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Moisture content during extrusion of oats impacts the initial fermentation metabolites and probiotic bacteria during extended fermentation by human fecal microbiota

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ABSTRACT

Extrusion exposes flour components to high pressure and shear during processing, which may affect the dietary fiber fermentability by human fecal microbiota. The objective of this study was to determine the effect of flour moisture content during extrusion on *in vitro* fermentation properties of whole grain oats. Extrudates were processed at three moisture levels (15%, 18%, and 21%) at fixed screw speed (300 rpm) and temperature (130 °C). The extrudates were then subjected to *in vitro* digestion and fermentation. Extrusion moisture significantly affected water-extractable β-glucan (WE-BG) in the extrudates, with samples processed at 15% moisture (lowest) and 21% moisture (highest) having the highest concentration of WE-BG. After the first 8 h of fermentation, more WE-BG remained in fermentation media in samples processed at 15% moisture compared with the other conditions. Also, extrusion moisture significantly affected the production of acetate, butyrate, and total SCFA by the microbiota during the first 8 h of fermentation. Microbiota grown on extrudates processed at 18% moisture had the highest production of acetate and total SCFA, whereas bacteria grown on extrudates processed at 15% and 18% moisture had the highest butyrate production. After 24 h of fermentation, samples processed at 15% moisture supported lower *Bifidobacterium* counts than those produced at other conditions, but had among the highest *Lactobacillus* counts. Thus, moisture content during extrusion significantly affects production of fermentation metabolites by the gut microbiota during the initial stages of fermentation, while also affecting probiotic bacteria counts during extended fermentation.

1. Introduction

Whole grain oats are widely consumed in the form of ready-to-eat (RTE) extruded breakfast cereals. Extrusion exposes flour components to high pressure and shear during processing, which affects the physicochemical properties of the extrudates (Brahma, Weier, & Rose, 2016; Camire & Flint, 1991; Zhang, Liang, Pei, Gao, & Zhang, 2009). For instance, Zhang et al. (2009) observed fragmentation of water-extractable β-glucan (WE-BG) upon extrusion of oat bran. They reported a decrease in the ratios of (1 → 3) and (1 → 4) linkages in oat bran from 1: 2.19 to 1: 0.85 accompanied by a decrease in (1 → 4) linkages from 72% in unprocessed flour to 48% in extruded samples. Another study reported an 18% increase in the WE-BG in extrudates processed at 15% moisture condition compared to 18% and 21% (Brahma et al., 2016). Camire and Flint (1991) reported around 33% increase in insoluble NSP and 14% in total NSP in extruded oatmeal as compared with the raw oatmeal.

Extrusion moisture is the most critical parameter in the extrusion process. Processing moisture content impacts the melting temperature, viscosity and shear stress of materials inside the extruder barrel (Brahma et al., 2016; Jongsutjarittam & Charoenrein, 2014; Sumargo, Gulati, Weier, Clarke, & Rose, 2016; Zhang, Bai, & Zhang, 2011). For instance, in our previous study, severe moisture played a critical role in increasing the resistant starch (RS), slowly digestible starch (SDS) fractions as well as WE-BG in extruded whole grain oats (Brahma et al., 2016). Moisture contents ranging from 20 to 29% in waxy rice flour and 16–25% in rice flour caused structural and physicochemical changes in the extrudates, with the lowest moisture condition causing more damage to the native crystalline structure accompanied by complete gelatinization of the starch granules compared to higher moisture processing conditions (Jongsutjarittam & Charoenrein, 2014). Another study reported a decrease in rapidly digestible starch (RDS)

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from 75% to 68% accompanied by an increase in resistant starch (RS) from 1.8 to 12.6% with an increase in moisture from 17.2 to 20.1% in extruded brown rice and pinto bean flours (Sumargo et al., 2016). Zhang et al. (2011) reported an increase in soluble dietary fiber (primarily β-glucan) in oat bran with a decrease in extrusion moisture from with 30–10%. Moreover, the authors also mentioned in this study that extrusion temperatures (100–160 °C) had less influence on the yield of the soluble dietary fiber.

Because extrusion affects the physicochemical properties of the extrudates it may affect the concentration of microbial accessible carbohydrates (MAC) during fermentation of the dietary fiber by human fecal microbiota (Connolly, Lovegrove, & Tuohy, 2010; Drzikova & Dongowski, 2005; Dust et al., 2004; Hernot, Boileau, Bauer, Swanson, & Fahey, 2008). For instance, Kim and White (2012) reported the *in vitro* fermentation properties of high (6.87×10^5 g/mol), medium (3.71×10^5 g/mol) and low molecular (1.56×10^5 g/mol) weight β-glucan from whole grain oats. The low molecular weight β-glucan resulted in higher amounts of propionate than its higher molecular weight counterparts during fermentation. On the other hand, Connolly et al. (2010) reported higher production of propionate and butyrate during later stages of fermentation of thick (0.85–1.00 mm) oat flakes compared with thin (0.53–0.63 mm) flakes. Butyrate and propionate are considered beneficial short chain fatty acids (SCFA) produced by gut bacteria during fermentation (den Besten et al., 2013). Low moisture (15%) coupled with low screw speed extrusion conditions (120 rpm) not only resulted in greatest extractability (around 3-fold) of non-starch polysaccharides in wheat bran, but also led to the highest production of SCFA (1.4-fold) compared to untreated bran (Arcila, Weier, & Rose, 2015). Extrusion processing made oat and barley extrudates more fermentable than wheat and corn (Hernot et al., 2008), with about a 58% increase in the production of total SCFA during fermentation with human fecal microbiota compared with the native unprocessed whole grain. In contrast, Moen et al. (2016) observed lower concentrations of SCFA and lower levels of the beneficial probiotic bacteria, *Bifidobacterium* and *Lactobacillus*, in feces collected from pigs after consuming extruded oat and barley diets compared to the unextruded diets. Thus, the purpose of this study was to investigate the influence of moisture content during extrusion on the *in vitro* fermentation of whole grain oats by human fecal microbiota.

2. Materials and methods

2.1. Starting material

Whole grain oat flour was obtained from General Mills (Minnesota, MN, USA). Moisture, non-starch polysaccharides (NSP) and total starch were measured in the whole grain oat flour following approved methods 44–15.02, 32–25.01 and 76–13.01 respectively (AACC International, 2016). A kit was used for the total starch assay (K-TSTA, Megazyme, Wicklow, Ireland). Amylose: amylopectin ratio was measured using the dual wavelength iodine binding method (Zhu, Jackson, Wehling, & Geera, 2008). Protein was measured following approved method 46–30.01 (AACC International, 2016) using a using a nitrogen analyzer (FP528, Leco, St. Joseph, WI USA).

2.2. Extrusion of whole grain oat flour

Extrusion of whole grain oat flour was carried out following the same procedure used previously (Brahma et al., 2016). In brief, extrudates were produced in duplicate at three moisture levels (wet basis): 15%, 18%, and 21%, at fixed screw speed (300 rpm) and temperature (130 °C). After drying, the extrudates were packaged in zip top bags and stored at 4 °C until further analysis.

2.3. In vitro digestion

Extrudates were milled using a cyclone mill (Model 4425, UDY, Fort Collins, CO, USA) equipped with a 1 mm screen. The milled samples were then subjected to *in vitro* digestion according Yang, Martínez, Walter, Keshavarzian, and Rose (2013). In brief, 25 g of sample was mixed with 300 mL of water, and boiled for 20 min with constant stirring. The mixture was cooled and the pH was adjusted to 2.5 with 1 M HCl followed by addition of 10 mL of 10% (*w/v*) pepsin (P-700; Sigma, St Louis, MO, USA) in 50 mM HCl. The mixture was then placed on an orbital shaker (150 rpm) at 37 °C for 30 min, whereupon 50 mL of 0.1 M sodium maleate buffer (pH 6, containing 1 mM $CaCl_2$) was added and the pH was adjusted to 6.9 with 1 M $NaHCO_3$. Fifty milliliters of 12.5% (*w/v*) pancreatin (P-7545; Sigma) in sodium maleate buffer and 2 mL of amyloglucosidase (3260 U/mL; Megazyme, Bray, Ireland) were then added and samples were kept in a shaking water bath at 37 °C for 6 h. The digested slurries were then transferred into dialysis tubing (molecular weight cutoff 12,000–14,000) (Spectrum Laboratories, Rancho Dominguez, CA, USA), and dialyzed for 3 d against distilled water at 4 °C with changing of the water every 3 h during the day. The retentate was frozen (− 20 °C) overnight and then freeze-dried. The freeze-dried samples were analyzed for total starch and NSP with a sample size of 150 mg following AACCI approved method 32–25.01 (AACC International, 2016). The total starch concentration in the freeze dried sample was used to calculate the resistant starch concentration.

2.4. In vitro fecal fermentation and analysis

In vitro batch fecal fermentation was performed according to the methods described by Arcila et al. (2015) using separately prepared tubes containing 15 mg of digested, freeze-dried material suspended in 1 mL of sterile fermentation medium and 0.1 mL of freshly prepared, pooled fecal inoculum from 3 healthy individuals with no record of gastrointestinal abnormalities or antibiotic administration in the last 6 months. The fecal slurry was prepared by mixing the fecal samples with the sterile phosphate buffered saline (PBS, pH 7.0) in the ratio 1:9 (*w/v*) using a hand blender for 1 min and then filtering through four layers of cheesecloth. The fermentation medium contained (per L) peptone (2 g; Fisher Scientific, Pittsburgh, PA USA), yeast extract (2 g; Alfa Aesar, Ward Hill, MA USA), bile salts (0.5 g; Oxoid), $NaHCO_3$ (2 g), NaCl (0.1 g), K2HPO4 (0.08 g), MgSO4·7H2O (0.01 g), CaCl2·6H2O (0.01 g), L-cysteine hydrochloride (0.5 g; Sigma), hemin (50 mg; Sigma), Tween 80 (2 mL), vitamin K (10 μL; Sigma), and 0.025% (*w/v*) resazurin solution (4 mL). The fermentation tubes were inoculated with 0.1 mL of fecal slurry, capped and incubated at 37 °C with orbital shaking (125 rpm) for 24 h. Samples were collected after 0 h, 8 h, and 24 h of fermentation and were immediately stored at − 80 °C. All steps were carried out inside an anaerobic hood (Bactron X, Sheldon manufacturing, Cornelius, Oregon USA) containing 5% H_2, 5% CO_2, and 90% N_2.

For analysis, fermentation tubes were thawed and centrifuged at 10000 g for 5 min. The supernatants (0.4 mL each) were used for analysis of short/branched chain fatty acids (S/BCFA) and water-extractable β-glucan (WE-BG), while the pellets were used for quantifying *Bifidobacterium* and *Lactobacillus* counts. SCFA were quantified by gas chromatography according to Arcila et al. (2015). WE-BG was analyzed following the European Brewery Convention method 8.11.1 using a kit (K-BGLU, Megazyme). *Bifidobacterium* and *Lactobacillus* were measured by quantitative real time PCR as described previously (Hartzell, Maldonado-Gómez, Hutkins, & Rose, 2013). Briefly, qPCR was performed using Mastercycler Realplex2 (Eppendorf AG, Hamburg, Germany), SYBR Green (Real master Mix, 5 PRIME Inc., Gaithersburg, MD, USA) with specific primers for *Bifidobacterium longum* ATCC 15705 (F: TCGCGTC(C/T) GGTGTGAAAG and R: CCACATCCAGC(A/G) TCC-AC) and *Lactobacillus reuteri* MM4 (F: AGCAGTAGGGAATCTTCCA and R: ATTYCACCGCTACACATG) and annealing temperatures of 58 °C and

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