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Enzymatic extraction of beta-glucan from oat bran cereals and oat crackers and optimization of viscosity measurement

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ABSTRACT

The viscosity of the soluble fibre, β-glucan, has been shown to influence its ability to lower serum cholesterol and postprandial blood glucose levels. The impact of various amylases, proteases and lipase on the solubility and resulting viscosity of β-glucan extracted from oat bran cereals with a range of β-glucan concentrations and molecular weights was investigated. Addition of enzymes increased the final viscosity of high molecular weight β-glucan in cereals by facilitating the release of β-glucan from the food matrix. For cereals with partially depolymerized β-glucan, the addition of digestive enzymes decreased the final viscosity by eliminating the contribution of starch and protein to viscosity. Final viscosity varied depending on enzyme combinations including pancreatin, salivary and microbial α-amylases, microbial protease, porcine protease, trypsin and α-chymotrypsin. Addition of lipase did not significantly affect viscosity or solubility of β-glucan extracted from oat crackers. Addition of lichenase showed that β-glucan was the major contributor of viscosity to the system, with negligible interference from other components. The viscosity of the optimized protocol was compared to physiological results previously obtained. The viscosity of β-glucan extracted with pancreatin plus microbial α-amylase (pH 6.9) was predictive of LDL-cholesterol reduction ($R^2 = 0.847$) and glycemic response ($R^2 = 0.883$).

1. Introduction

Extruded breakfast cereals and whole grain crackers are a major source of dietary fibre in the western diet (USDA, 2012). Products containing significant amounts of the soluble oat fibre, β-glucan, have been demonstrated to reduce serum LDL-cholesterol levels (Davy et al., 2002; Karmally et al., 2005) and glycemic response (Braaten et al., 1994; Granfeldt et al., 2008) in humans. However, processing of oat food products can influence the magnitude of the reductions (Kerckhoffs et al., 2003; Liljeberg et al., 1992) by changing the physicochemical characteristics of the β-glucan. Oat β-glucan consumed in a beverage was found to significantly reduce LDL-cholesterol by 0.26 mmol/L (Kerckhoffs et al., 2003). But, when the same oat fibre was used to produce bread and cookies the extent of reduction was not significant (0.12 mmol/L). Partial depolymerization of the β-glucan during bread production was observed.

Continuous cooking and forming are key process components in the production of direct-expanded breakfast cereals (Mościcki and Moster, 2011). The combination of pre-conditioner and extruder are key equipment components. During preconditioning, flours are heated rapidly by steam. Even distribution of water allows quick penetration and swelling of the starch granules for a high degree of pregelatinization (Miller and Mulvaney, 2000). The dough is then transported into the plasticization zone where it is exposed to shear forces. After the mixing and shearing zone, the material passes to the dwelling or reaction zone under increased temperature conditions. In the zone directly before the die, the pressure and temperature increases further, the dough is plasticized and the incorporated water is finely dispersed. The cooked mass is then

Abbreviations: 3H, 14% β-glucan cereal with high molecular weight (2180 kg/mol); 4M, 14% β-glucan cereal with medium molecular weight (921 kg/mol); 3M, 14% β-glucan cereal with medium molecular weight (627 kg/mol); 4L, 14% β-glucan cereal with low molecular weight (326 kg/mol); BP, bovine protease; HC5, 5% β-glucan cereal with high molecular weight (2300 kg/mol); HC10, 10% β-glucan cereal with high molecular weight (2270 kg/mol); HPLC, high performance liquid chromatography; HSA, human salivary amylase; MA, microbial α-amylase; MP, microbial protease; PN, porcine pancreatin; RVA, rapid visco analyzer; TC, Trypsin and chymotrypsin.

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forced by the screw through a die and immediately cut by a rotating knife. Under the abrupt release into atmospheric conditions, the water contained in the dough vaporizes, causing expansion of the elastic dough. Each finely dispersed droplet of water results in a steam bubble and a foamy structure appears. The evaporation of 5–10% of water removes the heat for quick cooling and hardening. The foam structure persists. Together, the process conditions prevailing inside the extruder, the die-hole geometry, the extrusion speed and the cutting mode determine the form and texture of the product.

The processing for crackers is quite different from extrusion cooking. The dough is mixed at ambient temperature and pressure (Faridi, 1994). The baking time is relatively short and is done at ambient pressure. Also the dough does not contain a lot of excess water. Therefore, the degree of starch gelatinization and β-glucan solubilization is not expected to be as high.

The solubility of β-glucans, together with their molecular properties, determines their viscosity in solutions and eventually their physiological effects. The effect of β-glucan in lowering postprandial blood glucose and insulin levels is viscosity-related (Brummer et al., 2012; Tosh et al., 2008; Wood et al., 1994), and the viscosity developed in the gut has been demonstrated to be of importance in lowering serum cholesterol levels (Wolever et al., 2010). This viscosity is linked to the molecular weight, structure, and concentration of the β-glucan in the intestinal fluid.

The amount of β-glucan which comes into solution depends on the solubilization method used. Dilute NaOH can be used to extract all of the β-glucan from cereal cell walls and hot water will remove most of the β-glucan (Li et al., 2006). But typically only a portion of the β-glucan in oat foods is dissolved during digestion in the upper gut (Gallaher et al., 1999). To be able to measure the viscosity of β-glucan resulting from ingested food, which is soluble in the upper intestine, it is extracted under conditions similar to those encountered in the digestive system.

A recent review by Hur et al. (2011) has shown that the most frequently used biological molecules included in the in vitro digestion protocols were digestive enzymes (pancreatin, pepsin, trypsin, chymotrypsin, peptidase, α-amylase and lipase) and bile salts. In all of the in vitro models surveyed, the digestion temperature was 37 °C, although varying types and concentrations of enzymes were used.

A specific enzymatic extraction protocol for barley and oat β-glucan was established by Beer et al. (1997) using α-amylase, pepsin and pancreatin to obtain a physiological extract containing soluble β-glucan. Based on this protocol, a simple method for direct measuring of β-glucan viscosity in oat and barley products using the rapid visco analyzer (RVA) was recently developed by Gamel et al. (2012). In this method, different digestive enzymes were added to oat or barley foods dispersed in phosphate buffer over a 2 h period at 37 °C. The rotating paddle provided effective mixing and the viscosity was measured directly by the RVA throughout the run. This method agreed well with the in vitro extraction protocol of Beer et al. (1997) which was indicative of the physiological response to oat cereal foods (Brummer et al., 2012; Lan-Pidhainy et al., 2007; Regand et al., 2009; Tosh et al., 2008; Wolever et al., 2010).

Amylases are widely distributed in plants, animals and microorganisms. For example, human saliva and pancreatic secretion containing a large amount of α-amylase are broadly used for starch digestion, while *Bacillus* species such as *B. stearothermophilus, Bacillus licheniformis* and *B. amyloliquefaciens,* are widely used for the commercial production of the enzymes for various applications (Sivaramakrishnan et al., 2006). On the other hand, different proteolytic enzymes have been used extensively in several in vitro digestion methods to mimic the action of the human body digestive system (Hur et al., 2011). The choice of proteolytic enzymes has considerable impact on the degree of protein degradation observed. Bacterial proteases can also be used to degrade proteins in food samples (AACC 32-07.01) and have the advantage of being more consistent in composition and more economical than extracts of animal origin.

The aim of this research was to investigate the viscosity and solubility of β-glucan in oat cereals with a range of β-glucan concentrations and molecular weights using the RVA method. In order to have a robust measurement of the viscosity of β-glucan and to achieve a viscosity similar to the luminal viscosity without interference of starch, proteins or other food constituents, the type and concentrations of amylolytic and proteolytic enzymes for the extraction of β-glucan were verified. For products with higher fat contents, there was a concern that the presence of oil droplets may affect the extractability of the β-glucan, so the influence of lipase on viscosity was determined for an oat cracker. Finally, the results of the optimized extraction method were compared to in vivo LDL-cholesterol and glycemic response data to confirm that the viscosity measured was related to the physiological effects observed.

2. Materials and methods

2.1. Materials

Six extruded oat cereals with β-glucan contents between 5 and 15% were used. Four of these cereals with codes 3H, 4M, 3M and 4L were used in a previous study and viscosities of their extracts obtained using the enzymatic extraction protocol (Beer et al., 1997) were shown to correspond with the LDL-cholesterol lowering effect (Wolever et al., 2010) and glycemic response (Brummer et al., 2012). The other two extruded cereals (HC5 and HC10) were obtained from (CreaNutrition, AG, Zug, Switzerland). The cereals contained high β-glucan oat bran, corn flour, fructose and salt. The cereals were produced in a Bühler BCTG 62/200 industrial twin-screw extruder with 5–25% added water and up to 7% of steam. Processing pressures ranged from 20 to 150 bar and temperatures were between 120 and 180 °C. Oat crackers (Nairn's, Edinburgh, Scotland), purchased locally, contained wholegrain oats, sunflower and palm oils, sea salt and sodium carbonate, used as an example of a high fat product (18%). All samples were milled with an M2 universal mill (IKA-Werke, Staufen, Germany) and screened through a 0.6 mm sieve.

2.2. Enzymes

Digestive enzymes were purchased from either, Sigma/Aldrich (St. Louis, MO., USA) or Megazyme International (Bray, Ireland). For starch digestion, human salivary α-amylase (Sigma A1031; EC.3.2.1.1, 100–120 U/mg solid) and microbial α-amylase (*B. licheniformis*) (Megazyme E-BLAAM100; EC.3.2.1.1, 3000U/ml) were used. For protein digestion, microbial protease (*B. licheniformis*) (Megazyme E-BSPRT100; EC.3.4.21.14, 300 U/ml); bovine pancreatic protease (Sigma P-4630; EC.3.4.21, 6.9 U/mg solid); porcine pancreatic trypsin (Sigma T0303; EC.3.4.21.4, 53.5 U/mg solid), bovine pancreatic α-chymotrypsin (Sigma C-4129; EC.3.4.21.1, 57 U/mg solid); and porcine pancreas pancreatin (Sigma P7545; activity equiv. 8 × USP) were used. Porcine pancreas lipase, type II (Sigma L-3126; EC.3.1.1.3, 30–90 U/mg protein) and lichenase (endo-(1 → 3)(1 → 4)-β-D-glucan 4-glucanohydrolase) (Megazyme-E-LICHN; EC.3.2.1.73, 1000 U/ml) were used for fat and β-glucan digestion, respectively.

2.3. Methods

2.3.1. β-Glucan viscosity measurement

The RVA method previously described (Gamel et al., 2012) was used for direct measurement of β-glucan viscosity of foods. Samples