



Impact of native form oat β -glucan on starch digestion and postprandial glycemia

Jie Zhang, Kaiyun Luo, Genyi Zhang*

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800, Lihu Ave, Wuxi, 214122, Jiangsu Province, PR China



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ABSTRACT

Whole grain oats, as a rich source of β -glucan, have been shown beneficial to glycemic control. In the current study, the impact of native form β -glucan in oat grains (NFO-glucan) on starch digestion and postprandial glycemia was investigated. The dry-milling prepared NFO-glucan sample was enriched with native form β -glucan (15.6%), and an in vitro starch digestion assay of NFO-glucan (0.5% starch equivalent) showed a significant decrease of starch digestion rate compared to oat starch (0.5%, w/v). However, pretreatment by either β -glucanase or pepsin significantly increased the starch digestion. Consistently, an in vivo examination on the postprandial glycemia of the cooked NFO-glucan sample using a mouse model displayed a significant decrease of postprandial glycemia compared to gelatinized oat starch. Further experiment on the pasting property of NFO-glucan sample by a rapid visco-analyser demonstrated both β -glucan and protein affected its viscosity profiles. Scanning Electronic Microscope (SEM) observation revealed a network-like native structure of β -glucan that might encapsulate protein and starch to reduce the enzyme accessibility and so the digestion of starch. Novel food processing technologies to maintain the native form of β -glucan in oat grains might be a better way to modulate the postprandial glycemia of oat-based whole grain foods.

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1. Introduction

As the prevalence of metabolic diseases such as type 2 diabetes continues to increase worldwide, dietary approaches have become important measures to prevent or delay the occurrence of these diseases. Whole grain foods, defined by the United States Food and Drug Administration (U.S. Food and Drug Administration, 2006) as “cereal grains that consist of the intact and unrefined, ground, cracked or flaked fruit of the grains whose principal components – the starchy endosperm, germ and bran – are present in the same relative proportions as they exist in the intact grain”, have been shown to be one of the efficient dietary approaches to reduce the incidences of metabolic diseases (Cho et al., 2013). Although bioactive phytochemicals (Okarter and Liu, 2010) and dietary fibers (Anderson et al., 2009) are commonly considered as the basis to the health benefits of whole grains foods (Fardet, 2010), the contribution of endosperm starch, as the major constituent of whole grain, to the health benefits of whole grain foods has been less

appreciated. With the known synergy between starch and phenolic compounds and/or dietary fibers to the health benefits of whole grain foods (Jonnalagadda et al., 2011), the nutritional property of endosperm starch and its impact on the health benefits of whole grain foods need to be studied in a whole grain food context (Wolever, 2013).

Whole grain oat, with a high content of (1 $\rightarrow$ 3,1 $\rightarrow$ 4)- β -glucan (Behall et al., 1997), is well known for its cholesterol-lowering function (Othman et al., 2011). Oat is also beneficial to glycemic control in diabetic patients with decreased postprandial glycemic and insulinemic responses (Hou et al., 2015), and the viscosity of oat β -glucan has been shown to be the deciding factor (Regand et al., 2011; Kim and White, 2013). Modified starch digestibility by oat β -glucan led to reduced glycemic response in human subjects (Regand et al., 2011), and incorporation of β -glucan also decreased the glycemic index of starchy foods (Makelainen et al., 2006). However, there exist non-positive reports on oat's effect on glycemic control (Zou et al., 2015) and impaired glucose tolerance (Belobrajdic et al., 2015) indicating there are other factors affecting oats' beneficial effect on glucose homeostasis. In fact, the intactness of botanical structure of whole grains plays an important

* Corresponding author. Tel.: +86 510 85328726.
E-mail address: genyiz@gmail.com (G. Zhang).

Abbreviations

AACC	American Association of Cereal Chemists
GOPOD	glucose oxidase/peroxidase
NFO-glucan	native form oat β -glucan
RVA	rapid visco-analyser

role in starch digestion and glycemic response, and severe processing to disrupt the physical structure of whole grains could result in a rapid digestion of endosperm starch (Holm et al., 1989) with increased serum glucose and augmented insulin response (Edwards et al., 2015). Thus, the food form might be another important factor influencing the starch digestion rate and related physiological responses, but the mechanism of food form of whole grain associated with starch digestion and glycemic response is still poorly understood. Due to the fact that most of the studies were focused on the functions of extracted β -glucan, the impact of β -glucan naturally presented in oat grain in its native physical forms (NFO-glucan) on starch digestion and related mechanism were studied to further the understanding of the relationship between the physical form of β -glucan and starch digestion.

2. Materials and methods

2.1. Native form oat β -glucan sample preparation and characterization of extracted β -glucan

Whole oat grains (Hebei, China) were milled into flour, and the milled flour was treated by 75% ethanol at 80 °C for 4 h to inactivate the indigenous enzymes (Beer et al., 1996). The treated oat flour was then fractionated by a sieve with a pore size of 250 μ m (mesh size of 60), and the flour fraction retained after passing through the sieve was used as the experimental sample, which was termed as the native form oat β -glucan sample (NFO-glucan) to differentiate it from extracted β -glucan. In the meantime, the β -glucan from the whole oat flour was extracted according to the method of Beer et al. (1996) under an alkaline condition of pH10 at 40 °C. The proximate analysis was carried out based on standard official methods (AACC method 08–01.01 for ash, 30–25.01 for fat, 44–16.01 for moisture, 46–10.01 for protein) from the 11th edition of AACC International. Starch content was measured by a heat-stable α -amylase and amyloglucosidase method using the Total Starch Measurement kit (Megazyme International Ireland), and the content of β -glucan was measured by AACC method 32–22.01 involving lichenase and β -glucosidase to convert extracted β -glucan into glucose, and then the glucose content was measured by a GOPOD kit (Sigma, Shanghai China) according to the manufacturer's instructions, and the total glucose content was converted to the content of β -glucan by a factor of 0.9.

The composition of extracted β -glucan was shown to contain 82.3% β -glucan (dry weight basis) and other minor component of protein (3.91%) and fat (2.13%). The molecular weight of β -glucan was measured by a HPSEC-MALLS system (Zhang et al., 2003). The apparent viscosity of extracted β -glucan was measured using an AR G2 rheometer (TA instrument Inc., USA) with a shear rate of 1–100 1/s at room temperature of 22 °C, and a cone plate (40 μ m diameter) with a specified loading gap distance (55 μ m) was selected for the measurement.

2.2. Extraction of oat starch

The oat grain was milled into fine flour (100 g), and fat was first

removed by extracting with petroleum ether (500 mL) with continuous stirring for 4 h. After centrifugation at 4400 $\times$ g for 20 min, the flour was air-dried in a fuming hood. Then, 1000 mL solution of 10 mM NaOH at a pH of 11.0 was used to soak the defatted oat flour at 30 °C for 2 h with continuous stirring. A sieve of 80 mesh size was used to remove the large particle materials, and the liquid portion passing through the sieve was centrifuged at 3050 $\times$ g for 15 min. The supernatant and the brown sediment were removed, and the white precipitate was collected and suspended in distilled water (400 mL) until there was no brown precipitate. The purified oat starch was obtained after the collected white precipitate was dewatered with ethanol and dried at 50 °C in an oven. The purity of extracted starch was measured based on the Total Starch Measurement kit (Megazyme International Ireland).

2.3. The effect of β -glucan on in vitro oat starch digestion

Starch hydrolysis kinetics was measured using an in vitro enzymatic hydrolysis method. Specifically, NFO-glucan samples (50.0 mg starch equivalent, dwb) were put into glass tubes containing 5 mL NaOAc buffer (100 mM, pH 5.2, CaCl₂ 4 mM) and cooked in a boiling water bath for 20 min with continuous stirring. After the tubes were cooled at 37 °C, 5 mL pre-heated (37 °C) dual enzyme solution (290 U/mL porcine pancreatic α -amylase, 15 U/mL *Aspergillus Niger* amyloglucosidase (Sigma, Shanghai China), dissolved in NaOAc buffer) was added for starch digestion with continuous stirring. Aliquot samples (100 μ L) were taken at 20, 40, 60, 80, 100 and 120 min, and the reaction was stopped with 900 μ L of absolute ethanol in a 1.5 mL microcentrifuge tube. After centrifugation (7000 $\times$ g, 3 min), glucose concentration of the supernatant was measured using a glucose oxidase/peroxidase (GOPOD) kit (Sigma, Shanghai China).

To test the effect of native form β -glucan on starch digestion, β -glucanase (Rui Yang biotechnology co. LTD, China, 20U/mg) dissolved in 5 mL NaOAc buffer (pH5.2) at a concentration of 0.02 mg/mL was added into tubes containing NFO-glucan samples (equivalent to 50 mg starch dwb) to hydrolyze β -glucan at 55 °C with continuous stirring for 20 min, and then heated in a boiling water bath for 20min to gelatinize the starch. The same procedure was followed to perform the starch digestion by adding 5 mL dual enzyme solution. Regarding the effect of extracted oat β -glucan on the digestion of purified oat starch, 50 mg oat starch (as the control) and starch (50 mg) mixed with extracted β -glucan (39.0% of starch, dwb) were used as the samples for digestion following the same procedure. The amount of β -glucan mixed with starch was based on its relative amount in NFO-glucan sample.

2.4. The effect of protein on in vitro oat starch digestion

Pepsin (Sinopharm Chemical Reagent co., LTD, China, $\geq$ 1200U/g) was first dissolved in a solution (NaCl 2.0 g/L, pH 2.5 by adding HCl) at a concentration of 1.0 mg/mL, then 5 mL pepsin solution was added into NFO-glucan sample (50 mg starch equivalence, dwb) to hydrolyze protein in a water bath held at 37 °C with a shaking speed of 160 rpm for 30 min. After that, the samples were neutralized with 100 mM NaOH and heated in a boiling water bath for 20 min, and cooled to 37 °C for starch digestion as the above by adding 5 mL dual enzyme solution prepared with 2 $\times$ buffer (200 mM NaOAc, pH 5.2, CaCl₂ 8 mM).

2.5. Scanning electronic microscopy (SEM) analysis

SEM analysis on the NFO-glucan sample after starch hydrolysis was carried out in order to visualize the physical structure of the β -glucan. Briefly, the residual of NFO-glucan digestion (500 mg oat

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