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Preparation of Xylooligosaccharides from Sesame Meal with Prebiotic Properties

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ABSTRACT:

Defatted sesame meals were extracted for ethanol soluble sugars and the effects of different conditions for extraction were compared for the composition and yield of the extracts. Xylooligosaccharides (XOS) were generated from the hemicellulose fraction (following deproteinization) using endo-xylanase from *Bacillus subtilis.* The condition which produced the highest yield for total soluble sugars was 40% ethanol at 60 ° C for 30 minutes for de-hulled (A) and hulled (B) sesame meal samples. Ultrasonication treatment of the samples had no significant benefit over water-bath. Sugar composition analysis of the samples showed the presence of glucose, arabinose, galactose and xylose with trace amounts of rhamnose. Prebiotic activity scores for *L. casie* (ATCC 393) were 0.87, 0.45, 0.13, on inulin, dehulled (A) and hulled (B) sesame meals, respectively. While *B. bifidum* (ATCC 29521) showed the following scores 0.77, -1.0, -0.43 on inulin, dehulled (A) and hulled (B) sesame meals, respectively. Dehulled sesame meal is a potential prebiotic source due to its content of xylooligosaccharides.

Keywords: Sesame meal, xylooligosaccharide, xylanase, prebiotic activity score

الملخص:

تم استخلاص كسبة السمسم منزوعة الدهن للحصول على السكريات الذائبة بالكحول وتم مقارنه الظروف المختلفة للاستخلاص على تركيب وكميه المستخلصات. الزايلو- عديد التسكر (XOS) تم انتاجها من الجزء شبه السيليلوزي (بعد ازاله البروتين) باستخدام انزيم الزايلانيز الخارجي من بكتيريا الباسيلس سابتيلس. الظرف الذي أنتج أعلى كميه من السكريات الذائبة كان عند ٤٠٪ كحول وعلى درجه حرارة ٦٠ درجه مئوية ولمده ٣٠ دقيقه لكل من العينات السمسم (ا) منزوع القشرة و(ب) غير منزوع القشرة. إن المعاملة بالأمواج فوق الصوتية للعينات لم تؤثر على نتائج الاستخلاص معنويا مقارنه بالحمام المائي. تحليل تركيبة السكريات للعينات المفحوصة أظهرت وجود الجلوكوز والاربينوز والجالاكتوز والزايلوز بالإضافة الى كميات قليلة من الرامنوز. مقياس النشاط المحفز للعصيات اللبنية (كيزي) أظهرت القياسات التالية:013 ,0.45 ,0.87 0.77 و - 1.0 و - 0.43 لكل من الانيولين (مؤشر إيجابي) و للكسبة منزوعة القشر ولغير منزوعه القشرعلى التوالي. بينما للبيفيدوبكتيريا (بيفيدم) كانت 0.43- ,1.0- ,0.77 لكل من الانيولين و للكسبة منزوعة القشر ولغير منزوعة القشرعلى التوالي. إن كسبة السمسم منزوعه القشر يمكن أن تكون ذات تآثير محفز للبكتيريا النافعة لاحتوائها على زايلو-عديد التسكر.

1. INTRODUCTION

Sesamum indicum, one of the traditional crops in Asia and Africa, is mainly cultivated for its oil (Lee, *et al.*, 2010), with by-product oilcake or sesame meal which is considered a source of dietary protein in animal feeds. Sesame crop has attracted more attention recently by entering the "omics" era which is characterised by complete genetic sequencing and other gene related functions (Dossa, *et al*., 2017). In 2014, more than 6 million tons of sesame seeds have been produced globally classifying sesame at the ninth rank among the major oil crops (FAO, 2013).

Monogastric animals are not able to digest the plant cell wall polysaccharides due to its complexity; therefore, sesame meal has a limited use in animal feeds, until it is further processed (Ghosh, *et al.*, 2005). One way to enhance the value of sesame meal would be the fractionation of the polysaccharide into fragments for different applications. Polysaccharides which help in maintaining a healthy

gut flora when mixed in food commodities are of commercial interest, such as functional foods. Non-digestible oligosaccharides that increase the health-promoting gut microbiota are known as ‘prebiotics’. The oligosaccharides must evade the action of digestive enzymes and juices in the stomach and the small intestine so that it gets metabolised in the colon (i.e. the major site of function of prebiotics) (Immerzeel, *et al.*, 2014).

A vast amount of research has been done in the past on the application of galactooligosaccharides and fructooligosaccharides as prebiotics. However, very few studies have been done on xylooligosaccharides (XOS) that have been reported to have a prebiotic effect on *Lactobacillus* and *Bifidobacterium* species. The release of lactic acid and short-chain fatty acids (SCFA), like butyric acid, propionic acid, and acetic acid, by the gut bacteria can be correlated with the beneficial effects in the host. The degree of substitutions and the polymerisation of the XOS may affect the fermentation rate (Immerzeel, *et al.*, 2014).

Moreover, Ghosh, *et al.* (2005) concluded that the total 2M H_2SO_4 hydrolysable sugar content in sesame meal was 19%, where the major monosaccharides were glucose, xylose, galactose, arabinose and mannose with trace amounts of rhamnose. The major neutral sugar in the extracted polysaccharide fraction was arabinose and the presence of galacturonic acid along with galactose and rhamnose suggested the presence of rhamnogalacturonan I. The occurrence of xylose, glucose and fucose suggested the possibility of xyloglucans. The sugar analysis of the hemicellulose fraction revealed more than 80% xylose residues in the 1*M* KOH-soluble fraction, suggesting the presence of xylan. The alkaline insoluble portion also showed around 80% glucose indicating the abundance of cellulose (Ghosh, *et al.*, 2005).

The procedures of hemicellulose extraction from plants affect the degree of substitution and the structure of the hemicellulose. As a result, the enzymatic hydrolysis and therefore the utilisation of XOS by probiotic bacteria may be affected. For instance, in a study for the uptake of arabinoxyloooligosaccharides

(AXOS), it was shown that two different strains of *Bifidobacteria* were able to utilise AXOS (although with different strategies), while the third strain was not able to utilise it. *Bifidobacterium adolescentis* utilised the XOS after the arabinose substituents were removed enzymatically whereas *Bifidobacterium longum* metabolised the released arabinose. (Immerzeel, *et al.*, 2014).

The objective of this study was to generate and compare variations of xylan from two different batches of sesame meal to have a deeper understanding of their enzyme hydrolysis products and their potential prebiotic properties using *Lactobacillus casei* (ATCC 393) and *B. bifidum* (ATCC 29521).

2. MATERIALS AND METHODS

2.1 Materials and preliminary treatments

Two batches of sesame meal were obtained from Altaj Tahini factory in Nablus, Palestine. One was de-hulled (Sample A) and the other contained only hulls and no seeds (Sample B). Both samples were oven-dried and milled to form fine powder. The sample B was desalted by vacuum filtration with de-ionized water, then re-dried. Both sesame meal batches were defatted by supercritical fluid extraction. All other experimental procedures were carried out at The University of Reading, UK.

2.2 Total acid hydrolysis for sugar composition analysis

Three-hundred *mg* of both defatted meals were weighed in duplicates and 3mL of 72% H_2SO_4 was added to each of the tubes. Tubes were incubated in water bath at 30 °C for 60 *mins*. followed by dilution of the acid to a 4% concentration by adding 84 *mL* of deionized water. A set of sugar recovery solution (SRS) was prepared to correct for sugar losses due to dilute acid hydrolysis. SRS included D (+) glucose, D (+) xylose, D (+) galactose, L (+) arabinose and D (+) mannose. All the sugars of SRS were put in a Duran bottles along with 10mL deionized water and 348 *µL* of 72% H_2SO_4. The samples and the SRS were autoclaved at 121°C for 30 *mins*. After cooling, $CaCO_3$ was added to each bottle until the pH

reached around 5-6 (Sluiter, *et al.*, 2012). The clear solution was filtered into HPLC vials and analysed using Dionex P680 HPLC system equipped with quaternary pump. The separation was done by a Dionex TCC-100 Column and a Dionex PDA-100 Photodiode array detector.

2.3 Optimisation of hemicellulose isolation

Two grams of both defatted meals were weighed in 8 bottles each and the extraction was carried out according to the scheme given in Fig. 1, in a ratio of 1:10 (w/v). Optimisation was based on temperature, time, ethanol concentration and mode of incubation. After incubation, all the samples were centrifuged (3,000 rpm for 10 min) and the supernatant was separated from the insoluble residue followed by evaporation of the ethanol by oven-drying overnight. The ethanol soluble fraction was freeze-dried and analysed for reducing sugars (3, 5-dinitrosalicylic acid method) and total sugars (phenol sulphuric acid method); whereas the alcohol insoluble fraction was analysed by total acid hydrolysis.

2.4 *3, 5*-dinitrosalicylic acid method for determination of reducing sugars

A hundred *μL* of 3, 5-dinitrosalicylic acid (DNS) was added to 100 *μL* of appropriately diluted sample and the mixture was boiled for 5 min at 100 °C. The tubes were cooled on ice for 5 min followed by addition of 1 *mL* deionised water. The contents were mixed properly and the absorbance was measured at 540 *nm*. The blank was prepared by substituting the sample with deionised water. All the reactions were done in duplicates. The amount of reducing sugars included in the samples was calculated using a reference standard curve.

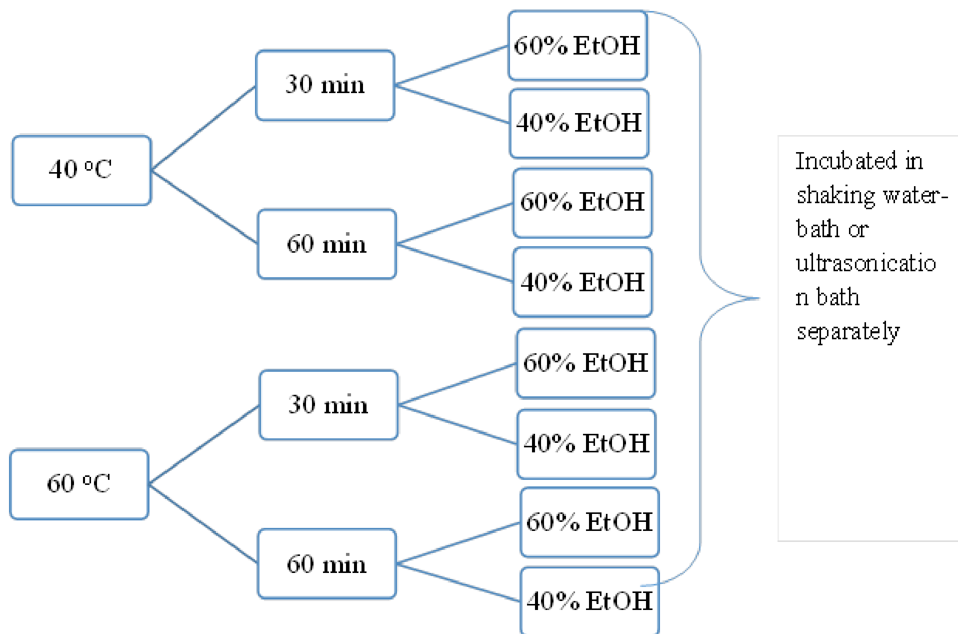

Figure 1: Scheme for the optimisation of the isolation of polysaccharides from defatted sesame meals using 40% and 60% ethanol, at 40 °C and 60 °C for 30min and 60 min. Extractions carried out in water-bath and ultrasonication bath.

2.5 Phenol sulphuric acid method for determination of total sugars

Two hundred *µL* of phenol (5% w/v) was added to 200 *µL* of the sample (100 times diluted) and the reaction mixture was left for 5 *mins*. With a 1 *mL* fast-delivery pipette (prepared by cutting the end of the 1 *mL* tip), 1 *mL* of concentrated H_2SO_4 was added rapidly at the centre of each tube and the reaction mixture was left to stand for 15 *mins*. The blank was prepared by substituting the sample for deionised water and the absorbance was measured at 480 *nm*. All the reactions were done in triplicates. The amount of total sugars present was calculated using a reference standard curve (Dubois, *et al.*, 1956).

2.6 Enzymatic hydrolysis of sesame protein

Fifty grams of defatted meals were mixed with 500 *mL* of 0.05 (mol. L^{-1}) citric acid-Na_2HPO_4 buffer, pH 3.0 and placed on a magnetic stirrer at 37°C for equilibration. The enzyme used was SIGMA Protease (Newlase ™) Type XVIII, from *Rhizopus* species (0.2-0.6 Units. mg^{-1} solid), and the enzyme solution was prepared in the same buffer at pH 3.0. The enzyme was added to the meal and the reaction was allowed to proceed for 2 hours. After the incubation, the reaction was stopped by immersing the bottle in hot water at 95° C for 15 min followed by centrifugation at 2800 *g* for 20 *mins*. The supernatant was discarded and the pellets were washed with deionised water and freeze-dried (Hrčková, *et al.*, 2002).

2.7 Enzymatic hydrolysis of xylan

The deproteinated sesame meals were hydrolyzed by xylanase from *Bacillus subtilis* (BIOCATALYSTS Depol™ 761P). The samples were weighed and suspended in 0.05 (mol. L^{-1}) Na_2HPO_4-NaH_2PO_4 buffer (pH 6.0), in the ratio of 1:10 $w.v^{-1}$. The reaction bottles were placed on a magnetic stirrer at 50 °C for equilibration and the enzyme was added as 5% based on the weight of the substrate (880 xylanase Units. g^{-1}). The reaction was allowed to proceed for 5 hours and it was terminated by immersing the bottles at 95 °C for 15 *mins*. The slurry was centrifuged at 4,000 rpm for 15 *mins*. and the supernatant was collected and oven-dried overnight to evaporate most of the water. The hydrolysis products were analysed by Dionex system using xylooligosaccharide standards from Megazyme (X1: xylose, X2: xylobiose, X3: xylotriose, X4: xylotetraose, X5: xylopentaose & X6: xylohexaose) and the amount of total sugar was estimated as per Dubois, *et al*. (1956).

2.8 Prebiotic Activity Score (PAS)

Prebiotic Activity Score (PAS) was determined using the procedures described by Huebner, *et al*. (2007) with a little modification. For the purpose of prebiotic

activity *Bifidobacterium bifidum* and *Lactocacillus casie* were used as probiotic representatives, while *E coli*. were used as the enteric bacteria. *L. casie* and *B. bifidum* were enumerated on MRS agar and MRS agar supplemented with 0.05 L-cystien HCl, respectively. *E. coli* were enumerated in tryptic soy agar. *L. casie* and *E coli*. were incubated aerobically while *B. bifidum* were incubated anaerobically using anaerobic jar (Oxoid, Basingstoke, UK) vacuumed and pumped with nitrogen gas. Incubation temperature was kept at 37° C for all bacterial cultures. After 24-48 hours of incubation a single colony of each culture was picked out and transferred to 10 *mL* broth of the corresponding media. The incubation conditions for 24 hr were remained as above. Then tubes contained 1% ($w.v^{-1}$) glucose or 1% ($w.v\text{-}^{1}$) sample A or sample B were also inoculated by the probiotics (1% $v.v^{-1}$) while E coli were transferred to a minimal medium broth with 1% ($w.v^{-1}$) glucose or 1% ($w.v^{-1}$) sample A or sample B and incubated overnight. Inulin was used as reference prebiotic for all bacterial cultures.

The prebiotic activity score (PAS) was calculated using the following equation:

$$PAS = \left[\frac{(probiotic\ log\frac{cfu}{ml}\ on\ prebiotic\ at\ 24\ hr) - (probiotic\ log\frac{cfu}{ml}\ on\ prebiotic\ at\ 0\ hr)}{\left(probiotic\ log\frac{cfu}{ml}\ on\ glucose\ at\ 24\ hr\right) - \left(probiotic\ log\frac{cfu}{ml}\ glucose\ 0\ hr\right)} \right]$$

$$- \left[\frac{(enteric\ \log\frac{cfu}{ml}\ on\ prebiotic\ at\ 24\ hr) - (enteric\ \log\frac{cfu}{ml}\ on\ prebiotic\ at\ 0\ hr)}{\left(enteric\ \log\frac{cfu}{ml}\ on\ glucose\ at\ 24\ hr\right) - \left(enteric\ \log\frac{cfu}{ml}\ glucose\ 0\ hr\right)} \right]$$

3. RESULTS AND DISCUSSION

3.1 Sugar composition

Sugar composition of the two sesame meals is shown in Table 1. Both meals had the same sugar composition pattern, however, there was a considerable difference seen in the individual proportions. Only the glucose content in sample B was much higher than sample A, whereas, rest of the sugars (Rhamnose, Arabinose, Galactose and Xylose) were less compared to sample A. The high level of

glucose in sample B can be attributed to the high content of cellulose and insoluble lignin, whereas rest of the sugars found in the seeds was higher in sample A.

Table 1: Sugar composition of defatted sesame meals after total acid hydrolysis

Sample		A. Defatted sesame meal (De-hulled)			B. Defatted sesame meal (with hulls & no seeds)		
	Detected Sugars	Retention Time (min)	Average Area	Percentage (%)	Retention Time (min)	Average Area	Percentage (%)
	Rhamnose	7.76	0.72	0.54	7.76	7.49	3.04
	Arabinose	8.66	22.99	17.00	8.66	25.00	10.15
	Galactose	10.93	14.87	11.00	10.93	16.33	6.63
	Glucose	11.9	46.57	34.45	11.9	148.34	60.20
	Xylose (+Mann)	12.87	50.04	37.02	13.33	49.26	20.0
Total			135.194 8	100		246.42	100

Legend Sugar analysis was done in duplicate. Sample A: Defatted sesame meal (de-hulled); Sample B: defatted sesame meal (containing hulls & no seeds). Abbreviation: Mann, Mannose. The retention time was compared with standard

3.2 Optimization of hemicellulose extraction

According to the total sugar analysis, the condition which gave the best extraction result was 40% ethanol at 60 °C for 30 *mins*. for both the samples A and B, when extracted in shaking water-bath (Table 2). Extraction with ultrasonication bath had no significant benefit over normal water-bath, probably due to the fact that in an ultrasonication bath, the cavitation phenomena is distributed uncontrollably through the tank and is non-conformable. The effect is unevenly spread and is of low intensity (Hielscher – Ultrasound Technology). In this case, even the temperature was hard to control. On the other hand, more sugars were extracted with 40% ethanol as compared to 60%. It has been found

that an increase in ethanol concentration from 50% to 90% significantly decreases the amount of stachyose and raffinose extracts from plants which can be correlated to the reduction in efficiency of ethanol to extract sugars with higher molecular weight (Giannoccaro et al., 2006) and sesame meal contains 0.59% raffinose, 0.38% stachyose, 0.23% planteose and 0.14% sesamose, out of the total carbohydrates content (Wankhede and Tharanathan, 1976). Boiling 80% ethanol is effective in extraction of soluble sugars, however, the extract would also contain contaminants such as pigments, ash, organic acids and even low-molecular weight peptides (Nielsen, 2010), in addition to some by-products from Millard reactions of some free amino acids, therefore, high temperatures were not favoured to avoid Maillard reaction. The compositional analysis of the alcohol-insoluble residue showed the same pattern as the raw materials, however, the glucose content increased in both the samples while rest of the sugars decreased in quantity (Table 3). This suggested the fact that sesame meal contained a lot of cellulosic polysaccharides. The second most abundant monosaccharide was xylose, whose quantity decreased in sample B but not in sample A, which suggested the presence of xylan in the material.

3.3 Enzymatic removal of protein and xylan hydrolysis

From the alcohol insoluble residues, proteins were partially removed. Due to some technical difficulties, the protein content of the samples could not be estimated before and after the proteolysis, and the loss of proteins was completely estimated from the loss of weight of the samples after proteolysis. Enzymatic hydrolysis of the xylan was performed to produce xylooligosaccharides using endo xylanase from *B. subtilis*. The hydrolysate composition after 5 hours of enzymatic hydrolysis is depicted in Figure 2 and calculated in Table 4.

Table 2: Quantification of total soluble sugars using phenol-sulphuric acid method

Conditions	Water-bath		Ultrasonication bath	
	Total sugar Sample A	Total sugar Sample B	Total sugar Sample A	Total sugar Sample B
40%, 40◦C, 30 min	10.30 mg/mL	2.15 mg/mL	10.21 mg/mL	2.32 mg/mL
40%, 40◦C, 60 min	10.20 mg/mL	2.68 mg/mL	10.11 mg/mL	2.77 mg/mL
40%, 60◦C, 30 min	16.53 mg/mL	3.29 mg/mL	17.81 mg/mL	3.87 mg/mL
40%, 60◦C, 60 min	13.27 mg/mL	2.67 mg/mL	11.70 mg/mL	1.47 mg/mL
60%, 40◦C, 30 min	15.10 mg/mL	1.71 mg/mL	14.40 mg/mL	1.49 mg/mL
60%, 40◦C, 60 min	12.38 mg/mL	1.77 mg/mL	9.26 mg/mL	1.19 mg/mL
60%, 60◦C, 30 min	12.79 mg/mL	1.56 mg/mL	15.49 mg/mL	1.26 mg/mL
60%, 60◦C, 60 min	14.22 mg/mL	2.44 mg/mL	19.18 mg/mL	8.90 mg/mL

Legend Sugar analysis was done in triplicate. Sample A: Defatted sesame meal (de-hulled); Sample B: defatted sesame meal (containing hulls & no seeds). The total sugar was quantified using glucose standard curve as the standard. The condition 40% ethanol at 40◦C for 30 minutes in water-bath gave the best yield of total soluble sugars in both the samples.

Table 3: Sugar composition of the alcohol-insoluble residues with the highest yield of ethanol soluble sugars

Sample	Sample A $_{Ins}$: 40% ethanol, 60◦C for 30 min			Sample B $_{Ins}$: 40% ethanol, 60◦C for 30 min		
Detected Sugars	Retention Time (min)	Average Area	Percentage (%)	Retention Time (min)	Average Area	Percentage (%)
Rhamnose	7.91	0.38	0.25	7.90	3.39	1.56

Arabinose	8.1	17.17	11.46	8.96	13.17	6.07
Galactose	10.76	16.59	11.07	10.46	18.75	8.63
Glucose	12.7	61.78	41.21	12.2	166.48	76.71
Xylose (+Mann)	13.63	53.97	36.01	13.23	15.23	7.02
Total		49.91	100		217.01	100

Table 4: Hydrolysate composition from defatted sesame meals and alcohol-insoluble residue after endoxylanase treatment for 5 hours.

Sugar	**Retention Time (min)**	**Sample A**		**Sample A $_{Ins}$**		**Sample B $_{Ins}$**	
		Percentage (Overall) (%)	Percentage (Known sugars) (%)	Percentage (Overall) (%)	Percentage (Known sugars) (%)	Percentage (Overall) (%)	Percentage (Known sugars) (%)
Unknown 1	1.767	38.31	-	63.57	-	1.04	-
Unknown 2	5.167	34.17	-	15.02	-	59.041	-
Xylose	5.834	0.78	13.03	0.28	14.59	0.51	49.00
Xylobiose	11.534	3.92	66.27	0.56	29.20	0.13	12.19
Unknown 3	15.100	21.59	-	19.44	-	35.67	-
Xylotriose	18.334	0.08	1.396	0.02	0.99	-	-
Xylotetraose	21.10	0.20	3.32	0.16	8.05	-	-
Xylopentaose	23.567	0.70	12.27	0.56	26.74	0.02	2.18
Xylohexaose	26.53	0.58	9.77	0.38	18.504	0.40	36.63

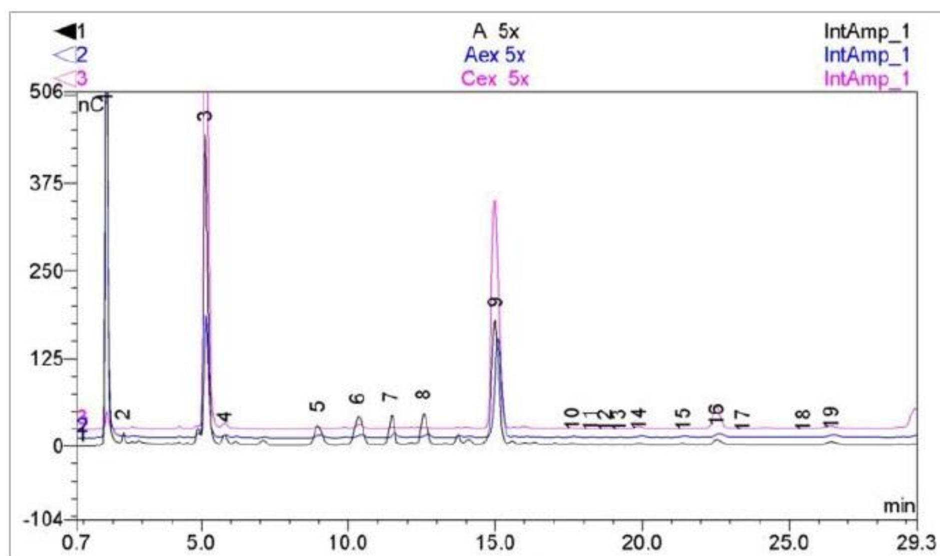

Figure 2: Chromatogram of xylan hydrolysis pattern after 5 hours of endoxylanse treatment at 50◦C analysed using Dionex HPLC system.

Legend

A 5x: Defatted sample A; **Aex 5x**: Defatted alcohol-insoluble sample A (40%, 60◦C, 30min); **Cex 5x**: Defatted alcohol-insoluble sample A (40%, 60◦C, 30min). All the samples were 5 times diluted. Sample C was not available for the experiment. (1: Unknown 1, 3: Unknown 2, 4: Xylose, 7: Xylobiose, 9: Unknown 3, 11: Xylotriose, 15: Xylotetraose, 16: Xylopentaose, 19: Xylohexaose). The hydrolysis was carried out for 5 hours and the sugar pattern indicates that more monosaccharides were produced as compared to oligosaccharides, suggesting the fact that the enzymes may be an exo-enzyme which was not specific to xylan but could hydrolyse other compounds as well.

Analysis of the hydrolysate composition from the xylan hydrolysis suggested the fact that the endo-xylanase enzyme had side activities and was not purely specific to xylan, which was expected as the enzyme is purified from *Bacillus subtilis*, which is a soil organism, and thus would have enzymes which are not specific to any one substrate, but can hydrolyze a wide range of substrates. By analysing the

retention times of the sugars, Unknown 2 was assumed to be arabinose, whereas, Unknown 3 was assumed to be a cellulosic oligosaccharide. The occurrence of such large quantities of arabinose is assumed to be from arabinans, which can either be present freely or as side chains of rhamnogalacturonan I, both of which are shown to be present in sesame meal along with arabinogalactan proteins. Sesame meal has also shown the presence of xyloglucan whose hydrolysis with endo-β-(1→4)-D-xylanase produced 40% xylose and 46% xylobiose along with other XOS (14%) (Ghosh, *et al.*, 2005). Also, the quantity of arabinose and cellulosic oligosaccharide was more in sample B, which contained the hulls, i.e. more cell wall polysaccharides, which justifies the composition. Another interesting observation was that, because the concentration of monosaccharides was more as compared to the middle-range products, it suggested that the enzyme might have an exo-hydrolysis activity more than the endo-hydrolysis activity, which can again be expected as the bacteria in the environment would prefer to hydrolyze substrates into simple sugars for utilization. Moreover, the structural diversity of XOS produced have been shown to be dependent on the type of substrate(s), pretreatment and structure/function of enzyme or enzymes utilized (Javier, *et al*., 2018).

3.4 Prebiotic Activity Score

The sesame meals derived hydrolysate containing XOS were subject to fermentation by the chosen bacteria. Probiotic bacteria showed higher growth on prebiotic (inulin) and sesame meal hydrolysates than that of enteric bacteria as shown in Table 5.

The growth of probiotics was the highest on inulin followed by dehulled sesame meal hydrolysate. The prebiotic activity score for dehulled sesame meal hydrolysate was significantly higher than that of hulled sesame meal hydrolysate ($p \leq 0.05$) Fig 3. This is expected as the major composition of the hulled

fractions was glucose. On the other hand, *L. casie* showed higher score than *B. bifidum* when grown on dehulled sesame meal hydrolysate. The explanation of that can be due to differences in transport systems and/or metabolic pathways between bacterial species. On the other hand, it was reported that many gut microbiota can ferment XOS, however, human faecal analysis after ingestion of XOS had increased bifidobacteial count (Karlsson, *et al*., 2018).

Table 5: measurement of cell count of probiotics and enteric bacteria grown on glucose, inulin, sample A (dehulled sesame meal), and sample B (hulled sesame meal). Cell count was taken at 0 and 24 hr of incubation

Bacterial culture	**Glucose**		**Inulin**		**Sample A (dehulled)**		**Sample B (hulled)**	
	Log Cfu /*mL* (±SD)		**Log Cfu /*mL* (±SD)**		**Log Cfu /*mL* (±SD)**		**Log Cfu /*mL* (±SD)**	
	0	24 hr	0	24 hr	0	24 hr	0	24 hr
L. casie	0.67 *(0.09)*	2.40 *(0.03)*	0.74 *(0.26)*	2.98 *(0.15)*	0.59 *(0.13)*	2.67 *(0.07)*	0.64 *(0.27)*	2.53 *(0.51)*
B. bifidum	0.72 *(0.06)*	2.43 *(0.12)*	0.66 *(0.34)*	2.70 *(0.19)*	0.69 *(0.09)*	2.20 *(0.33)*	0.71 *(0.40)*	2.0 *(0.29)*
E .coli	0.68 *(0.07)*	2.55 *(0.14)*	0.75 *(0.16)*	1.55 *(0.04)*	0.70 *(0.15)*	2.10 *(0.26)*	0.65 *(0.29)*	2.45 *(0.23)*

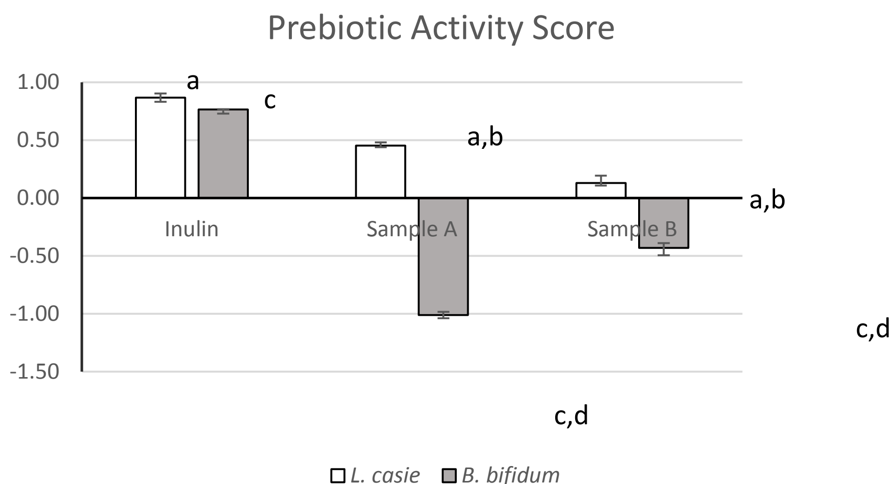

Fig 3: Prebiotic activity score of *L. casie* and *B. bifidum* grown on sample A (dehulled sesame meal) and sample B (hulled sesame meal) compared with inulin as a reference prebiotic. Columns of the same series having different letters are significantly different ($p \leq 0.05$).

4. THE CONCLUSION

The study showed that the conditions which led to the highest extraction of soluble sugars were 40% ethanol concentration at 60 °C when incubated for 30 min in shaking water-bath. Ultrasonication bath had no significant benefit over water-bath, although it would be interesting to compare the results by using Probe-type sonication method as well. The sugar composition analysis showed the presence of high amount of glucose in sample B which contained only hulls, whereas, the rest of the sugars like xylose, arabinose, galactose were less compared to sample A (de-hulled sample). Enzymatic hydrolysis of the xylan produced more monosaccharides (probably arabinose and xylose) than xylooligosaccharides, suggesting that the enzyme was not very specific and was exo-enzyme. *L.casie* showed better prebiotic score than *B. bifidum* for both samples. This study has given an insight into the probable future studies,

wherein, the enzymatic treatment could be modified to get more XOS than monosaccharides.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest with any part of this research.

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