content**

The moisture content was determined using oven at 105 °C, 2 g of sample placed in drying oven until gaining a constant weight (1).

**Determination of hydroxymethylfurfural (HMF)**

HMF content was determined using White (41) method. The yield of HMF was calculated using the following formula:

\[ \text{HMF (mg /100 g)} = (A_{284} - A_{336}) \times 21.39 \times W \times D/W \]
\[ A_{284} = \text{absorbance at 284 nm} \]
\[ A_{326} = \text{absorbance at 336 nm} \]
\[ 21.39 = \text{Constant} \]
\[ D = \text{dilution factor, in case dilution is necessary} \]
\[ W = \text{Weight in g of the sample} \]

HPLC analysis
Sample analysis was performed using RP-HPLC Model LC-2010 a HT. UV-detector 190 nm. Equipped with a quaternary pump and Empower software. An NUCLEODUR 100 – 5 NH2 column (4.6 x 250 mm i.d.) was used at a column temperature of 40°C. The mobile phase was mixture of acetonitrile/water (75:25) (v/v) and flow rate of 1.4 ml/min. The sample injection volume was 10 μl. Using (Fructose, Glucose, Sucrose, Cellobiose, raffinose, 1-Kestose and Inulin from Chicory root) as standards (31).

Thin-layer chromatography identification
The qualitative identification of inulin and FOS was performed by (TLC) before and after hydrolysis of inulin. Using (Fructose, Glucose, Sucrose, 1-Kestose and Inulin from Chicory root) as standards. Phase ascension is composed of a mixture of solvents: (butanol–ethanol–water) (5:3:2) (v: v: v), after drying the plate, the spots were visualised by spraying (aniline-diphenyl-amine–phosphoric acid–acetone) (1:1:5:50) (v: w: v: v), followed by drying at 80 °C for 10 min until the spots appear clearly (29).

Statistical analysis
The data were statistically analyzed by the (LSD) value at 0.05 probability level as described in (13)

RESULTS AND DISCUSSION

Chemical composition of JAT
Table 1 shows the chemical composition of local JAT (fresh & powder) which included (moisture, total solid, total carbohydrates, inulin, sucrose, reducing sugar and pectin). The total carbohydrate percentages were (14.20% & 61.74%) in fresh and dry JAT respectively. These amounts are consistent with Bekers et al. (6) findings who reported that the total carbohydrate content in different varieties of JAT powder was ranged (62.8% - 64.5%), whereas in fresh JAT was 14.97% (5). The percentage of inulin in experimental JAT (fresh & powder) were (9.66 % & 42%) respectively. This is in line with Dias et al. (8) findings who reported that JAT powder contains a high percentage of inulin ranging from (40% - 60%). Sahar (34) stated that inulin content in fresh JAT was 9.60%. The differences in inulin percentages are due to the varieties, degree of polymerization, harvest date, storage and the postharvest extraction methods. The high content of inulin makes JAT an important plant source for the production of inulin and FOS at the commercial level. The results in Table 1 also indicate that the moisture content of JAT (fresh & powder) were (77.0% & 6.37%) respectively, these results are consistent with Munim et al. (24) finding who stated that the moisture content in fresh JAT was 78.01%. Gaafar et al. (13) found that the moisture content in JAT powder was 6.36%. The percentage of pectin in JAT (fresh & powder) were (3.29 % & 14.30%) respectively. Our results are in line with those of Toshkov et al. (39) who found that the pectin content in JAT powder was 14.80 %. In recent study the percentage of reducing sugar and sucrose in JAT powder were (2.13% & 3.31%) respectively. While in fresh JAT, those values were (0.49% & 2.13%) respectively. Our findings were lower than Barta & Patkai (5) results who reported that JAT powder contained (1.72% to 2.55%) reducing sugar and (3.50% to 9.45%) sucrose.

Table 1. Chemical composition of JAT

<table>
<thead>
<tr>
<th>Constituents</th>
<th>(%) Fresh JAT</th>
<th>(%) JAT Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>77.00</td>
<td>6.37</td>
</tr>
<tr>
<td>Total solid</td>
<td>23.00</td>
<td>93.63</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>14.20</td>
<td>61.74</td>
</tr>
<tr>
<td>Inulin</td>
<td>9.66</td>
<td>42.00</td>
</tr>
<tr>
<td>Pectin</td>
<td>3.29</td>
<td>14.30</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>0.49</td>
<td>2.13</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.76</td>
<td>3.31</td>
</tr>
</tbody>
</table>

The experiment runs in duplicate

Effect of microwave power and extraction time on extractable inulin (%):
Figure 1 shows the effect of microwave power and extraction time on the inulin yield and extraction efficiency. The highest yield (39.61 and 36.38%) of purified inulin observed with (700W\ 5min & 450W\ 14 min) respectively, with no significant differences between them (P<0.05). However, there were significant differences in inulin yield between the 700W\
5 min. treatments and the treatment with 350W/14 min (33.87%). These results were close to the results of Ruo-ling (32) who found that the extraction efficiency using 700W was better than 600W and 500W, the highest yield of inulin was achieved with microwave power 700W/3 min. Concerning the extraction efficiency (EF), Fig. 1 showed that the highest EF (94.34 %) achieved with 700w/5min., followed by 450w/8min (86.62 %) and 350w/14 min (80.64%). Gaafar et al. (13) reported that the highest EF of inulin was noticed at 80°C/90 min using conventional heat reflux method. The high temperatures in closed vessels MAE resulted in improved extraction efficiency, since the motivation and solubility of analytes from the JAT powder will increases. While the surface tension and solvent viscosity will decrease as temperature increases, which will eventually improve solvent penetration, consequently the efficiency of the inulin extraction will increases, (11).

![Figure 1. Effect of microwave power and extraction time on inulin yield and extraction efficiency (%) from JAT powder](image)

Carbohydrate profiles and DPn of inulin obtained by RP-HPLC

The purified Inulin analyzed by RP-HPLC and compared with native chicory inulin, Fig. 2 shows that the fructose is a first monomer peak appeared, followed by glucose peak and disaccharide (sucrose), then the fructooligosaccharides peaks, which including (1-kestose GF2 and nystose GF3) etc. Results of current study shows that the DP of inulin ranging from (2 - 35) unit. These results are consistent with Panchev et al. (27) who stated that the DP for inulin extracted from JAT ranging from (2 - 33) units. Based on the generally accepted presumption that the retention time of a inulin units increased as the degree of polymerization increased, and that each sequential peaks appeared in chromatographic pattern, represents an inulin moiety which had a fructose more than that of the previous peak. Additionally the well resolved peaks suggesting that inulin and FOS were linear units, as expected. However, it is not possible to distinguish between Fn or GFn. In most of the published studies, each sharp peak represents inulin units either of type of GFn or Fn, they appear after of sucrose peak (7). Furthermore Fig. 2 shows that the FOS DP ranging between (2 - 10) units, begging to appear after the sucrose unit. The FOS content in inulin extracted from JAT powder reaches 16 %, this refers to high content of JAT powder from FOS. Our results are in agreement with those of Judprasong et al. (16) who found that the content of FOS in inulin extracted from JAT was 19.18 % as well as the percentage of reducing sugar reached to 3.22% (purified inulin). These results are consistent with Khuenpet et al. (18) who found that the reducing sugar content in JAT inulin was 3.45 %. The percentage of remaining carbohydrates represents the pure inulin with DP more than 10 units.

![Figure 2. High-performance liquid chromatographic of inulin extracted from JAT. (FOS) fructo-oligosaccharides. (DP), degrees of polymerization](image)

Qualitative analysis TLC of inulin extracted from JAT powder

TLC analysis of MAE inulin (Fig. 3), shows a polydisperse carbohydrate with different DP ranging from (3 - 9) units. The analysis of a TLC chromatogram of a homologous series of
carbohydrates is based on the generally accepted assumption that each spots represents an inulin units with a certain DP value and that a higher DP leads to a decrease in the retention factor (RF). Therefore, it is assumed that each spot has one more fructose unit than the previous spot. Results of thin layer chromatography corresponded with RP-HPLC analysis in the inulin units separation, showed that the inulin extracted from JAT powder contains FOS appears as a separated spots on the glass silica gel. Then the spots overlap with each other gradually due to increasing of molecular weight, make it immobile with the mobile phase and remains close to the baseline. Our results are in line with those of Walz et al. (40) who found the first spots on TLC in line belongs to monosaccharides (fructose or glucose) with the lowest molecular weight, then sucrose and gradually other components of FOS or polysaccharide polymer chain with increasing molecular weight.

Optimization of inulin hydrolysis to produce FOS and high fructose syrup

Acid hydrolysis of inulin was carried out using different pH values and different hydrolysis period at 90°C (Fig. 4, 5 & 6). Fig. 5 shows that the percentage of reducing sugars was increased with time from (5 - 30) min. at pH 1.5. The percentage of (remaining inulin and produced FOS mixture) significantly (p<0.05) decreased from (56.06% to 8.47%) during hydrolysis time (5 - 30) min. respectively. Fig. 4 also demonstrate that the percentage of reducing sugars was significantly (p<0.05) increased from (43.94% to 87.39%) at the reaction time (5 - 30) min. respectively, Glibowski et al. (14) stated that using low pH values ranged from (1 - 2)\ 90°C accelerated hydrolysis of the glycosidic bond, resulting in high percentage of free fructose.

**Figure 3. Thin layer chromatography of inulin extraction from JAT powder. (DP, degrees of polymerization; FOS, Fructooligosaccharides).**

**Figure 4. Percentages of (remaining inulin & produced FOS mixture), reducing sugar and HMF during acid hydrolysis of inulin (pH 1.5, 90°C)**

At pH 2.5 the hydrolysis of inulin was lower than that at pH 1.5. Fig. 5 shows that the percentage of (remaining inulin and produced FOS mixture) were (74.48, 63.32, 51.24, 45.02, 39.61, 29.33) % at (5, 10, 15, 20, 25 & 30) min. respectively. While, percentage of reducing sugars was increased as the reaction time increased at this pH value. The percentage of reducing sugars was (25.52%) after 5 min. hydrolysis and it reached, (68.36%) after 30 min. of reaction time. Our results agreed with the study of Szambelan et al. (37) who stated that the low pH and high temperature enhanced the acid hydrolysis of inulin.
Fig. 5. Percentages of (remaining inulin & produced FOS mixture), reducing sugar and HMF during acid hydrolysis of inulin (pH 2.5\ 90˚C)

Fig. 6 shows the products of inulin hydrolysis at pH 3.00 the depolymerization were slow, the percentage of (remaining inulin and produced FOS mixture) was (80.87, 75.50, 64.74, 57.82, 48.57, 37.01) % at (5, 10, 15, 20, 25 & 30) min. hydrolysis respectively. The reducing sugars percentage were increased from (19.13% to 37.01%) at (5 - 30) min. hydrolysis respectively.

Fig. 7. RP-HPLC profile of FOS obtained by inulin hydrolysis with citric acid at pH 2.5/15 min

Fig. 8 shows that the more acidic environment (pH 1.5) resulted in higher hydrolysis degree of inulin as the time proceeded from (5 - 30) min. Consequently, the amount of free fructose increased, so these conditions can be applied for producing high fructose syrup. However, at pH (2.5) the hydrolysis of inulin was lower than that at (pH 1.5). The hydrolysis at (pH 2.5) recorded the highest percentage of FOS with low amount of free fructose through (5 - 20 min) after 30 min. of reaction time. The amounts of HMF formed at pH (2.5 & 3.0) were low as compared with pH 1.5. Figure 7 demonstrates the same trend in HMF formation at the pH values. The co-generated HMF in small amount can be easily removed by adsorption with activated charcoal (1%–2%) (3, 12). RP-HPLC and TLC indicated a wide range of modulation in the qualitative profile of the partial hydrolysis products for inulin. Optimal conditions of partial hydrolysis of inulin were studied to determine highest yield of FOS and lowest amount of free fructose. The optimum result for FOS production with less amounts of reducing sugars and HMF, achieved at pH 2.5\ 90˚C\ 15 min., as presented in (Fig.7). Under these conditions, the highest FOS (degree of polymerization, 2 to 9) yield was 39%. Fontana et al. (12) stated that the best condition for acid hydrolysis of inulin were with citric acid catalyst (pH 2.5), temperature 85˚C and reaction time 15 min, which resulted in high amount of FOS with DP ranging from (2 - 9) units.

The results of acidic hydrolysis of inulin at 90 °C and pH values (1.5, 2.5, and 3.0) shows that the amount of HMF increased as the reaction time increased. Fig. 5 shows that the highest percentage of HMF was 4.14 % at pH 1.5 after 30 min. Fig. 6 also demonstrates that the HMF not formed during the initial stages of acid hydrolysis (pH 3) at 90°C up to 20 min. of hydrolysis (0.27%) then gradually increased to (2.31 %) after 30 min. of reaction time. The amounts of HMF formed at pH (2.5 & 3.0) were low as compared with pH 1.5. Figure 7 demonstrates the same trend in HMF formation at the pH values. The co-generated HMF in small amount can be easily removed by adsorption with activated charcoal (1%–2%) (3, 12). RP-HPLC and TLC indicated a wide range of modulation in the qualitative profile of the partial hydrolysis products for inulin. Optimal conditions of partial hydrolysis of inulin were studied to determine highest yield of FOS and lowest amount of free fructose. The optimum result for FOS production with less amounts of reducing sugars and HMF, achieved at pH 2.5\ 90˚C\ 15 min., as presented in (Fig.7). Under these conditions, the highest FOS (degree of polymerization, 2 to 9) yield was 39%. Fontana et al. (12) stated that the best condition for acid hydrolysis of inulin were with citric acid catalyst (pH 2.5), temperature 85˚C and reaction time 15 min, which resulted in high amount of FOS with DP ranging from (2 - 9) units.

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30) min. The Qualitative analysis indicates an increase in the amount of FOS through initial stages (hydrolysis time 15 min) with continues increases of free fructose (up to 30 min). Whereas hydrolysis of inulin at pH 3.0 was less effective in depolymerization of inulin. Thus, for better hydrolysis of inulin at this pH required higher temperature and longer hydrolysis times.

Figure 8. Thin-layer chromatographic analysis of the reaction products in the hydrolysis of inulin extracted from JAT with citric acid at pH (1.5, 2.5 & 3.0) for (5, 10, 15, 20, 25, 30) min. DP, degrees of polymerization; FOS, fructooligosaccharides

Purified inulin was prepared from JAT powder through Microwave-assisted extraction has been considered as a potential alternative to traditional solid-liquid extraction for the increasing of inulin yield from JAT powder. Optimum conditions for extracted inulin were using microwave power 700\% 95 °C for 5 min. Under these conditions high amount of inulin could be extracted (39.61\%) with better extraction efficiency (94.31 \%). This inulin polymer was efficiently and partially depolymerized with aqueous citric acid to release FOS, with a degree of polymerization ranging from (2 – 9), at pH 2.5, the temperature was 85 - 90 °C, and the hydrolysis time was 5 – 30 min. There is no need for catalyst removal in most industrial applications of the FOS syrups that are produced with citric acid.

REFERENCES


