The chemistry and sources of fructose and their effect on its utility and health implications.

Thomas Barclay[a], Milena Ginic-Markovic[a], Peter D. Cooper[b,c], Nikolai Petrovsky[c,d*]

[a] School of Chemical and Physical Sciences, Flinders University, Adelaide, Australia 5042
[b] Cancer Research Laboratory, ANU Medical School at The Canberra Hospital, Australian National University, Canberra, Australia 2605
[c] Vaxine Pty Ltd, Flinders Medical Centre, Adelaide, Australia 5042
[d] Department of Endocrinology, Flinders Medical Centre/Flinders University, Adelaide, Australia 5042

Received: 17 April, 2012; *Accepted*: 16 May, 2012

Review Article

ABSTRACT

Fructose is a significant component in unprocessed food and has become one of the most commonly sweeteners used in food manufacturing. Fructose is also a useful pharmaceutical excipient and derivatives of fructose are exploited as renewable chemical building blocks. Fructose based polysaccharides have extensive pharmaceutical and dietary functions. We discuss here the chemistry and physical behaviours of this saccharide and how these factors affect the utility and health implications of fructose.

KEY WORDS: Fructose, 5-hydroxymethylfurfural, HMF, excipients, inulin, health food, pharmaceutical, sugar chemistry

INTRODUCTION

Fructose is a naturally occurring mono-saccharide first isolated from cane sugar in 1847 (1). It is found in many fruits and vegetables, being the predominant sugar in apples, grapes, oranges and watermelon, as well as comprising of up to half of the total sugars in honey (2-5). Up until 40 years ago the vast majority of dietary fructose intake was from natural sources. However, industrial scale production of fructose from corn starch has resulted in a dramatic increase in the fructose consumption. Fructose rich products are now used extensively by manufacturers to sweeten foods and beverages instead of sucrose (6) such that most fructose in the Western diet is now from added sources (4). Fructose is also used by the pharmaceutical and chemical industries. The use of fructose as an excipient is mostly to make medicines more palatable (7, 8), but it also serves as a cryprotectant (9, 10), an aid for the solubility of hydrophobic active ingredients (11, 12) and a component to alter the osmolarity of injectable solutions (13). Fructose is also a useful starting material in the formation of bio-based alternatives to petrochemicals (14-16).

*Corresponding author: Professor Nikolai Petrovsky, Dept. of Diabetes and Endocrinology, Flinders Medical Centre, Adelaide, SA, Australia 5042, Tel: 61-8-82044572, Fax: 61-8-82045987, E-mail: nikolai.petrovsky@flinders.edu.au

Fructose is a component of other carbohydrates, such as the disaccharide sucrose, and is thus a constituent in many oligosaccharides and polysaccharides that contain sucrose (4). Additionally, fructose is the chief component in the fructan polysaccharides, levan and inulin (3-5). Such fructan polysaccharides are being increasingly utilised in the health food and pharmaceutical industries. Given the importance of fructose as a dietary component, key metabolite, pharmaceutical element and chemical building block, it is timely to review the physical behavior and chemistry of fructose and how these factors influence its commercial use.

CHEMISTRY

While the chemistries of monosaccharides are closely related, the differences in chemical behavior of these sugars are relevant to the uses to which they are applied. Specifically, glucose and fructose are structural isomers, having the same molecular formula. Together with di-saccharide sucrose, which contains both glucose and fructose and is cleaved into the monosaccharide components in the body (17), glucose and fructose are the most commonly used natural sweeteners. Their chemistries vary in that glucose is an aldose that is found almost exclusively in two anomeric, pyranose forms and crystallises in either of these forms. In contrast, fructose is a ketose that crystallises exclusively in the β-D-fructopyranose form (18) and mutarotates into at least five different tautomers in solution (shown in Figure 1) (19-22).

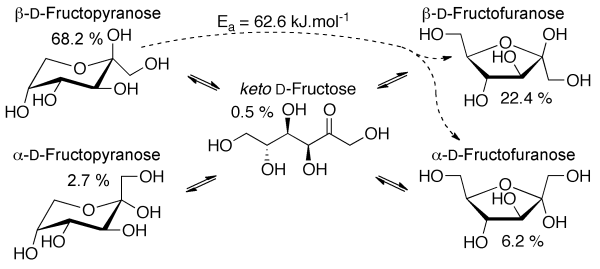

Figure 1 Tautomeric Forms of D-Fructose in Solution (19)

Mutarotation of fructose

At equilibrium, in solution at 20 ºC, β-D-fructopyranose is the preponderant tautomer of fructose comprising 68.2% of the total, followed by β-D-fructofuranose (22.4%), α-D-fructofuranose (6.2%), α-D-fructopyranose (2.7%) and the linear *keto* form of fructose (0.5%) (19). The mutarotation to achieve equilibrium is complicated by varying rates of transformation. The pyranose to pyranose transformation occurs between two stable chair conformations and is consequently slow (18, 23-25). In contrast, the transformation between pyranose and furanose forms is quick (18) and the furanose to furanose transformation occurs effectively instantaneously between high energy envelope and twist forms (26-29). Despite this complexity, the mutarotation of fructose can usually be approximated as a simple first-order process (18-20), with the kinetics represented by the conversion of β-pyranose to the furanose forms (30). Using this kinetic assumption the mutarotation of fructose has recently been demonstrated to have an activation energy of 62.6 kJ.mol^{-1} (19).

Interconversion of the cyclic forms of fructose occurs through the linear *keto* form. The concentration of this *keto* form is relatively high, being 2-3 orders of magnitude more concentrated than the linear *aldehydo* form of glucose (31, 32). This is significant because these carbonyl tautomers are much more reactive than the ring forms of sugars (33), and can undergo transformations under relatively mild conditions in food production and within the body (34).

Non-enzymatic browning

As a reducing sugar, fructose can undergo non-enzymatic browning reactions such as the Maillard reaction and caramelisation. Due to the increased concentration of the carbonyl-containing form in solution, non-enzymatic browning reactions occur quicker with fructose than with glucose and require less energy (34-37). The products of non-enzymatic browning

reactions that occur during food processing alter the appearance, aroma, flavour and texture of the food, as well as, nutritional value and shelf life (34, 37-42). While the sensorial aspects of food can often be improved by the influence of non-enzymatic browning, this is not always the case (42), and the nutrition and shelf-life of foods affected by non-enzymatic browning is usually reduced (34, 35, 37-41). Non-enzymatic browning, specifically the Maillard reaction, can also occur within the body, leading to toxic by-products described as advanced glycation end-products (43, 44). As such the increased reactivity of fructose to these types of reactions is pertinent to its commercial use.

The Maillard reaction

The Maillard reaction is actually a series of chemical modifications that occur between reducing sugars and compounds containing free amino groups, such as proteins and amino acids commonly found in food, within the body and in biopharmaceuticals (34, 44, 45). In the case of fructose, the *keto* carbonyl group and the amine condense to a Schiff base, which is subsequently isomerised to fructosylamine. Then a new carbonyl bond is generated in the sugar, resulting in the formation of species known as Heyns products in a process related to the Amadori rearrangement of glucose. These products then fragment into reactive intermediates that take part in complex polymerisation reactions, crosslinking proteins and resulting in brown, fluorescent, high molecular weight melanoidins as shown in Figure 2 (34, 45-47).

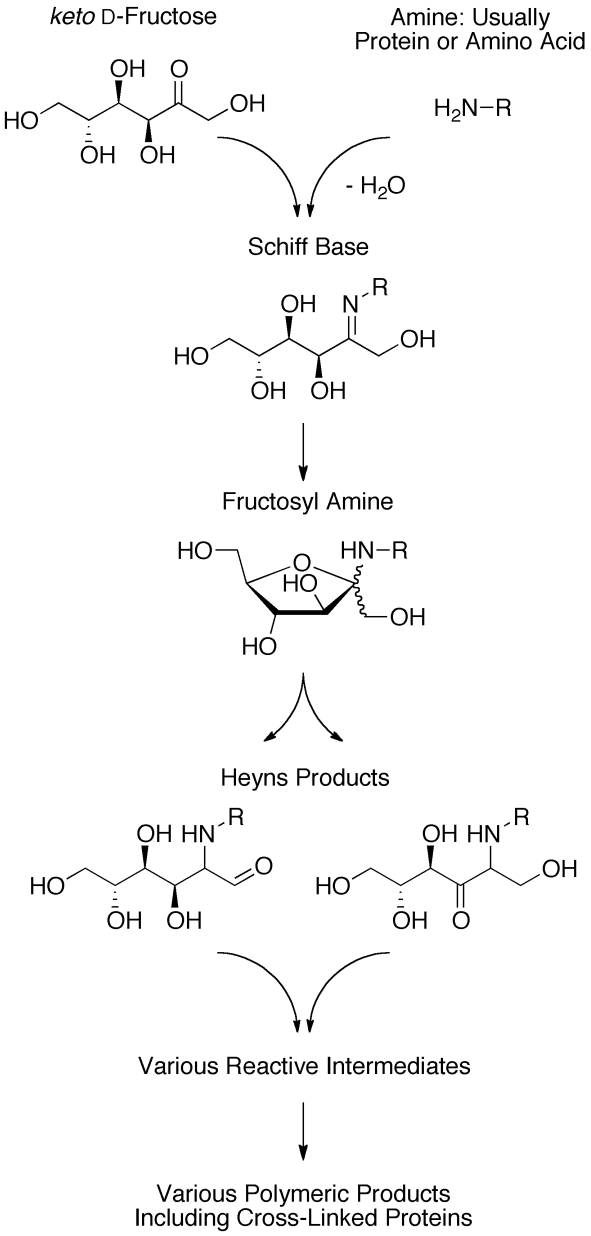

Figure 2 The Maillard Reaction of Fructose

Caramelisation

The term caramelisation refers to a group of reactions, related to the Maillard reaction, that occur when carbohydrates are heated in the absence of amino groups (48). In caramelisation the carbohydrate undergoes 1,2-enolisation and the resulting enol is susceptible to β-elimination of water, forming anhydro- rings or other reactive intermediates (Figure 3). Typical products of this include furans, which in the case of fructose is predominantly 5-hydroxymethylfurfural (HMF) (34, 40, 48-51). Such intermediate products provide some of the caramel aroma and flavor (49, 51) and are the precursors to polymerisations that lead to the brown color of caramel (48, 50). Many of the reactions and products of caramelisation are the same as, or are similar to, those of the Maillard reaction (34, 50). However, caramelisation requires more energy than the Maillard reaction (34, 49) and does not usually occur below the melting point of fructose.

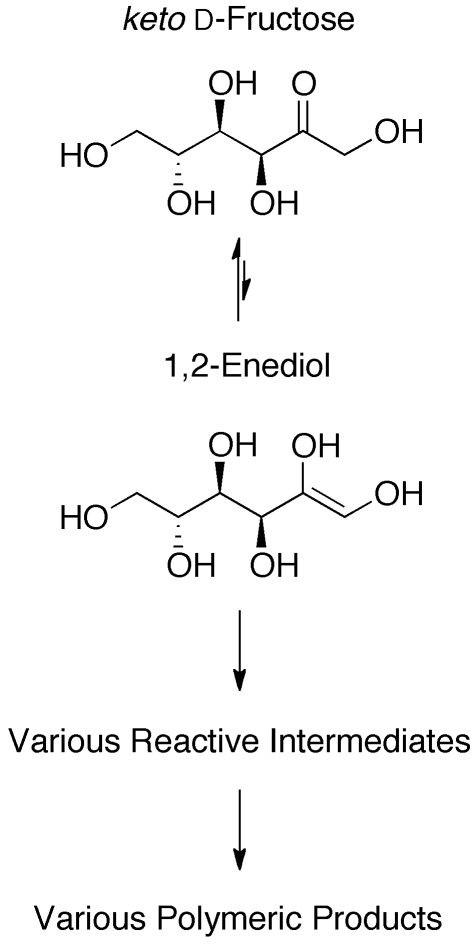

Figure 3 Caramelisation of Fructose

Thus, Maillard products will typically dominate whenever amino groups are abundant.

Nonetheless, the presence of the accelerators in the form of carboxylic acids, and their salts, as well as, phosphates and metal ions, can reduce the temperature required for the caramelisation reaction significantly (34).

HMF formation

5-Hydroxymethylfurfural (HMF) is not only a byproduct of non-enzymatic browning reactions, but is also an important product in its own right, as discussed in more detail below. HMF can be produced from a number of starting materials, including various hexoses and polysaccharides (52). Fructose is generally

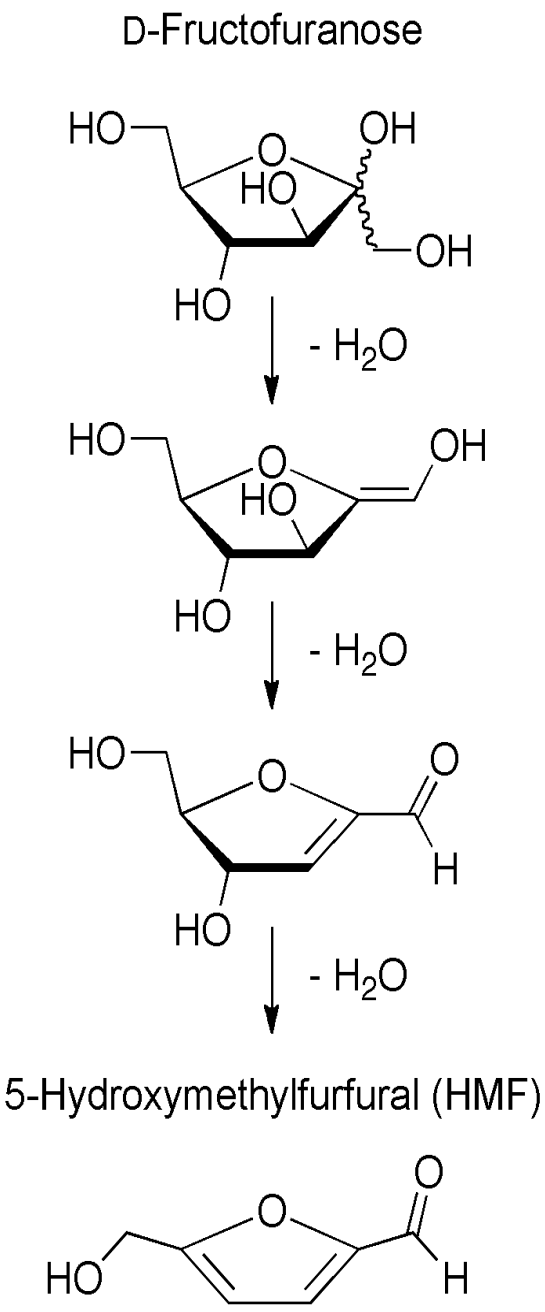

Figure 4 The most likely pathway for the formation of HMF from fructose (52)

an efficient and selective starting material for the production of HMF, enabling more rapid conversion with higher yields than for glucose (49, 53). Nevertheless glucose is often used in HMF production in industry due to its lower cost (52). HMF is produced by the dehydration of fructose, induced by an acid or metal catalyst

(14, 16, 54). The dehydration involves the loss of three water molecules through a complex, as yet not fully defined reaction mechanism (14, 52, 55) to create the final furfural ring system (Figure 4).

SOURCES OF FRUCTOSE

Though the fructose monosaccharide is found in many natural sources, the relatively low concentrations of fructose dictate that commercial quantities of fructose are usually manufactured from sucrose or polysaccharides. These methods of production are, however, complex, often using enzymatic processes for chemical conversion. Additionally, there are difficulties in isolating fructose from mixtures of sugars and in crystallising the highly soluble sugar from aqueous solutions (30, 41, 56). This means that fructose, in its pure crystalline form, is still a relatively high cost sugar (5), part of the reason why pure fructose is still used sparingly in the food industry (41). Much more prevalent is the use of mixtures of fructose and other sugars, mostly glucose and short chain oligosaccharides, in high fructose syrups (HFS) (3, 41).

Fructose from starch

HFS are primarily made from the starch obtained from corn, to make high fructose corn syrup (HFCS). This source is used because it is a readily available, cost-effective source of starch in high concentration (56, 57), though other sources such as rice, wheat, tapioca and potato are used where these crops are predominant (41). The starch is separated from other components of source material and enzymatically hydrolysed to glucose, which in turn is enzymatically isomerised to form fructose. The equilibrium constraints of this isomerisation process mean the concentration of fructose is limited in the initial product (58), commercially generating syrups containing 42% fructose by mass. This syrup, HFCS-42, is utilised mostly in baked goods. The concentration of fructose can be increased by using chromatography (59) to produce a syrup containing 90% fructose, HFCS-90, which is combined with HFCS-42 to form a 55% syrup, HFCS-55, which is used extensively for sweetened drinks such as carbonated beverages (56, 57).

Fructose from sucrose

HFS can also be produced from sucrose isolated from cane sugar or sugar beets, involving a process of heating sucrose solutions to induce hydrolysis, most often in the presence of an acid or enzymatic catalyst. This produces a syrup containing equal quantities of fructose and glucose, known as invert sugar syrup (57, 60). As with HFS produced from starch, increasing the concentration of fructose in the mixture relies on expensive chromatography techniques (59), or potentially toxic additives (5, 61).

Fructose from fructans

Another method to produce fructose is by the hydrolysis of fructan polysaccharides. Fructan polymers, such as inulin and levan, occur as storage carbohydrates in plants and contain predominantly D-fructose residues linked together, with a glucose end cap at the reducing end of the fructosyl chain. Plant-derived inulin is constructed almost exclusively of $\beta(2\rightarrow1)$ glycosidic linkages between fructose units (62-65). In contrast, levan is made up of $\beta(2\rightarrow6)$ glycosidic linkages and is more significantly branched than inulin, with the branches being created from $\beta(2\rightarrow1)$ linkages (4, 66, 67). These biopolymers are used in commercial fructose production (4, 68). Inulin is a particularly valuable source of the simple sugar as plants such as chicory and Jerusalem artichoke can contain inulin carbohydrate in concentrations comparable with other sources of carbohydrate used in fructose production (61).

Fructose can be produced from a simple acid hydrolysis of inulin (69), but concomitant production of undesirable colouring and flavouring components requires costly post-production purification (59). Therefore,

enzymatic hydrolysis is generally used for fructose production from inulin (57, 68). A significant advantage to using inulin as the source material of fructose production is that the concentration of fructose generated can be as high as 95% (70).

APPLICATIONS OF FRUCTOSE

Uses of fructose in food

The predominant commercial application of fructose is in the food industry where it is used extensively in beverages, dairy products and baked goods (40, 41). Sugars in general are important elements of food, providing much more than simply a sweet taste. They also have an impact on the overall flavor of food and are active food components, influencing parameters such as the look, feel and shelf-life of food (40). As an example, sugars affect mouthfeel by providing body and texture themselves while also aiding the formation and retention of food texture influencing aerosols and emulsions. The phase of the sugar is also important to food texture, with dissolved or amorphous sugar contributing to making a product soft and smooth, while crystallised sugar and sugar products of non-enzymatic browning reactions can provide hard or crispy textures. Non-enzymatic browning reactions also influence the look of food, often providing an appetising appearance (40, 41).

Fructose is a valuable sugar in the food industry because, amongst other, it is the sweetest natural sugar (71) and it is less glucogenic than glucose or sucrose (3-5, 57, 61, 71-73). These two factors mean that for a given quantity, it provides less energy than the other sugars, and compounding this benefit, less can used to achieve the same sweetness. Additionally, fructose is synergistic in terms of sweetness when used in combination with other natural and synthetic sweeteners (40, 74).

The way fructose interacts with water is particularly useful in food manufacturing. First, fructose is the most soluble of the monosaccharide sugars which means that it is less prone to crystallization, thus able to maintain the desired texture of high sugar foods and drinks (3, 5, 57). Second, the lower the molecular weight of a sugar, the lesser the amount required to lower the freezing point of water, thus reducing the likelihood of crystallization due to temperature variation. This leads to greater smoothness of ice creams when monosaccharides like fructose are used in manufacture (40). Finally, fructose is also particularly effective at lowering the water activity of foods, retaining moisture in the food and also inhibiting microbial growth (40, 41, 57, 75).

As mentioned previously, pure fructose is used sparingly in the food industry. Where crystalline fructose is used, its low volume to high sweetness ratio is often exploited, such as in dry mix beverages. Another example of a useful application of crystalline fructose is in the production of low calorie foods where particular advantage can be taken of fructose's sweetness synergy with other sweeteners and its relatively low energy relative to its sweetness (41).

In general, manufacturers prefer sugars in syrups for ease of use (57). HFSs are now used almost exclusively to sweeten carbonated beverages, and extensively in other sweetened drinks and in baked products, being more economical and easier to handle than sucrose or sucrose syrups (41). Part of the reason for this is that HFS takes up less space for equivalent quantity of sweetening solids (due to greater solubility and high sweetness of fructose). HFS is also less susceptible to microbial spoilage than sucrose syrups (41). For these reasons, fructose is also used widely in dairy products such as yoghurt, flavoured milks and ice cream. Fructose also aids color stabilisation of jams and jellies (41).

Use of fructose as an excipient

Fructose is used as an excipient in pharmaceutical tablets, syrups and solutions (41, 57, 76). It is unsurprising, given the high sweetness to volume ratio of fructose and its safe history of use in food, that it is often used to make medicines more palatable (7, 8). A mixture of fructose with a small amount of starch (Advantose® FS 95) can also be made compressible, making a palatable excipient suitable for tablet manufacture (8). The way fructose interacts with water is important in its use as an excipient. The high solubility of fructose enables it to aid the solubility of therapeutic agents (11, 12), and in adjusting osmolarity of solutions to make them compatible with parenteral administration (13). The ability to inhibit water crystallisation allows it to serve as a cryoprotectant (9, 10). Moreover, the reluctance of fructose to crystallise itself allows it to sympathetically replace the water hydrogen bonding of labile active ingredients during lyophilisation, stabilising their structure (9, 10, 77). The ability of fructose to protect against the fundamentally different stresses of freezing and subsequent dehydration is an invaluable property for lyoprotectants (78). Of course, fructose and fructose degradation products, such as HMF, have been identified as undesirable side products of using sugar based excipients that need to be heated (79). Heating results in high concentrations of aldehyde leading to a faster formation of coloured, ill defined Maillard products (79). This means that the presence of fructose is not desirable in drug formulations that require sterilization.

Use of fructose in the production of HMF

Another growing use for fructose is in the synthesis of 5-hydroxymethylfurfural (HMF) and other chemicals derived from HMF. These products are a significant bio-based alternative to petrochemicals, being important precursors in chemical and polymer manufacture and the production of biofuels (14-16, 80). It is the functional groups of HMF, comprising the furan ring, a primary aromatic alcohol and an aldehyde, that provide the varied synthetic opportunities that make it a valuable, renewable chemical building block (14-16). A weakness to this point is that high production costs limit its use on an industrial scale (15, 16), but as the cost of petroleum-based products increases, HMF is likely to become an important renewable alternative.

HEALTH IMPLICATIONS OF CONSUMING FRUCTOSE

Fructose is 'generally recognised as safe' (GRAS) by the United States Food and Drug Administration (FDA) (81) and is less likely to induce dental cavities than sucrose (82). Once ingested, the specifics of the health implications of fructose are related to the distinct differences between the metabolism of glucose and fructose. For example, fructose present in the gut is absorbed more slowly than glucose, with fructose specific transporters moving it through the enterocytes to the portal bloodstream and thereafter to the liver (83-85). Due to the slow absorption of fructose, consumption of large amounts can exceed the capacity of the intestinal absorption, though co-consumption with glucose can enhance fructose absorption (86). Nevertheless, when consumed in excess of dietary glucose, fructose may be malabsorbed (86-88), leading to abdominal discomfort and diarrhoea (89).

While the liver only accounts for approximately 20-30% of glucose metabolism, it is responsible for 50-70% of fructose metabolism (90). This metabolism of fructose leads to different products than glucose, fructose being metabolised more like fat than other carbohydrates (85), being converted to relatively high amounts of lactate (91, 92). Lactate is a precursor for lipid synthesis, so that fructose is more lipogenic than other carbohydrates and can encourage unfavourable lipid profiles (3, 57, 83, 85, 93-95). Lactate production also enhances the formation of uric acid (96), which may be detrimental for individuals prone to gout (6).

Fructose has a lower glycaemic index compared with other natural sweeteners (72, 75, 83, 94), and therefore from the late 17th century it has been considered a valuable replacement sugar for diabetics (3, 97). In particular, because it does not induce insulin secretion and does not require insulin to be transported and metabolised, fructose has been recommended for individuals with insulin resistance and type 2 diabetes (6). However, scientific data is still inconclusive as to the benefits of fructose as a sucrose substitute for the long term management of diabetes (1, 6, 83). For patients with hypertriglyceridemia it can also lead to further increased insulin levels (98).

As fructose does not stimulate insulin secretion from pancreatic β-cells, less insulin is released with fructose than if a glucose-containing food was consumed (3, 99). The lower insulin levels, the consequent lower leptin concentrations and attenuation of the suppression of ghrelin may increase the likelihood of obesity in individuals with diets high in dietary fructose (94, 100-103). It has also been suggested that consumption of fructose contributes to insulin resistance syndrome (85, 93, 104). Nonetheless, the evidence of a link between consumption of HFS and obesity is weak (56, 105). Part of the reason for this is that many of the health issues associated with fructose consumption have been investigated using pure fructose. However, the bulk of fructose added to food is as HFS that, on average, contains almost 50% glucose. Thus, in practice the metabolism and energy consumption of fructose in HFS is similar to that found for the consumption of sucrose (56, 83, 105, 106), and so health studies conducted on pure fructose may be irrelevant to the effects of HFS (105).

The relatively high concentration of the reactive *keto* form of fructose at equilibrium, means that it can more readily be involved in reactions that may lead to the formation of toxic by-products (36, 43). Given that fructose is often used in food products subject to conditions in which non-enzymatic browning can occur, such as cooking, the by-products of these reactions have to be considered as well. Generally the latter products of the Maillard reaction are not energetically available to animals, so there is a loss of the nutritional value of the sugars and amino acids involved in these reactions (40, 42, 44, 107). Also, the Maillard reaction products affect the absorption of other intact nutrients, mostly from derivatised proteins in which the digestibility is influenced (38, 107). Maillard products that can influence other metabolic functions, such as the absorption of minerals, create further nutritional impact (38, 107). Other health effects are generated by Maillard products, which can have limited mutagenic and anti-mutagenic properties (44). These effects can be difficult to isolate as the same conditions lead to both types of products (108). HMF is one of the intermediate species created during the Maillard and caramelisation reactions and consequently can be found in many foods (14). Investigations have been conducted into the effects of HMF on humans, generally finding little evidence of adverse effect in concentrations correlated to the amounts found in normal human diets (14). There has been some weak evidence of a mutagenic effect, but the compounds responsible for the effect were not isolated, so other compounds related to HMF may be responsible (109, 110). Moreover, HMF is found in Asian natural remedies and may be beneficial for the liver and for therapy of sickle cell disease (111-113). Further benefit of HMF specifically, and caramelisation products in general, is that they have an antioxidant effect (51). Fructose caramelisation products have stronger antioxidant activity compared to caramelisation products of glucose, ribose or xylose (51, 114).

Advanced glycation end-products of the Maillard reactions occurring within the body can accelerate the aging process through protein cross-linking reactions (43, 44). High dietary intake of fructose may lead to increased fructose concentration in tissues, with fructose undergoing faster Maillard reactions than other simple carbohydrates. This could increase the detrimental effects on body proteins (36) and lead to increased triggering of the receptor for

advanced glycation end products, through which cells recognise and respond to these Maillard products, often in a detrimental fashion (115, 116).

Fructose in fructans

Fructans, as well as being a source of the monosaccharide fructose, can also be considered a way of using fructose in oligomeric or polymeric form. These species are used in diverse applications including the chemical, pharmaceutical and food industries (62, 117). In the chemical industry fructans are used as precursors in the synthesis of a wide range of compounds (117, 118). In the pharmaceutical industry their applications includes use as stabilisers, excipients, clinical tools and therapeutics (62). In the food industry inulin and fructose oligosaccharides are one of the largest classes of bifidogenic foods for health (119). This is because humans cannot digest the glycosidic linkages of fructans, making them low energy foods. These linkages, however, can be digested by bifidobacteria in the gut, but not generally by pathogenic bacteria (120), thereby encouraging the growth of health promoting intestinal microflora (3, 57, 62, 119, 121-123).

Fructooligosaccharides

Fructooligosaccharides are essentially short chain inulin species that are enzymatically hydrolysed from inulin (119) or enzymatically synthesised from sucrose, and are water soluble, non-caloric, non-cariogenic and indigestible sweeteners (119, 123, 124). An important feature of these sweeteners is that fructooligosaccharides produced from sucrose have no reducing ends and cannot reorganise into carbonyl containing forms. Consequently, such sweeteners are stabilised to non-enzymatic browning reactions (57).

Inulin

Long chain inulin is essentially tasteless, but is nonetheless of value in the food industry because of its probiotic promoting features and its ability to gel aqueous solutions. The volume of the gel makes it an ideal bulking agent, replacing fat and flour. Additionally the texture of the gel mimics the mouthfeel of fat making it an important component in low calorie foods (118, 121, 125). After consumption, by-products of the metabolite of inulin have been shown to be protective against colon cancer (126) and suppress appetite (127) thereby creating health benefits. More specific health benefits are also generated through the pharmaceutical use of inulin. For example, solubilised inulin can be used for testing kidney function (128), gelled and solid amorphous inulins can be used as drug delivery systems, capable of stabilising labile therapeutics (77, 129) and providing controlled and targeted drug release (130-132). Additionally, crystalline forms of inulin have an immunomodulatory effect relevant to its use as a vaccine adjuvant. Advax™ adjuvant, currently in advanced stages of clinical development, is produced from plant-derived inulin by crystallising it into a lower solubility delta polymorphic type (133, 134). Intriguingly, while soluble inulin has no measurable effects on immune cells, once precipitated into particles of semi-crystalline delta inulin, it becomes highly immunologically active, binding to mononuclear cells, up-regulating co-stimulatory molecules and enhancing adaptive immune responses. This is consistent with the observation that several plant-derived carbohydrates have vaccine adjuvant activity (135). Inulin adjuvants have been shown to successfully increase the effectiveness of a broad range of inactivated or recombinant protein antigens, including vaccines against pandemic and seasonal influenza, Japanese encephalitis, the West Nile virus, hepatitis B, and malaria (136-140). The demonstrated safety of inulin in humans provides a major advantage for its use in the design of vaccine adjuvants, particularly those that are likely to be used in childhood vaccines where safety considerations are paramount. Another important aspect of inulin adjuvants is their excellent tolerability as assessed in a recent camel immunization study which showed that

Advax™ delta inulin adjuvant was better tolerated than a range of commercially available veterinary adjuvants (141).

Interestingly, another crystalline form of inulin, gamma inulin, has been shown to have direct anti-cancer effects, thought to be mediated via its ability to modululate immune function via activation of the alternative complement pathway (142, 143). These anti-cancer effects of gamma inulin synergise with other cancer therapies, such as phototherapy, raising the possibility of use as an adjunct to standard cancer therapy (144).

CONCLUSION

Fructose is a valuable sugar and its use is expected to continue to grow as its commercial production expands and it becomes more cost effective to manufacture. One way of lowering the cost of manufacture is to change the source of the raw material to fructose-containing polysaccharides, such as inulin. This would reduce the number of steps in the production of fructose. However, more research in the use of inulin in fructose production and in maximizing the generation of inulin from cost effective crops is required. Less expensive sources of fructose will support its continued use as an excipient and could also make HMF a real, renewable alternative to petrochemicals in the short to medium term.

More cost effective production of fructose will lead to its increased use in food manufacturing. If this is to occur, more research must be undertaken into the health implications of dietary and pharmaceutical fructose. In particular, the ambiguity in perceived health concerns attributed specifically to fructose must be resolved, not only for pure fructose, but also for the high fructose/glucose mixtures commonly used in food. By contrast the dietary use of fructan polysaccharides is likely to continue to expand given their proven health promoting benefits. Finally, exciting new applications of fructan polysaccharides as drug stabilisers, controlled release drug delivery systems and vaccine adjuvants herald expansion of pharmaceutical applications of this highly versatile plant-derived sugar.

ACKNOWLEDGEMENTS

This work was supported by the National Institute of Allergy and Infectious Diseases, NIH [Contracts U01-AI061142 and HHSN272200800039C]. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health or the National Institute of Allergy and Infectious Diseases. This work was also supported by The Australian Research Council through a Linkage Grant [LP0882596].

REFERENCES

1 Wang Y, Vaneys J. Nutritional significance of fructose and sugar alcohols. Annu Rev Nutr, 1: 437-475. 1981.

2 Wrolstad R, Shallenberger R. Free sugars and sorbitol in fruits: A compilation from the literature. J Assoc Off Ana Chem, 64: 91-103, 1981.

3 Hallfrisch J. Metabolic effects of dietary fructose. FASEB J, 4: 2652-2660, 1990.

4 Rumessen J. Fructose and related food carbohydrates: Sources, intake, absorption, and clinical implications. Scand J Gastroentero, 27: 819-828 1992.

5 Ricca E, Calabrò V, Curcio S, Iorio G. The state of the art in the production of fructose from inulin enzymatic hydrolysis. Crit Rev Biotechnol, 27: 129-145, 2007.

6 Henry R, Crapo P, Thorburn A. Current issues in fructose metabolism. Annu Rev Nutr, 11: 21-39 1991.

7 Kumar A, Rawlings RD, Beaman DC. The mystery ingredients: Sweeteners, flavorings, dyes, and preservatives in analgesic/antipyretic, antihistamine/decongestant, cough and cold, antidiarrheal, and liquid theophylline preparations. Pediatrics, 91: 927-933, 1993.

8 Mulderrig K. Placebo evaluation of selected sugar-based excipients: in pharmaceutical and nutraceutical tableting. Pharm Technol, 24: 34-44, 2000.

9 Date PV, Samad A, Devarajan PV. Freeze thaw: A simple approach for prediction of optimal

cryoprotectant for freeze drying. AAPS Pharmscitech, 11: 304-313, 2010.

10 Toegel S, Salar-Behzadi S, Horaczek-Clausen A, Viernstein H. Preservation of aerial conidia and biomasses from entomopathogenic fungi *Beauveria brongniartii* and *Metarhizium anisopliae* during lyophilization. J Invertebr Pathol. 105: 16-23, 2010.

11 Schmidt E, Dooley N, Ford SJ, Elliott M, Halbert GW. Physicochemical investigation of the influence of saccharide-based parenteral formulation excipients on L-*p*-boronphenylalanine solubilisation for boron neutron capture therapy. J Pharm Sci, 101: 223-232, 2011.

12 Mallard C, Coudane J, Rault I, Vert M. In vitro delivery of a sparingly water soluble compound from PLA50 microparticles. J Microencapsul, 17: 13-28, 2000.

13 Evans R, Nawrocki D, Isopi L. Development of stable liquid formulations for adenovirus-based vaccines. J Pharm Sci, 93: 2458-2475, 2004.

14 Rosatella AA, Simeonov SP, Frade RFM, Afonso CAM. 5-Hydroxymethylfurfural (HMF) as a building block platform: Biological properties, synthesis and synthetic applications. Green Chem, 13: 754-793, 2011.

15 Zakrzewska ME, Bogel-Łukasik E, Bogel-Łukasik R. Ionic liquid-mediated formation of 5-hydroxymethylfurfural: A promising biomass-derived building block. Chem Rev, 111: 397-417, 2011.

16 Tong X, Ma Y, Li Y. Biomass into chemicals: Conversion of sugars to furan derivatives by catalytic processes. Appl Catal A- Gen, 385: 1-13, 2010.

17 Krüger D, Grossklaus R, Herold M, Lorenz S, Klingebiel L. Gastrointestinal transit and digestibility of maltitol, sucrose and sorbitol in rats: A multicompartmental model and recovery study. Experientia, 48: 733-740, 1992.

18 Shallenberger R. Intrinsic chemistry of fructose. Pure Appl Chem, 50: 1409-1420, 1978.

19 Barclay T, Ginic-Markovic M, Johnston M, Cooper P, Petrovsky, N. Observation of the keto tautomer of D-fructose in D_2O using ^1H NMR spectroscopy. Carbohyd Res, 347: 136-141, 2011.

20 Cockman M, Kubler D, Oswald A, Wilson L. The mutarotation of fructose and the invertase hydrolysis of sucrose. J Carbohyd Chem, 6: 181-201, 1987.

21 Yaylayan V, Ismail A, Mandeville S. Quantitative determination of the effect of pH and temperature on the *keto* form of D-fructose by FT IR spectroscopy. Carbohyd Res, 248: 355-360, 1993.

22 Mega T, Cortes S, Vanetten R. The ^{18}O isotope shift in ^{13}C nuclear magnetic resonance spectroscopy: 13. Oxygen exchange at the anomeric carbon of D-glucose, D-mannose, and D-fructose. J Org Chem, 55: 522-528, 1990.

23 Zhu Y, Zajicek J, Serianni A. Acyclic forms of [1-^{13}C]aldohexoses in aqueous solution: Quantitation by ^{13}C NMR and deuterium isotope effects on tautomeric equilibria. J Org Chem, 66: 6244-6251, 2001.

24 Angyal S, Dawes K. Conformational analysis in carbohydrate chemistry: II. Equilibria between reducing sugars and their glycosidic anhydrides. Aust J Chem, 21: 2747-2760, 1968.

25 Rudrum M, Shaw D. The structure and conformation of some monosaccharides in solution. J Chem Soc, 1965: 52-57, 1965.

26 Angyal S. The composition and conformation of sugars in solution. Angew Chem Int Edit, 8: 157-166, 1969.

27 Angyal S. The composition of reducing sugars in solution: Current aspects. Adv Carbohyd Chem Biochem, 49: 19-35, 1991.

28 Goux W. Complex isomerisation of ketoses: A ^{13}C NMR-study of the base-catalyzed ring-opening and ring-closing rates of D-fructose isomers in aqueous solution. J Am Chem Soc, 107: 4320-4327, 1985.

29 Bubb W. NMR spectroscopy in the study of carbohydrates: Characterizing the structural complexity. Concept Magn Reson A, 19: 1-19, 2003.

30 Flood A, Johns M, White E. Mutarotation of D-fructose in aqueous-ethanolic solutions and its influence on crystallisation. Carbohyd Res, 288: 45-56, 1996.

31 Avigad G, Englard S, Listowsky I. Evaluation of the proportion of the *keto* form of D-fructose and related 2-*keto*hexoses present in aqueous solution. Carbohyd Res, 14: 365–373, 1970.

32 Hayward DL. A Symmetry rule for the circular dichroism of reducing sugars, and the proportion of carbonyl forms in aqueous solutions thereof. Carbohyd Res, 53: 13-20, 1977.

33 Kuszmann J. Introduction to carbohydrates, in Levy D E; Fügedi P (eds), The organic chemistry of sugars. *Taylor & Francis Group*, Boca Raton, pp 25-52, 2006.

34 Hodge JE. Dehydrated foods: chemistry of browning reactions in model systems. J Agr Food Chem 1: 928-943, 1953.

35 Maillard LC. Action des acides aminés sur les sucres: Formation des mélanoidins par voine méthodologique. CR Acad Sci, 154: 66-68, 1912.

36 Bunn H, Higgins P. Reaction of monosaccharides with proteins: Possible evolutionary significance. Science, 213: 222-224, 1981.

37 Buera M, Chirife J, Resnik SL, Lozano RD. Nonenzymatic browning in liquid model systems of high water activity: Kinetics of color changes due to caramelization of various single sugars. J Food Sci, 52: 1059-1062, 1987.

38 Hurrell R. Influence of the Maillard reaction on the nutritional value of foods, in Finot PA, Aeschbacher HU, Hurrell RF, Liardon R (eds), The Maillard reaction: Advances in Life Sciences. *Bikhäuser Verlag*, Basel, pp 245-258, 1990.

39 Buera MDP, Chirife J, Resnik SL, Wetzler G. Nonenzymatic Browning in Liquid Model Systems of High Water Activity: Kinetics of Color Changes due to Maillard's Reaction Between Different Single Sugars and Glycine and Comparison with Caramelization Browning. J Food Sci, 52: 1063-1067, 1987.

40 Davis E. Functionality of Sugars: Physicochemical Interactions in Foods. Am J Clin Nutr, 62: S170-S177, 1995.

41 Hanover L, White J. Manufacturing, Composition, and Applications of Fructose. Am J Clin Nutr, 58: S724-S732, 1993.

42 Mauron J. The Maillard reaction in food: A critical review from the nutritional standpoint. Prog Food Nutr Sci, 5: 5-35, 1981.

43 Gaby AR. Adverse effects of dietary fructose. Altern Med Rev, 10: 294-306, 2005.

44 Dills W. Protein fructosylation: Fructose and the Maillard reaction. Am. J. Clin. Nutr. 58: 779S-787S, 1993.

45 Adams A, Borrelli R, Fogliano V, De Kimpe N. Thermal degradation studies of food melanoidins. J Agric Food Chem, 53: 4136-4142, 2005.

46 Suarez G, Maturana J, Oronsky AL, Raventós-Suárez C. Fructose-induced fluorescence generation of reductively methylated glycated bovine serum albumin: Evidence for nonenzymatic glycation of Amadori adducts. Biochim Biophy Acta, 1075: 12-19, 1991.

47 Suarez G, Rajaram R, Oronsky A, Gawinowicz M. Nonenzymatic glycation of bovine serum-albumin by fructose (fructation): Comparison with the Maillard reaction initiated by glucose. J Biol Chem, 264: 3674-3679, 1989.

48 Quintas M, Brandão T, Silva C. Modelling colour changes during the caramelisation reaction. J Food Eng, 83: 483-491, 2007.

49 Kroh L. Caramelisation in Food and Beverages. Food Chem, 51: 373-379, 1994.

50 Kovats L, Orsi F. Some observations on caramelization. Period Polytech Chem, 17: 373-385, 1973.

51 Chen S-L, Yang D-J, Chen H-Y, Liu S-C. Effect of hot acidic fructose solution on caramelisation intermediates including colour, hydroxymethylfurfural and antioxidative activity changes. Food Chem, 114: 582-588, 2009.

52 Lewkowski J. Synthesis, chemistry and applications of 5-hydroxymethyl-furfural and its derivatives. Arkivoc, 17-54, 2001.

53 Theander O, Nelson D. Aqueous, high-temperature transformation of carbohydrates relative to utilization of biomass. Adv Carbohyd Chem Biochem, 46: 273-326, 1988.

54 Cottier L, Descotes G. 5-Hydroxymethylfurfural syntheses and chemical transformations. Trends Heterocycl Chem, 2: 233-248, 1991.

55 Antal M, Mok W, Richards G. Kinetic-studies of the reactions of ketoses and aldoses in water at high-temperature: 1. Mechanism of formation of 5-(hydroxymethyl)-2-furaldehyde from D-fructose and sucrose. Carbohyd Res, 199: 91-109, 1990.

56 White JS. Straight talk about high-fructose corn syrup: What it is and what it ain't. Am J Clin Nutr, 88: 1716S-1721S, 2008.

57 Lima DM, Fernandes P, Nascimento DS, Ribeiro RCLF, de Assis SA. Fructose syrup: A biotechnology asset. Food Technol Biotech, 49: 424-434, 2011.

58 Zittan L. Enzymatic-hydrolysis of inulin: An alternative way to fructose production. Starch-Stärke, 33: 373-377, 1981.

59 Kochhar A, Gupta A, Kaur N. Purification and immobilisation of inulinase from Aspergillus candidus for producing fructose. J Sci Food Agr, 79: 549-554, 1999.

60 Zhang Y, Hidajat K, Ray AK. Optimal design and operation of SMB bioreactor: Production of high fructose syrup by isomerization of glucose. Biochem Eng J, 21: 111-121, 2004.

61 Fleming S, Grootwassink J. Preparation of high-fructose syrup from the tubers of the jerusalem artichoke (*Helianthus tuberosus L.*). CRC Crit Rev Food Sci, 12: 1-28, 1979.

62 Barclay T, Ginic-Markovic M, Cooper P, Petrovsky N. Inulin: A versatile polysaccharide with multiple pharmaceutical and food chemical uses. J Excipients Food Chem, 1: 27-50, 2010.

63 Andre I, Mazeau K, Tvaroska I, Putaux J-L, Winter WT, Taravel FR, Chanzy H. Molecular and crystal structures of inulin from electron diffraction data. Macromolecules, 29: 4626-4635, 1996.

64 Carpita N, Housley T, Hendrix J. New features of plant-fructan structure revealed by methylation analysis and ^{13}C NMR spectroscopy. Carbohyd Res, 217: 127-136, 1991.

65 Oka M, Ota N, Mino Y, Iwashita T, Komura H. Studies on the conformational aspects of inulin oligomers. Chem Pharm Bull, 40: 1203-1207, 1992.

66 French A. Chemical and physical properties of fructans. J Plant Physiol, 134: 125-136, 1989.

67 French A. Accessible conformations of the β-D-(2→1)- and β-D-(2→1)-linked D-fructans inulin and levan. Carbohyd Res, 176: 17-29, 1988.

68 Ricca E, Calabrò V, Curcio S, Iorio G. Fructose production by chicory inulin enzymatic hydrolysis: A kinetic study and reaction mechanism. Process Biochem, 44: 466-470, 2009.

69 Barclay T, Ginic-Markovic M, Johnston M.R, Cooper PD, Petrovsky N. Analysis of the hydrolysis of inulin using real time ^{1}H NMR spectroscopy. Carbohyd Res, 352: 117-125, 2012

70 Kim C, Rhee S. Fructose production from Jerusalem artichoke by inulinase immobilized on chitin. Biotechnol Lett, 11: 201-206, 1989.

71 Bean MM, Setser CS., Polysaccharides, sugars, and sweeteners in Bowers J (ed), Food theory and applications. *Macmillan Publishing*, New York, pp 69-198, 1992.

72 Crapo PA, Kolterman OG, Olefsky JM. Effects of oral fructose in normal, diabetic, and impaired glucose tolerance subjects. Diabetes Care, 3: 575-582, 1980.

73 Gupta A, Kaur N. Fructan storing plants: A potential source of high fructose syrups. J Sci Ind Res India, 56: 447-452, 1997.

74 Van der Heijden A, Brussel L, Heidema J, Kosmeijer J, Peer H. Interrelationships among synergism, potentiation, enhancement and expanded perceived intensity vs concentration. J Food Sci, 48: 1192-1196, 1983.

75 Schved F, Hassidov B. Fructose: a high quality sweetener - Flavour enhancing, calorie-reduction and impact on glycemic load. Agro Food Ind Hi Tec, 19: 26-28, 2008.

76 Enterprise Directorate-General. Guidelines: Medicinal products for human use. Safety, environment and information. *European Commision,* Brussels, 2003.

77 Hinrichs W, Prinsen M, Frijlink H. Inulin glasses for the stabilization of therapeutic proteins. Int J Pharm, 215: 163-174, 2001.

78 Crowe, J., Carpenter J, Crowe L, Anchordoguy T. Are freezing and dehydration similar stress vectors: A comparison of modes of interaction of stabilizing solutes with biomolecules. Cryobiology, 27: 219-231, 1990.

79 Crowley P, Martini LG. Drug-excipient interactions. Pharm Technol, 4: 7-12, 2001.

80 Huber GW, Iborra S, Corma A. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. Chem Rev, 106: 4044-4098, 2006.

81 Duffy V, Sigman-Grant M. Position of the American Dietetic Association: Use of nutritive and nonnutritive sweeteners. J Am Diet Assoc, 104: 255-275, 2004.

82 Frostell GG, Keyes PHP, Larson RHR. Effect of various sugars and sugar substitutes on dental caries in hamsters and rats. J Nutr, 93: 65-76, 1967.

83 Havel PJ. Dietary fructose: Implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. Nutr Rev, 63: 133-157, 2005.

84 Corpe C, Burant C, Hoekstra J. Intestinal fructose absorption: Clinical and molecular aspects. J Pediatr Gastr Nutr, 28: 364-374, 1999.

85 Lustig RH. Fructose: Metabolic, hedonic, and societal parallels with ethanol. J Am Diet Assoc, 110: 1307-1321, 2010.

86 Truswell A, Seach J. Incomplete absorption of pure fructose in healthy subjects and the facilitating effect of glucose. Am J Clin Nutr, 48: 1424-1430, 1988.

87 Rumessen J, Gudmandhoyer E. Absorption capacity of fructose in healthy-Adults: Comparison with sucrose and its constituent monosaccharides. Gut, 27: 1161-1168, 1986.

88 Kneepkens C, Vonk R. Incomplete intestinal absorption of fructose. Arch Dis Child, 59: 735-738, 1984.

89 Rumessen J, Gudmandhoyer E. Functional bowel-disease: malabsorption and abdominal distress after ingestion of fructose, sorbitol, and fructose-sorbitol mixtures. Gastroenterology, 95: 694-700, 1988.

90 Bizeau ME, Pagliassotti MJ. Hepatic adaptations to sucrose and fructose. Metabolism, 54: 1189-1201, 2005.

91 Tappy L, Le KA. Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev, 90: 23-46, 2010.

92 Bjorkman O, Crump M, Phillips R. Intestinal metabolism of orally-administered glucose and fructose in Yucatan miniature swine. J Nutr, 114: 1413-1420, 1984.

93 Elliott S, Keim N, Stern J, Teff K, Havel P. Fructose, weight gain, and the insulin resistance syndrome. Am J Clin Nutr, 76: 911-922, 2002.

94 Teff K, Elliot SS, Tschöp M, Kieffer TJ, Rader D, Heiman M, Townsend RR, Keim NL, D'alessio D, Havel PJ. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. J Clin Endocr Metab, 89: 2963-2972, 2004.

95 Herman RH, Zakim D, Stifel FB. Effect of diet on lipid metabolism in experimental animals and man. Fed Proc, 29: 1302-1307, 1970.

96 Van den Berghe G. Fructose: Metabolism and short-term effects on carbohydrate and purine metabolic pathways. Prog Biochem Pharmacol, 21: 1-32, 1986.

97 Külz E., Beiträge zur pathologie und therapie der diabetes mellitus. *Elwert Verlag*, Marburg, pp 130-146, 1874.

98 Thorburn A, Crapo P, Beltz W. Lipid metabolism in non-insulin-dependent diabetes: Effects of long-term treatment with fructose-supplemented mixed meals. Am J Clin Nutr, 50: 1015-1022, 1989.

99 Curry DLD. Effects of mannose and fructose on the synthesis and secretion of insulin. Pancreas, 4: 2-9, 1988.

100 Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature, 404: 661-671, 2000.

101 Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature, 443: 289-295, 2006.

102 Havel P. Control of energy homeostasis and insulin action by adipocyte hormones: Leptin, acylation stimulating protein, and adiponectin. Curr Opin Lipidol, 13: 51-59, 2002.

103 Havel P, Townsend R, Chaump L, Teff K. High-fat meals reduce 24-h circulating leptin concentrations in women. Diabetes, 48: 334-341, 1999.

104 Beck-Nielsen H, Pedersen O, Lindskov HO. Impaired cellular insulin binding and insulin sensitivity induced by high-fructose feeding in normal subjects. Am. J. Clin. Nutr, 33: 273-278, 1980.

105 Forshee RA, Storey ML, Allison DB, Glinsmann WH, Hein GL, Lineback DR, Miller SA, Nicklas TA, Weaver GA, White JS. A critical examination of the evidence relating high fructose corn syrup and weight gain. Crit Rev Food Sci, 47: 561-582, 2007.

106 Bray G, Nielsen S, Popkin B. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. (vol 79, pg 537, 2004). Am J Clin Nutr, 79: 537-543, 2004.

107 Finot PA. Metabolism and physiological effects of Maillard reaction products (MRP) in Finot PA, Liardon R, Hurrell RF, Aeschbacher HU (eds) The Maillard reaction: Advances in Life Sciences, Bikhäuser Verlag, Basel, pp 259-272, 1990.

108 Aeschenbacher HU. Anticarcinogenic effect of browning reaction products. in Finot PA, Liardon R, Hurrell RF, Aeschbacher HU (eds) The Maillard reaction: Advances in Life Sciences, Bikhäuser Verlag, Basel, pp 335-348, 1990.

109 Brands C, Alink G, van Boekel M, Jongen W. Mutagenicity of heated sugar - Casein systems: Effect of the Maillard reaction. J Agr Food Chem, 48; 2271-2275, 2000.

110 Surh Y, Liem A, Miller J, Tannenbaum S. 5-Sulfoxymethylfurfural as a possible ultimate mutagenic and carcinogenic metabolite of the Maillard reaction-product, 5-hydroxymethylfurfural. Carcinogenesis, 15: 2375-2377, 1994.

111 Abdulmalik O, Safo MK, Chen Q, Yang J, Brugnara C, Ohene-Frempong K, Abraham DJ, Asakura T. 5-hydroxymethyl-2-furfural modifies intracellular sickle haemoglobin and inhibits sickling of red blood cells. Brit J Haematol, 128: 552-561, 2005.

112 Lin A-S, Qian K, Usami Y, Lin L, Itokawa H, Hsu C, Morris-Natschke SL, Lee K-H. 5-Hydroxymethyl-2-furfural, a clinical trials agent for sickle cell anemia, and its mono/di-glucosides from classically processed steamed *Rehmanniae Radix*. J Nat Med, 62: 164-167, 2008.

113 Ding X, Wang M-Y, Yao Y-X, Li G-Y, Cai B-C. Protective effect of 5-hydroxymethylfurfural derived from processed *Fructus Corni* on human hepatocyte LO2 injured by hydrogen peroxide and its mechanism. J Ethnopharmacol, 128: 373-376, 2010.

114 Phongkanpai V, Benjakul S, Tanaka M. Effect of pH on antioxidative activity and other characteristics of caramelization products. J Food Biochem, 30: 174-186, 2006.

115 Wu C-H, Huang S-M, Lin J-A, Yen G-C. Inhibition of advanced glycation endproduct formation by foodstuffs. Food Funct, 2: 224-234, 2011.

116 Unoki H, Yamagishi S-I. Advanced glycation end products and insulin resistance. Curr Pharm Design 14: 987-989, 2008.

117 Fuchs A. Potentials for non-food utilization of fructose and inulin. Starch-Stärke, 39: 335-343, 1987.

118 Stevens C, Meriggi A, Booten K. Chemical modification of inulin, a valuable renewable resource, and its industrial applications. Biomacromolecules, 2: 1-16, 2001.

119 Crittenden R, Playne M. Production, properties and applications of food-grade oligosaccharides. Trends Food Sci Tech, 7: 353-361, 1996.

120 Tomomatsu H. Health effects of oligosaccharides. Food Technol, 48: 61-65, 1994.

121 Roberfroid M. Introducing inulin-type fructans. Brit J Nutr 93, S13-S25, 2005.

122 Vanloo J, Coussement P, Deleenheer L, Hoebregs H, Smits G. On the presence of inulin and oligofructose as natural ingredients in the western diet. Crit Rev Food Sci, 35: 525-552, 1995.

123 Yun J. Fructooligosaccharides: Occurrence, preparation, and application. Enzyme Microb Tech, 19: 107-117, 1996.

124 Sangeetha P, Ramesh M. Recent trends in the microbial production, analysis and application of Fructooligosaccharides. Trends Food Sci Tech, 16: 442-457, 2005.

125 Franck A, De Leenheer L. Inulin in Steinbuchel A, De Baets S, Vandamme E. (eds) Biopolymers, Polysaccharides II: Polysaccharides from Eukaryotes, *Wiley-VCH Verlag GmbH*, Berlin, Volume 6, pp 439-479, 2002.

126 Hague A, Manning AM, Hanlon KA, Hart D, Paraskeva C, Huschtscha LI. Sodium butyrate induces apoptosis in human colonic tumour cell lines in a p53-independent pathway: Implications for the possible role of dietary fibre in the prevention of large-bowel cancer. Int. J. Cancer, 55: 498-505, 1993.

127 Delzenne NM, Cani PD, Neyrinck AM. Modulation of glucagon-like peptide 1 and energy metabolism by inulin and oligofructose: Experimental data. J Nutr, 137: 2547S-2551S, 2007.

128 Phelps C. The physical properties of inulin solutions. Biochem J, 95: 41-47, 1965.

129 Amorij J-P, Huckriede A, Wilschut J, Frijlink HW, Hinrichs WLJ. Development of stable influenza vaccine powder formulations: Challenges and possibilities. Pharm Res, 25: 1256-1273, 2008.

130 Vervoort L, Van den Mooter G, Augustijns P, Bousson R, Toppet S, Kinget R. Inulin hydrogels as carriers for colonic drug targeting: I. Synthesis and characterization of methacrylated inulin and hydrogen formation. Pharm Res, 14: 1730-1737, 1997.

131 Castelli F. Sarpietro MG, Micieli D, Ottimo S, Pitarresi G, Tripodo G, Carlisi B, Giammona G. Differential scanning calorimetry study on drug release from an inulin-based hydrogel and its interaction with a biomembrane model: pH and loading effect. Eur J Pharm Sci, 35: 76-85, 2008.

132 Maris B, Verheyden L, Van Reeth K, Samyn C, Augustijns P, Kinget R, Van den Mooter G. Synthesis and characterisation of inulin-azo hydrogels designed for colon targeting. Int J Pharm, 213: 143-152, 2001.

133 Cooper P, Petrovsky, N. New Polymorphic Form of Inulin and Uses Thereof. Patent W.I.P. WO 2006/024100 A1, pp 1–53, 2006.

134 Cooper PD, Petrovsky N. Delta inulin: a novel, immunologically active, stable packing structure comprising β-D-[2 → 1] poly(fructo-furanosyl) alpha-D-glucose polymers. Glycobiology, 21: 595-606, 2011.

135 Petrovsky N, Cooper PD. Carbohydrate-based immune adjuvants. Expert Rev Vaccines, 10: 523-537, 2011

136 Silva D, Cooper PD, Petrovsky N. Inulin-derived adjuvants efficiently promote both Th1 and Th2 immune responses. Immunol Cell Biol, 82: 611-616, 2004.

137 Petrovsky N. Novel human polysaccharide adjuvants with dual Th1 and Th2 potentiating activity. Vaccine, 24: S26-S29, 2006

138 Lobigs M, Pavy M, Hall RA, Lobigs P, Cooper P, Komiya T, Toriniwa H, Petrovsky N. An inactivated Vero cell-grown Japanese encephalitis vaccine formulated with Advax, a novel inulin-based adjuvant, induces protective neutralizing antibody against homologous and heterologous flaviviruses. J Gen Virol, 91: 1407-1417, 2010

139 Cristillo AD, Ferrari MG, Hudacik L, Lewis B, Galmin L, Bowen B, Thompson D, Petrovsky N, Markham P, Pal R. Induction of mucosal and systemic antibody and T-cell responses following prime-boost immunization with novel adjuvanted human immunodeficiency virus-1-vaccine formulations. J Gen Virol, 92: 128-140, 2010

140 Layton RC, Petrovsky N, Gigliotti AP, Pollock Z, Knight J, Donart N, Pyles J, Harrod KS, Gao P, Koster F. Delta inulin polysaccharide adjuvant enhances the ability of split-virion H5N1 vaccine to protect against lethal challenge in ferrets. Vaccine, 29: 6242-6251

141 Eckersley AM, Petrovsky N, Kinnel J, Wernery R, Wernery U. Improving the dromedary antibody response: The hunt for the ideal camel adjuvant. J Camel Pract Res, 18: 35-46, 2011

142 Cooper PD, Steele EJ. The adjuvanticity of gamma inulin. Immunol Cell Biol, 66: 345-352, 1988.

143 Cooper PD, Carter M. The anti-melanoma activity of inulin in mice. Mol Immunol, 23: 903-908, 1986.

144 Korbelik M, Cooper PD. Potentiation of photodynamic therapy of cancer by complement: The effect of gamma-inulin. Brit J Cancer, 96: 67-72, 2007.