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Please cite this article as: Roye, C., Bulckaen, K., De Bondt, Y., Liberloo, I., Van De Walle, D., Dewettinck, K., Courtin, C.M., Side-by-side comparison of composition and structural properties of wheat, rye, oat and maize bran and their impact on in vitro fermentability, Cereal Chem. 2019;00:1-14. <https://onlinelibrary.wiley.com/doi/full/10.1002/cche.10213>

Side-by-side comparison of composition and structural properties of wheat, rye, oat and maize bran and their impact on in vitro fermentability

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Word count body text:

Abstract

Background and objectives: Compared to wheat bran, less information on physicochemical characteristics and health related effects is available for bran from other cereals and results are often confounded by residual endosperm. Therefore, the objective of this study was to compare structure and composition of endosperm depleted bran of wheat, rye, oat and maize, and link these characteristics to fecal fermentation characteristics.

Findings: Compared to wheat, endosperm depleted rye bran contained twice the amount of fructan (8.0% dm) and water-extractable arabinoxylan (1.4% dm). Oat bran had a more open structure and was rich in (1,3:1,4)- β -glucan (17.0% dm). For rye and oat bran, and to a lesser extent wheat bran, fermentation with fecal microbiota resulted in short chain fatty acid production, with full metabolization of fructan and β -glucan after 2 and 24 hours of fecal fermentation, respectively. For maize bran, a less accessible structure containing over 70% of arabinoxylan and cellulose, was coupled to a lower degree of fecal fermentation.

Conclusion: Removal of endosperm as a confounding factor when studying bran properties allowed us to obtain more accurate estimates of the composition, structure and some physicochemical characteristics of bran of wheat, rye, oat and maize. Bran fecal fermentation characteristics were mainly determined by composition and to a lesser extent by accessibility of the structure.

Significance and novelty: This is the first study that compares composition, structure and fecal fermentation characteristics of wheat, rye, oat and maize bran, after eliminating endosperm as a confounding factor. The knowledge obtained will allow a more goal oriented choice of bran type for incorporation into food products, depending on targeted physiological effects and nutritional impact.

KEYWORDS

Bran-water interaction, bran structure, *in vitro* fermentation, cereal bran

ABBREVIATIONS

EFSA: European Food Safety Authority, WB: wheat bran , RB: rye bran, OB: oat bran, MB: maize bran, ED: endosperm depleted, WE-AX: water-extractable arabinoxylan, WU-AX: water-unextractable arabinoxylan, TOT-AX: total arabinoxylan, TFA: trifluoroacetic acid, cryo-scanning electron microscopy: cryo-SEM, SCFA: short chain fatty acids, dm: dry matter.

1. Introduction

Due to the growing demand for healthy food, there is an increasing interest in foods with a high dietary fiber content. In most Western countries, the average daily intake of fiber lies beneath the recommended intake (EFSA 2010a; Stephen et al. 2017). Cereal bran incorporation in food systems as an ingredient or as part of whole meal may be a solution for this problem because it is one of the most concentrated natural sources of dietary fiber in the Western diet.

Most bran in our diets is wheat (*Triticum aestivum* L.) derived. Wheat bran is commercially available as a byproduct of the wheat milling process, during which the endosperm is separated from the bran to produce refined wheat flour. The bran fraction is mostly discarded as animal feed, but is increasingly used to reconstitute whole meal. Commercial bran consists of the botanical bran, the aleurone layer, and residual endosperm due to incomplete separation during milling (Hemdane et al. 2016). The European Food Safety Authority (EFSA) approved two health claims stating that the consumption of wheat bran can increase the fecal bulk and decrease transit time (EFSA 2010b). A characteristic that is important for both effects is the high water holding capacity of wheat bran (Jacobs et al. 2015).

Compared to wheat bran (Marcotuli et al. 2016), less information on physicochemical characteristics and health related effects is available for other bran types. It can be hypothesized, though, that the high amount of unextractable dietary fiber in maize (*Zea mays* L.) bran, the high (1,3:1,4)- β -glucan content of oat (*Avena sativa* L.) bran (Wu et al. 2018) and the high fructan content of rye (*Secale cereal* L.) bran (Kamal-Eldin et al. 2009) can induce similar positive health effects as seen with wheat bran.

The properties of bran hinder its widespread use. Bran incorporation in foods gives rise to technofunctional and organoleptic problems (Hemdane et al. 2016). In this context it is shown that wheat bran has an adverse effect on bread making (Hemdane et al. 2016) and the production of biscuits (Sozer et al. 2014). Bran of rye, oat and maize might have other technofunctional properties in food products and can hence be used as an alternative source of dietary fiber. Thus, more in depth information on the characteristics of these dietary fiber sources could lead to a more goal oriented choice of bran type for incorporation into food products.

Brans of wheat, rye, oat and maize have different amounts of endosperm still attached, which will influence the analysis of the physicochemical characteristics. Literature results are often difficult to compare. Especially for maize bran, the reported chemical composition is variable due to the existence of dry and wet milling methods (Rose et al. 2010). The wet milling procedure is not focusing on the production of refined flour but rather on corn starch isolation, and it results in starch, protein, bran and germ fractions (Haros and Suarez 1997). It is clear that the milling technique will have an influence on the composition and physical characteristics of maize bran. The above shows that more in depth information on the characteristics of endosperm free bran would be useful, allowing a better side-by-side comparison of the characteristics of bran of different grains.

Next to the difference in endosperm content, bran samples of different cereal sources are unique in their fermentable and unfermentable dietary fiber composition (Knudsen et al. 2017). Although extensive research has been carried out on the fermentation of individual dietary fiber components by colon microbiota (Damen et al. 2011), studies on the fermentation of bran from different cereals are rather hard to find. For the physical characteristics and the structure of bran, Bäckhed et al. (2005) suggested that undigested food components such as bran particles can act as 'dinner table' to which bacteria can attach to efficiently ferment soluble dietary fiber. Thus, also the accessibility and structure of bran seems to determine the fermentation characteristics. In spite of this, information on the relation between the composition, structure and fermentation characteristics of bran is lacking.

Furthermore, determination of the *in vitro* fermentation characteristics of foods mostly starts with a predigestion step, used to mimic the digestion in the stomach and small intestine (Minekus et al. 2014). This step leads to the removal of digestible starch and proteins, but unavoidably also fructan and part of water-extractable arabinoxylan (WE-AX) are lost in the process of dialysis (Karppinen et al. 2003). The predigestion step for rye and wheat bran was shown to remove 48% and 34% of the fructan content during dialysis, respectively (Karppinen et al. 2003). This influences the fermentation characteristics of bran as these dietary fibers are readily fermentable by the microbiota (Damen et al. 2011). Because bran of different sources has different amounts of these small dietary fiber components, the impact of the predigestion procedure can be thought to be grain dependent.

The aim of this study was to perform a side-by-side comparison of the structure and chemical composition of bran of wheat, rye, oat and maize, and investigate their impact on *in vitro* fermentability. In view of the above arguments, endosperm content of the different bran materials was strongly reduced prior to further use of the bran and no predigestion was conducted prior to *in vitro* fermentation.

2. Materials and methods

2.1 Materials

Wheat bran was obtained from Dossche Mills (Deinze, Belgium). Oat bran was provided by AVEVE (Merksem, Belgium) and dry milled maize bran by Maselis (Roeselare, Belgium). Rye kernels (cultivar Dukato) were obtained from AVEVE (Merksem, Belgium), conditioned to 16.0% moisture and milled into flour, shorts and bran with a Bühler MLU-202 laboratory mill (Bühler AG, Uzwil, Switzerland). One batch of native wheat bran (WB), rye bran (RB), oat bran (OB) and maize bran (MB) was used. All chemicals, solvents and reagents were of analytical grade and purchased from Sigma-Aldrich (Bornem, Belgium), unless specified otherwise.

2.2 Determination of the chemical composition

Ash and moisture contents were determined according to AACC International methods 08-01.01 and 44-15.02 (AACC International). Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7700x, Santa Clara, CA, USA) was used to determine the elemental composition of the bran samples. Samples (0.05 g) were weighed into glass tubes and dispersed with 1.0 ml ultrapure 25% v/v HNO₃, followed by 2.0 ml *aqua regia* (75% v/v ultrapure HCl and 25% v/v ultrapure HNO₃), and followed by a dilution step with Milli-Q water to 10.0 ml (Milli-Q Plus, Merck Millipore, Darmstadt, Germany). Also the NIST1567 wheat reference with a known elemental composition was included. Starch content was determined using the Megazyme Total Starch Kit (Bray, Ireland). (1,3:1,4)- β -glucan content was analyzed using the Megazyme β -glucan Assay Kit (Bray). Fructan concentrations were determined using a mild acid hydrolysis, followed by glucose and fructose determination with high performance anion-exchange chromatography with pulsed amperometric detection on a Dionex ICS 5000 chromatography system (Sunnyvale, CA, USA) as described by Verspreet et al. (2012). Total arabinoxylan (TOT-AX) and water-extractable arabinoxylan (WE-AX) contents were determined using the procedure described by Courtin, Van den Broeck and Delcour (2000). The steps in this procedure are (i) hydrolysis to monosaccharides using 2.0 M trifluoroacetic acid (TFA), (ii) reduction of the monosaccharides with NaBH₄ to alditols, (iii) acetylation with acetic anhydride, and (iv) extraction of the acetylated products with ethyl acetate followed by (v) GC analysis. TOT-AX and WE-AX are determined as the sum of arabinose and xylose, multiplied by 0.88 to account for the polymeric nature of AX. Water-unextractable arabinoxylan (WU-AX) was estimated as the difference between TOT-AX and WE-AX. Cellulose was determined with the same procedure in essence, with some adaptations. Starch in the samples was first hydrolyzed in a boiling water bath using 1.5 mL of a diluted thermostable α -amylase (100 U/mL, Megazyme, Ireland), followed by hydrolysis to glucose with amyloglucosidase (330 U, Megazyme, Ireland) at 50°C for 30 minutes. 6.7 mL of water was added, vortexed and centrifuged (10 min, 2500 g, 20°C) to remove glucose. This washing step was repeated three times and samples were dried in the oven (overnight, 130°C). To determine glucose coming from residual starch

and β -glucan, hydrolysis with TFA was performed as described by Courtin et al. (2000). To determine glucose present in the form of cellulose, samples were pre-hydrolyzed using 13 M H_2SO_4 (1.0 mL) for 1 hour at room temperature. Afterwards, 5.0 mL of water was added and hydrolysis was performed for 2 hours at 110°C. Next, a reduction and acetylation were performed. Cellulose was determined as the amount of glucose formed during hydrolysis with H_2SO_4 subtracted with the amount of glucose that is formed during the hydrolysis with TFA and multiplied with a factor 0.9 to account for the polymeric nature of cellulose. Protein content was measured using an adaptation of the AOAC Method 990-03 (AOAC 1995) using the Dumas combustion method with an automated protein analysis system (Vario MAX CN, Elt, Gouda, Netherland). The amount of detected nitrogen was converted to protein content using a conversion factor of 6.25. Total lipid content was gravimetrically determined as described by Gerits, Pareyt et al. (2013), after extraction with water-saturated butanol. The determination of the amount of free P and P present in phytic acid was done with the K-Phyt assay from Megazyme (Bray, Ireland) and started with an extraction of myo-inositol phosphates with 0.66 M HCl (15h, 150 rpm, room temperature). Free P was measured after the extraction procedure by adding a color reagent solution, which contained ammonium molybdate, ascorbic acid and sulfuric acid. Total P was determined after degradation of phytate into free P and inositol with phytase and alkaline phosphatase, after which phosphate was measured colorimetrically. Phytase content was determined with a procedure that uses an extraction of the enzyme, an incubation with phytic acid and measurement of the released phosphate by addition of ammonium molybdate (Megazyme, Bray, Ireland). All analyses were conducted in triplicate.

2.3 Bran endosperm removal

Residual endosperm attached to native bran was largely removed to allow (i) a more correct estimation of the chemical composition and bran-water interactions and (ii) comparison of the *in vitro* fermentation characteristics of the different bran samples without the removal of bran components as result of an *in vitro* digestion procedure. Endosperm was removed using a combination of dry and wet methods. Preliminary results showed that for every bran sample, distinct dry procedures were

needed to reduce the endosperm content as much as possible. WB and MB were passed through the break rolls of a Chopin CD1 mill (Villeneuve-la-Garenne Cedex, France) to remove endosperm. The reduction rolls of the same Chopin mill were used for native OB. For RB, a bran finisher, developed and described by Jacobs, Hemdane, et al. (2018) was used to remove endosperm (20 minutes, 550 rpm, 180 μ m sieve). Afterwards, the bran samples with reduced endosperm content were washed. Bran (20 g) and water (70 ml) were mixed for 10 minutes using a Hobart N50 5-Quart Mixer (Troy, Ohio, USA). The hydrated bran was transferred to a sieve (250 μ m) and washing water [1L (for WB, RB, MB) or 2L (for OB)] was gradually added, resulting finally in washing water and washed bran. The washing water was centrifuged (10 min, 2500g, 7 °C) and the pellet kept for analysis. The supernatant and washed bran were combined and lyophilized together. A Cyclotec 1093 Sample mill (FOSS, Högenäs, Sweden) was used to reduce the particle size of the dried samples to the same size (D_{50} between 750 μ m and 900 μ m). In this way, interference of particle size on the physicochemical characteristics was reduced to a minimum. The dry endosperm removal procedure followed by the wet procedure and particle size reduction resulted in a batch of endosperm depleted wheat bran (WB^{ED}), rye bran (RB^{ED}), oat bran (OB^{ED}) and maize bran (MB^{ED}).

2.4 Visualization of the structure of bran

The structure of native bran and endosperm depleted bran was visualized using light microscopy. Native bran and endosperm depleted bran were embedded in agar (2%) and fixed in 3.0% paraformaldehyde and 1.0% glutaraldehyde in 0.20 M sodium potassium phosphate buffer pH 7.0. Samples were dehydrated by washing with different ethanol solutions and infiltrated with a Leica Histoiresin Embedding Kit and fixed with the Leica Histoiresin mounting medium (Leica Microsystems, Bensheim, Germany). Finally, the embedded samples were cut into thin sections (4 μ m) with a Leica RM2255 microtome equipped with a tungsten carbide edge knife (16 cm, profile d) and transferred to thin slides. Next, samples were stained with lugol to visualize starch and with light green to visualize proteins. Lugol's solution was prepared by dissolving 0.33 g I₂ and 0.68 g KI in 100 mL water. Light green solution was prepared by dissolving 0.10 g of Light green SF Yellowish in 100 mL of water. Afterwards,

the samples were rinsed with water and dried overnight. Multiple microscopic picture were taken of every sample with a Nikon ECLIPSE 80i Epifluorescence Microscope (Nikon Inc., Melville, New York, USA) equipped with a Nikon Digital Sight DS-U2 camera and an Intensilight C-HGFI lamp (Nikon).

The structure of endosperm depleted bran was also visualized with cryo-scanning electron microscopy (cryo-SEM). The endosperm depleted bran samples were frozen under vacuum with liquid nitrogen and transferred to a PP3000T cryo-SEM preparation system (Quorum Technologies, East Sussex, UK) where they were sublimated for 15 minutes at -90 °C. Next, the samples were coated with platinum and visualized using a JSM-7100 F TTLS TFEG-SEM (Jeol Europe, Zaventem, Belgium).

2.5 Particle size reduction and analysis

Endosperm depleted bran samples were milled to the same average particle size with a Cyclotec 1093 Sample mill (FOSS, Högenäs, Sweden). The particle size distribution of the different bran samples was measured in duplicate with a laser diffraction Coulter LS320 particle size analyzer equipped with the wet module (Coulter Corporation, Miami, USA). Bran (50 mg) was suspended in water (20 mL) and sonicated for 1 min (20 kHz, amplitude 40%) and subsequently measured in the laser diffractor.

2.6 Determination of bran water binding, extractability and extract viscosity

2.6.1 Water retention capacity and water extractability

The water retention capacity and extractability determination procedures were adapted from the procedure described by Jacobs, Hemdane et al. (2015) and was based on AACC International method 56.11.02 (AACC International). Water (30.0 mL) was added to bran (1.0 g) and shaken (150 rpm) for 30 min at 7 °C. Next, the samples were centrifuged (4000 g, 10 minutes, 7°C) after which the supernatant was removed. The residue was weighed and the water retention capacity was calculated as the amount of water held by the bran material (dry matter, dm). To determine the extractability, the pellet was overnight dried in the oven (90 °C) and the extractability was calculated as follows:

$$217 \quad \text{Extractability (\% dm)} = \frac{\text{Mass}_{\text{initial}}(g) - \text{Mass}_{\text{after drying}}(g)}{\text{Mass}_{\text{Initial}}(g)} * 100$$

218 *2.6.2 Strong water retention capacity*

219 The strong water retention capacity measures the amount of water that can be bound by bran when
 220 an external force is applied and when bran and water are not in contact anymore after application of
 221 the external force. A QIAprep Spin Miniprep Column (Qiagen, Hilden, Germany) consisting of an
 222 Eppendorf tube at the lower part and a filter at the upper part was used. Bran (50.0 mg) was weighed
 223 in the upper part of the column and water (700 µL) was added. After 60 min of hydration,
 224 centrifugation (10 min, 20 °C) was performed at 4000 g for endosperm depleted wheat, rye and maize
 225 bran and at 10 000 g for endosperm depleted oat bran. A higher speed was needed for oat bran as this
 226 sample displayed a high extract viscosity and water could not pass through the filter at 4000 g. After
 227 centrifugation, the solvent was unable to make contact with the bran, as it is located in the lower part
 228 of the column. The strong water retention capacity was afterwards calculated as the amount of solvent
 229 that was still present in the bran material after centrifugation. Three columns without bran were
 230 included in the procedure to correct for the amount of water that was taken up by the filter.

231 *2.6.3 Extract viscosity*

232 An extract of bran was made (4.0 g in 30.0 mL of deionized water) by shaking (150 rpm, 30 min, 7°C),
 233 centrifugation (10 min, 4000 g, 7 °C) and decantation. The supernatant was used to measure the
 234 extract viscosity with the Brookfield DV-III Ultra Viscometer (Brookfields Ametek, Middleboro,
 235 Massachusetts, USA). A sample volume of 6.7 mL was transferred to the sample holder and the spindle
 236 was rotated at 250 rpm. Viscosity was measured every 15 s over a time range of 3 min. For WB^{ED}, RB^{ED}
 237 and MB^{ED}, the SC4-18 cylindrical spindle (Brookfield) was used to measure extract viscosities. For OB^{ED}
 238 the SC4-34 cylindrical spindle (Brookfield) was used as the viscosity was too high to be measures with
 239 the SC4-18 spindle.

2.6 Analysis of bran *in vitro* fermentation by colon microbiota

Fecal samples of seven healthy volunteers (no history of antibiotics use for at least 6 months) were collected and used to make a pooled fecal slurry (10% w/v) with phosphate-buffered saline (pH 7.3). The suspension was shaken thoroughly, decanted and flushed with nitrogen gas to remove oxygen. For every endosperm depleted bran sample, an amount of sample containing 100 mg dm of dietary fiber was weighed by accounting for starch, protein, lipid and ash content. Fecal slurry (25.0 mL) was added to the bran as described by De Preter, Falcony et al. (2010). Afterwards the samples were flushed with nitrogen gas and the tubes were closed to exclude oxygen. The samples were incubated at 37 °C for 2, 4, 6, 8 and 24 hours. A blank, without addition of bran, was included in the experimental set-up to correct for short chain fatty acid (SCFA) production from fermentable components in the fecal slurry. At every time point, the pH of the fecal slurry was measured with a pH meter (Hanna Instruments HI 9025, Woonsocket, USA). SCFA (acetate, propionate, butyrate, isobutyrate and isovalerate) concentrations were measured by addition of 200 µl of the fecal slurry with/without added bran to a test tube and subsequent addition of (i) 10 µl of 0.016% 2-ethylbutyric acid (v/v, internal standard), (ii) 300 µl of 25% NaCl, (iii) 200 µl 9.38 M sulfuric acid and (iv) 800 µl diethyl ether. Also a blank (in triplicate), consisting of phosphate buffered saline, and a calibration solution (in triplicate), consisting of 140 µl buffer and 60 µl volatile free acid mix (429.0 µL acetate, 149.6 µL propionate, 91.4 µL butyrate, 92.8 µL isobutyrate, 69.0 µL isovalerate and 78.9 µL 2-ethylbutyrate diluted to 500 mL with MilliQ-water), were included in the analysis. The test tubes were closed, rotated for 2 minutes and centrifuged (2000g, 5 minutes, 20 °C). The diethyl phase, in which the SCFA are present, was analyzed with GC using a flame ionization detector. Therefore, a capillary FFA packed column [EC-1000 Econo-Cap (Alltech Laarne, Belgium), 25 m x 0.53 mm, film thickness 1.2 µm] was used, with Helium (flow rate 20 ml/min) the carrier gas. The columns, the injector and the flame ionization detector temperatures reached 130 °C, 195 °C and 195 °C, respectively. Moreover, the residual fecal slurry with/without added bran was lyophilized at every time point and analyzed for fructan and (1,3:1,4)-β-glucan content. Measurement of WE-AX and TOT-AX as a function of fermentation time was not

possible in a correct way due to the presence of AX in the fecal slurry. This resulted in an inaccurate measurement.

3. Results and discussion

3.1 Bran endosperm removal

The chemical composition of native WB, RB, OB and MB was measured and is summarized in Table 1. Large differences in chemical composition, and especially in starch content, can be observed. Results for oat bran starch content are comparable with results found in literature (Doehlert and Moore 1997; Luhalo et al. 1998). For maize bran, the reported chemical composition in literature is dependent on the milling procedure, as dry and wet milling procedures exist (Rose et al. 2010). A starch content between 2 and 40 % has been reported (Ebringerova and Hromadkova 2002; Hespell 1998; Rose et al. 2010). It has to be remarked that starch values for maize bran in this article are high compared to most articles in literature. This shows the high variability in native maize bran composition and the need to remove the endosperm to do a side-by side comparison. Endosperm remnants attached to the native WB, RB, OB and MB were to large extent removed using an endosperm removal procedure. The starch content of bran, used as a marker for residual endosperm (Hemery et al. 2009), decreased from 12% to 1% for WB, 40% to 6% for RB, from 54% to 12% for OB and from 65% to 4% for MB. The need for different bran endosperm removal procedures can be attributed to the difference in adhesion between the endosperm and the bran layer. The extent of endosperm removal was evaluated by determining total bran yield (see table S1) after endosperm removal, as well as characterization of the discarded pellet during the wet endosperm removal procedure (see table S2). The starch content of the pellet for the different bran samples varied between 62% dm and 76% dm, the protein content between 6 and 17% dm and the residual fraction between 12 and 21% dm. As flour has a comparable composition, it was concluded that mostly endosperm material was removed during the procedure. The proposed endosperm removal procedures therefore seemed well adapted to reduce the content of attached endosperm remnants to bran with limited removal of intrinsic bran components. The same

conclusions were drawn with light microscopy. Only small amounts of starch were still visible after the endosperm removal procedure (see figure S1). Finally, bran average particle size and particle size distribution were equalized using a Cyclotec mill (D_{50} between 750 μm and 900 μm) to exclude the effect of particle size on the characterization experiments. This resulted in the endosperm depleted samples (WB^{ED} , RB^{ED} , OB^{ED} and MB^{ED}).

It should be noted that also water extractable compounds derived from the endosperm will be present in the washing water and will thus become part of the endosperm depleted bran using this procedure. The error introduced in the estimate of the composition of bran will be small because (i) a dry endosperm removal procedure is used in a first instance to remove endosperm, including the extractable compounds and (ii) the amount of water-extractable compounds in cereal endosperm is low compared to the amount of water-extractable material in bran. Moreover, it should also be noted that the not characterized fraction increased, in part due to the increased concentration of lignin. Finally, as water is used during the bran endosperm removal procedure, enzymes can for a short time act on their substrates during the procedure itself. This will be further discussed in section 3.3, where the effect of phytase activity on phytate content is discussed. In spite of these disadvantages, we believe this procedure presents an added value to the side-by-side comparison of the physicochemical characteristics of cereal brans. The procedure also allows us to perform an *in vitro* fermentation experiment without doing an *in vitro* digestion step, which is important for comparison of the *in vitro* fermentation characteristics of bran of different cereals.

3.2 Determination of the structure of bran

The structure of WB^{ED} , RB^{ED} , OB^{ED} and MB^{ED} is visualized in Figure 1. For WB^{ED} and RB^{ED} , comparable structures were observed. In both bran samples, a distinct porous pericarp layer ($\pm 30\text{-}40\ \mu\text{m}$) and aleurone layer ($\pm 50\ \mu\text{m}$) were seen. OB^{ED} , on the other hand, contains a thin pericarp layer of $\pm 10\ \mu\text{m}$ with a thick single, double or triple aleurone layer underneath (80-100 μm), as visualised on the cryo-SEM and light microscopy pictures. MB^{ED} is characterized by a completely different structure,

containing a porous pericarp layer with a cross section of 100 to 250 μm . The thickness of this layer depends on the location in the kernel where the sample is taken from. Bran originating from the attachment between plant and kernel is thinner as bran is more squeezed within the corn cob. Moreover, no aleurone layer was seen on the microscopy pictures of MB^{ED}, although maize contains a single aleurone layer (Delcour and Hoskeney 2010). It was seen with light microscopy that the aleurone layer was already detached from maize bran during industrial milling and was thus not part of the endosperm depleted bran. This shows that the adhesion between aleurone and pericarp is weak for maize, in comparison to the other cereals. As the detachment occurred during dry industrial milling, it can be assumed that detachment would also occur during wet milling. The endosperm removal procedure thus allows a good estimation of the chemical composition of maize bran both after dry and wet milling.

3.3 Comparison of bran chemical composition

As the endosperm mainly consists of starch, but also contains proteins, AX and other compounds, expressing the composition of native bran on starch free basis would lead to incorrect estimations. Moreover, the higher the amount of residual endosperm, the larger the error. If the endosperm is completely removed or residual endosperm content is low, expressing the composition on starch free basis only leads to a small error. Therefore, endosperm was removed as much as possible and the composition of WB^{ED}, RB^{ED}, OB^{ED} and MB^{ED} was only then expressed on starch free basis (Table 2). This allows a side-by-side comparison of the chemical composition of bran of different cereals. Wheat bran is already extensively studied in literature and will be used here as bench mark for comparison. RB^{ED} contains more than double the amount of the soluble polysaccharides WE-AX (1.4 % dm) and fructan (8.0% dm), and less WU-AX (27.7% dm) and cellulose (5.4 % dm) compared to WB^{ED}. Moreover, it contains double the amount of (1,3:1,4)- β -glucan (4.9% dm) than WB^{ED} (2.4% dm), but comparable amounts of ash (6.9% dm), proteins (21.3% dm) and lipids (5.8 % dm). OB^{ED} on the other hand, contains a large amount of β -glucan (17.0% dm) and low amounts of cellulose and AX (2.9% dm and 13.2% dm, respectively) compared to WB^{ED}, RB^{ED} and MB^{ED}. Moreover, OB^{ED} contains no to a very limited amount

of fructan (0.3% dm) and has a high content of lipids (14.0% dm). MB^{ED} contains high amounts of water-unextractable dietary fiber, i.e. WU-AX (42.7% dm) and cellulose (19.6% dm) compared to WB^{ED} (34.7% dm and 10.9% dm, respectively). MB^{ED} contains low amounts of ash (1.1% dm) and lipids (2.0% dm) compared to the other bran samples. It is known that 80% of the minerals and 85% of lipids in maize are present in the germ (Nuss and Tanumihardjo 2010) that is removed during industrial milling. This explains the low mineral and lipid content in MB^{ED} compared to the other bran samples, where minerals and lipids are mainly located in the aleurone layer (Xiong et al. 2013). Based on the composition of native maize bran and endosperm depleted bran, it can be assumed that minerals are distributed equally in the pericarp layer, aleurone layer and endosperm, as only minimal changes in mineral content were seen after the endosperm removal procedure. Moreover, AX and cellulose content is increased more than proportional with the removal of starch due to the increased concentration of the pericarp fraction.

For the elemental composition (Table 3), it was seen that the most abundant element in WB^{ED}, RB^{ED} and MB^{ED} was potassium (16070 ppm, 19411 ppm and 4051 ppm, respectively), compared to phosphorous in OB^{ED} (16337 ppm). In general, MB^{ED} contained the lowest ash content and thus the lowest concentration of all different elements in comparison with the other bran samples. Moreover, no calcium or iron were present in the MB^{ED}. Manganese was more present in WB^{ED} and RB^{ED} (128 ppm and 152 ppm, respectively) compared to the OB^{ED} (84.5 ppm) and MB^{ED} (14.5 ppm). Finally, iron was only present in low amounts for RB^{ED} (2.03 ppm) compared to WB^{ED} (49.1 ppm) and OB^{ED} (44.3 ppm).

Phytate (inositol hexaphosphate) levels were low in MB^{ED} (0.2% dm) in comparison to WB^{ED} (4.1% dm), RB^{ED} (1.2% dm) and OB^{ED} (2.4% dm) due to removal of the phytate rich germ during industrial milling. As phytate is present in the aleurone layer in wheat, rye and oat bran, the higher phytate content can be explained (Reddy 2001). Furthermore, adding water during the endosperm removal procedure resulted in partial degradation of phytate for WB and RB due to phytase activity. This is shown by the ratio of free to total P, that increased for the wheat bran sample from 0.09 (± 0.01) to 0.27 (± 0.01), and

for the rye bran sample from 0.16 (± 0.01) to 0.54 (± 0.01). Also the determination of the phytase activity supports this conclusion, as the phytase activity was 329 PU in WB^{ED} and 1523 PU in RB^{ED}. The presence of phytase activity can result in phytate breakdown during the production process of end product e.g. bread or cookies and thus can increase the mineral bio-accessibility. As the phytase activity is the highest in RB^{ED}, this phenomena will be most dominant for this bran sample. On the other hand, no phytate degradation was seen for OB^{ED} during endosperm removal due to kilning prior to milling. Kilning, a high temperature steam treatment, is performed as oat contains a high amount of unsaturated fatty acids as well as high levels of lipid-digesting enzymes, which could lead to rancidity (Decker et al. 2014). No enzyme activity was thus present in OB^{ED}. Nevertheless, OB^{ED} contains lower amounts of phytate and comparable amounts of minerals than WB^{ED} and RB^{ED}. Thus, it can be hypothesized that the mineral bio-accessibility in OB^{ED} will be higher before processing. Furthermore, MB^{ED} contains low amounts of phytate and low amounts of minerals, so maize bran is a less interesting source of minerals compared to the other bran samples.

It can be concluded that removing the endosperm prior to characterization allows a more correct side-by-side comparison of the chemical composition of bran of different cereals. In this context, there are large differences in chemical composition and structure between the WB^{ED}, RB^{ED}, OB^{ED} and MB^{ED} which can lead to different bran-water interactions.

3.4 Water binding, extractability and extract viscosity

Water binding, water extractability and the aqueous extract viscosity of the bran samples were determined to compare the interactions between bran and water of endosperm depleted bran of different origins.

Jacobs et al. (2015) hypothesized that water can be bound strongly in nanopores and due to hydrogen bridges, while water can also be bound in micropores and through stacking effects (weakly bound water). The sum of the strongly and weakly bound water is the total bound water. Large differences exist in the water binding capacities between WB^{ED}, RB^{ED}, OB^{ED} and MB^{ED} (Figure 2). The porous

pericarp structure of WB^{ED} and RB^{ED} and the flake like structure of both bran types leading to stacking effects, were visualized using microscopic techniques and could explain the high total water binding capacity of WB^{ED} and RB^{ED}. Moreover, WB^{ED} and RB^{ED} have comparable water binding capacities, possibly due to their comparable structure. OB^{ED} has the highest total water binding capacity (5.99 g/g dm) and strong water binding capacity (1.48 g/g dm). MB^{ED} has the lowest total water binding capacity (3.58 g/g dm) of the four bran types, but a higher strong water binding capacity (0.89 g/g dm) than WB^{ED} (0.78 g/g dm) and RB^{ED} (0.71 g/g dm). As MB^{ED} contains 19% dm cellulose and OB^{ED} contains 17% dm β -glucan, both known for their ability to form hydrogen bounds with water (Deepak 2017; Himmel et al. 2007; Zhao et al. 2012), the higher amount of strongly bound water can be explained. For MB^{ED} it was seen that the micropores are larger compared to WB^{ED}, possibly leading to less water retention in the micropores and thus a lower total water binding capacity.

Looking at the extractability and extract viscosity (Table 4), the higher amount of soluble polysaccharides WE-AX and fructan in RB^{ED} compared to WB^{ED} is in agreement with the extractability, as the extractability of the RB^{ED} (34.5% dm) is higher compared to WB^{ED} (18.7% dm). OB^{ED} had a comparable extractability as RB^{ED}, which is in agreement with the results of Karppinen et al. (2000). Moreover, it was seen that MB^{ED} had the lowest extractability (4.3% dm). This is in agreement with the chemical composition, as MB^{ED} contains very low amounts of WE-AX and fructan.

RB^{ED} displayed a higher extract viscosity than WB^{ED} and MB^{ED}. The higher WE-AX content in RB^{ED} can be partly responsible for this, as it is known that this component induces viscosity in solution (Courtin and Delcour 2002). The extract viscosity of OB^{ED} was much higher compared to the other cereal brans. This can be ascribed to the presence of high amounts of β -glucan, which is known for its high viscosity inducing capacity (Lazaridou and Biliaderis 2007). Apart from the concentration, the viscosity of aqueous WE-AX and β -glucan suspensions also depends on the molecular weight of these components and their degree and pattern of substitution (Karppinen et al. 2000; Lazaridou and Biliaderis 2007).

It can be concluded that the interaction of bran with water, the extractability and the extract viscosity is grain dependent and dependent on the bran structure and the chemical composition.

3.5 Determination of the *in vitro* fermentation characteristics of bran

In most *in vitro* fermentation experiments in literature, e.g. in Karpinnen et al. (2000), predigestion is performed to remove digestible compounds (Minekus et al. 2014). In this way, interference of starch as fast fermentable substrate is eliminated (Christl et al. 1995). Nevertheless, during this procedure also components with low molecular weight like fructans (Karpinnen et al. 2003) and maybe part of WE-AX are removed. Using no predigestion can also influence the results as Lebet, Arrigoni et al. (1998) showed that large amounts of starch during *in vitro* fermentation can mask the fermentation patterns of dietary fiber. The approach followed in this study was the removal of the endosperm fraction without the loss of small dietary fiber components. In this way, a more correct side-by-side comparison of the *in vitro* fermentation characteristics can be performed. pH, SCFA production and consumption of β -glucan and fructan of WB^{ED}, RB^{ED}, OB^{ED} and MB^{ED} were measured as a function of fermentation time. A blank sample with no addition of bran was also included in the analysis. It was not possible to measure TOT-AX and WE-AX as a function of time during *in vitro* fermentation due to large quantities of these components in the fecal slurry. This resulted in large standard deviations which did not allow accurate measurements.

All sample were fermented to some extent by the colon microbiota during 24 hours of *in vitro* fermentation, and SCFA were produced (Figure 3). The fermentation rate and fermentation degree differed among the bran samples. In the first two hours of fermentation, the pH dropped the most for RB^{ED} (5.3 ± 0.0) and OB^{ED} (5.2 ± 0.0) compared to WB^{ED} (6.0 ± 0.0) and MB^{ED} (6.4 ± 0.0). Comparable trends were seen for the SCFA production after 2 hours of fermentation (18.8 mmol/l for RB^{ED} and 19.1 mmol/l for OB^{ED} versus 15.7 mmol/l for WB^{ED} and 11.8 mmol/l for MB^{ED}). After 24 hours of fermentation, RB^{ED} and OB^{ED} had a comparable pH (5.2 and 5.1, respectively) and SCFA concentration (44.0 ± 0.1 mmol/l and 39.5 ± 1.6 mmol/l, respectively). The fermentation degree at 24 hours of

441 fermentation of WB^{ED} was lower compared to RB^{ED} and OB^{ED}, but was higher than MB^{ED}. The pH and
442 SCFA concentration of WB^{ED} at that moment were 5.5 (± 0.1) and 33.3 (± 2.6) mmol/l, compared to 5.8
443 (± 0.0) and 23.8 (± 2.8) mmol/l for MB^{ED}. Moreover, after 6 hours of fermentation of MB^{ED}, there is no
444 difference in SCFA production and only a small difference in pH with the blank. This suggests depletion
445 of fermentable carbohydrates after 6 hours. Comparing the fermentation results to literature,
446 Karpinnen et al. (2000, 2003) found that oat bran was better fermentable compared to rye bran.
447 Differences with this study could possibly be explained by the digestion procedure used in literature.
448 The removal of fructan and part of WE-AX in rye bran during predigestion could lead to an
449 underestimation of the fermentation rate and degree. The low fermentability of maize bran is
450 consistent with results in literature (Hernot et al. 2008).

451 The percentage of branched SCFA (isovaleric acid and isobutyric acid) on total SCFA (Table 5) can be
452 used as a marker for protein fermentation, as they are metabolic end products of the protein
453 fermentation. When good fermentable carbohydrate sources are depleted, some microorganisms can
454 switch to protein fermentation to meet their energy needs (Macfarlane et al. 1992). In literature,
455 protein fermentation is linked to the production of toxic products like phenols and is therefore an
456 unwanted phenomenon (Hughes et al. 2000). It was seen that addition of bran, independent of the
457 type, always resulted in lower percentages of branched SCFA on total SCFA compared to the blank.
458 Moreover, after 2 hours of fermentation, RB^{ED} and WB^{ED} showed comparable percentages of branched
459 SCFA on total SCFA (3.2% and 3.1%, respectively). This percentage increased to 3.7% for RB^{ED} and to
460 5.0% for WB^{ED} after 24 hours of fermentation. OB^{ED} had a low percentage of branched SCFA on total
461 SCFA at 2 hours of fermentation (1.7%) and this only increased to 2.5% after 24 hours of fermentation.
462 The percentage of branched on total SCFA for MB^{ED} was comparable to WB^{ED} and also increased as a
463 function of time. After 2 hours of fermentation, 3.2% of the produced SCFA were branched for MB^{ED}.
464 After 24 hours this amount increased to 4.8%.

The differences in fermentation characteristics by colon microbiota between the different bran samples can be (partly) attributed to the differences in highly fermentable dietary fibers and the fermentation thereof. Therefore, the fermentation of β -glucan (Figure 4) as well as fructan (results not shown) was measured as a function of fermentation time. For OB^{ED}, 7.11 g β -glucan per 100 g slurry (dm) was metabolized after 2 hours, leading to a 56% decrease in β -glucan content. Between 2 and 4 hours, 3.65 g β -glucan per 100 g slurry (dm) was metabolized and this value decreased to 0.38 (between 4 and 6 hours) and 0.31 (between 6 and 8 hours), until all β -glucan was fermented after 24 hours. It was moreover seen that the protein fermentation in OB^{ED} increased when the fermentable β -glucan substrate was depleted. For WB^{ED} and RB^{ED}, initial β -glucan content was lower compared to OB^{ED}. After 2 hours, 1.1 and 2.1 g β -glucan per 100 g slurry (dm) was fermented for WB^{ED} and RB^{ED}, decreasing the initial β -glucan content with 63% and 60%, respectively. Also for these samples, the amount of metabolized β -glucan decreased as a function of time until after 24 hours, all β -glucan was metabolized. For MB^{ED}, the initial β -glucan content was very low, and was already completely metabolized after 2 hours. Moreover, no β -glucan was detected in the fecal slurry (blank). The differences in β -glucan metabolization rate can be due to the characteristics and accessibility of the β -glucan structure within the different bran samples, as described by Wood et al. (2002).

Also fructan content was measured as a function of fermentation time (results not shown). After 2 hours, all fructan was depleted in all bran samples. The initial high fructan concentration of RB^{ED} (8.0%) explains the fast pH drop between 0 and 2 hours of fermentation. These results show that fructan is fermented fast.

When trying to explain the observed differences in fermentation characteristics between the different bran samples, the following observations can be made. RB^{ED} and OB^{ED} contained the highest levels of seemingly readily available dietary fiber - WE-AX, fructan and β -glucan - which resulted in the highest degree and rate of fermentation. This led to a lower pH and higher SCFA production already after 2 hours of fermentation by colon microbiota than what was observed for WB^{ED}. This also explains the

positive correlation between extractability and fermentability that was seen in this study and also by Guillon and Champ (2000). MB^{ED} mainly consists of unextractable dietary fibre, like cellulose and WU-AX, known to be only fermentable to a limited extend (Cummings 1984). Therefore, the lower fermentability for MB^{ED} compared to the other bran samples can in part be explained by the chemical composition.

One can wonder whether cereal bran structure and water-binding as a rough measure of accessibility could also be able to partially explain the *in vitro* fermentation results. The high water holding capacity of OB^{ED} could indicate that it is well accessible for microbiota, and hence, is a good “dinner table” that can induce efficient fermentation. On top of composition, the accessibility could in part explain the high short chain fatty acid production for OB^{ED}. MB^{ED}, on the other hand, had a low total water retention capacity compared to the other cereal brans. Therefore it could be hypothesized that MB^{ED} is a less hospitable “dinner table” for the microbiota, leading to a less efficient fermentation. Nevertheless, based on the comparable structure and water retention capacities of WB^{ED} and RB^{ED}, mainly the difference in chemical composition can be expected to be the main driver for the differences in *in vitro* fermentation characteristics. As far as viscosity of the fermentation medium is concerned, it was seen by Nordlund et al. (2012) that high viscosities can lead to a delayed fermentation. This was not observed in this study. The high viscosity induced by β -glucan in OB^{ED} did not seem to hinder the fermentation process, as OB^{ED} was fermented the fastest.

Overall, the *in vitro* fermentation characteristics in this study mainly reflect the chemical composition and to a lesser extent possibly the accessibility of the structure of the different cereal bran types we investigated. A high amount of WE-AX, fructan and β -glucan, good fermentable dietary fibers and a highly accessible structure lead to a fast and high short chain fatty acid production.

4. Conclusion

In this study, removal of endosperm as confounding factor when studying bran properties allowed to obtain more accurate estimates of the composition, structure and some physicochemical

characteristics of bran of wheat, rye, oat and maize. It allowed a side-by-side comparison of these bran samples. Differences in each of the studied aspects were observed. The much higher extract viscosity of OB^{ED} compared to the other bran samples, related to the high (1,3:1,4)- β -glucan in OB^{ED}, was notable. Incorporation of oat bran in food products can possibly increase the viscosity in the gastrointestinal tract and reduce the blood glucose peak height after a meal and induce a faster and longer feeling of satiety (Natarajan and Pluznick 2014). OB^{ED} also displayed the highest water binding capacity, which can possibly lead to a normalized transit time and increased fecal bulk, which is linked to positive health effects (Monro 2001). Nevertheless, clinical studies are needed to confirm this. OB^{ED} contained a high mineral content and low levels of phytate, suggesting a high mineral bio-accessibility. WB^{ED} and RB^{ED} contained comparable amounts of minerals than the OB^{ED}, but higher amounts of phytate. Nevertheless, phytase activity was detected in WB^{ED} and RB^{ED} which can lead to the degradation of phytate during the production process of end products that contain these bran samples as ingredient.

The endosperm removal procedure also allowed a side-by-side comparison of the *in vitro* fermentation characteristics without performing a predigestion procedure, in which soluble dietary fiber components would have been removed. Also *in vitro* fermentation characteristics of the samples were clearly grain dependent. RB^{ED} and OB^{ED} showed the highest fermentation rate and -degree and thus yielded high concentrations of SCFA. Addition of oat bran and rye bran to food products can lead to a high SCFA production to which different positive health effects are attributed (Dalile et al. 2018). Moreover, less protein fermentation was observed for these samples, which can be positive for health as protein fermentation is linked to the production of toxic compounds (Hughes et al. 2000). The high fermentability of these cereal brans was linked to a high amount of WE-AX, fructan and β -glucan, good fermentable dietary fibers, and an accessible structure that as “dinner table” promotes efficient fermentation.

The differences in properties between the different bran samples suggest that they can be implemented in end products in a targeted way, depending on technofunctional implications and

targeted physiological effects and nutritional impact. Clinical studies should be performed to understand whether these differences in physicochemical characteristics and *in vitro* fermentation characteristics also lead to differences in physiological effects in humans.

Acknowledgements

This study was part of the SBO BRANDING project (grant no. 130028). We gratefully acknowledge the “Fonds voor Wetenschappelijk Onderzoek Vlaanderen” (FWO, Brussels, Belgium) for the financial support. Furthermore, we also acknowledge the Hercules foundation for its financial support in the acquisition of the scanning electron microscope JEOL JSM-7100F equipped with the cryo-transfer system Quorum PP3010T (grant no. AUGÉ-09-029) used in this research.

Conflict of interest

The authors declare that they do not have any conflict of interest

579 Figure 1: Cryo-SEM pictures (left) and light microscopy pictures (right), where starch is stained with
580 lugol solution (purple) and proteins are stained with light green of endosperm depleted bran of (A)
581 wheat, (B) rye, (C) oat and (D) maize. P=Pericarp, A= aleurone layer

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Table 1: Chemical composition of native bran of wheat, rye, oat and maize.

Standard deviations are indicated between brackets. The data represent means of a triplicate measurement starting from one batch of bran. Abbreviations: AX, arabinoxylan; WE-AX, water-extractable arabinoxylan; dm, dry matter.

	Wheat bran	Rye bran	Oat bran	Maize bran
Ash (% dm)	6.4 (0.1)	4.6 (0.0)	2.8 (0.1)	1.0 (0.0)
Proteins (% dm)	20.9 (0.3)	18.1 (0.2)	15.3 (0.3)	6.2 (0.2)
Starch (% dm)	11.8 (0.4)	40.3 (1.0)	53.6 (0.7)	64.7 (2.6)
AX (% dm)	25.0 (1.6)	17.8 (0.3)	3.5 (0.0)	10.7 (0.8)
WE-AX	0.6 (0.0)	1.1 (0.1)	0.1 (0.0)	0.2 (0.0)
β-glucan (% dm)	2.1 (0.0)	2.9 (0.0)	5.4 (0.1)	0.1 (0.0)
Fructan (% dm)	3.3 (0.1)	5.3 (0.1)	0.3 (0.1)	0.2 (0.0)
Phytate (% dm)	4.3 (0.1)	1.7 (0.1)	1.3 (0.0)	0.1 (0.0)
Cellulose	9.1 (0.6)	2.6 (0.9)	1.4 (0.7)	7.2 (0.9)
Lipids (% dm)	5.4 (0.4)	3.5 (0.3)	11.8 (0.1)	1.6 (0.2)
Free	3.3 (0.2)	1.1 (0.2)	9.8 (0.1)	1.1 (0.2)
Bound	2.1 (0.4)	2.4 (0.2)	2.0 (0.1)	0.5 (0.1)
Residue (% dm)	11.6. (1.8)	3.2 (1.4)	4.6 (1.0)	8.2 (2.8)

Table 2: Chemical composition of endosperm depleted bran of wheat, rye, oat and maize expressed on a starch free basis.

Standard deviations are indicated between brackets. The data represent means of triplicate measurements starting from one batch of bran. Abbreviations: AX, arabinoxylan; WE-AX, water-extractable arabinoxylan, dm, dry matter. 1% dm of starch was present in endosperm depleted (ED) wheat bran, 6% dm in ED rye bran, 12% dm in ED oat bran and 4% dm in ED maize bran.

	Wheat bran ^{ED}	Rye bran ^{ED}	Oat bran ^{ED}	Maize bran ^{ED}
Ash (% dm)	7.4 (0.1)	6.9 (0.1)	6.8 (0.1)	1.1 (0.0)
Proteins (% dm)	17.6 (0.1)	21.3 (0.2)	22.1 (0.2)	7.5 (0.2)
AX (% dm)	35.4 (2.5)	29.1 (1.8)	13.2 (0.7)	42.7 (2.1)
WE-AX	0.7 (0.0)	1.4 (0.0)	0.6 (0.0)	0.4 (0.0)
β-glucan (% dm)	2.4 (0.0)	4.9 (0.1)	17.0 (0.1)	0.1 (0.0)
Fructan (% dm)	3.4 (.0)	8.0 (0.1)	0.3 (0.1)	0.3 (0.1)
Phytate (% dm)	4.1 (0.1)	1.3 (0.1)	2.7 (0.2)	0.2 (0.1)
Cellulose	10.9 (0.3)	5.4 (0.6)	2.9 (0.8)	19.6 (0.5)
Lipids (% dm)	6.3 (0.2)	5.8 (0.4)	14.0 (0.4)	2.0 (0.2)
Free	3.4 (0.2)	2.3 (0.1)	10.6 (0.3)	1.3 (0.1)
Bound	2.9 (0.1)	3.5 (0.4)	3.4 (0.2)	0.7 (0.2)
Residue (% dm)	12.5 (2.5)	17.3 (0.8)	21.0 (1.4)	26.5 (2.4)

Table 3: Concentrations of different elements in endosperm depleted (ED) wheat, rye, oat and maize bran, expressed on a starch free basis. Results are expressed in parts per million (ppm). Standard deviations are indicated between brackets. The data represent means of a triplicate measurements starting from one batch of bran. Abbreviation: n.d.: not detectable.

Element (ppm)	Wheat bran ^{ED}	Rye bran ^{ED}	Oat bran ^{ED}	Maize bran ^{ED}
Mg	6587 (±39)	3981 (±75)	5736 (±292)	540 (±5)
P	15303 (±148)	13256 (±119)	16337 (±396)	791 (±38)
K	16070 (± 136)	19411 (±588)	14906 (±854)	4051 (±100)
Ca	814 (±26)	1038 (±31)	2489 (±31)	n.d.
Mn	128 (±0)	152 (±2)	84.5 (±1)	14.5 (±0)
Fe	49.1 (±9.0)	2.03 (±3.1)	44.3 (±13.5)	n.d.
Cu	11.7 (±0.6)	10.6 (±0.2)	7.99 (±0.1)	2.28 (±0.3)
Zn	97.2 (±0.5)	121 (±1.6)	73.9 (±4.5)	18.9 (±0.9)

Figure 2: Total water binding capacity (left) and strong water binding capacity (right) of endosperm depleted (ED) wheat, rye, oat and maize bran, expressed as g/g dm. Standard deviations are indicated with bars. The data represent means of triplicate measurements starting from one batch of bran.

Table 4: Extractability (% dm) of an extract prepared by extracting 1 gram of bran in 30 mL of water, and extract viscosity (mPa*s) of an extract prepared by extracting 4 gram of bran in 30 mL of water of endosperm depleted (ED) wheat, rye, oat and maize bran

Standard deviations are indicated between brackets. The data represent means of triplicate measurements starting from one batch of bran.

	Extractability (% dm)	Viscosity (mPa*s)
Wheat bran^{ED}	18.7 (0.8)	1.33 (0.01)
Rye bran^{ED}	34.5 (1.9)	7.33 (0.04)
Oat bran^{ED}	30.7 (1.4)	976.81 (6.39)
Maize bran^{ED}	4.3 (0.6)	1.36 (0.03)

Figure 3: (A) pH and (B) concentration of short chain fatty acids (sum of acetate, propionate, butyrate, isobutyrate, isovalerate; mmol/l), as a function of time obtained during an *in vitro* fermentation experiment in a 10% fecal slurry of a blank sample (no bran addition), and of endosperm depleted (ED) wheat, rye, oats and maize bran.

Table 5: Amount (%) of branched short chain fatty acids (isovalerate acid and isobutyrate) relative to the total amount of short chain fatty acids (acetate, propionate, butyrate, isovalerate and isobutyrate) obtained during an *in vitro* fermentation experiment in a 10% fecal slurry of a blank sample (no bran addition), and endosperm depleted (ED) wheat, rye, oat and maize bran.

Fermentation time (h)	Blank	Wheat bran ^{ED}	Rye bran ^{ED}	Oat bran ^{ED}	Maize bran ^{ED}
2	4.2 (±0.4)	3.2 (±0.2)	3.1 (±0.2)	1.7 (±0.1)	3.6 (±0.2)
4	4.9 (±0.2)	3.0 (±0.2)	2.8 (±0.3)	1.2 (±0.1)	3.2 (±0.3)
6	4.8 (±0.3)	3.2 (±0.4)	3.1 (±0.3)	1.6 (±0.5)	3.5 (±0.3)
8	6.8 (±0.4)	5.1 (±0.2)	3.6 (±0.3)	1.6 (±0.2)	5.6 (±0.4)
24	6.3 (±0.2)	5.0 (±0.3)	3.7 (±0.1)	2.5 (±0.3)	4.8 (±0.4)

Figure 4: β -glucan content (dry matter) of the feces and bran in the beginning of the *in vitro* fermentation experiment and after 2 hours, 4 hours, 6 hours, 8 hours and 24 hours of fermentation of endosperm depleted (ED) wheat, rye, oats and maize bran, expressed in gram β -glucan per 100 gram dry matter of feces and bran.

Supplementary material

Figure S1: Microscopy picture of wheat bran showing (A) native wheat bran sample containing 12% of starch, (B) dry teated wheat bran sample containing 2% of starch and (C) wet treated wheat bran sample containing 1% of starch. Starch is colored with lugol (purple) and proteins are colored light green (green). P=pericarp; A=aleurone; E=endosperm.

650 Table S1: Bran yield (%) and amount of removed fraction (%) after the dry and wet endosperm removal
651 procedure.

	Bran yield (%)	Removed fraction (%)
Wheat bran	57.9	42.1
Rye bran	49.2	50.8
Oat bran	20.1	79.9
Maize bran	40.2	59.8

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657 Table S2: Starch content (% dm), protein content (% dm) and content (% dm) of residual non-
658 characterized fraction of the pellet that is removed after centrifugation during the wet endosperm
659 removal procedure from wheat, rye, oat and maize bran

	Starch (% dm)	Protein (% dm)	Residue (% dm)	660
Wheat bran pellet	62 (±2)	17 (±2)	21 (±3)	661
Rye bran pellet	76 (±0)	13 (±1)	12 (±1)	
Oat bran pellet	73 (±3)	13 (±1)	14 (±3)	662
Maize bran pellet	76 (±1)	6 (±0)	17 (±1)	663

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