Preparation of Inulin from Chicory Roots

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Inulin has immense applications in health and nutrition as well as in food industries. Inulin is isolated from chicory roots by differential precipitation with ethanol (0-20, 20-40 and 40-60 per cent) from the aqueous extract of sun-dried roots, by melting the frozen chicory root extract and by removing the oligo-fructosachharides from chicory roots with 20 per cent ethanol. Different inulin preparations so obtained are compared with some of the commercial inulin preparations. Sun-dried roots are as good as the fresh roots for isolation of inulin but roots dried in an oven (80-90°C) have lower inulin content due to hydrolysis of fructans during drying. For better inulin yield, chicory roots should be extracted with water having pH adjusted to 7. pH of water on acidic side results in lower inulin recovery.

Keywords: Inulin, Chicory roots, Oligo-fructosachharides

Introduction

Inulin and oligo-fructose (fructans with fructose polymerization from 2 to 10) are officially recognized as natural food ingredients in most European countries and have a self-confirmed GRAS (generally regarded as safe) status in the US. Inulin and oligo-fructose are prebiotics, are considered as functional foods as they alter physiological and biochemical processes of the body for better health and prevent risk of many diseases. Inulin has been reported to stimulate the immune system of body, decrease pathogenic bacteria in intestine, relieve constipation, reduce risk of atherosclerosis and modulates hormonal level and hence blood glucose level especially in diabetics. It also lowers blood urea and uric acid levels, decreases the risk of osteoporosis by increasing absorption of minerals, especially calcium and above all inulin is reported to decrease the incidence of colon cancer. Inulin and oligo-fructose have wide applications in various types of foods like, confectionery, fruit preparations, milk deserts, yogurt, fresh cheese, baked goods, chocolates, ice-cream, and sauces. Inulin is less soluble in water and forms micro-crystals when sheared in water and milk and hence can replace fat in table spreads. A detailed review on uses of inulin in health and nutrition is given by Kaur and Gupta'.

Consumer is health conscious and demands foods, which have low fat and low calories with additional health benefits. In present day society the leading health concerns are heart disease, cancer, high cholesterol, obesity, osteoporosis, and diabetes. Inulin and oligofructose are widely used in functional foods throughout the world for their health promoting properties'.”

Jerusalem artichoke (Helianthus tuberosus) and chicory (Cichorium intybus) are the two species currently used by the industry to produce inulin and belong to compositae, the latter being so far the most commonly used source'.

The roots of chicory store fructans as a reserve material, which constitutes 15-20 per cent by fresh weight (70 to 80 per cent of dry weight) of root5. The yield of chicory root on dry weight basis has been reported to be 11-16 t/ha which can give 8-12 t/ha of inulin if there is no loss during extraction and purification6.

In spite of so many applications of inulin in health and nutrition, beside its applications in food industry, no work has been done on the isolation of inulin from inulin storing plants in India. The present

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research has been undertaken to develop a cost-effective way of isolating inulin from chicory roots.

**Experimental Procedure**

*Cichorium intybus* (Chicory) variety Kalpa No. 1, was sown in the third week of October and roots were harvested starting in the last week of March to first week of April. Roots were cleaned with tap water and processed in three ways after peeling. In some experiments, fructans were extracted from the fresh roots, in others roots were cut with a knife into small pieces and dried under sun between 30-35°C. Some of the roots were dried in hot air oven at 80-90°C. The roots dried in oven were brownish in colour, whereas sun dried roots were of light yellow off-white colour.

**Extraction of Fructans for Isolating Inulin**

Sun-dried and oven-dried roots were crushed manually in an iron pestle and mortar and passed through a sieve (100 μm) to have chicory powder of uniform size. Fresh roots (100 g) were crushed in a blender. Five volumes of water were added to crushed chicory roots. Fructans were solubilized by keeping the contents in a boiling water bath for 20 min and the contents were passed through a double layered muslin cloth. The extraction procedure was repeated 3-4 times and all the filtrates were pooled for isolating inulin.

**Quantitative Extraction of Sugars**

The total sugars were extracted with hot water, as described earlier. Reducing sugars were estimated by the procedure, described by Nelson, whereas fructan was determined after destroying the free fructose with NaOH and estimating fructan by resorcinol and HCl. 

**Differential Precipitation with Ethanol**

From the fructan extract obtained from chicory roots, various grades of inulin were obtained by precipitation with 20, 20-40 and 40-60 per cent ethanol. After separating inulin precipitated with 20 per cent ethanol by centrifugation, the ethanol concentration in the supernatant was raised from 20 to 40 per cent. The inulin thus obtained was separated by centrifugation. Another inulin fraction was obtained by raising the ethanol concentration in the supernatant from 40 to 60 per cent. The precipitates were washed twice with respective aqueous ethanol solvents and then dried at 45°C.

**Separation of Inulin and Oligo-fructosaccharide Fraction by Using 20 Per Cent Ethanol and Aqueous Extraction**

The chicory roots were extracted with 20 per cent ethanol at 25°C for 3 h with intermittent shaking. The process was repeated three-times. From the remaining roots, more fructans were extracted with boiling water. Both these fractions were subjected to biochemical analysis and descending chromatography on Whatman chromatography paper 1MM in Butanol: acetic acid: water (4:1:5) The fructans were identified, using fructose specific urea orthophosphoric acid spray reagent.

All experiments were conducted independently three times and three values for each parameter were obtained. Student’s t test was applied for determining the significance of difference between two treatments.

**Results and Discussion**

**Comparison of Oven-dried, Sun-dried and Fresh Chicory Roots for Inulin Extraction**

From the composition of fresh, oven-dried and sun dried chicory roots, it was observed that during drying in an oven between 80 to 90°C, some of inulin is hydrolyzed, leading to higher fructose content. However, sun dried roots had almost the same fructan content as in fresh chicory roots. Sun-dried roots can, therefore, be used for extraction and isolation of inulin because fresh roots are available only in March and April (Table 1).

**Effect of Temperature on Precipitation of Inulin by Ethanol**

Gupta et al. have shown that inulin can be precipitated from the chicory root extract with ethanol. The precipitation of inulin from the aqueous extract of sun-dried chicory root with ethanol at -15,

| Table 1 — A comparison of carbohydrate composition of oven-dried, sun-dried and fresh chicory roots |
|---|---|---|
| Roots | Per cent carbohydrate (Dry weight basis) |  |
| | Fructan | Fructose |
| Fresh | 72.4 ± 2.3<sup>a</sup> | 1.4 ± 0.2<sup>d</sup> |
| Oven-dried | 58.5 ± 0.8<sup>b</sup> | 12.4 ± 0.8<sup>c</sup> |
| Sun-dried | 70.8 ± 1.0<sup>c</sup> | 1.8 ± 0.2<sup>c</sup> |

Values are mean ± SD of data of three experiments.
a > b; p < 0.01 and c > d; p < 0.01 (student’s t test)
5 and 25 °C after 12 h showed that the low temperature did not result in increased inulin precipitation (Table 2). Therefore, inulin can be precipitated at 25°C. Data also showed that supernatant obtained after precipitation of chicory aqueous root extract with 50 per cent ethanol is a rich source of fructo-oligosaccharides.

**Effect of pH of the Extraction Medium on the Isolation of Inulin**

In this experiment, inulin was extracted by adjusting the pH of water to 6, 7, or 9. The data showed that maximum inulin is extracted at pH 7. Therefore, it is better to check the pH of water whereas slight increase of pH on alkaline side did not affect the inulin recovery, but small decrease in pH from 7 to 6 could lead to almost 35 per cent decline in inulin recovery (Table 3).

**Effect of Using Different Concentrations of Ethanol on Inulin Precipitation**

Maximum inulin was precipitated with 20 per cent ethanol. Inulin obtained by 20 per cent and 20-40 per cent ethanol hardly contained any oligosaccharides and these inulin preparations did not move from its origin during paper chromatography, however 40-60 per cent ethanol precipitates showed the presence of some fructo-oligosaccharides. High reducing sugar content precipitates obtained by 40-60 per cent ethanol (Table 4) also reflected this.

Inulin precipitated by 20 per cent ethanol was compared with commercially available inulin samples. The inulin obtained from Kingherb International China contained almost all the fructo-oligosaccharides found in chicory root extract, whereas inulin of Sigma USA was free from any fructo-oligosaccharides (Figure 1).

### Table 2 — Effect of temperature on inulin precipitation and supernatant composition by 50 per cent ethanol from the aqueous extract obtained from 100 g of sun-dried chicory roots

<table>
<thead>
<tr>
<th>Precipitation temperature, °C</th>
<th>Dry weight of inulin, g</th>
<th>Fructan, g</th>
<th>Reducing sugar, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>-15</td>
<td>21.6 ± 0.4</td>
<td>47.8 ± 0.7</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>21.7 ± 0.5</td>
<td>45.9 ± 0.6</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>25</td>
<td>22.0 ± 0.5</td>
<td>48.2 ± 0.6</td>
<td>4.5 ± 0.2</td>
</tr>
</tbody>
</table>

Inulin was determined on gravimetric basis by drying the precipitates at 45°C. Values are mean ± SD of data of three experiments.

### Table 3 — Effect of pH of water on the precipitation of inulin by 50 per cent ethanol from the aqueous extract obtained from 100 g of chicory roots

<table>
<thead>
<tr>
<th>pH of water</th>
<th>Inulin, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>21.6 ± 0.7</td>
</tr>
<tr>
<td>7.0</td>
<td>33.4 ± 0.7</td>
</tr>
<tr>
<td>9.0</td>
<td>32.0 ± 0.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD of data of three experiments. a > b; p < 0.01 (student’s t test)

### Table 4 — Effect of differential ethanol fractionation on inulin precipitation from 100 g of sun-dried roots

<table>
<thead>
<tr>
<th>Per cent ethanol</th>
<th>Inulin, g</th>
<th>Per cent composition of isolated inulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fructan</td>
<td>Reducing sugar</td>
</tr>
<tr>
<td>0-20</td>
<td>20.5 ± 1.2</td>
<td>93.6 ± 0.8 3.4 ± 0.2</td>
</tr>
<tr>
<td>20-40</td>
<td>15.6 ± 1.1</td>
<td>96.0 ± 1.1 3.7 ± 0.1</td>
</tr>
<tr>
<td>40-60</td>
<td>12.1 ± 1.4</td>
<td>90.3 ± 0.7 5.3 ± 0.2</td>
</tr>
</tbody>
</table>

Values are mean ± SD of data of three experiments.

Figure 1—Comparison of inulin isolated from chicory roots with other commercially available inulins by descending paper partition chromatography. Sugars with Rf less than sucrose are fructo-oligosaccharides. (1) Aqueous extract of chicory roots; (2) Inulin from Kingherb International China; (3) Inulin from Sigma Chemical company, USA; (4) Inulin isolated from sun-dried roots with 20 per cent ethanol, and (5), Standard sugars fructose + sucrose.
Inulin and oligo-fructosaccharide fractions have been successfully separated from chicory roots by extracting fructo-oligosaccharides with 20 per cent ethanol from the chicory root powder, whereas the residue contained mainly inulin. The compositional analysis showed that 20 per cent ethanol fraction had a significantly higher reducing sugars as compared to water extract containing mainly inulin (Table 5).

Precipitation of Inulin at Low Temperature

On melting the frozen aqueous extract of chicory roots, kept at about -10°C for about 3 d at 25°C, fine inulin precipitates settle at the bottom. The comparison of sun-dried and oven-dried roots showed that higher amount of inulin was present in sun-dried roots. However the supernatant left after isolation of inulin of oven-dried roots had higher mass than that of sun-dried roots because of higher amount of fructo-oligosaccharides formed due to hydrolysis of inulin during drying at 80-90°C (Table 6).

By using this procedure, inulin was also isolated from fresh roots. It was observed that preparations obtained by precipitating at -10°C from sun-dried and fresh roots were comparable with respect to their fructo-oligosaccharide composition, as observed by paper chromatography. Both these preparations were better than the two inulin preparations of Sensus-Netherlands (Fructafit HD & IQ), however the third preparation of inulin (Fructafit TEX) had low levels of oligosaccharides (Figure 2). Bubnik et al. have observed that when unrefined chicory root extract was vacuum thickened at 55-60°C to 43 per cent (W/W), crystallization of inulin occurred following cooling for 2 d. Berghofer et al. have also reported that cooling of concentrated aqueous chicory root extract over a period of 3 to 4 d to 4°C resulted in inulin precipitation.

Conclusions

A cost-effective procedure for isolating inulin form chicory roots has been developed. On keeping an aqueous extract of chicory roots at -10°C for 3 d and melting the frozen extract at 25°C, fine inulin precipitates were separated.
References
12 Berghofer E, Cramer A, Schmidt U & Vogel M, Pilot-scale production of inulin from chicory roots and its use in food, In Inulin and inulin containing crops, edited by Fuchs (Elsevier Amsterdam, The Netherlands) 1993, pp 784.