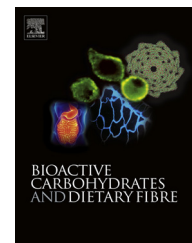


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Physicochemical evaluation of fenugreek gum and extrusion modified fenugreek gum and effects on starch degradation in bread

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ABSTRACT

The inclusion of extruded and non-extruded fenugreek gum into bread at 5% and 10% supplementation levels caused a dose-response increase in pasting profiles compared with control bread. Fresh breads containing extruded fenugreek gum at 5% and 10% were produced and *in vitro* starch digestion conducted. D-glucose released from digestion of breads with fenugreek gum was less than that for a control bread at all time points except 60 min for 5% fenugreek bread. At 120 min glucose released was 18.7% and 33% less than the control while at 300 min glucose released was 17.9% and 57.3% less than the control for 5% and 10% fenugreek gum breads respectively. Scanning Electron Microscopy showed extrusion of fenugreek gum may have caused morphological changes to the polysaccharide which may account for an increased absorption rate compared with non-extruded gum, also this change in morphology may affect the nature of the interaction of the fenugreek gum with starch.

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1. Introduction

The rise in chronic non-communicable diseases (CNCD) such as cardiovascular disease, Type 2 diabetes mellitus (T2DM) and cancer, have been linked to the over consumption of high fat, high calorie foods. The carbohydrate content of foods though is also a cause for concern, due to the high postprandial glycemic effect seen with the consumption of foods with high levels of available carbohydrate. Studies have shown this elevation of blood glucose may be tempered through the addition of dietary fibre to food, particularly soluble dietary fibre. This reduction in glycemic response is desirable as elevated postprandial glycaemia has been associated with an increased risk factor for

T2DM (Cui & Roberts, 2009; Brennan, Kuri, & Tudorica, 2004; Brennan, Blake, Ellis, & Schofield, 1996; Lehmann & Robin, 2007).

Mechanisms for the beneficial postprandial glycemic effects of soluble dietary fibre have been attributed to its viscosity, while viscosity in itself is related to the molecular weight, solubility and concentration of fibre when applied to a food product (Cui & Roberts, 2009). Polysaccharides are hydrophilic and their abundant free hydroxyl groups form hydrogen bonds with water. Therefore, the pH of a medium affects WHC. Both soluble and insoluble polysaccharides exhibit WHC, however, soluble fibres, for example pectins and soluble gums have a higher WHC than cellulose fibres (Schneeman, 2001; Borderias, Sanchez-Alonso, & Pérez-Mateos, 2005).

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These properties including viscosity effects cause an increase in the volume of intestinal contents. Consequently, absorption of nutrients like glucose and lipids is slowed (Oakenfull, 2001; Schneeman, 2001). Viscosity is the ability of particular polysaccharides to thicken on mixing with liquid. The extent of thickening is affected by the chemical composition of the polysaccharides. In the food industry gums from plants are usually used as thickeners (Schneeman, 2001; Borderias et al., 2005).

Over the years numerous scientists have added high soluble fibre to traditionally low fibre foods with detrimental organoleptic results due to the viscous properties of soluble fibre. However, great strides have been made and it is not uncommon to have products such as high fibre breads (insoluble), yoghurt and cookies. Still, more research is needed in the incorporation of various types of dietary fibre into a broad range of foods, in ways that do not interfere with palatability, to encourage the consumption of healthy foods. Research is also needed to understand exactly what aspects of the process convey health benefits. From a chemical standpoint dietary fibre is well understood. However, because dietary fibre polysaccharides are complex and their physiological functions and physical properties change in the gastrointestinal tract, it cannot be assumed that a compound that is chemically defined as fibre would lend to meaningful physiological benefits for consumers; (Spiller, 2001).

Fenugreek (*Trigonella foenum-graecum* L.) (FEN) is a legume grown annually and used extensively as a spice worldwide. This crop is grown in India, Ethiopia, Egypt and Turkey. In India, the seeds are generally used as a spice while in Europe and North America the seeds are also used for their pharmaceutical properties. Some medicinal properties attributed to FEN include cholesterol lowering, anti-inflammatory and anti-diabetic. These properties have been related to components in FEN seeds and leaves such as galactomannans, diosgenin and trigonelline (Acharya, Thomas, & Basu, 2008; Im & Maliakel, 2008 and Emerald Seed Products, downloaded 2008). Fenugreek gum is obtained from the endosperm of the seeds of the plant, being composed of galactose and mannose, at a ratio of 1:1 (Meghwal & Goswami, 2012) Chang, Cui, Roberts, Ng, & Wang 2011 found fenugreek gum had a protein content ranging from 1.86–1.94%, while according to Meghwal & Goswami, 2012 crude fenugreek gum had a protein content of 13.9%. Fenugreek was also found to be high in choline, vitamin A, the vitamin B complex and alkaloids, with saponins being the most abundant of the saponins in fenugreek. Fenugreek has GRAS (generally regarded as safe) status with Health Canada, as it is registered on the Licensed Natural Health Products database.

In this study, Fenugreek gum (FG) (galactomannan), which is the soluble dietary fibre component was used to determine its physicochemical effects in bread. It was hypothesised that there would be blunted starch digestion of bread containing FG, in a dose–response manner.

2. Materials and methods

2.1. Bread

Breads were baked as outlined in our previous work Roberts, Cui, Chang, Ng, and Graham (2012), with no FG (control), 5%

and 10% extruded FG (Ext) and non-extruded FG (NE). Breads were freeze dried, milled and stored in air tight containers for RVA and molecular weight analyses, while fresh breads were baked, cooled and used for SEM and *in vitro* starch digestion.

2.2. RVA pasting properties

RVA provides information on the rheological properties of starch such as viscosity. The pasting properties of the milled breads, that is, control and breads substituted with 5% and 10% Ext and NE was determined by a Rapid Visco Analyser (RVA-4, Newport Scientific Inc., Warriewood, Australia). Samples were subjected to standard 1 profile and the computer software Thermocline for Windows was used to analyse the pasting profiles obtained. All samples were analysed in triplicate.

2.3. *In vitro* starch digestion

In vitro studies were conducted based on the methods of Brighenti, Pellegrini, Casiraghi, and Testolin (1995) and Brennan et al. (2004) with minor modifications. Samples of bread equivalent to 2 g of available starch were weighed. Breads were diluted with 20 ml of 20 mM sodium phosphate buffer (pH 6.9) and pre-incubated for 5 min with 25 U of human salivary α -amylase per gram of starch. This was reduced to pH 1.5 with 8-M HCL. Breads were digested with pepsin (from porcine stomach mucosa) 115 U/g starch for 30 min at 37 °C. The pH of the mixture was readjusted to pH 6.9 with 8-M NaOH. This mixture was diluted to 50 ml with sodium phosphate buffer and porcine pancreatic α -amylase 110 U/g starch was added. The mixture was transferred to prepared dialysis tubing, 25 cm strips and 15,000 Da. Tubing was placed in 450 ml of sodium phosphate buffer for 5 h at 37 °C. Containers with tubes were agitated every 15 min to simulate gut movements. Duplicate aliquots (1 ml) were removed every 30 min for 5 h, replacing the volume each time with fresh buffer. The collected dialysate was boiled for 10 min to inactivate the enzymes. All samples were analysed in duplicate. Samples were then analysed for D-glucose using the megazyme GOPOD assay procedure.

2.4. Molecular mass distribution

Size exclusion chromatography (SEC) is a technique used to separate polymers based on their molecular size, instead of their chemical properties. Polymer solutions are placed in a SEC column and separated based on size, with the larger molecules exiting the column before the smaller molecules. Therefore, the retention time or volume of the different fractions from a chromatogram gives the molecular mass distribution (Wang & Cui, 2005). The molecular mass distribution of the NE, Ext and Ext and NE bread at 5% and 10% were evaluated by a high performance size exclusion chromatograph (HPSEC) equipped with a refractive index detector (RI) (Model Dual 250, Viscotek, Houston, TX, USA). The chromatographic system included a Shimadzu SCL-10Avp pump, automatic injector (Shimadzu Scientific Instruments Inc., Columbia, MD, USA), and two columns in series: a Shodex OHpak KB-806M (Showa Denko K.K., Tokyo, Japan) and an Ultrahydrogel linear (Waters, Milford, CT, USA). The

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