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Screening and Estimation of Pre-biotic Oligosaccharides in Fruits and Vegetables

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Abstract

An attempt was made to estimate the oligosaccharides content in fruits, vegetables and edible roots and to find the effect of drying on different oligosaccharides (stachyose, raffinose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose) considering their importance as prebiotics (substrate for the growth of probiotics in human colon). Quantification involved hot water extraction (98 ^{0}C/ 2 h) of oligosaccharides, different filtration steps, followed by high performance liquid chromatography (HPLC) analysis using 70 % acetonitrile as eluent with refractive index (RI) detector and oligosaccharide standards as reference. Raffinose content was observed (mg / g of DM) in okra (0.089), onion (0.3015) and potato (in trace amounts). Stachyose content was noticed (in mg / g of DM) in apple (traces only) Maltotriose content was observed (mg / g of DM) in onion (0.1148). Maltotetraose content was observed (mg / g of DM) in beet root (0.2394), okra (traces), onion (0.105), garlic (0.3075), guava (0.0678) and banana (0.177). Maltopentaose content was found in three samples (mg / g of DM) in yam (traces), garlic (0.3929) and tomato (0.549). Maltohexaose content was present (mg / g of DM) in carrot but only in traces. Maltoheptaose content was not found in any analysed samples (fruits, vegetables and roots). Drying had no effect on enzymatic hydrolysis of different oligosaccharides compared to fresh samples. Carrot, okra, onion, garlic, beet root, yam, guava, apple, tomato and banana appear to be good sources for commercial extraction of prebiotics by using membrane processing technology.

Keywords

Fruits, Vegetables, Roots, Oligosaccharides, Prebiotics, HPLC

Introduction

Over the last 25 years, the relationship between man and food has been particularly focused on the ability of foods to modulate physiology and biochemistry and thereby conferring protection against a range of human diseases, as also the aging process itself. The term 'functional food' seems to have been coined in Japan in the late 1980s. It is used to denote 'a food imparting health benefits beyond that of basic nutrition and making a claim about this benefit'.

For effective utilization of nutrients of foods consumed, gut microflora play an important role. The presence of probiotics increases functional efficiency of these microflora. All fermented milk products are generally probiotic in nature, more so when they contain active Lactobacilli and Bifidobacteria species of organisms. Oligosaccharides are prebiotic food ingredients, which are nondigestable and which do not interfere in digestive process, but stimulate the growth of probiotics. Hence the practice of using oligosaccharides as prebiotics to stimulate

probiotic growth and multiplication is gaining popularity and acceptance in food and healthcare industries. An oligosaccharide is formed from 2 – 10 monosaccharides and is a component stored in various agro products as an energy source (8). The oligosaccharides with a degree of polymerisation (DP) of 3 and over can be distinguished from monosaccharide (saccharide of DP 1) and disaccharide (saccharide of DP 2) by their physiological functions (3). The oligosaccharides used so far in food and health care industries are obtained either through enzymatic synthesis of monomers or through hydrolysis of complex starches. Since a number of oligosaccharides naturally occur in certain fruits and vegetables, there is a scope to separate them with their innate characteristics and use them as prebiotics to probiotic production to be incorporated in functional foods and neutraceuticals by the industry.

The primary objective of the study was to estimate the oligosaccharides content in natural state in different fruits, vegetables and edible roots and to quantify the levels of different types of oligosaccharides. Effect of drying on the levels of oligosaccharides was also studied, drying being a popular means of increasing the shelf life of fresh produce.

Material and Methods

The following locally available fruits, vegetables and edible roots were procured from the local fruit and vegetable markets

Fruits	**Vegetables**
Apple	Okra
Mango	Beet root
Sapota	Radish
Banana	Carrot
Grapes	Karrela (bitter gourd)
Pineapple	Corm
Beal fruit	Onion
Guava	Garlic
Papaya	Potato
——	Pumpkin
——	Tomato
——	Yam

The following oligosaccharides standards were procured from Sigma Aldrich

Chemicals (U.S.A) and used as reference for quantification.

Standard oligosaccharides
Raffinose
Stachyose
Maltotriose
Maltotetriose
Maltopentaose
Maltohexaose
Maltoheptaose

HPLC grade acetonitrile and water were used for analysis**.** The sample preparation sequence is outlined in the flow diagram. For dried samples the procedure was similar except that the samples were dried at 45^0 C in hot air oven for 72 hours. The prepared samples were used for analysis by HPLC (Waters model No.2695 and Refractive Index detector of 'Waters' No.2414) with 70 per cent acetonitrile as eluent as described (6). HPLC analysis was carried out using Waters Spherisorb (5µm), ammonia column (Dimensions: 4.6 mm x 250 mm) at a temperature of 65^0C and flow rate of 1.6 ml/ min. Quantification of oligosaccharides

was done by taking the peak areas in relation to the corresponding standards. Empower software was used to integrate the peaks for the purpose of calculation of peak areas.

Collection of commonly available fruits and vegetables

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Maceration and Twitchuration

(By mortar-pestle)

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Heat treatment (water bath at 98^{0}C / 2 h)

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Cooling to 45^{0}C

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Initial filtration (soya cloth)

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Secondary filtration (ordinary filter paper)

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Tertiary filtration (whatman No.42 paper)

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Final filtration through Millipore (syringe) membrane (0.25µ)

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Analysis for concentration of oligosaccharides by HPLC

Flow diagram for sample preparation

The moisture content of the samples was determined as per the method described (1). The mean values of data from two replications were tabulated. For standardizing HPLC analysis of oligosaccharides five replications were taken and from which mean and SD were calculated.

Results and Discussion

Moisture content in fruits, vegetables and roots: Moisture content is an important parameter, which reflects the actual oligosaccharide content in fruits, vegetables and roots on dry matter (DM) basis. Since the oligosaccharides are present as minor fractions in fruits, vegetables and roots, the moisture content is an important consideration of the oligosaccharide availability. The results of moisture content obtained from fruits, vegetables and roots are presented in Table 1. The observed results are in confirmation with the published results (6).

Table 1: Moisture (%) content in fruits, vegetables and roots

Sample	Moisture	Dry matter
Carrot	89.08	10.92
Beet Root	88.25	11.75
Yam	82.42	17.58
Radish	94.10	5.9
Okra	88.16	11.84
Karrela	91.64	8.36
Corm	78.60	21.4
Onion	87.10	12.9
Garlic	61.25	38.75
Potato	80.05	19.95
Guava	78.15	21.85
Pumpkin	94.47	5.53
Apple	84.60	15.4
Tomato	93.05	6.95
Mango	83.63	16.37
Sapota	72.10	27.9
Banana	72.55	27.45
Papaya	86.13	13.87
Grapes	82.85	17.15
Pineapple	85.54	14.46
Bael fruit	65.81	34.19

Standardizing HPLC analysis: Generally, analysis of sugars by HPLC gives poor quantification. The peak areas of repetitive injections of either samples or standard sugars differ to some extent due to concentration variation. The errors associated with repetitive injection are: (a) the precise injection of small quantities of samples is very difficult, and (b) detector responses fluctuate with time. In the

following experiment an auto sampler was used to avoid the first error and an experiment with different levels of standards was carried out to check the second factor. Precisely 10.0 µl of water based standard solutions containing different concentrations (1.2, 1.0, 0.7, 0.5, 0.4 % (w/v)) of maltotriose and maltohexaose were injected five times into HPLC with similar operating conditions.

Maltotriose: The standard solution of different maltotriose at different concentrations was injected for five times. Maltotriose was eluted at retention time as shown in the Fig - 1. The Mean, Standard Deviation and Coefficient of Variation of the observed concentration are presented in Table – 2. It indicates that coefficient of variation at 1.2 % concentration of maltotriose is minimum. This can be considered as standard for further calculations.

Maltoheptaose : The standard solutions of maltoheptaose at different concentrations were injected for five times. Maltoheptaose was eluted at the retention time as shown in the standard Fig - 2. The mean, standard deviation and coefficient of variation of the observed concentration are presented in Table 3. It indicates that coefficient of variation at 1.2 % concentration of maltoheptaose is minimum. This can be considered as standard for further calculations. Similarly, all other standard oligosaccharides namely, Raffinose, Stachyose, Maltotriose, Maltotetriose, Maltopentaose, Maltohexaose and Maltoheptaose retention times were recorded.

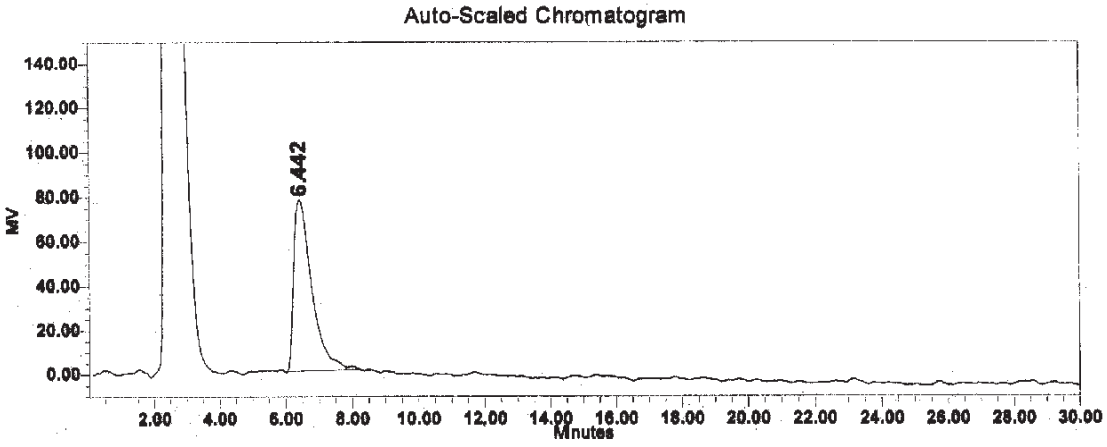

Fig 1 : Chromatogram of Maltotriose standard

Table 2 : Observed concentration of maltotriose by HPLC analysis.

Known Concentration %	Observed concentration		
	Mean*	Standard Deviation (±)	Coeff. Variation
1.2	1.186	0.009	0.758
1.0	0.989	0.008	0.808
0.7	0.693	0.012	1.731
0.5	0.492	0.007	1.422
0.4	0.394	0.006	1.522

* Mean of five observations

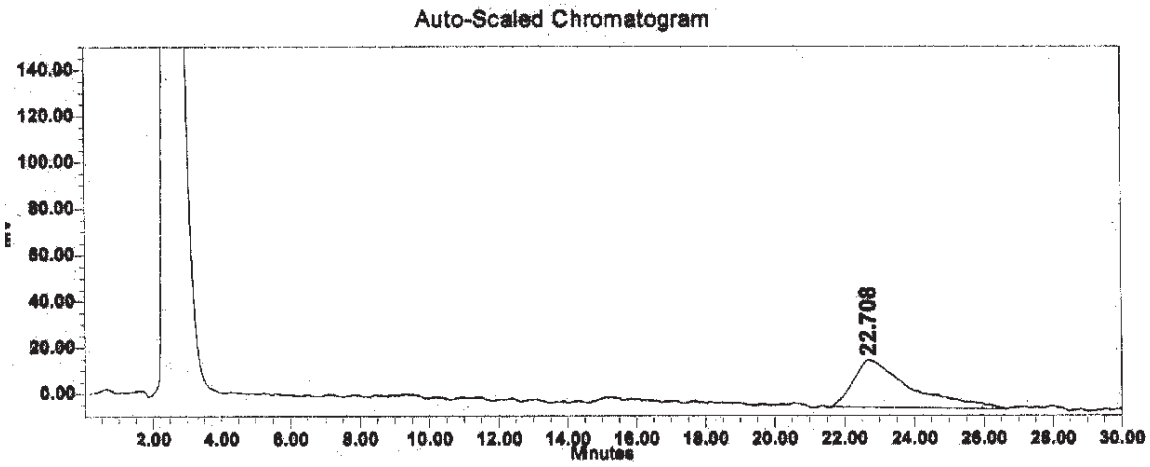

Fig 2 : Chromatogram of Maltoheptaose standard

Table 3 : Observed concentration of maltoheptaose by HPLC analysis

Known Concentration %	Observed concentration		
	Mean*	Standard Deviation (±)	Coeffe. Variation
1.2	1.198	0.008	0.667
1.0	0.997	0.008	0.802
0.7	0.696	0.008	1.149
0.5	0.494	0.007	1.417
0.4	0.395	0.007	1.772

* Mean of five observations

The results obtained by repetitive injections of the selected standard sugars at different concentrations has shown that there is no significant variation in the observed concentration, indicating that detector response is valid at various concentrations of sugars. Such validation of detector response is essential to extrapolate the results in samples with different levels of sugars.

Estimation of oligosaccharides in samples: After standardizing the quantification of standard sugars by HPLC, samples extracted from fruits, vegetables and roots were analysed. The concentration of oligosaccharides was calculated on dry matter basis taking into account the moisture content of fruits and vegetables estimated and the mean results of two replications of analysis are presented in Table 4. Variation observed in oligosaccharide content between the two replications was not significant.

Research workers who estimated sugars earlier reported mostly the concentration of inulin and oligofructose in fruits, vegetables and roots. Most of the work on determining oligosaccharides content was done to assess the degree of flatulence that they may cause after ingestion due to their non-digestibility.

Researchers reported the presence of inulin in raw samples of banana (0.3-0.7 g/100g), garlic (9.0-16.0 g/100g), onion (1.1-7.5 g/100g) and oligofructose content in raw banana (0.3-0.7 g/100g), garlic (3.6-6.4 g/100g), onion (1.1-7.5g/100g) (10). They also reported inulin content in

Table 4: Oligosaccharides content in fresh fruits, vegetables and roots (mg/g)

Sample Name	Raf	Sta	M.Tri	M.Tet	M.Pen	M.Hex	M.Hep
Carrot	0	0	0	0	0	Tr	0
Beet root	0	0	0	0.2394	0	0	0
Yam	0	0	0	0	Tr	0	0
Radish	0	0	0	0	0	0	0
Okra	0.089	0	0	Tr	0	0	0
Karrela	0	0	0	0	0	0	0
Corm	0	0	0	0	0	0	0
Onion	0.3015	0	0.1148	0.105	0	0	0
Garlic	0	0	0	0.3075	0.3929	0	0
Potato	Tr	0	0	0	0	0	0
Guava	0	0	0	0.0678	0	0	0
Pumpkin	0	0	0	0	0	0	0
Apple	0	Tr	0	0	0	0	0
Tomato	0	0	0	0	0.5419	0	0
Mango	0	0	0	0	0	0	0
Sapota	0	0	0	0	0	0	0
Banana	0	0	0	0.177	0	0	0
Papaya	0	0	0	0	0	0	0
Grapes	0	0	0	0	0	0	0
Pineapple	0	0	0	0	0	0	0
Bael fruit	0	0	0	0	0	0	0

* The values presented are means of two replications.

Tr- Traces, Sta – Stachyose, Raf- Raffinose, M. tri- Maltotriose, M.tet- Maltotetraose, M.pen- Maltopentaose, M.hex- Maltohexaose, M.hep- Maltoheptaose.

dried samples of banana (0.9-2.g/100g), garlic (20.3-36.1g/100g), and onion (4.7-31.9g/100g) and oligofructose content in banana (0.9-2.0g/ 100g), garlic (8.1-14.5g/100g), and onion (4.7-31.9g/ 100g).

Carrot: Only a trace amount of maltohexaose was observed in fresh carrot sample as presented in Table -4. No published literature on oligosaccharide content of carrot is available for comparison. No other oligosaccharide was noticed in carrot samples, except maltohexaose. It is also interesting to note that no other fruit or vegetable or root had even traces of maltohexaose. Therefore, it may form an important source for extraction of maltohexaose by membrane separation.

Beetroot: Beetroot appears to be a very good source of maltotetraose only after the garlic (0.3075mg/g). The other carbohydrates of the beetroot reported include sucrose, glucose, fructose and small amounts of raffinose (5).

Yam: No other oligosaccharide was noticed in yam samples, except traces of maltopentaose. No published literature on oligosaccharide content of yam is available for comparison.

Okra: In ladies finger (okra) two oligosaccharides were observed were namely raffinose (content of 0.089mg/g) and trace amounts of maltopentaose. No published literature on oligosaccharide content of Ladies finger is available for comparison.

Onion: Compared to any other fruits and vegetables studied, onion had three oligosaccharides with fairly good proportion of raffinose (0.03015mg/ g), maltotriose content was 0.1148mg/g and maltotetraose was 0.105 mg/ g. It was found that 2.8 per cent of oligofructose is seen on fresh weight basis of onion (2). Similarly the estimated oligofructose content of several onion samples are found in range of 1.1 and 7.1 per cent on fresh weight basis (10). However, no reports are available regarding the presence of maltotriose and maltotetraose in onion.

Garlic: In the present study, garlic showed a maltotetraose content of 0.3075 mg/ g and maltopentaose content of 0.3929-mg/g of dry matter. In related studies, It was reported that inulin content of garlic was in the range of 1.1 to 7.5 g/ 100g (10). Inulin is a precursor to produce a number of oligosaccharides by hydrolysis. He also reported that oligofructose is also present (1.1 to 1.7 g/100g) in garlic. It is not clear whether they analysed the garlic for presence of maltosugars. General reports suggest that garlic is a good source of oligosaccharides.

Potato: In the current study, trace amount of raffinose was observed in potato. No published literature on oligosaccharide content of potato is available for comparison. No other oligosaccharide was noticed in potato samples. Although potato is a good source of starch, it is surprising that no malto oligosaccharides were found in it.

Guava: In the present study, guava fruit had maltotetraose (0.0678mg/g) only. No published literature on oligosaccharide content of guava is available for comparison. It is interesting to note that no other fruit except guava and banana contained the maltotetraose, among the fruits studied.

Apple: A trace amount of stachyose was observed in apple. Published reports on oligosaccharide content of apple are not available for comparison.

Tomato: Maltopentaose content of tomato was 0.5419 mg/g. It is interesting to note that maltopentaose is present only in tomato out of all the vegetables studied and the proportion of it is also very high considering that moisture content in tomato is also very high (93%). Therefore, it may be a good option to extract maltopentaose as a source of prebiotic by application of membrane processing. No published literature on oligosaccharide content of tomato is available for comparison.

Banana: The maltotetraose content in banana was 0.177 mg/g. Some researchers observed the conversion of starch into glucose, fructose and sucrose during the ripening of bananas (4). It was reported that the inulin content is present at the rate of 0.3 per cent and 0.7 per cent respectively on fresh weight basis (2) (10). The degree of ripening may influence the breakdown of inulin into simpler sugars including oligosaccharides. However, as these researchers did not analyse the oligosaccharides there is no scope for comparison of results.

In Karrela, radish, corm, pumpkin, mango, sapota, papaya, grapes, pineapple and bael fruit, the oligosaccharides was not found. Further studies are needed to find out other oligosaccharides content such as fructooligosaccharides.

Effect of oven drying on oligosaccharides content in fruits and vegetables

If any rich source of the perishable produce such as fruits, vegetables and roots is identified

for the purpose of extraction of oligosachharides, drying is a convenient option, considering their short shelf life. The results of oligosaccharide content in fresh fruits and vegetables on dry matter basis are presented in Table 4.

In dried samples, the content of oligosaccharides was proportionate to the dry matter present in fresh samples (Table 5). All the fresh fruits, vegetables and roots, which had oligosaccharides, also had similar type of oligosaccharides in dried samples. It suggests that drying had no influence on enzymatic hydrolysis of sugars to produce any different oligosaccharides for which study was made.

Out of all the raw foods studied, carrot is the exclusive source of maltohexaose and it may be useful to extract in natural state in dried carrots by membrane processing for commercial use. Tomato seems to be an excellent source for maltopentaose and absence of other oligosaccharides is a very good opportunity to separate it in pure form by nanofiltration technology. Onion and garlic also have fairly good levels of three (raffinose, maltotriose and maltotetraose) and two (maltotetraose and maltopentaose) oligosaccharides respectively, which may be exploited commercially by extracting in their natural state. Beetroot also

Table 5: Oligosaccharide content in dried fruits, vegetables and roots (mg/g)

Sample Name	Raf	Sta	M.Tri	M.Tet	M.Pen	M.Hex	M.Hep
Carrot	0	0	0	0	0	0.3396	0
Beet root	0	0	0	2.0396	0	0	0
Yam	0	0	0	0	0.1365	0	0
Radish	0	0	0	0	0	0	0
Okra	0.7644	0	0	0.2203	0	0	0
Karrela	0	0	0	0	0	0	0
Corm	0	0	0	0	0	0	0
Onion	2.3209	0	0.8809	0.8098	0	0	0
Garlic	0	0	0	0.7930	0.3929	0	0
Potato	0.2020	0	0	0	0	0	0
Guava	0	0	0	0.3099	0	0	0
Pumpkin	0	0	0	0	0	0	0
Apple	0	0.0840	0	0	0	0	0
Tomato	0	0	0	0	7.7533	0	0
Mango	0	0	0	0	0	0	0
Sapota	0	0	0	0	0	0	0
Banana	0	0	0	0.6458	0	0	0
Papaya	0	0	0	0	0	0	0
Grapes	0	0	0	0	0	0	0
Pineapple	0	0	0	0	0	0	0
Bael fruit	0	0	0	0	0	0	0

* The values presented are means of two replications.

Tr- Traces, Sta – Stachyose, Raf- Raffinose, M. tri- Maltotriose, M.tet- Maltotetraose, M.pen- Maltopentaose, M.hex- Maltohexaose, M.hep- Maltoheptaose.

appears a promising source of maltotetraose with its high level and exclusive presence.

The effectiveness of a membrane process combining ultra filtration (UF) and nanofiltration (NF) for purifying and concentrating non-digestible saccharides (NDS) from yacon rootstock was studied (7). The other studies (6) also reveal that effectiveness of combined membrane processing with ultrafiltration (UF) and nanofiltration (NF) for purifying and concentrating oligosaccharides from chicory rootstock. The identified sources in the present study may be further studied to recover prebiotics in their natural state to be used on a commercial scale.

Conclusion

Out of the 21 samples of fruits and vegetables evaluated for the composition of pre-biotic oligosaccharides, beet root (maltotetraose), onion (for raffinose, maltotriose and maltotetraose), garlic (for maltotetraose and maltopentaose) tomato (maltopentaose), banana (maltotetraose) and carrot (maltohexaose) were found to have reasonably high proportion of pre-biotic oligosaccharides, which may be exploited for commercial extraction to be used as substrate together with probiotics for the development of functional foods. Earlier Kamada also found good source of oligosaccharides in Yakon root stocks and suggested for commercial extraction (6).

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