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Inulin

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DFAI di-D-fructofuranose 2’; 1; 2,1’-dianhydride
DFAII di-D-fructofuranose 2’; 1; 2,3’-dianhydride
DP degree of polymerization
DP$_5$ + or DP $\geq$ 5 fructan molecules with a DP of 5 or more
DP$_n$ a fructan with a degree of polymerization of n
EFA European Fructan Association
F fructose (only in reactions)
F$_2$ to F$_9$ fructan molecule consisting of only fructofuranosyl units (2 to 9 indicates the number of units present)
FEH fructan exohydrolase
FFT fructan:fructan fructosyltransferase
F<sub>m</sub> fructofuranosyl-only fructan molecule with a DP of m
F-G-F neo-kestose (only in reactions)
FOS fructo-oligosaccharide
Fru fructose
G glucose (only in reactions)
GF sucrose
GF2,GF8…GF<sub>n</sub> fructan molecule consisting of 2, 8…n fructofuranosyl units and containing one terminal glucose
G-F sucrose (only in reactions)
G-F-(F)<sub>n</sub> fructan molecule with a DP of n + 2 and containing one terminal glucose
G-F-F 1-kestose (only in reactions)
Glc glucose
6G-FFT fructan:fructan 6G-fructosyltransferase
PAD pulsed amperometric detector
PED pulsed electrochemical detector
RI refractive index
SST sucrose:sucrose fructosyltransferase

1 Introduction

Inulin, a nondigestible carbohydrate, is a fructan that is not only found in many plants as a storage carbohydrate, but has also been part of man’s daily diet for several centuries. It is present in many regularly consumed vegetables, fruits and cereals, including leek, onion, garlic, wheat, chicory, artichoke, and banana. Industrially, inulin is obtained from chicory roots, and is used as a functional food ingredient that offers a unique combination of interesting nutritional properties and important technological benefits. In food formulations, inulin significantly improves the organoleptic characteristics, allowing an upgrading of both taste and mouthfeel in a wide range of applications. In particular, this taste-free fructan increases the stability of foams and emulsions, as well as showing an exceptional fat-like behavior when used in the form of a gel in water. By contrast, as an ever-increasing amount of information becomes available on inulin, its nutritional attributes continue to amaze both researchers and nutritionists alike. Consequently, fat and carbohydrate replacement with inulin offers the advantage of not having to compromise on taste and texture, while delivering further nutritional benefits. Hence, inulin represents a key ingredient that offers new opportunities to a food industry which is constantly seeking well-balanced, yet better tasting, products of the future.

2 Historical Outline

Rose, a German scientist, first isolated a “peculiar substance of plant origin” from a boiling water extract of *Inula helenium* in 1804, and the substance was later called inulin by Thomson (1818). The German
plant physiologist Julius Sachs (1864) was a pioneer in fructan research and, by using only a microscope, was able to detect the spherocrystals of inulin in the tubers of *Dahlia, Helianthus tuberosus* and *Inula heli- nium* after ethanol precipitation.

Although today, chicory is the major crop used for the industrial production of inulin, the first reference to chicory being consumed by humans was made during the first century by Pedanios Dioscoride (Leroux, 1996) who, as a physician in the Roman army, praised the plant for its beneficial effects on the stomach, liver, and kidneys. Much later, Baillargé (1942) stated that in about 1850, Jerusalem artichoke (*Helianthus tuberosus*) pulp, when prepared by cooking and drying the tubers, was added in a 50:50 ratio to flour when baking bread to provide cheap food for laborers.

On a more physiological basis, Külz reported in 1874 that no sugar appeared in the urine of diabetics who ate 50–120 g of inulin per day, and by the end of the nineteenth century the feeding of diabetic patients with pure inulin in doses of 40–100 g daily was reported to be “with much benefit” (Von Mehring, 1876). The first studies on the effects of inulin in healthy humans appeared during the early twentieth century (Lewis, 1912), whilst the nontoxicity of inulin was demonstrated dramatically some years later (Shannon and Smith, 1935) when one of the authors injected himself intravenously with 160 g inulin. In particular, during the past 10 years there has been a spectacular increase in the number of publications relating to the functional and nutritional benefits of inulin.

Subsequently, as inulin changed from a subject of mere scientific interest into an industrial product with many applications, there was a major stimulation of research related to its production and use.

### 3 Chemical Structure

Inulin has been defined as a polydisperse carbohydrate material consisting mainly, if not exclusively, of $\beta(2 \rightarrow 1)$ fructosyl-fructose links (Waterhouse and Chatterton, 1993). A starting glucose moiety can be present, but is not necessary. In contrast, levan – which is formed by certain bacteria – consists mainly or exclusively of $\beta(2 \rightarrow 6)$ fructosyl-fructose links. As is the case for inulin, glucose can be present, but again it is not necessary. Fructan is a more general name which is used for any compound in which one or more fructosyl-fructose links constitute the majority. The term “fructan” therefore covers both inulin and levan.

When referring to the definition of inulin, both $G_n$ and $F_m$ compounds are considered to be included under this same nomenclature. In chicory inulin, $n$ (the number of fructose units linked to a terminal glucose) can vary from two to 70 (De Leenheer and Hoebregs, 1994). This also means that inulin is a mixture of oligomers and polymers. The molecular structure of inulin compounds is shown in Figure 1.

The degree of polymerization (DP) of inulin, as well as the presence of branches, are important properties since they influence the functionality of most inulin to a striking extent. Thus, a strict distinction must be made between inulin of plant origin and that of bacterial origin. The DP of plant inulin is rather low (maximally <200) and varies according to the plant species, weather conditions and the physiological age of the plant (see Section 9).

Native inulin always contains glucose, fructose, sucrose, and small oligosaccharides. The term “native” refers to inulin that, before its analysis, is extracted from fresh roots, taking precautions to inhibit the plant’s own inulinase activity as well as acid
hydrolysis. Moreover, no fractionation procedure is applied to eliminate the smaller oligosaccharides and monomers that are naturally present. In this respect, the commercially available inulin (Sigma Chemical Co.) that is derived from *Dahlia*, Jerusalem artichoke or chicory is not considered to be "native" as these products barely represent the inulin typical of the plants from which it is extracted, the average DP being 27–29 (for all three products). This DP value is not only very high, but chains of < 10 units are also absent (De Leenheer, 1996); this difference is shown clearly in Figure 2.

Until recently, (plant) inulin was considered to be a linear molecule, but by using optimized permethylation analysis it was possible to show that even native chicory inulin (DP 12) has a very small degree of branching (1–2%), and this was also the case for inulin from *Dahlia* (De Leenheer and Hoebregs, 1994).

In contrast to plant inulin, bacterial inulin has a very high DP, ranging from 10,000 to over 100,000; moreover, this inulin is highly branched (≥ 15%). Although Harada et al. (1993) reported that the inulin derived from the spores of *Aspergillus sydowi* was linear, this could not be confirmed by permethylation analysis. The fact that inulin has a small intrinsic viscosity in spite of its high molecular weight (as do levans), and that it appears to adopt a compact, globular shape rather than a coil is another indication of its nonlinearity.

From a structural/polymeric viewpoint, (linear) inulin can be considered as a polyoxyethylene backbone to which fructose moieties are attached, as are the steps of a spiral staircase. Inulin crystallizes along a pseudohexagonal, six-fold symmetry with an advance of 0.24 nm per monomer. Moreover, two inulin crystalline allomorphs exist: semi-hydrated and hydrated. The difference between the unit cells seems not to correlate with any change in the conformation of the six-fold helix, but rather to a variation in water content (Andre et al., 1996).

Oligomers with a DP up to 5 can adopt structures resembling the conformation of cyclo-inulohexaose. Oligomers with DP between 7 and 8 most likely adopt a conformational change because they form helical structures that become more rigid as the
DP increases (Timmermans et al., 1997). This hypothesis of changing conformation also provides a very reasonable explanation for the observation that, at DP values between 6 and 9, the elution sequence of the oligomers on a reversed-phase C<sub>18</sub> Nucleosil column is completely reversed (De Leenheer and Hoebregs, 1994).

### Natural Occurrence

After starch, fructans are the most abundant nonstructural polysaccharides found naturally, being present in a wide variety of plants, and in some bacteria. Most reports on the natural occurrence of fructans do not
differentiate between levan and inulin, however.

Fructan-producing plants are commonly present among the grasses (1200 species), whereas 15% of flowering plants produce fructans in significant amounts. They are widely spread within the Liliaceae (3500 species), and most frequently among the Compositae (25,000 species) (Hendry and Wallace, 1993). Strictly speaking, \( \beta(2 \rightarrow 1) \)-defined inulin is typical of the Compositae.

Inulin-containing plants that are commonly used for human nutrition belong mainly to either the Liliaceae, e.g., leek, onion, garlic and asparagus, or the Compositae, e.g., Jerusalem artichoke, dahlia, chicory and yacon (Table 1).

The occurrence of endogenous inulin in fungi appears doubtful: inulin-type molecules can be produced by incubating spores in sucrose (Harada et al., 1993), although as sucrose is not a true fungal carbohydrate it is unlikely that sufficient starting material is present for the synthesis of inulin via the sucrose:sucrose fructosyltransferase route (Lewis, 1991).

In bacteria, the presence of fructan-producing genera is common. Bacterial fructans are almost by definition of the levan type, and are found among the Pseudomonaceae, Enterobacteriaceae, Streptococcaceae, Actinomycetes and Bacillaceae. Recently, fructans were also detected within the Lactobacillli, more specific in Lactobacillus reuteri (van Geel-Schutten, 2000). Two fructosyltransferase genes have been described: one gene product is excreted by the bacterium during growth and is of the levan-type, while the second fructosyltransferase gene could only be brought to expression in Escherichia coli and produces an inulin-type fructan. Full characterization of these fructans is ongoing.

Until 1999, Streptococcus mutans was the only bacterium known to produce inulin-type molecules of which the linearity is, to date, unclear (Ponstein and Van Leeuwen, 1993).

One plant of special status is the Agave Azul Tequila Weber (Liliaceae). Although tequila is an alcoholic drink that is known world-wide, few people realize that it is made

### Tab. 1 Inulin content (% of fresh weight) of plants that are commonly used in human nutrition (Van Loo et al., 1995)

<table>
<thead>
<tr>
<th>Source</th>
<th>Edible parts</th>
<th>Dry solids content</th>
<th>Inulin content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onion</td>
<td>Bulb</td>
<td>6–12</td>
<td>2–6</td>
</tr>
<tr>
<td>Jerusalem artichoke</td>
<td>Tuber</td>
<td>19–25</td>
<td>14–19</td>
</tr>
<tr>
<td>Chicory</td>
<td>Root</td>
<td>20–25</td>
<td>15–20</td>
</tr>
<tr>
<td>Leek</td>
<td>Bulb</td>
<td>15–20*</td>
<td>3–10</td>
</tr>
<tr>
<td>Garlic</td>
<td>Bulb</td>
<td>40–45*</td>
<td>9–16</td>
</tr>
<tr>
<td>Artichoke</td>
<td>Leaves-heart</td>
<td>14–16</td>
<td>3–10</td>
</tr>
<tr>
<td>Banana</td>
<td>Fruit</td>
<td>24–26</td>
<td>0.3–0.7</td>
</tr>
<tr>
<td>Rye</td>
<td>Cereal</td>
<td>88–90</td>
<td>0.5–1*</td>
</tr>
<tr>
<td>Barley</td>
<td>Cereal</td>
<td>NA</td>
<td>0.5–1.5*</td>
</tr>
<tr>
<td>Dandelion</td>
<td>Leaves</td>
<td>50–55*</td>
<td>12–15</td>
</tr>
<tr>
<td>Burdock</td>
<td>Root</td>
<td>21–25</td>
<td>3.5–4.0</td>
</tr>
<tr>
<td>Camas</td>
<td>Bulb</td>
<td>31–50</td>
<td>12–22</td>
</tr>
<tr>
<td>Murnong</td>
<td>Root</td>
<td>25–28</td>
<td>8–13</td>
</tr>
<tr>
<td>Yacon</td>
<td>Root</td>
<td>13–31</td>
<td>3–19</td>
</tr>
<tr>
<td>Salsify</td>
<td>Root</td>
<td>20–22</td>
<td>4–11</td>
</tr>
</tbody>
</table>

NA, data not available. *Estimated value.
by the fermentation of a type of “inulin”. In fact, the fructan molecule is highly branched (24%), containing β(2 → 1) linkages as well as β(2 → 6) linkages (L. De Leenheer, unpublished results).

5 Physiological Function

Despite major advances having been made in the elucidation of the metabolism of fructans, their precise physiological function remains a subject of debate. The most documented role is that of a long-term reserve carbohydrate stored in underground, over-wintering organs. Two other functions are often quoted: first, as a cryoprotectant, and second as an osmotic regulator. Together, these roles allow not only survival but also growth under conditions of water shortage, whether induced by drought or low temperatures (Hendry and Wallace, 1993).

De Roover et al. (2000) have reported on the effects of drought on inulin metabolism in chicory. Glucose, fructose and sucrose contents were increased in the roots and leaves of stressed plants, whereas the inulin concentration was found to be ten-fold higher than in control plants, with inulin content being normal in roots but absent in leaves. In a cold environment (3 weeks at 4°C), chicory inulin is clearly degraded, and this results in lower-DP inulin and mainly fructose which, osmotically, is more active than inulin.

The role of fructans as true cryoprotectors is under discussion, as the increase in hexoses and sucrose upon depolymerization of the fructan would only account for a freezing point decrease of 0.2–0.5°C (Van Den Ende, 1996). In contrast, inulin was seen to interact directly with membrane lipids upon freeze-drying, thereby preserving the membranes in a liquid-crystalline phase at room temperature and preventing phase transition and solute leakage during rehydration (Hincha et al., 2000).

6 Chemical Analysis and Detection

Although several techniques are available for the analysis of inulin, no single method provides a complete and quantitative analysis of all the compounds present; hence, a combination of different methods is often necessary.

6.1 High-performance Liquid Chromatography (HPLC)

For HPLC analysis, two columns in series in the K+ form are used (Aminex HPX-87 K+) for optimal separation. The separations into fructose, glucose, difructose-dianhydride (DFA), sucrose (GF), F2 and F3 are optimal, but further separation into DP 3, DP 4 and DP ≥ 5 is not very precise. DP 3 and DP 4 fractions are not pure, but include GF2 plus F4 and GF3 plus F5, respectively. The DP ≥ 5 fraction is the integrated sum of DP 5 and higher-DP molecules. As this fraction might include small oligosaccharides as well as high-DP inulins, a fixed response factor cannot be determined, which makes this analysis unsuited for quantitative inulin determination. The method is well adapted to evaluate the relative amounts of the different compounds present, especially the amounts of the non-inulin compounds glucose, fructose and sucrose (Table 2).

6.2 Gas Chromatography

A high-temperature capillary gas chromatographic method was developed for the
quantitative determination of fructo-oligosaccharides (FOS) with DP \(\leq 10\) (Joye and Hoebregs, 2000). Sample preparation involves oxymation and silylation of the extracted sugars. The oximetrimethylsilyl derivatives are analyzed on an apolar capillary aluminum-clad column with temperature programming up to \(440°C\) and detection by flame ionization. The method is accurate and specific, as malto-, isomalto- and galacto-oligosaccharides, all of which are commonly present in foods, do not interfere. Moreover, \(\beta(2\rightarrow6)\) oligosaccharides (levan) can be clearly distinguished from \(\beta(2\rightarrow1)\) compounds, and GF\(_n\) compounds from F\(_n\) compounds (Figure 3; see also Table 2).

### 6.3 HPAEC Analysis (Dionex)

High-pressure anion exchange chromatography (HPAEC) is another technique which can be used to differentiate between GF\(_n\) and F\(_n\) compounds; moreover, the method also provides a “fingerprint” of the molecular weight distribution of inulin (see Figure 2). This analytical technique uses a Dionex series 4000 ion chromatograph (Carbo-Pac PA-1 column) coupled with a pulsed amperometric detector (PAD). During the analysis, the carbohydrates are eluted with a NaOH/NaAc gradient; the high pH (13–14) of the NaOH converts the hydroxyl groups into oxy-anions. The degree of oxy-anion interaction with the anion-exchange resin determines the carbohydrate retention times. To reduce the retention times, a competing ion such as acetate is added to the eluant. The PAD system oxidizes and detects the now separated carbohydrates as they pass through the detector.

The major drawback of HPAEC-PAD is that it is very difficult to quantify the high-DP oligomers, due on the one hand to the lack of appropriate standards and on the other hand to the reduced sensitivity of the PAD detector for high-DP polymers. The detector measures in fact the electrons released during oxidation of the carbohydrates at the gold electrode. Chatterton et al. (1993) have suggested that, as carbohydrates become larger, then proportionally fewer electrons are released per fructosyl unit, and so the PAD output per \(\mu\)g sugar decreases as the DP is increased.

Timmermans et al. (1993) also used HPAE-chromatography, but coupled with a pulsed electrochemical detector (PED). The sensitivity of the PED detector decreased clearly from DP 2 to DP 5, but these authors observed only a slow decrease for DP 10–17. From this, they calculated the PED respons-
es for inulin oligomers with different DPs relative to sucrose, and this enabled quantification of oligomers up to DP 17. Based on these relative responses, it was then possible to calculate the weight fraction of each compound present.

In a further study (Timmermans et al., 1997), the same group developed a HPAEC method with modified gradient elution in combination with a refractive index (RI) detector. In order to allow RI and PAD detection, the gradient was adapted to give a constant RI: the sodium acetate concentration was increased in order to obtain the desired fractionation, and the sodium hydroxide concentration decreased to keep the