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Isolation, Characterization and Antioxidant Activity of Oligosaccharides from Corn Meal

Shahzad A. Shahid Chatha[*1], Iqra Iram[1], Abdullah Ijaz Hussain[1] and Syed Makhdoom Hussain[2]*

1-Natural Products & Synthetic Chemistry Lab, Department of Applied Chemistry & Biochemistry, Government College University, Faisalabad, Pakistan

2-Department of Zoology & Fisheries, Government College University, Faisalabad, Pakistan

Abstract

This research was carried out to isolate and investigate the antioxidant activity of corn meal oligosaccharides. Oligosaccharides (3.2g/100g), extracted by soxhlet extraction technique, preliminary confirmed by Molisch and Barfoed's tests were further identified and quantified by HPLC. The HPLC profile showed the separation of galactose (84.5%), glucose/xylose (6.0%), rhamnose (5.6%) and arabinose (3.9%) as major corn meal oligosaccharides. The extracted oligosaccharides showed mild percent inhibition of linoleic acid oxidation (24.5%) and DPPH radical scavenging capacity (16.9%) than that of synthetic antioxidant, butylated hydroxytoluene (BHT). From the above results it can be concluded that corn meal is good source of some important oligosaccharides which can be used in food products.

Keywords: Corn meal, Oligosaccharides, Galactose, Antioxidants, Food Products

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**Corresponding Author: E.mail*: chatha222@gmail.com Phone : 0092-41-9200061-70 ext. 344

1. Introduction

Modern food consumers are becoming more conscious in their personal health and expect beyond taste and flavor, a safe, healthy and wholesome food. As interest in the link between diet and health is becoming popular, many consumers seek ways to feel well and stay healthy by consuming nutritionally designed foods (Mussatto and Mancilha, 2007). Oligosaccharides are relatively newly discovered functional food ingredients having good potential to improve the quality of many foods. In addition to providing useful modifications to food flavor and physicochemical characteristics, many of these sugars possess good defensive action against carcinogenicity. Oligosaccharides have low calorific value and ability to stimulate the growth of beneficial bacteria in colon (Crittenden and Playne, 1996; Crittenden and Playne, 2002; Mussatto and Mancilha, 2007).

Oligosaccharides, owning the important physicochemical and physiological properties associated with positive health benefits, are getting attention as healthy food ingredients. The functional oligosaccharides are intermediate in nature between simple sugars and polysaccharides and are claimed to behave as dietary fibers and prebiotics (Crittenden and Playne, 1996). These macromolecules are considered as non-absorbable food ingredients working as microbial food supplements, thereby benefit the host by selectively stimulating salutary bacteria in the large intestine and promote a good balance of intestinal micro flora and decrease gastrointestinal infections (Courtois, 2009; Qiana *et al*., 2014). The oligosaccharides are claimed to be stimulant for intestinal absorption of minerals, such as calcium, magnesium and iron. Moreover, consumption

of higher amounts of functional oligosaccharides reduces the risk of urbanization diseases such as cardiovascular diseases, colon cancer and obesity (Qiana *et al*., 2014). In last two decades, research precedence of scientists is focused on biological activity of natural polysaccharides (Courtois, 2009).

Pakistan, being an agricultural country is blessed with medicinally and economically important flora and agricultural crops. There are a number of agro wastes, fruit processing byproducts and cereal residues (Sultana *et al*., 2008). A high proportion of such waste material is comprised of carbohydrate and phenolic in nature (Katapodis and Christakopoulos, 2008). Corn meal is an agricultural by-product having low-value utilization as animal feed; scientific findings have established the concept that it is a rich source of natural compounds, therefore, can be used for value addition of products. In Pakistan, there exists a gap in scientific knowledge regarding the composition of these types of agricultural wastes. Therefore, this research work was designed to carry out isolation and quantification of oligosaccharides from corn meal, as an effort to find out potential for better utilization of agro waste. Furthermore, antioxidant activity and DPPH radical scavenging capacity of isolated oligosaccharides were also studied.

2. Materials and Methods

2.1 Chemicals and reagents

All the chemicals and reagents used in this research work, including hydrogen peroxide, butylated hydroxytoluene (BHT), linoleic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), β–carotene, chloroform, Molisch's reagent and methanol were of analytical grade and purchased from Sigma Aldrich, otherwise specified and were used without further purification.

2.2 Collection of materials

Corn meal (by-product of dry milling process of corn seeds) was collected from local expeller units of Faisalabad. Meal was dried under shade at room temperature and ground to 80-mesh size and preceded for extraction.

2.3 Extraction of oligosaccharides from corn meal

Extraction of oligosaccharides from corn meal was performed by following the reported procedure (Jiang and Quin, 2014). Briefly, 50 g corn meal was soaked in the 450 mL distilled water at 60 °C for 24 hours. Then it was homogenized in a blender and 1% (w/v) suspension was made with water. H_2O_2 was added into a flask containing 500 mL of corn seed meal suspension to yield a final concentration of 2.5% and then the flask was maintained in the soxhlet extractor at 70 °C for 4 hours. After 4 hours, residual mixture was filtered and six volumes of the absolute ethanol were added to get the oligosaccharides precipitate. Oligosaccharide precipitates were filtered and dried. The yield of oligosaccharides of corn meal was calculated using the following equation

$$\text{Yield (\%)} = \{W1/ W2\}(100)$$

W1 and W2 represent the weights of the extracted oligosaccharides and the original corn seed meal, respectively.

2.4 Identification and quantification of oligosaccharides

2.4.1.*Molisch's Test*

The test solution containing oligosaccharides was combined with a small amount of Molisch's reagent (α-naphthol dissolved in ethanol) in a test tube and mixed thoroughly. Then a small amount of concentrated sulfuric acid was slowly added down the sides of the sloping test-tube, without mixing, to form a layer. A positive reaction was indicated by appearance of a purple ring at the interface between the acid and test layers (Saini *et al*., 2016).

2.4.2*Barfoed's Test*

Sample solution (1 mL) was placed in a test tube followed by addition of 3 mL of Barfoed's reagent (a solution of cupric acetate and acetic acid) and heating on a boiling water bath for 3 minutes. The formation of reddish precipitates showed the positive indication for the presence of sugars (Saini *et al*., 2016).

2.4.3 HPLC analysis

The oligosaccharides contents were measured by using a Shimadzu HPLC LC-20A (Singapore). The HPLC system consisted of a pump (model LC20AT Prominence), a solvent degasser (model G1322A), a C-8 column in a column oven (model CT 020A/20AC), and refractive index detector (model RID10A) the system was controlled by means of Shimadzu LC Solution software. The device was assisted by means of CBM 20A/20A light system controller. An amount of 50 g sample was blended with 100 mL double distilled water for 3 minutes. After filtering through Whatman No. 1 filter paper, the filtrate was dashed by filtering through cation and anion resin. Before injecting to HPLC, the sample was filtered through syringe filter of 0.22 µm. an amount of 20 µL sample was injected in HPLC system. The mobile phase was simply deionized water at 0.5 mL / min flow rate and detector temperature was 80 °C. The LC solution software was used for integration. Glucose, galactose, rhamnose and arabinose were identified and quantified on the basis of retention times, peak areas and comparison with calibration curve obtained by corresponding standards.

2.5 Antioxidant activity

2.7.1 Bleach ability of β-carotene in linoleic acid system

Antioxidant activity of the oligosaccharides was also assessed by bleaching of β-carotene / linoleic acid emulsion system (Kulisic *et al*., 2004). A stock solution of β-carotene-linoleic acid mixture was prepared by dissolving 10 mg β-carotene, 200 mg linoleic acid and 1000 mg Tween 40 in 10 mL of chloroform (HPLC grade). The chloroform was removed under vacuum in rotary evaporator at 50 0C. Then, 50 mL of distilled water, saturated with oxygen (30 min, 100 mL / min), was added. A 5.0 mL of this reaction mixture was dispensed to test tubes containing 200 µL of the sample solution of 10 mg/mL concentration, and the absorbance as t = 0 measured at 490 nm against a blank (emulsion without β-carotene). Then emulsion was incubated for 50 h at room temperature and the absorbance was recorded at different time intervals. The same procedure was applied for BHT and blank.

2.5.2 DPPH radical-scavenging assay

The antioxidant activity of the corn meal oligosaccharides was also assessed by measuring their scavenging abilities to 2, 2-diphenyl-1-picrylhydrazyl radicals. The DPPH assay was performed following the previously established method (Bozin *et al*., 2006). The sample solutions (10 mg/mL) were mixed with 1 mL of 90 µM DPPH solution and final volume of each was made 4 mL with 95% MeOH. Butylated hydroxytoluene (BHT) was used as a positive control. Each test sample was prepared in three replicates and stored for 1 h at room temperature before measurement. The disappearance of DPPH was studied at 515 nm using a spectrophotometer (Hitachi U-2001p model 121-0032, Japan). Percentage inhibition of free radical by DPPH was calculated using formula:

$$I\ (\%) = 100\times (\text{Blank} \_ \text{Sample}) / \text{Blank}$$

2.6 Statistical analysis

All the experiments were conducted in triplicate and statistical analyses of the data were performed by analysis of variance (ANOVA) using computer based statistical package, STATISTICA 5.5 (Stat Soft Inc., Tulsa, OK, USA). Data are presented as mean values ± standard deviation of triplicate determinations and probability value $p<0.05$ was considered statistically significant.

3. Results and Discussion

The present research work was conducted for the extraction and biological activities of oligosaccharides from corn meal using Soxhlet's extraction apparatus. For better extraction yield H_2O_2 and distilled water were used. Rapid evaluation of oligosaccharides was carried out by using different assays.

3.1 Yield of oligosaccharides and evaluation of antioxidant activity

The data regarding the yield and antioxidant activity of oligosaccharides extracted from corn meal have been presented in Table 1. The yield of oligosaccharides extract was found to be 1.6g / 50g of corn meal which is 3.2%. The reports available in literature regarding the oligosaccharides contents of different plant materials / agro-waste differ in varying degree from present investigation. The young croziers of *Pteridium aquilinum* contain 5.29% (Wang and Shengjun, 2013), Fruit of *Lycium barbarum* contained 21.05% (Jiang, 2014) and shoots of Dandelion (*Taraxacum officinale*) consist of 25.43% (Qian *et al*., 2014) oligosaccharides. Dandelion (*Taraxacum officinale*) owning the highest known oligosaccharides contents (Qian *et al*., 2014). Oligosaccharides were confirmed by the Molisch's test following the protocol mention in Hendrickson *et al*., 2001. Barfoed's test (used for presence of reducing sugars) was done for the oligosaccharides following the protocol described by Pasto *et al*., 1992. In our present research, oligosaccharides give a positive Molisch's test and negative Barfoed's test, indicating that non-reducing oligosaccharides are present in the extracted contents.

Results showing the antioxidant activities of oligosaccharides of corn meal as presented in the Table 1 were measured by DPPH assay following the previously established method (Bozin *et al*., 2006). In the DPPH assay, the ability of the examined

Table 1. Yield and antioxidant activity of oligosaccharides extract from corn meal

Material	Yield (%)	DPPH radical scavenging activity (%)	Inhibition of Linoleic acid oxidation (%)
Oligosaccharides	3.2 ± 0.2	16.9 ± 0.4	24.5 ± 1.1
BHT	------	28.5 ± 0.6	92.3 ± 2.3

Values are mean ± standard deviation of triplicate analysis

oligosaccharides to act as donor of hydrogen atoms or electrons in transformation of DPPH• into the reduced form DPPH-H was investigated. All of the assessed oligosaccharides were able to reduce the stable, purple radical DPPH into yellow DPPH-H. Corn meal oligosaccharides showed poor radical scavenging activity, (16.9%) as compared to the synthetic antioxidant BHT (24.5%). Percentage inhibition of linoleic acid oxidation as exhibited by the corn meal oligosaccharides was found 28.5%. The antioxidant activity of oligosaccharides was measured by the bleachability of β-carotene in linoleic acid system (Kulisic *et al*., 2004). The emulsion was incubated for 50 hour at room temperature and the absorbance was recorded at different time intervals (Figure 1). The same procedure was applied for BHT and blank. When the inhibitions of linoleic acid oxidation of corn seed meal were compared with BHT, oligosaccharides exhibited significantly ($p < 0.05$) lower antioxidant activity than that shown by BHT (92.7%).

The reports available in literature on the subject of the antioxidant behavior and free radical scavenging activity of oligosaccharides content in different plant materials/agro-waste differs in varying degree from present investigation. The oligosaccharides derived from the fruit of L. japonica showed high hydroxyl radical scavenging activity (91.31%) at the concentration of 100 µg/mL (Jun-Wu, 2014) whereas the oligosaccharides derive from the young croziers *Pteridium aquilinum* shows higher hydroxyl radical scavenging activity (82%) at the concentration of 80 µg/mL (Wang and Shengjun, 2013). The work on the alginate oligosaccharides obtained by the facile enzymatic treatment of the alginate from marine algae was

found to be an excellent natural antioxidant as suggested to find applications in the food industry (Falkeborg *et al*., 2014). In present study, although oligosaccharides content of corn meal showed poor antioxidant behavior, however it has good potential for use in in food industry as functional-food ingredients.

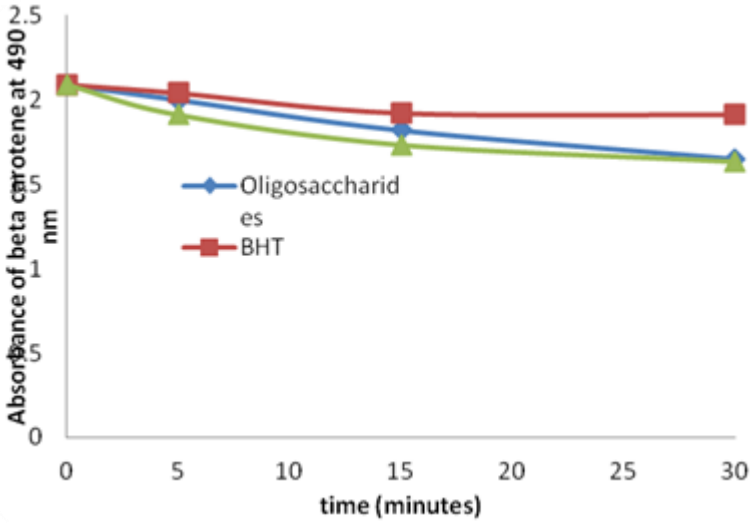

Figure 1. Bleachability of β-carotene by corn meal oligosaccharides and BHT

3.3 HPLC analysis of oligosaccharides of corn meal

The results of high performance liquid chromatography of oligosaccharides of corn meal are shown in the Figure 2 and list of compounds present in it are presented in the Table 2. The major oligosaccharides in corn meal were identified as Galactose (84.5%), Glucose/Xylose (6%), Rhamnose (5.6%) and Arabinose (3.9%). Galactose is the major oligosaccharide in corn meal

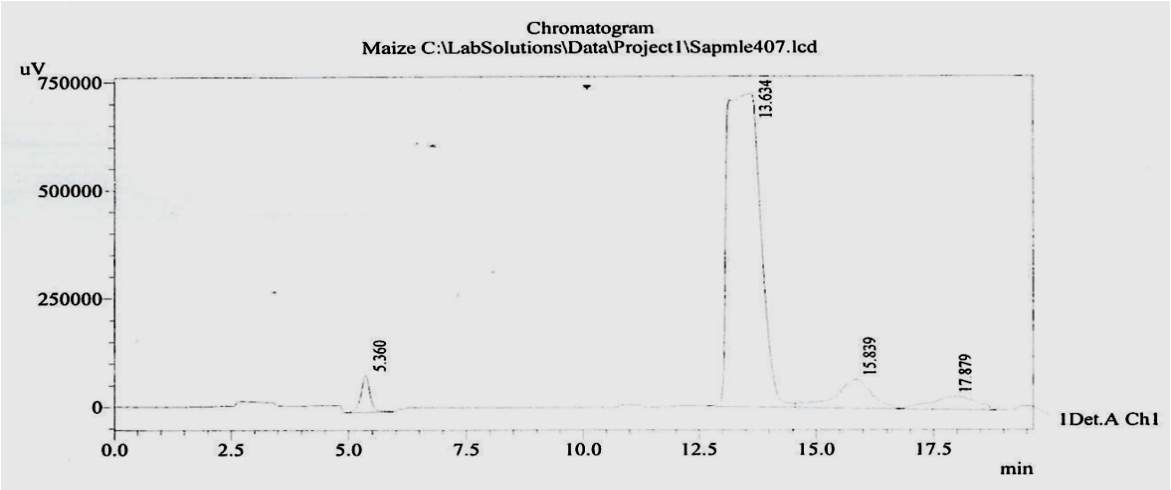

Figure 2. Typical HPLC chromatogram of corn meal oligosaccharides

with 84.5% concentration and Arabinose with lowest concentration of 3.9%. Reports are available in literature regarding the chromatography of oligosaccharides from different sources. The retention times generally increased with increasing oligosaccharide chain length, linkage of fucose α-(1 → 2) to galactose and by fucose α-(1 → 3) or fucose α-(1 → 4) to glcNAc may decrease the retention times of both the alditols and the reducing oligosaccharides. Branching generally increased the retention times for oligosaccharide alditols. The

retention times of isomers differing in the position of fucose substitution (LNF-1 vs LNF-2) differed greatly while those of the linkage isomers LNF-2 and LNF-3 were similar but distinct (Reddy and Bush, 1991).

Table 2 . Concentration of corn meal oligosaccharides determined by HPLC.

Compound Name	Retention time (min)	Concentration (g/100g)
Glucose / Xylose	5.360	6.0 ± 0.5
Galactose	13.634	84.5 ± 4.0
Rhamnose	15.839	5.6 ± 0.3
Arabinose	17.879	3.9 ± 0.2

Conclusion

The present research was conducted for the extraction of oligosaccharides from agriculture waste and their characterization. Corn meal oligosaccharides extract showed moderate antioxidant activity and free radical scavenging capacity. Agricultural waste (corn meal) proved to be a good source of useful compounds like galactose (84.5%), glucose/xylose (6.0%), rhamnose (5.6%) and arabinose (3.9%) having recognized health benefits. It would be important to utilize the agriculture waste to extract oligosaccharides which can be used in food industry as food preservative or as an ingredient of functional foods.

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