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Stable isotope ($\delta^{13}C$) profiling of xylitol and sugar in South Africa

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Xylitol is an alternative sweetener to sucrose, glucose and fructose, and is available under a number of brands in South Africa. Carbon stable isotope values ($\delta^{13}C$) of a selection of commercially available xylitol products ($n=28$) were analysed and compared with sugar samples ($n=29$). Sugarcane (C_4) and beet sugar (C_3) derived sugar samples aligned with published values of source, although two samples that indicated a sugarcane origin suggested a beet sugar origin. Control corn-derived samples defined a stepwise xylose to xylitol discrimination of +0.7‰. The distinction between C_3- and C_4-derived xylitol was less clear with three samples difficult to define (range = -14.8 to -17.1‰). The values for a suite of xylitol samples (-22.3‰ to -19.7‰; $n=8$) that aligned closely with a suspected C_3-derived xylose, were ~8‰ more positive than known birch isotope values. Some xylitol samples may thus represent (1) a mixture of C_3- and C_4-derived products, (2) derivation from a CAM species source or (3) different processing techniques in which the discrimination values of xylose from corn, and xylose from birch, may differ because of the respective chemical processing techniques. No samples that claimed a birch bark origin were within the range of samples suggested to be corn derived (i.e. -13.0‰ to -9.7‰, $n=16$). We suggest that the threshold values provided are relatively robust for defining the origins of xylitol and sugar, and can be used in determining the authenticity and claims of suppliers and producers.

Significance:

- Stable isotope ($\delta^{13}C$) profiles of commercially available xylitol and sugar products in South Africa will enable the determination of authenticity.

Introduction

Sucrose or 'table sugar', together with its disaccharide constituents – fructose and glucose – are important in the global economy as the major sweeteners of food and beverages.[1-5] The intake of free sugars modifies both energy intake and body weight, and has been shown to be harmful by fuelling the development of obesity.[1-3] Added sugars in processed foods and beverages are linked to the development of diabetes, obesity and metabolic syndrome.[6,7] A positive behavioural response to these health risks, especially healthy eating and increased physical activity is important.[2,8-10] The intake of alternative sweeteners (artificial and non-nutritive) may reduce these health risks[9,11] and consequently the alternative sweetener market has increased in recent times[5,12].

Xylitol (D-erythro-pentitol), a reduced-calorie sweetener, is a five-carbon sugar alcohol or polyol ($C_5H_{12}O_5$), also known as wood or birch sugar[10,13], that occurs naturally in small quantities in a variety of plants[9,14-16], fruits and vegetables[4,17-19] and is even produced in the human body[10]. It has been used as a sweetening agent in food since the 1960s[20,21], in chewing gums, mints, sweets and toothpaste and also as a sugar substitute in confectionery and drinks[4,18]. Evidence suggests that it may prevent dental caries[15], ear and upper respiratory tract infections[4,18], the development of obesity[22], and can be safely consumed by sufferers of diabetes type I and II, as it has the capacity to stabilise blood sugar levels[4,22]. In comparison with other alternative sweeteners, xylitol is similar in sweetness to sucrose with a lower calorie content (2.4 vs 4.0 cal/g).[16,22]

On an industrial scale, xylitol is currently produced by chemical reduction of xylose (D-xylose), traditionally derived from the bark of birch trees (*Betula pendula*) and other hardwoods.[4,23,24] More recently, corncobs (*Zea mays*)[25], sugarcane bagasse, and wheat, sorghum and rice straw[26-28] have been used as sources of xylose; in China, xylitol production from corncob reached 50 000 tons in 2008[25,29]. Production from corncob is favoured as it is less expensive than production from birch.[30] Alternative methodologies to produce xylitol utilise yeasts (e.g. *Candida* sp.), bacteria and fungi.[4,17,31,32] The annual demand for xylitol is over 100 000 tons worldwide, with a selling price of USD4–5/kg and an economic value of up to USD537 million per year.[4,25,33] Asia produces 50% of the world's total xylitol, with the balance produced in Europe, the USA and Australia.[25] The largest producer of xylitol in the world has production plants in Finland, the USA and China.[25,34]

Food adulteration (or fraud) occurs when external substances are added to a food product and it is economically motivated when added or substituted (with lower-valued ones) to increase a product's value or to reduce production costs.[35-38] This has the overall negative effect of raising health concerns, reducing consumer confidence, and decreasing the sale of authentic products.[36] Stable isotope analysis is one method to identify food adulteration, where the botanical origin, geographical origin, and specific farming regime or production system of a product can potentially be determined.[35,36,38] Stable isotope analysis has been successfully utilised to detect the adulteration of honey with cheaper sugars[37,39-41] and olive oil with pomace oil[42], and CO_2 in apple cider (C_3) with a C_4 carbon isotope value may suggest the incorporation of C_4 sugars[43].

The fundamental variation in the ratios of stable carbon isotopes in terrestrial food webs stems from differences in the photosynthetic pathways of plants.[44-48] The majority of plants utilise the Calvin cycle (C_3) and have tissues with a mean $\delta^{13}C$ value of approximately -26.5‰. The $\delta^{13}C$ values of plants relying upon the Hatch-Slack pathway (C_4) – mainly tropical grasses including maize (corn), millet and sugarcane – are much higher, with a mean stable

isotope value of approximately -12.5‰. Plants utilising a third pathway, crassulacean acid metabolism (CAM), present intermediate $\delta^{13}C$ values ranging from -27‰ to -12‰.[49] The $^{13}C/^{12}C$ isotope ratio can thus be used to identify a product's botanical origin.[38]

In South Africa, xylitol is available from local retailers, supermarkets and health stores, with a number of brands available to the public. Xylitol is one of the more recent alternatives to sugar (sucrose, fructose, glucose) and claims on the origins of products vary, from those indicating a birch bark or corn origin, to those for which the source is unknown or not disclosed. The aim of this study was to isotopically profile xylitol products available in South Africa, in an attempt to define their likely origin: from C_3 (e.g. birch bark) or C_4 (e.g. corn) plants. For comparison, we analysed sugar samples that were expected to reflect distinct origins from either sugarcane *Saccharum* spp. (C_4) or sugar beet *Beta vulgaris* (C_3). By doing so we attempt to present a critical assessment of these commercially available sweeteners that adds to consumer transparency and the integrity of food products currently available on the South African market.

Materials and methods

Samples

Samples of xylitol products ($n=28$; including duplicates for two brands) available in South Africa were purchased from various retailers (January 2015 – November 2016), mostly in the Gauteng Province. Information was collected from each product regarding (1) country of origin and (2) claimed source (if disclosed). Of the 28 samples, 10 disclosed the source on the packaging. Anonymity of the selected brands is assured through assigned control numbers to each sample. In all instances we assumed products to be 100% xylitol, as indicated by the packaging.

To understand the relationship between xylose and xylitol we sourced two laboratory xylose samples, a sample of xylose from a Chinese supplier and derived xylitol (from the supplier of one of the brands for which we sourced repeat samples). To understand the likely source species we compared them to published isotope values of corn and birch – the two most common sources of xylitol. In addition, we measured the carbon isotope value of a birch bark and a birch leaf sample collected on the University of the Witwatersrand east campus (in Johannesburg) in December 2016.

We obtained additional sugar (fructose, glucose and sucrose) samples as controls ($n=29$) because South African table sugar (sucrose) is mainly derived from sugarcane (C_4) and European sugar from sugar beet (C_3). Some of these samples also included laboratory samples ($n=8$), of which three samples were indicated to be derived from *Agave* (a CAM photosynthesiser) and two samples were indicated to be derived from coconut, *Cocos nucifera*.

To assess the likely mixing of xylitol from different sources, in which the different sources may contain different size xylitol crystals, we selected three samples (X1, X3, X7) and separated them into $>500\,\mu$m or $<500\,\mu$m constituents (Labotec™, Star Screens, Booysens, Johannesburg, South Africa, sieve conforms to SABS197-ISO9002 specifications) for stable isotope analysis. A 28–33 g amount of each sample was separated into the different constituent size classes and prepared for isotope analysis.

Sample preparation for $\delta^{13}C$ analyses

Duplicate subsamples (0.4–0.5 mg) of all xylitol, xylose and sugar samples were weighed into tin cups (pre-cleaned in toluene) for analysis. For every four samples we weighed a gelatine (Merck) laboratory working standard (0.2, 0.4, 0.6 mg). Standards were of variable mass in order to determine if there was any sample size effect. The samples were combusted at 1020 °C on an EA1112 Elemental Analyser coupled to a DeltaV Plus stable light isotope mass spectrometer by a Conflo IV interface (all equipment supplied by Thermo Scientific, Bremen, Germany). These analyses were performed in the Mammal Research Institute stable light isotope laboratory at the University of Pretoria. Precision on the standard analyses was 0.11‰ and no sample size effect was noted. The stable isotopic values are expressed in delta (δ) notation in parts per thousand (per mille, ‰), relative to the international standard Vienna PeeDee belemnite (VPDB).

Results

The $\delta^{13}C$ values of the 28 branded xylitol samples ranged from -26.5‰ to -9.7‰ (Figure 1). The xylitol sample (X33) sourced from a Chinese supplier was 0.7‰ more positive than the xylose (Xy33=-11.5‰) from which it was derived (Figure 1). Fourteen xylitol values were more positive than the corn average of -12.4‰[48,50-53] (within 2.7‰), and two xylitol values were more negative but within 0.6‰.The two laboratory xylose samples had $\delta^{13}C$ values (-21.5‰ and -22.3‰) concomitant with eight xylitol samples (-22.3‰ to -19.7‰) (Figure 1). Three xylitol samples (-13.7‰, -15.2‰, -17.1‰) appeared unassociated with either of these apparent endpoints and a single xylitol sample (X14=-26.5‰) was 2.0‰ more positive than the mean birch isotope value (-28.5‰)[54-58] derived from the literature (Figure 1). The locally sampled birch bark and leaves measured -27.0‰ and -25.5‰, respectively. Seven samples (X14,X11=-20.4‰, X27=-20.2‰, X28=-20.0‰, X26=-19.9‰, X15=-15.2‰, X5=-13.7‰) had birch bark as the origin indicated on the package; with X26–X28 being the samples sourced from Finland. The packages of three xylitol samples indicated that they were corncob derived (X29=-11.0‰, X20=-10.7‰, X30=-10.5‰). For the three samples tested (X1, X3, X7), the isotope values of the different separated particle size classes ($<$ or $>500\,\mu$m) were similar (Table 1).

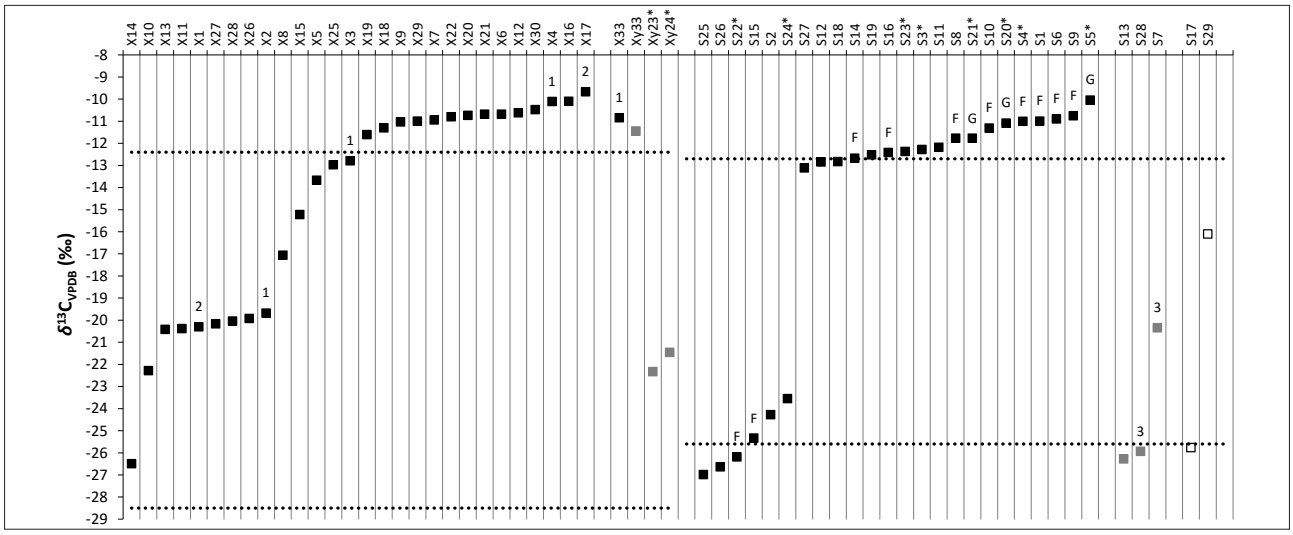
The sugar samples were clearly separated into two clusters: those C_4 derived (range: -13.1‰ to -10.0‰, $n=18$; cf. mean literature cane value of -12.7‰[41,48]), and those C_3 derived (range: -27.0‰ to -23.5‰, $n=6$; cf. mean sugar beet value of -25.6‰[41,59]). Three of the four C_3 commercially available sugar samples were sourced from Europe (one from the UK and two from Finland), i.e. they were of sugar beet origin. Two of the C_3 samples were indicated to be sugarcane derived (laboratory sucrose sample S24=-23.5‰ and fructose sample S15=-25.3‰). The samples that indicated an *Agave* origin (CAM) had a range of values (-26.3‰, -25.9‰, -20.3‰), and the two samples (S17 and S29) indicating a coconut blossom origin had contrasting values (-25.8‰ and -16.1‰, respectively; Figure 1).

Discussion

The hexose sugars sampled in this study suggest that stable isotope analysis is able to clearly define the origin of C_3- and C_4-derived sugars, with $\delta^{13}C$ values ranging from 27.0‰ to -23.5‰ and -13.1‰ to -10.0‰, respectively. These values in turn align, respectively, with the published $\delta^{13}C$ values for sugarcane and beet sugar. Sugars derived from *Agave* (a CAM photosynthesiser) yielded expected intermediate isotope values, but this finding does not affect the analysis, as CAM plants are not routinely used for xylitol production.

The xylitol isotope values are less clear. Because the chemical processes involved in the production of xylitol may vary, and are more complex than those for sugar production, we cannot be sure that stable isotopes unambiguously link source and product. In one of the manufacturing processes, xylose in hemicellulosic hydrolysate fractions is converted to xylitol by a chemical catalytic reaction,[24] but other processes may be used and it is unclear if different chemical processes are the reason for the range of values observed. If the processing method is discounted, then the variation in isotope signatures in commercially available xylitol in South Africa may indicate many different sources.

To understand the isotopic relationship between xylitol and its precursor, namely xylose, we sourced xylose and the derivative xylitol from a Chinese manufacturer (and the supplier of one of the brands sampled). In this assessment, we found very little difference between the two samples (xylitol ‰ = xylose + 0.7 ‰), suggesting that 14 samples, with $\delta^{13}C$ values ranging from -13.0‰ to -9.7‰, are most likely derived from corn (or some other C_4 plant). In support of this conclusion is a mean corn value of -12.4‰ reported in the literature. If this $\delta^{13}C$ discrimination from xylose to xylitol is minimal for C_4-derived xylitol, it is difficult to align the group of eight xylitol samples (-22.3‰ to -19.7‰) to a C_3 origin if the mean published birch (-28.5‰) and the local birch (mean=-26.3‰) values are significantly more negative than the xylitol samples claimed to be derived from birch.



Dotted lines indicate endpoints for (1) xylitol; mean maize (corn) Zea mays *$\delta^{13}C$=-12.4‰, includes seeds and cob values[48,50-53], mean birch* Betula pendula *$\delta^{13}C$=-28.5‰, includes leaves and wood[54-58] and (2) sugar; mean sugarcane* Sacharrum *spp. $\delta^{13}C$=-12.7‰[41,48], mean sugar beet* Beta vulgaris *$\delta^{13}C$=-25.6‰[41,59].*

**indicates laboratory samples.*

For xylitol samples: Xy indicates xylose samples (grey markers), similar numbers indicate same brand name.

For sugar samples: sucrose unless indicated; F, fructose; G, glucose. Grey sugar markers indicate Agave *(CAM photosynthesiser) derived sugars; similar numbers indicate same brand name; open sugar markers indicate sugar samples derived from coconut blossom.*

Figure 1: The $\delta^{13}C$ values of 29 xylitol, 3 xylose and 29 sugar samples. See Supplementary tables 1 and 2 for isotope values of samples presented in the figure, and values and references for calculated mean endpoint values for xylitol and sugar.

Table 1: $\delta^{13}C$ values of different xylitol granule size components (>500 μm and <500 μm), with proportions (%) of each size category indicated

Sample	Combined	>500 μm		<500 μm	
	$\delta^{13}C_{VPDB}$ (‰)	%	$\delta^{13}C_{VPDB}$ (‰)	%	$\delta^{13}C_{VPDB}$ (‰)
X1	**-20.3**	54.7	**-19.9**	45.3	**-19.8**
X3	**-13.3**	91.0	**-12.8**	9.0	**-12.4**
X7	**-10.7**	95.9	**-10.8**	4.1	**-10.8**

However, two forms of evidence suggest that these samples are birch authentic; first, three of the samples (within 0.3‰ of each other) are from Finland and we suggest that we are less likely to observe any adulteration in the country in which this process was developed, and second, two xylose values (albeit laboratory samples) that are similar to these samples lend support to the fact that they may be pure C_3 derived (in the same way that we recognise a close isotopic link between C_4 xylose and the derived xylitol in the factory control). The different discrimination values between xylose (and xylitol) and the plants from which they are thought to be derived, i.e. C_3 or C_4, may be a consequence of different chemical processes, and this remains to be investigated further. If not, then an entire suite of available C_3 xylitol products may be consistently adulterated with C_4 xylitol.

Only one sample (X14) was within 2.0‰ of the published values for birch. This sample warranted further investigation and for this and a number of other products, contact with the supplier was made to obtain further insight on their products. It was ascertained that xylitol in the product X5 was supplied by the packager of X15. Also, X15's supplier supplied the xylitol in X14, but further communication revealed that X14 had been withdrawn from the market because of suspected mistrust with X15. Because X14 was 'suspicious', one of us (C.S.) tasted it. The lack of a subtle cooling effect in the mouth (a characteristic of xylitol)[5] and an aftertaste reminiscent of other sweeteners (e.g. stevia) added basis to the idea that we were dealing with a likely adulterated food product. Analysing the contents of all samples is beyond the scope of this study, but given this outcome, it is an investigation that should be undertaken.

Two brands are represented more than once (Figure 1), and in each case the within-product isotope values are different; which may be explained by a change in supplier.

To test the possibility that sample contents may comprise mixed origins we analysed samples separated by size (< or >500 μm; n=3 samples). For each (although the proportions of each size class were different between products), we found no difference in the isotope values of the two fractions. This analysis included one sample for which the source (C3 or C4) was not clear. This finding suggests, at least at this scale, that the sample was not derived from separate sources.

In 36% (n=10) of xylitol samples, the country of origin was not disclosed on the packaging, and information regarding the origin and source was sometimes vague, e.g. 'fibrous parts of plant', or lacking. The *South African Foodstuffs, Cosmetics and Disinfectants Act* (*Act no. 54 of 1972)*, which includes regulations on the labelling and advertising of foods (Government Gazette No 37695, May 2014), prescribes numerous requirements that do not appear on a number of the products. These inconsistencies, in what appear to be standard labelling requirements, may result in consumers being mis- or uninformed regarding the products they are purchasing. Xylitol available in South Africa is not produced locally but, to the best of our knowledge, imported. In China, the waste biomass available after preparing corncobs (C_4) for the food industry provides a substrate for xylitol production[25,29], yet negative perceptions regarding the source (and country) of certain products may persist for consumers. In the USA, hydrosylate from birch trees (C_3) is utilised as a substrate for xylitol production[25] and it is also potentially produced from other hardwood (C_3) species[26]. Even though xylitol produced from birch may appeal to a discerning market, although it may be more expensive, there is no other major difference between birch-sourced and corn-sourced xylitol. Therefore, both information that is provided and a lack of information, can be misleading for the consumer.

Xylitol is traded all over the world. South Africa is simply one example of an importer vulnerable to adulteration and/or misinformation originating at either the source or locally; making this work of both national and international relevance. The carbon isotope data prima facie suggest that most xylitol samples represent origins suggesting either a C_3 or a C_4 source. For four samples, we question a single C_3 or C_4 source (intermediate isotope values; X8, X15, X5), or authenticity as xylitol

(X14). However, other reasons may explain these findings, including that: (1) the samples represent both C_3 and C_4 origins (mixture of different products; although this was not apparent in the three samples for which we measured the isotope value of different size granules), (2) different processing techniques result in fractionation processes that result in isotope values of the product not aligned with the source (we suggest that this is unlikely because the wide range of isotope values obtained would then suggest many processing techniques), (3) the isotope values of the samples do align with the source, but it is rather the source that (for whatever reason) does not have a clearly distinguishable C_3 or C_4 signature, (4) these intermediate products represent xylitol with a CAM origin (although in no sample was this disclosed, and we are not aware of any xylitol derived from a CAM species), or (5) adulteration with non-xylitol additives (e.g. flow agents, other artificial sweeteners like stevia or erythritol) affected the final $\delta^{13}C$ of the marketed product. Because marketers are not obliged to disclose the origins or methods of processing, the intention is thus not to challenge the authenticity of those marketing these products, but rather to challenge the information (or lack thereof) in the marketing process. Stable isotope analyses may be a suitable method to distinguish the origin of commercially available xylitol, especially when this is not clearly stated on the packaging or label. This study thus provides information that may be used to profile C_3- or C_4-derived sugar and xylitol products, and to lay a foundation for further investigations regarding these products in the food market. However, a priori information may be required regarding chemical processes that present source-product variation in isotope signatures, before conclusive origins (i.e. C_3 or C_4) can be defined, and before any isotope interpretation can be applied in a forensic assessment.

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Authors' contributions

C.S. conceived the project; E.L. and C.S. collected and prepared samples; S.W. analysed samples; all authors contributed to data interpretation and the write-up.

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