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Fructans – Analytical approaches to a fibre that ferments

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FRUCTANS ARE DEFINED as any compound where one or more fructosyl–fructose linkages constitute a majority of the linkages.[1] This refers to polymeric material as well as oligomers as small as the disaccharide inulobiose. Material included in this definition may or may not contain attached glucose. The terms oligomer and polymer are used by fructan researchers to distinguish between materials that can be specifically characterised and those that cannot.[1] Fructans are widely distributed in the plant kingdom. They are present in monocotyledons, dicotyledons and in green algae.

Fructans differ in molecular structure and in molecular weight. They may be classified in three main types: the inulin group, the phlein group and the branched group.[2] The inulin group consists of material that has mostly or exclusively the (2 → 1) fructosyl–fructose linkage. Phlein (or levan) is material that contains mostly or exclusively the (2 → 6) fructosyl–fructose linkage. The branched group has both (2 → 1) and (2 → 6) fructosyl–fructose linkages in significant amounts (e.g. graminan from Gramineae).

Onions, leeks and garlic are rich in fructan and are the most common source of this polysaccharide in the Australian diet. Onions with a high dry matter content are grown specifically to be dehydrated into onion powder. These cultivars contain higher molecular weight (up to DP 10) as well as higher concentrations (50% dry wt) of fructan.[3] Other food plants that store fructan as their reserve polysaccharide include Jerusalem artichoke, Salsify, Yacon, a South American yam-like tuber now also grown in Japan, and chicory roots. These are all rather specialist vegetables, only Jerusalem artichokes are commonly available in Australia although roasted chicory root was once important as a coffee substitute and extender.

Australian Aborigines once ate several fructan-containing roots[4] and Murnong (*Microseris scapigera*). The yam daisy was an important food[5] widely collected throughout the Victorian grasslands. Fructan-containing tubers required long cooking in excess water to improve digestibility and reduce flatulence problems.[6] Fructans are very easily hydrolysed and prolonged cooking probably results in depolymerisation.

Harold McGee in a review[6] of the culinary uses of Jerusalem artichokes quotes John Goodyear (1617), an early English grower of this crop as stating: "in my judgment whichsoever way they be dressed and eaten they stir and cause a filthy loathsome stinking wind within the body, thereby causing the belly to be pained and tormented and one a meal more fit for swine, than men: yet some say they have usually eaten them and have found no such windy quality in them ...". Other food writers have been less critical, however, with the exception of onions fructan-rich crops have not become popular everyday foods. This is due to the fact that fructans are not broken down by the human digestive system and contribute only about 6 kJ/g through indirect colonic fermentation.[7,8] Fructans, due to this resistance to hydrolysis by the endogenous secretions of the human digestive tract, meet the definition of dietary fibre.[9]

Fructans as dietary fibre

Industrially the fructan inulin is extracted from chicory root, a biennial plant grown commercially in Holland. It is similar to the sugar beet and consequently there are some similarities in the agronomic practices and extraction technologies for beet sugar. The inulin extraction process involves extraction, purification, and finally spray drying of the juice to inulin powder.

Inulin extracted from chicory is a mixture of oligomers with different degrees of polymerisation with a modal chain length of approximately 9. Inulin extracted from chicory has the following typical formulation: monosaccharides, 2%; disaccharides, 5%; and inulin (GF3-GF60), 93%.[10]

Dry inulin powder is white, amorphous, hygroscopic, and has a specific gravity of about 1.35. It is soluble in water with the solubility dependent on the temperature of the water, thus at 10°C the solubility is only about 6%, although it is completely soluble in hot water. Inulin has a water-binding capacity of about 2:1. In solution, inulin reduces the freezing point of water and increases the boiling point.[11] Current world production is only about 7000 tonnes. Another commercial product derived from dehydrated, ground Jerusalem artichokes and sold as Jerusalem artichoke flour is produced in the United States.

Other recent interest in fructans has been as a texture modifier to replace fat in such foods as icings, cakes and sweet goods.[11] In these cases high concentrations of fructan in water can form a creamy fat-like gel. The use of fructan in a high dry weight glue has also recently been patented.[12]

Recently interest in fructans has been stimulated in Australian industry and food laboratories, not only by scientific and media publicity concerning its nutritional benefits, but by an application (A277) to the Australia New Zealand Food Authority (ANZFA) that inulin and oligofructose should be incorporated into the present official definition of dietary fibre. The application was made on behalf of ORAFTI, a Belgian food manufacturer of inulin and oligofructose from chicory root. Following consideration of public submissions, ANZFA has agreed in princi-

ple that oligofructose and inulin deserve recognition as dietary fibre, but has deferred further consideration of approval until an internationally recognised and validated analytical method becomes available.

The need for such analytical methods arises because of limitations in the presently accepted method of total dietary fibre analysis for food labelling[13] (AOACI method 985.29).[14] This method employs three standardised enzymic hydrolysis stages and then relies on precipitation of soluble dietary fibre occurring in 80% ethanol, before gravimetric measurement of combined insoluble and soluble dietary fibre material. Under these conditions, physiologically "unavailable" carbohydrates that are largely soluble in 80% ethanol are not measured, which includes oligosaccharides (e.g. oligofructose and the raffinose series) and most of inulin.

Fructan analysis

Analytical methods that can specifically or preferentially detect ketohexoses have been popular for the analysis of fructose in fructan preparations.[15] A colorimetric method based on the reaction of 2-thiobarbituric acid with 5-hydroxymethylfurfural,[16] formed from dehydration, in hot acid, of fructose or fructose-containing carbohydrates. This method has proven useful for fructan assay in plant materials[17] but is unspecific in the presence of sucrose.

Several other procedures have been described for the measurement of fructan in plant material and food products. It is generally accepted that fructans are best measured after hydrolysis to fructose (and glucose). This introduces the problem of independently removing or measuring sucrose, fructose and glucose. Pontis[18] reported the removal of sucrose, glucose and fructose by hydrolysis with a crystalline yeast invertase and destroying the resulting glucose and fructose as well as existing monosaccharides by boiling with sodium hydroxide. It was claimed that the action of invertase on the lower molecular weight members of the inulin series is slow and can be rendered insignificant by judicious selection of the incubation conditions. In testing currently available pure yeast invertases, we have found that it is extremely difficult, if not impossible, to achieve these conditions. 1-kestose is hydrolysed at approximately 20% the rate for sucrose, and 1,1-kestotetraose is hydrolysed at about 10% the rate for sucrose.

An alternative approach[19] involves the use of capillary gas chromatography (CGC) or HPLC to analyse extracts of samples either non-treated, or treated with amyloglucosidase or amyloglucosidase plus inulinase (fructanase). By measuring sucrose, fructose and glucose in the various samples, and with appropriate calculations, it is possible to get an estimate of free glucose and fructose, sucrose, starch and fructan. The possible interference of raffinose-series oligosaccharides (which may be present in some samples) was not considered. The fructanase enzyme preparation used in this work contains a very active α-galactosidase, consequently, any raffinose-series oligosaccharides present in the sample will also be hydrolysed to fructose and glucose (and galactose). Separate from the possible problems with raffinose-series oligosaccharides, this method is quite complex, and requires the use of expensive equipment. However at about the same time as the application to ANZFA by ORAFTI, an international collaborative study was underway of a high performance anion exchange chromatography (HPAEC) procedure for assay of inulin and oligofructose as fructose, incorporating hydrolysis of food materials with a fructanase enzyme.[20] This method has now been approved as official First Action method 997.08 by AOACI in June 1997, so further ANZFA consideration is imminent on expansion of the analytical definition of dietary fibre for food regulatory purposes.

New simple analytical method

We have developed a non-instrumental method for fructose which is available in kit form.[21] It makes fructan analysis easy to perform, uses standard laboratory equipment, and is accurate, reproducible and specific. This procedure employs highly purified and specific enzymes to hydrolyse sucrose, starch and fructans. The sucrase enzyme used in this method rapidly hydrolyses sucrose but has negligible activity on 1-kestose and other fructo-oligosaccharides.[22] At substrate concentrations of 10 mg/mL, the relative rate of hydrolysis of sucrose and 1-kestose is 3,800:1.

The method is applicable to the measurement of fructan in plant materials and food mixtures. Hydrolysis of fructans from chicory (polymeric frac-

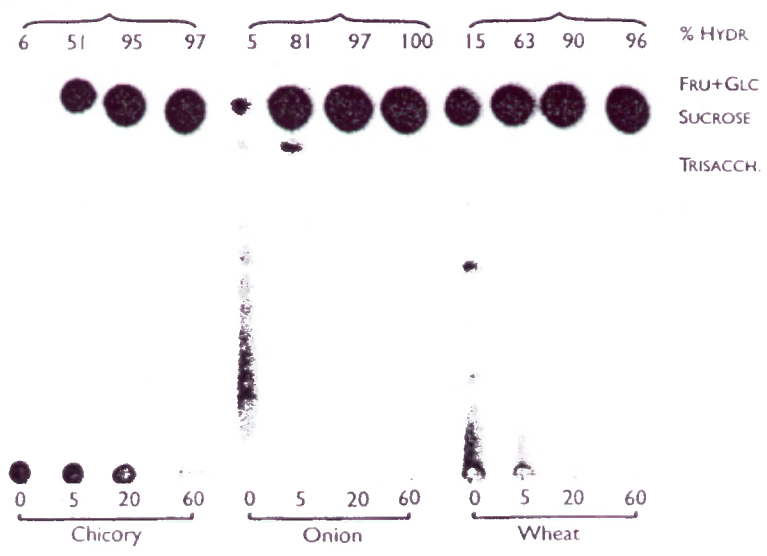

Figure 1. Thin layer chromatography of the sugars produced on hydrolysis of chicory, onion and wheat fructans by fructanase (conditions as described in the text). TLC plates developed once with *n*-propanol-ethanol-water (7:1:2).

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tion), onion and wheat leaves is shown in the thin layer chromatography (TLC) results in Figure 1. Fructan (5 g/100 mL of 10 mM sodium acetate buffer (pH 4.5)) was incubated with 4,000 U fructanase (exo-inulinase) at 40°C. Aliquots were removed at 0, 5, 20 and 60 min, incubated at 100°C to inactivate the enzyme and analysed by TLC, and by the *p*-hydroxybenzoicacid hydrazide (PAHBAH) reducing sugar method. Reducing-sugar values were calculated as a percentage of total carbohydrate, and are shown in Figure 1.

Sucrose is hydrolysed to glucose and fructose using a specific sucrase. Concurrently, starch and maltosaccharides (if present in the sample) are hydrolysed to glucose by the combined action of highly purified β-amylase, pullulanase and maltase present in the enzyme mixture. These reducing sugars are then reduced to the sugar alcohols by treatment with alkaline borohydride.[23] The solution is neutralised and excess borohydride is removed by treatment with dilute acetic acid. The fructan is hydrolysed to fructose and glucose with purified fructanase (exo-inulinase) and the reducing sugars produced (fructose and glucose) are measured with the PAHBAH reducing-sugar method. This method is simple to use and the colour response with fructose and glucose is the same. For samples containing raffinose-series oligosaccharides, we recommend the inclusion of *A. niger* α-galactosidase in the initial incubation step. The new method described here has been submitted to an AOACI ring test and should provide the basis for the simple analysis of fructan in foods.

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