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Viscosity of β-glucan from oat products at the intestinal phase of the gastrointestinal model

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ABSTRACT

The physiological functionality of cereal beta-glucan (β-glucan) has been mainly attributed to its ability to form viscous solutions in the gastrointestinal (GI) tract. The viscosity is dependent on the concentration, extractability and molecular weight of β-glucan, and to enable maximal functionality, these factors should therefore be acknowledged and their role in the physiological functionality of cereal β-glucan further studied. An *in vitro* GI simulation with separate oral, gastric and small intestine phases was used to model the state of β-glucan from various oat products in the GI tract. A rather large variation (from 26% to 99%) was observed in the extractabilities between product categories, with the highest extractabilities observed in spoonable products. The viscosities also varied highly within categories. When the comparison was done at similar concentration levels, the highest viscosities were observed in the products produced through dry processes, and moisture content during processing was suggested to be essential to the extent of β-glucan degradation. The viscosity in samples that were likely to exhibit enzymatic activity was shown to be rather low, and thus the physiological functionality of β-glucan may be threatened if the product also contains grain ingredients other than kiln-dried oat. Clear differences were observed in the functionality of β-glucan in the GI tract model depending on a product type, and these were explained by differences in ingredients and processes. However, further studies are needed to specify the influence of each factor and to clarify the factors determining the physiological functionality of β-glucan in food products.

1. Introduction

The attractiveness of oat as a food ingredient has increased, probably because of its high nutritional value and suitability for most persons with celiac disease or gluten sensitivity. Additionally, one of the possible reasons behind the increased interest towards the food-use of oat is the knowledge on the functionality of oat beta-glucan. Intensive research on this topic during the past decades has led to the approval of health claims concerning cereal beta-glucan by both the US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA). The FDA was the first to declare in 1997 that oat soluble fibre, beta-glucan, may reduce the risk of coronary heart disease, and the EFSA approved a similar kind of claim first in 2009 stating the connection between oat beta-glucan and the maintenance of normal blood cholesterol and one year later, in 2010, approving the claim on the reduction of cholesterol through regular consumption of oat beta-glucan (EFSA, 2009, 2010; FDA 1997). Additionally, the EFSA has approved a health claim on the attenuating effect of oat and barley beta-glucan on postprandial blood glucose levels (EFSA, 2011).

Cereal beta-glucan refers to mixed-linkage (1 → 3)(1 → 4)-β-D-glucan (hereafter β-glucan), which is the major non-starch polysaccharide in both oat and barley. The structure consists of cellulosic blocks of β–(1 → 4)-linked glucose units, linked by single β-(1 → 3) linkages, which differentiate its structure from that of cellulose and enable the water solubility of the structure. This is essential for the ability of β-glucan to form viscosity in aqueous solutions.

Mälkki and Virtanen (2001) reviewed different mechanisms of the viscosity of β-glucan affecting nutrient absorption in the small intestine and found that its high viscosity both hinders digestive enzymes from encountering their substrates and decreases the rate of nutrient transportation to surfaces where absorption occurs. The blood glucose-attenuating effect has been quite unquestionably linked to the viscosity of β-glucan in the small intestine, as reviewed by Wood (2010), but for cholesterol-lowering effect the mechanism has not been yet unambiguously explored. Nevertheless, studies on the topic show the possibility of a correlation between the viscosity of β-glucan and the

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extent of reduction of cholesterol levels (EFSA, 2009, 2010; Othman, Moghadasian, & Jones, 2011; Wolever et al., 2010). Othman et al. (2011) reported that cholesterol reduction may occur through the formation of a viscous layer in small intestine hindering the absorption of cholesterol and bile acids. This leads to the excretion of bile acids in the faeces, thus increasing the synthetisation of new bile acids from cholesterol in the liver, which results in lowered serum cholesterol (EFSA, 2009, 2010; Othman et al., 2011). Therefore, it seems quite undisputed that the state of β-glucan in the small intestine is essential for physiological functionality.

The rheological properties of β-glucan are altered by several factors. The solubility of β-glucan is a requirement for its ability to increase viscosity in aqueous systems, and this solubility is affected by structural features, such as the length of the cellulosic blocks and the ratio of cellotriosyl and cellotetraosyl units in the structure (Anttila, Sontag-Strohm, & Salovaara, 2004; Cui & Wood, 2000). However, solubility alone does not ensure the formation of viscosity; the concentration and molecular weight also affect viscosity. The effects of concentration and molecular weight on viscosity are connected, as the critical concentration for viscosity enhancement is increased with a decreasing molecular weight, which means that with a decreasing molecular weight, the amount of molecules needed for the formation of viscosity-causing entanglements is increased (Lazaridou, Biliaderis, & Izydorczyk, 2003; Skendi, Biliaderis, Lazaridou, & Izydorczyk, 2003; Vaikousi, Biliaderis, & Izydorczyk, 2004). From this perspective, in addition to the total β-glucan in products, both the extractability and the molecular weight of β-glucan in processed products are clearly critical for physiological functionality. Extractability can be altered, for example, by the particle size of the product and by different factors affecting water penetration (Mälkki & Virtanen, 2001), and the factors influencing the molecular weight of β-glucan include enzymatic hydrolysis, mechanical processes, heat treatments, acid hydrolysis or chemical oxidation (Andersson et al., 2004; Johansson et al., 2006; Kivelä, Gates, & Sontag-Strohm, 2009; Kivelä, Henniges, Sontag-Strohm, & Potthast, 2012; Lazaridou, Marinopoulou, Matsoukas, & Biliaderis, 2014; Mäkelä, Sontag-Strohm, & Maina, 2015; Pérez-Quirce, Ronda, Lazaridou, & Biliaderis, 2017; Tosh, Wood, Wang, & Weisz, 2004; Vaikousi & Biliaderis, 2005).

The physicochemical properties of β-glucan are likely to be altered during the processing of oat products, and possibly, these alterations lead to changes in the functionality of oat β-glucan. Thus far, studies on the effects of food processes on the physiological functionality of β-glucan are scarce, and as of the moment, the health claim approved by the EFSA in 2010 does not state any requirements for the state of β-glucan in the final product, but only declares the needed β-glucan content and daily dose. More studies are therefore needed to evaluate the actual physiological functionality of β-glucan after different processing steps. In this study, the aim was to investigate the state of β-glucan in the small intestine by using *in vitro* gastrointestinal (GI) simulation to study commercial oat products from different product categories. This study also aimed to shed light on the processes that are likely to influence β-glucan functionality.

2. Material and methods

2.1. Material

All oat products (N = 22) were purchased locally from supermarkets in the Helsinki area and were selected from different manufacturers. The samples were divided into the following six categories: oat breads, oat crisps and chips, oat porridge, spoonable oat products, extruded oat products and meat analogues (Table 1). All breads had oat as 100% of their grain ingredients, but some of them also included added hydrocolloids. In all products, oat was the main cereal grain ingredient.

For the *in vitro* upper GI tract model, Na_2HPO_4 buffer was prepared by dissolving 20 mM Na_2HPO_4 (di-Sodium hydrogen phosphate anhydrous for analysis, Meck, Germany) in MQ water, adjusting the pH to 6.9 and adjusting the volume, after which 10 mM sodium chloride was added to the buffer solution. Amylase solution was prepared from Termamyl (Termamyl® 300L, Novozymes, Denmark) by inactivating enzyme activities other than thermostable α-amylase by heating the enzyme solution to 80 °C for 15 min, after which the solution was allowed to cool prior to centrifuging and discarding the precipitate. Pepsin solution was made by dissolving 0.5 mg pepsin (pepsin from porcine stomach mucosa, 1:60,000, Sigma, USA) per ml of 0.9% NaCl. For the pancreatin solution, 150 mM $NaHCO_3$ (sodium hydrogen carbonate for analysis, Merck, Germany) solution was first prepared and 0.938 g pancreatin (pancreatin from porcine pancreas, Sigma, USA) was dissolved in 50 ml of the 150 mM $NaHCO_3$ solution at 37 °C for 20 min after which the solution was centrifuged (5810 R, Eppendorf, Germany) at 1000 rcf for 10 min. Bile acid solution was prepared by mixing 150 mg bile acids (Bile from bovine and ovine, Sigma, USA) per ml of 150 mM $NaHCO_3$ at 37 °C for 30 min. All buffers and enzyme preparates used for the *in vitro* upper GI tract model were stored at +4 °C.

For the β-glucan content analysis, the following buffers were prepared: 20 mM NaH_2PO_4 (sodium dihydrogen phosphate dehydrate, J.T. Baker, Holland) (pH 6.5), 200 mM sodium acetate (Sigma-Aldrich, Germany) (pH 4.0) and 50 mM sodium acetate (pH 4.0). Other reagents (lichenase, β-glucosidase, GOPOD reagent buffer, GOPOD reagent enzymes) were provided in the β-glucan assay kit (β-glucan Assay Kit [Mixed Linkage], Megazyme, Ireland) and were prepared, stored and used according to kit instructions. The assay kit also provided the

Table 1
Samples categorised and the ingredients essential for the work described.

Sample name	Sample description
Bread 1	100% of the grain ingredients from oat. Includes added hydrocolloids.
Bread 2	100% of the grain ingredients from oat. Includes added hydrocolloids and seeds.
Bread 3	100% of the grain ingredients from oat. Includes added hydrocolloids.
Bread 4	100% of the grain ingredients from oat. Includes added hydrocolloids.
Bread 5	100% of the grain ingredients from oat.
Bread 6	100% of the grain ingredients from oat, except for the rye flour used as flour on the belt conveyor.
Crisps and chips 1	Contains also grain ingredients other than oat.
Crisps and chips 2	100% of the grain ingredients from oat. Includes added hydrocolloids.
Crisps and chips 3	100% of the grain ingredients from oat. Includes added hydrocolloids.
Porridge 1	Regular oat flakes. 100% of the grain ingredients from oat.
Porridge 2	Regular oat flakes and bran. 100% of the grain ingredients from oat.
Porridge 3	Regular oat flakes and muesli flakes. 100% of the grain ingredients from oat.
Extruded product 1	Contains also grain ingredients other than oat.
Extruded product 2	Other grain ingredients from oat, except added starch.
Extruded product 3	Other grain ingredients from oat, except added starch.
Extruded product 4	100% of the grain ingredients from oat.
Spoonable product 1	100% of the grain ingredients from oat. Includes added hydrocolloids. Fermented.
Spoonable product 2	Other grain ingredients from oat, except added starch. Includes added hydrocolloids. Fermented.
Spoonable product 3	100% of the grain ingredients from oat. Includes added hydrocolloids. Fermented.
Meat analogue 1	100% of the grain ingredients from oat. Includes legume ingredients.
Meat analogue 2	100% of the grain ingredients from oat. Includes legume ingredients.
Meat analogue 3	100% of the grain ingredients from oat. Includes legume ingredients.

glucose standard (D-Glucose standard solution [1.0 mg/mL] in 0.2% (w/v) benzoic acid), as well as the standardised barley and oat flour controls with known β-glucan and dry matter contents.

2.2. Methods

2.2.1. Sample pre-treatments

The samples in powder form, as well as the spoonable samples, were used as such for the *in vitro* upper gut model. The solid samples (breads, crisps, chips, extruded breakfast cereals) were homogenised with a kitchen blender (Bamix, Switzerland). The oat flakes were cooked to porridge to better model the common way of consuming such a product. All porridges were prepared by weighing 40 g of oat flakes and adding 250 ml of MQ water. The porridge samples were heated until boiling by using heating plates and boiling was continued for 5 min prior to letting the sample cool.

For analyses other than the *in vitro* analysis, the samples were stored at −20 °C. Prior to freezing the bread samples, the homogenised samples were air-dried and the dried samples were milled to a particle size of 0.5 mm (Retsch, ZM 200, Haan, Germany).

2.2.2. Moisture content analysis

The moisture content of the samples analysed with the *in vitro* upper GI tract model was determined. Additionally, all the samples that were air-dried were also analysed for moisture content after the drying process. The moisture content of the solid samples was analysed with an air-oven according to the AACC 44–15.02 method, and the moisture content of spoonable and semi-solid (porridge) samples was analysed using the quartz sand method AACC 44–60.01.

2.2.3. Upper gastrointestinal tract model

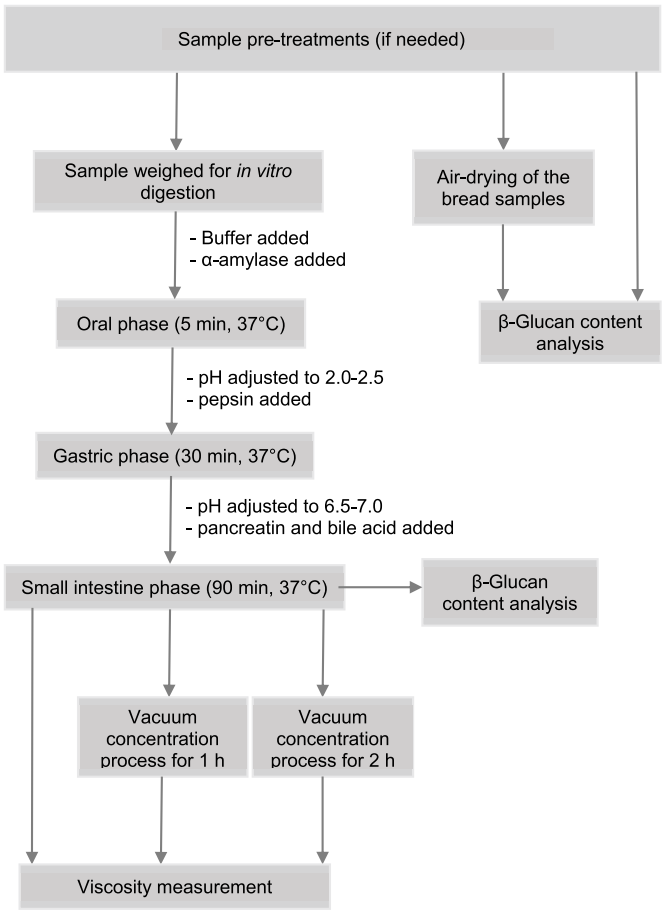
The *in vitro* method models upper GI tract digestion, including the oral phase, gastric phase and small intestine phase (Scheme 1). The repeatability of the method has been tested with oat flour prior to using the method for modelling the state of β-glucan from other food products in the small intestine.

2.2.3.1. Oral phase. The samples were weighed to 85 ml centrifugation tubes as two replicates, and the weighed amounts were calculated by estimating the final β-glucan content of the *in vitro* digestion extract to be 0.6%. The actual β-glucan content was later analysed, and the results are expressed according to the actual analysed β-glucan contents. Twenty millilitres of the extraction buffer (20 mM Na_2HPO_4 + 10 mM NaCl) was added after pre-heating the buffer to about 37 °C. The samples were homogenised using a homogeniser (Ultra Turrax T25, Janke&Kunkel, IKA, Germany) at a speed of 8000 min^{-1} for 5 s. The powdery samples were not homogenised. For the spoonable samples, no buffer was added, as the β-glucan content of the product itself was already lower than 0.6%. Thus, for these samples no buffering to pH 6.9 occurred during the oral phase. For the semi-solid samples (porridge), 2 ml buffer addition was conducted to reach the total of 20 ml of liquids in the sample.

One millilitre of α-amylase solution (inactivated Termamyl) was added to all the samples, after which the samples were mixed thoroughly and incubated at 37 °C for 5 min using a shaking incubator (Unimax 1010 combined with Inkubator 1000, Heidolph, Germany). All incubation steps of the upper GI tract model were conducted by shaking the sample tubes horizontally.

2.2.3.2. Gastric phase. After the oral phase, the pH of the samples was adjusted to 2.0–2.5 with 2.5 M HCl. Then, 2 ml of pepsin was added, and the samples were mixed thoroughly and incubated at 37 °C for 30 min using a shaking incubator.

2.2.3.3. Small intestine phase. The pH of the samples was adjusted to 6.5–7.0 with 4 M NaOH. Two millilitres of pancreatin solution and 1 ml of bile acid solution were added to each sample, after which the samples were thoroughly mixed. The samples were incubated at 37 °C for 90 min using a shaking incubator. After the incubation, the samples were centrifuged (12,900 rcf, 10 min; 5810 R, Eppendorf, Germany). Further analyses were conducted for the supernatant, which included the solubilised β-glucan.



Scheme 1. Outline of the upper gastrointestinal tract model with separate oral, gastric and small intestine phases.

From each sample, one part of the supernatant was used as such for viscosity measurement. From each sample tube, part of the supernatant was also weighed in a test tube, which was placed in a vacuum concentrator (SpeedVac Plus, SC110A combined with Refrigerated Condensation Trap RT100, Savant, Savant Instruments Inc. U.S.). From one replicate, the sample was concentrated for 1 h, and from the other replicate, the sample was concentrated for 2 h. Thus, in total, three concentration steps were gained: 0 h as two replicates and 1 h and 2 h as one sample but all measured twice. This concentration step was done to mimic the concentration process that occurs in the small intestine, whilst water is absorbed from the chyme.

2.2.4. Viscosity measurements

The viscosities of the *in vitro* extracts were measured with a HAAKE MARS 40 rheometer (Thermo Scientific, Germany). The flow profile was measured with a rotational measurement program in which the shear rate was increased step-wise from 0.3 s^{-1} to 300 s^{-1}, with each step including stabilisation of 3 s prior to the measurement for 4 s. All viscosities were measured at 20 °C. In the results, the apparent viscosities at a shear rate of 25 s^{-1} are reported as a function of the β-glucan concentration of the extract.

2.2.5. β-glucan content analysis

The β-glucan content was analysed using a β-glucan assay kit for mixed linkage β-glucan (β-glucan Assay Kit [Mixed Linkage], Megazyme, Ireland), with some modifications. The β-glucan content of the original food product samples was measured directly from the semi-solid (porridge) and spoonable samples, from the samples in powder-form and from the *in vitro* extracts. For those solid samples that underwent pre-treatments, such as air-drying and/or homogenisation, the β-glucan content was analysed after the pre-treatments.

For the dry homogenised samples, 100 mg (80–120 mg) of the sample was accurately weighed into the test tube. For the spoonable and semi-solid samples and for the *in vitro* extracts, the weighed amount was 1 g. All samples were weighed as two replicates. The samples were placed into boiling water for 5 min prior to ethanol treatment, which was done to remove free sugars and wash out some fats and oils. This step was done by adding 3 ml of 95% ethanol, after which the samples were mixed and centrifuged (10 min; 3220 g). Ethanol was discarded and 8 ml of 66% ethanol was added. Again the samples were thoroughly mixed and centrifuged (10 min; 3220 g). After ethanol was discarded, the samples underwent β-glucan content analysis, which was done according to the kit instructions. In all sample sets, the oat and barley control flours provided in the assay kit were also analysed, but for the control flours, the ethanol washing steps were not conducted; the control flours were instead moistened by adding 0.2 ml of 50% ethanol.

2.2.6. Statistical analysis

The analyses were conducted with either two or three replicates (specified in the description of each method) and the results are reported as averages ± standard error of mean (SEM). Statistical analyses were accomplished with the Statistical Package for the Social Science (SPSS Statistics version 24, IBM, USA) using ANOVA with post-hoc Tukey test. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Extractability of β-glucan from varying oat products

The extractability of β-glucan was determined as the proportion of β-glucan dissolved from the oat matrix to the aqueous extract during the *in vitro* extraction. The values ranged from 26% to 99%, and although the highest differences were observed between product categories the variation within categories was also large.

In the bread samples, the extractabilities ranged from 36% to 54% and the extractability of bread 2 differed significantly from that of all the other bread samples (Fig. 1). Breads 5 and 6 did not contain added hydrocolloids, but this was not shown to significantly affect the extractability of β-glucan. The extractabilities of β-glucan from the crisp and chip samples differed significantly from those of the bread samples, but within the crisp and chip samples, the extractabilities were similar. The β-glucan extractability of this product category was rather high, ranging from 63% to 64%.

The extractability of β-glucan from oat porridge was shown to be surprisingly low, as all three samples had an extractability lower than or equal to 35% (29%–35%). The meat analogue samples had β-glucan extractabilities (26–32%) that were comparable with those of porridge. Porridge 1 and meat analogue 3 differed significantly, but the extractabilities were otherwise similar in all the samples in these two product categories.

The highest variations in β-glucan extractabilities within a category were found in extruded products and spoonable products. Extruded products had two subclasses, as extruded products 1 and 4 had significantly higher extractabilities (both 64%) than extruded products 2 and 3 (both 43%). The overall highest extractabilities were found in the spoonable product category, as β-glucan was almost completely extracted from spoonable products 1 and 2 (100% and 94%, respectively). However, the trend was not common for all spoonable products, as the third spoonable product had a significantly lower β-glucan extractability than those with a rather complete extraction, and in this sample only 74% of β-glucan was extracted in the *in vitro* model. Still, the extractability here was higher than that in any other sample category.

3.2. Viscosity of β-glucan at intestine phase in vitro

The viscosities of the *in vitro* extracts were measured after the GI simulation and after 1 h and 2 h concentration steps to obtain three different concentration levels. Thus, the viscosities are presented as a function of β-glucan concentration. It should be kept in mind that the extracts also contain other components (e.g. hydrocolloids, such as CMC and psyllium), and the effects of these on viscosity need to be considered when interpreting the data.

The viscosities in the bread extracts varied, although within this category, some samples behaved similarly (Fig. 2a). Extracts from breads 2, 4 and 6 had a rather similar rheological behaviour, with the viscosity increasing exponentially with an increasing concentration. The

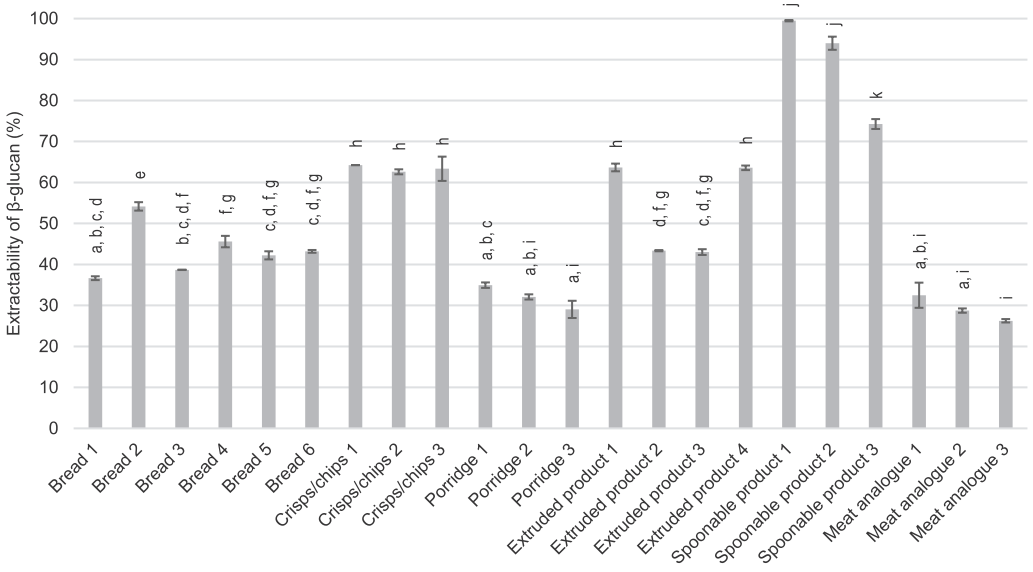


Fig. 1. Extractability of β-glucan (%) from different oat products in the *in vitro* gastrointestinal model. Significant differences shown with indices from a to k.

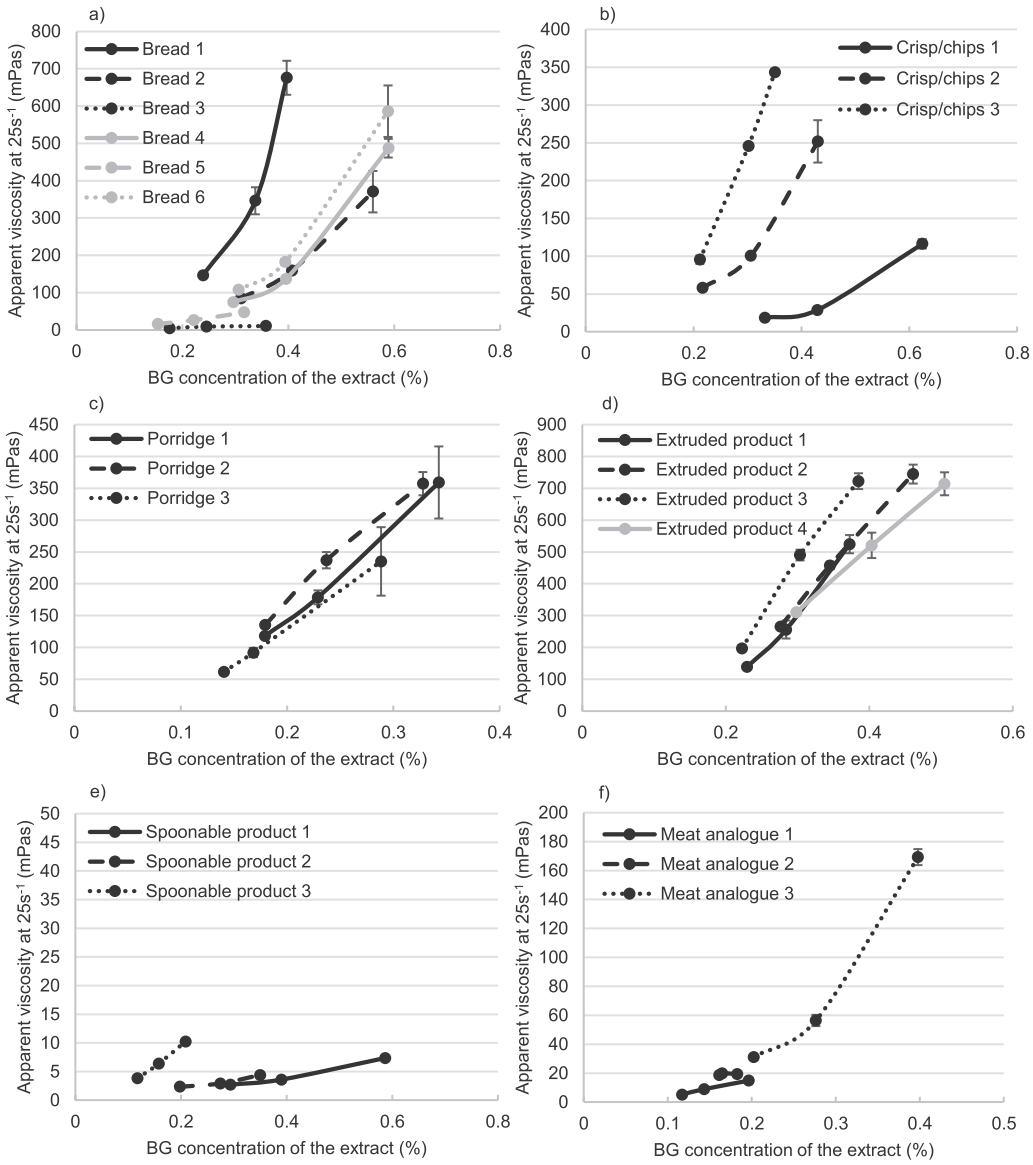


Fig. 2. Apparent viscosities at 25 s^{-1} of the *in vitro* extracts of oat samples from different product categories, including breads (a), crisps and chips (b), porridges (c), extruded products (d), spoonable products (e) and meat analogues (f).

concentrations of these extracts were 0.56%–0.59% after a 2 h concentration process and the viscosities varied from 370 mPa s to 590 mPa s. Bread 1 had a high viscosity already at relatively low concentrations, with the viscosity increasing sharply from 150 mPa s to 680 mPa s when the concentration was increased from 0.24% to 0.40%. Only two of the breads – breads 3 and 5 – had rather insignificant viscosities: only 11 mPa s and 47 mPa s, respectively, after the 2 h concentration process.

For the *in vitro* extracts of crisp and chip products the values of viscosity varied largely between the samples (Fig. 2b). The viscosity of crisp and chip sample 1 was only 120 mPa s after the 2 h concentration process, although the β-glucan concentration was already 0.62% at that point. The two other crisp and chip samples had higher viscosities, and the viscosity increased at lower concentration values compared to sample 1.

In both the porridge and the extruded product categories, the variation in viscosities was rather low within the categories. The concentrations varied slightly, but at each concentration level, the viscosities were quite similar, as seen from Fig. 2c and d. The viscosities in the extruded products increased to much higher levels (extruded products 2, 3 and 4 having final viscosities ranging from 710 mPa s to 750 mPa s) than in the porridge samples, because of the much greater extractabilities in extruded products. However, the viscosities were similar when comparing these samples within similar concentrations.

The lowest viscosity levels were found in the spoonable product and meat analogue categories. During the *in vitro* extraction, the sample amount was adjusted to gain a sufficient β-glucan concentration, but the spoonable products had lower β-glucan concentrations than the other analysed products, so they were analysed as such without concentration adjustment. The viscosities in spoonable products 1 and 2 did not have a significant viscosity increase even though the concentrations increased to 0.59% and 0.35%, respectively, and the viscosities remained at values lower than 8 mPa s for all measurement points (Fig. 2e). The viscosity of spoonable product 3 increased with an increasing concentration, but the concentration was quite low (only 0.2%), so the actual effect of β-glucan on viscosity is difficult to interpret from these data. In the meat analogue samples, the adjustment of the concentration was rather difficult. Meat analogue 1 contained high amounts of legume ingredients, and thus to gain sufficient β-glucan content in the sample, the solid material content would have been too high for the extraction process. To address this, the concentration was increased to the point in which the extraction was

still possible, but as seen from Fig. 2f, the β-glucan concentration remained low. In meat analogue 2, the evaporation process did not efficiently concentrate the sample, which may be attributed to the oil at the surface of the sample preventing water evaporation.

4. Discussion

Several studies have indicated a correlation between the ability of β-glucan to form viscosity and its physiological functionality related to cholesterol-lowering and postprandial blood glucose-attenuating effects (Mälkki & Virtanen, 2001; Wood, 2002, 2010). In some cereal β-glucan-containing products, the ability of β-glucan to increase viscosity in the small intestine was minimal, but the cholesterol-lowering ability in human intervention was still shown (Wood, 2010), raising a question on whether other mechanisms for functionality are involved. However, the physiological functionality of cereal β-glucan is still mainly explained by the entrapment of nutrients by the viscous matrix, preventing their transportation to absorption surfaces in the gut and restricting the mixing of digestive enzymes and their substrates (Grundy, Fardet, Tosh, Rich, & Wilde, 2018).

The viscosity of β-glucan in the digestive tract is affected by the original concentration of the β-glucan in the product, by extractability and solubility during digestion, and by molecular weight (Wood, 2010). Some health claims require products to be minimally processed, but the EFSA health claims from years 2010 and 2011 for the cholesterol-lowering and blood glucose-attenuating effects of oat are valid as long as the β-glucan dose is sufficient, without any specifications about the processing or the form of β-glucan (EFSA, 2010, 2011). Information on the factors affecting the physiological functionality of cereal β-glucan is lacking, and in this study, the extractability and state of β-glucan in small intestine conditions were studied *in vitro* in order to gain more knowledge about the potential variations in physiological functionality depending on the product or processing type of the food.

One factor to be considered is that in this study, the variation between product batches was not examined. The β-glucan content may fluctuate significantly between different oat ingredient batches or different cultivars used for the ingredient production, and this makes the oat processing difficult, as the quality and processability may vary. In this study, the effect of different processes was analysed based on single batches but still by having several samples from each product category, which gives an indication of the possible variations within product category.

4.1. Factors affecting the extractability of β-glucan in oat products

The solubility and extractability of β-glucan can be affected by the extraction temperature, moisture content, particle size of the matrix and factors influencing water penetration (Mälkki & Virtanen, 2001). In this study, the temperature during extraction was kept constant, so the extraction temperature did not cause any of the observed differences. However, the thermal history during the processing of each food product is different between categories and potentially also within the categories, so this may have caused some differences in extractability, along with the other factors listed above.

Wood, Siddiqui, and Paton (1978) showed the increased extractability of oat gum from oat flour when the extraction temperature was increased from 5 °C to 80 °C, and therefore the temperature affects the extractability of oat β-glucan to some extent. However, in our study, the extractability from the porridge samples was rather low compared with that from the other samples, and thus it was concluded that the cooking alone has limited influence on the increase in extractability. According to Yiu, Wood, and Weisz (1987), the water content during cooking significantly affects the solubilisation of β-glucan, and therefore the extractability of β-glucan from the porridge samples could have been increased if larger volumes of water were used during cooking. However, in this study, the amount of water used was based on the cooking instructions of the porridge, which is therefore in accordance with the actual solubilisation when consuming porridge.

In addition to the temperature and moisture content during extraction, there are also other factors affecting to the extractability of β-glucan. Anguita, Gasa, Martín-Orúe, and Pérez (2006) showed an increased *in vitro* solubility of non-starch polysaccharides from oat after cooking, but the effect was even more pronounced when the oat material was extruded. The higher increase in solubility after the extrusion was proposed to be caused by the mechanical treatment in the presence of heat and pressure, which leads to the formation of a uniform structure. According to Mälkki and Virtanen (2001), the extractability of β-glucan can be enhanced by decreasing the particle size of the matrix. This leads to an increased surface area, allowing more efficient water penetration. In the samples used in this study, the particle size of the oat ingredients visibly varied between products, and therefore this is one cause of the observed differences in extractability. The samples in the bread category had significantly lower extractabilities than those in the crisps and chips category. In breads, oat was added as different kinds of fractions, such as flour, bran, flakes and groats, whereas in crisps and chips, only flour and bran were used, and the structure of these products seemed to be more homogeneous, which could indicate also the smaller particle size of the oat ingredients. Furthermore, the extruded products had high extractabilities, which is consistent with the above-mentioned observations of Anguita et al. (2006). The differences in extractability of β-glucan within the extruded product category are linked to the differences in extrusion conditions, because for example the moisture content of oat flour in extrusion has been shown to affect β-glucan extractability significantly (Brahma, Weier, & Rose, 2016).

In meat analogues, the sample matrix was quite homogenised, and no large oat ingredient particles were observed in any of the analysed meat analogue samples. However, the extractabilities were the lowest in this category. In all of the meat analogues, the protein content was reported to be relatively high (at least 20% in all of these samples) and this may have affected the extractability of other compounds. The structure of plant protein-based meat analogues usually relies on the proteineous matrix, which provides a meat-like mouthfeel and texture. To produce a meat-like structure, extrusion cooking is often used (Lin, Huff, & Hsieh, 2000; Liu & Hsieh, 2008; Wenger, Osterhaus, & Smith, 1976). In the formation of the protein structure, cross-linking by disulphide bonds is essential, although non-covalent bonds also probably affect the structuration (Lin et al., 2000; Liu & Hsieh, 2007, 2008). In this study, the processing history of the sample products is not known, but some kind of structuration process, such as extrusion, is likely involved in the production of all the meat analogue samples. The formed tight matrix with structured proteins decreases the extractability of other components as water penetration, which affects the extractability, is altered. Additionally, protein structures may lead to encapsulation of polysaccharides as indicated by Colonna et al. (1990), who showed the decreased enzymatic hydrolysis of starch in pasta when proteins are cross-linked.

4.2. State of β-glucan in small intestine conditions

As indicated in the introduction, the viscosity of β-glucan solution is affected by the solubility, concentration and molecular weight of β-glucan. The concentration of β-glucan in the GI tract is affected by the original β-glucan content of the food, the amount of both the fluid secreted to the digesta and the fluid absorbed from the GI tract, and the extractability of β-glucan. The digesta dilutes significantly when as much as about 7 L of fluid are secreted to the digesta per day, as reviewed by Masyuk, Marinelli, and LaRusso (2002), leading to such low β-glucan concentrations in the early parts of the digestive tract that the viscosity of the digesta is probably not influenced by β-glucan. However, most of this fluid is absorbed in the small intestine and the colon, so the components (such as β-glucan) in the digesta are concentrated (Masyuk et al., 2002). As the exact amount of fluids secreted and absorbed depends on various factors and thus cannot be estimated, we aimed to have

a similar fluid amount in all the samples in the GI model used in this study. In all the samples, the β-glucan concentration was set to the estimated 0.6% (or slightly lower in the spoonable product samples in which the original concentration was lower than this) by calculating from the concentration given by the manufacturer or by estimating the β-glucan amount from the reported total dietary fibre content of the product. Thus, these results can be used to compare the state and potential physiological functionality of β-glucan *in vitro* at given concentrations. However, it has to be borne in mind that the actual β-glucan concentration in the small intestine may vary from these concentrations, depending on the amount of fluids excreted and absorbed during the digestion of food.

As the viscosity of β-glucan is altered by its molecular weight, the original molecular weight of β-glucan in oat ingredients affects the physiological functionality of oat products. The variation in the molecular weights of oat β-glucan can be high, as reported in several studies (Beer, Wood, & Weisz, 1997; Skendi et al., 2003; Wood, 2010), and this may contribute to the differences observed in this study when comparing the samples both between and within the sample categories. For example, according to a review by Wood (2010), the molecular weight of oat β-glucan varied from 1×10^6 g/mol to 3×10^6 g/mol even when the possibility of varying results because of differences in extraction and analysis conditions was excluded. This kind of difference in molecular weight would also be seen as differences in *in vitro* extract viscosities. Additionally, the potential degradation of β-glucan during the processing or storage of products might be a threat to the physiological functionality of oat products.

The viscosities of the porridge samples were all rather similar, which agrees with the results of Åman, Rimsten, and Andersson (2004) indicating no degradation in the production of rolled oat. The low extractabilities led to rather low β-glucan concentrations in the porridge sample extracts, but still, the viscosities were quite high compared with those of most of the bread samples at similar concentration levels. A similar observation was made in the extruded samples, although in such samples, the extractability was also high. One possible reason for the β-glucan remaining quite intact, which is seen as high viscosity, is the low moisture content of these products. At low water content the reaction rates decline leading to decreased amount of deterioration (Labuza, 1980). A low water activity prevents the reacting molecules from dissolving and, consequently, for example both oxidation and enzymatic reactions are slow or even negligible. Accordingly, the low water activity of the food product during processing and storage is most likely an efficient way of preserving β-glucan in its physiologically active state.

In several food processes, the action of enzymes can result in the degradation of β-glucan. Andersson et al. (2004) concluded that during the baking of bread from barley and wheat flour mixtures, no degradation of β-glucan as a result of heat treatment occurred. Instead, a significant degradation occurred during the preparation of the dough, and the effect was also seen without yeast addition. This result indicates that the degradation most likely occurred because of the endogenous enzymes in flour. One of the samples in the crisps and chips category (crisp and chip sample 1) of our study contained other grain ingredients in addition to oat, and this may partly explain the lower viscosity of this specific sample compared with the other samples in both the crisps and chips category and the bread category. The bread samples in our study, however, did not contain other cereal grain ingredients (except for the flour on the conveyor belt used in one bread sample), and as most of the endogenous enzymes in oat ingredients are inactivated during the conditioning (increasing the moisture content) and the subsequent kilning process (Anttila et al., 2004), the significance of endogenous enzymes for the viscosity of such samples should be rather low. The viscosities of *in vitro* extracts were quite high in most of the bread samples, indicating no drastic degradation during bread production. However, breads 3 and 5 had low viscosities, and this should not result from the activity of endogenous enzymes, so the low viscosity could be caused by the originally lower molecular weight of the β-glucan in the oat ingredients of these two breads compared with the oat ingredients used in breads 1, 2, 4 and 6.

The lowest viscosities were shown in the extracts of spoonable products and meat analogues. In spoonable product 3, the concentration was very low, so the increase in viscosity with an increasing concentration resulted from a component other than β-glucan. In two other spoonable products, practically no viscosity (viscosities <10 mPa s) was observed. All the tested spoonable products contained a fermentation step, and as bacteria use carbohydrates as a source of energy, they can produce some enzymes necessary for the cleavage of carbohydrates (Mayo, van Sinderen, & Ventura, 2008). Depending on the starter culture used, the enzyme activities vary, but some starter cultures containing lactic acid bacteria have been analysed to contain β-glucanase activity (Marklinder, Haglund, & Johansson, 1996). Hence, some enzymatic hydrolysis of β-glucan may have occurred when using fermentation in the production of spoonable oat products. Furthermore, in spoonable products an enzymatic hydrolysis step may be used in processing to alter the structure of the product, if the aim is to obtain a lower viscosity in the final product.

In meat analogues 1 and 2, the β-glucan concentrations were not sufficient for viscosity formation. Unfortunately, these concentrations could not be increased in the *in vitro* extraction. In one of these samples, the original β-glucan content of the product was low and the dry matter content during extraction would have increased too much for the optimal handling of the samples. Furthermore, in another sample the evaporation process did not properly concentrate the sample because of to the oil layer formed on top of the extract during evaporation. In the third meat analogue sample in which the β-glucan concentration of the extract was higher, the viscosity increased sharply when the concentration increased because of the evaporation treatment. Nonetheless, the viscosity of this sample was also lower than that in the samples of bread, porridge or extruded products. Both Kivelä, Sontag-Strohm, Loponen, Tuomainen, and Nyström (2011) and Wang, Wood, Cui, and Ross-Murphy (2001) showed the degradation of β-glucan when it is heated in solution form to 120 °C, a temperature that is also relevant for extrusion processes, for example. This indicates that in meat analogues, thermal degradation can alter the physiological functionality of oat β-glucan.

5. Conclusion

In this study, the upper GI tract model was used to mimic the state of β-glucan during the digestion of oat products. This method is applicable for reviewing the factors that affect both the extractability of β-glucan in physiological conditions and the state of β-glucan in small intestine conditions, which further determines its possibility of influencing the viscosity of the digesta. The method does not consider the actual fluid amount during the digestion of different kinds of products, so the extractability and viscosity somewhat differs from the true situation. However, as the concentration is kept similar in all the samples, the method succeeds in differentiating those factors that are significant for the behaviour of β-glucan during the digestion of oat products.

Significant differences were observed in the extractability and state of β-glucan in the upper GI tract model when differently processed oat products were compared. The structure of the food matrix was shown to affect extractability, as a finer particle size and a more porous/loose structure led to better water penetration. The high moisture content and elevated temperature during the production of oat products increase β-glucan solubility and extractability, but these factors are also risks for the physiological functionality of β-glucan, as they can enhance the reactions leading to its degradation. Thus, some of the highest viscosities were obtained in products whose the moisture content was limited, such as in breads and extruded products.

This study indicated that between the product categories there are differences in the physicochemical properties of β-glucan that also determine its physiological functionality. Thus, further studies are

needed to identify the exact factors that alter the extractability and structure of β-glucan, which, in turn, affect luminal viscosity.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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References

Åman, P., Rimsten, L., & Andersson, R. (2004). Molecular weight distribution of β-glucan in oat-based foods. *Cereal Chemistry, 81*(3), 356–360.

Andersson, A. A. M., Armö, E., Grangeon, E., Fredriksson, H., Andersson, R., & Åman, P. (2004). Molecular weight and structure units of (1 → 3, 1 → 4)-β-glucans in dough and bread made from hull-less barley milling fractions. *Journal of Cereal Science, 40*, 195–204.

Anguita, M., Gasa, J., Martín-Orúe, S. M., & Pérez, J. F. (2006). Study of the effect of technological processes on starch hydrolysis, non-starch polysaccharides solubilization and physicochemical properties of different ingredients using a two-step *in vitro* system. *Animal Feed Science and Technology, 129*, 99–115.

Anttila, A., Sontag-Strohm, T., & Salovaara, H. (2004). Viscosity of beta-glucan in oat products. *Agricultural and Food Science, 13*, 80–87.

Beer, M. U., Wood, P. J., & Weisz, J. (1997). Molecular weight distribution and (1→3)(1→4)-β-D-glucan content of consecutive extracts of various oat and barley cultivars. *Cereal Chemistry, 74*(4), 476–480.

Brahma, S., Weier, S. A., & Rose, D. J. (2016). Effects of selected extrusion parameters on physicochemical properties and in vitro starch digestibility and β-glucan extractability of whole grain oats. *Journal of Cereal Science, 70*, 85–90.

Colonna, P., Barry, J. L., Cloarec, D., Bornet, F., Gouilloud, S., & Galmiche, J.-P. (1990). Enzymatic susceptibility of starch from pasta. *Journal of Cereal Science, 11*, 59–70.

Cui, W., & Wood, P. J. (2000). Relationships between structural features, molecular weight and rheological properties of cereal β-D-glucan. In K. Nishinari (Ed.), *Hydrocolloids, Part 1* (pp. 159–168). Amsterdam: Elsevier.

EFSA, European Food Safety Authority. (2009). Panel on dietetic products, nutrition and allergies (NDA). Scientific Opinion on the substantiation of health claims related to beta-glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal, 7*(9), 1254.

EFSA, European Food Safety Authority. (2010). Panel on dietetic products, nutrition and allergies (NDA). Scientific Opinion on the substantiation of a health claim related to oat beta-glucan and lowering blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006. *EFSA Journal, 8*, 1885.

EFSA, European Food Safety Authority. (2011). Panel on dietetic products, nutrition and allergies (NDA). Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal, 9*, 2207.

FDA, Food and Drug Administration, Department of Health and Human Services (HHS). (1997). Food labeling: Health claims; Oats and coronary heart disease. Final Rule. *Federal Register, 62*(15), 3584–3601.

Grundy, M. M.-L., Fardet, A., Tosh, S. M., Rich, G. T., & Wilde, P. J. (2018). Processing of oat: The impact on oat's cholesterol lowering effect. *Food & Function, 9*, 1328–1343.

Johansson, L., Virkki, L., Anttila, H., Esselström, H., Tuomainen, P., & Sontag-Strohm, T. (2006). Hydrolysis of β-glucan. *Food Chemistry, 97*, 71–79.

Kivelä, R., Gates, F., & Sontag-Strohm, T. (2009). Rapid communication. Degradation of cereal beta-glucan by ascorbic acid induced oxygen radicals. *Journal of Cereal Science, 49*, 1–3.

Kivelä, R., Henniges, U., Sontag-Strohm, T., & Potthast, A. (2012). Oxidation of oat β-glucan in aqueous solutions during processing. *Carbohydrate Polymers, 87*, 589–597.

Kivelä, R., Sontag-Strohm, T., Loponen, J., Tuomainen, P., & Nyström, L. (2011). Oxidative and radical mediated cleavage of β-glucan in thermal treatments. *Carbohydrate Polymers, 85*, 645–652.

Labuza, T. P. (1980). The effect of water activity on reaction kinetics of food deterioration. *Food Technology, 34*(4), 36–41.

Lazaridou, A., Biliaderis, C. G., & Izydorczyk, M. S. (2003). Molecular size effects on rheological properties of oat β-glucans in solution and gels. *Food Hydrocolloids, 17*, 693–712.

Lazaridou, A., Marinopoulou, A., Matsoukas, N. P., & Biliaderis, C. G. (2014). Impact of flour particle size and autoclaving on β-glucan physicochemical properties and starch digestibility of barley rusks as assessed by *in vitro* assays. *Bioactive Carbohydrates and Dietary Fibre, 4*, 58–73.

Lin, S., Huff, H. E., & Hsieh, F. (2000). Texture and chemical characteristics of soy protein meat analog extruded at high moisture. *Journal of Food Science, 65*(2), 264–269.

Liu, K. S., & Hsieh, F.-H. (2007). Protein–protein interactions in high moisture-extruded meat analogs and heat-induced soy protein gels. *Journal of the American Oil Chemists' Society, 84*, 741–748.

Liu, K. S., & Hsieh, F.-H. (2008). Protein–protein interactions during high-moisture extrusion for fibrous meat analogues and comparison of protein solubility methods using different solvent systems. *Journal of Agricultural and Food Chemistry, 56*, 2681–2687.

Mäkelä, N., Sontag-Strohm, T., & Maina, N. H. (2015). The oxidative degradation of barley β-glucan in the presence of ascorbic acid or hydrogen peroxide. *Carbohydrate Polymers, 123*, 390–395.

Mälkki, Y., & Virtanen, E. (2001). Gastrointestinal effects of oat bran and oat gum, a review. *Lebensmittel-Wissenschaft und -Technologie, 34*, 337–347.

Marklinder, I., Haglund, Å., & Johansson, L. (1996). Influences of lactic acid bacteria on technological, nutritional, and sensory properties of barley sour dough bread. *Food Quality and Preference, 7*, 285–292.

Masyuk, A. I., Marinelli, R. A., & LaRusso, N. F. (2002). Water transport by epithelia of the digestive tract. *Gastroenterology, 122*, 545–562.

Mayo, B., van Sinderen, D., & Ventura, M. (2008). Genome analysis of food grade lactic acid-producing bacteria: From basics to applications. *Current Genomics, 9*, 169–183.

Othman, R. A., Moghadasian, M. H., & Jones, P. J. H. (2011). Cholesterol-lowering effects of oat β-glucan. *Nutrition Reviews, 69*(6), 299–309.

Pérez-Quirce, S., Ronda, F., Lazaridou, A., & Biliaderis, C. G. (2017). Effect of microwave radiation pretreatment of rice flour on gluten-free breadmaking and molecular size of β-glucans in the fortified breads. *Food and Bioprocess Technology, 10*, 1412–1421.

Skendi, A., Biliaderis, C. G., Lazaridou, A., & Izydorczyk, M. S. (2003). Structure and rheological properties of water soluble β-glucans from oat cultivars of *Avena sativa* and *Avena bysantina*. *Journal of Cereal Science, 38*, 15–31.

Tosh, S. M., Wood, P. J., Wang, Q., & Weisz, J. (2004). Structural characteristics and rheological properties of partially hydrolyzed oat β-glucan: The effects of molecular weight and hydrolysis method. *Carbohydrate Polymers, 55*, 425–436.

Vaikousi, H., & Biliaderis, C. G. (2005). Processing and formulation effects on rheological behaviour of barley β-glucan aqueous dispersions. *Food Chemistry, 91*, 505–516.

Vaikousi, H., Biliaderis, C. G., & Izydorczyk, M. S. (2004). Solution flow behavior and gelling properties of water-soluble barley (1→3,1→4)-β-glucans varying in molecular size. *Journal of Cereal Science, 39*, 119–137.

Wang, Q., Wood, P. J., Cui, W., & Ross-Murphy, S. B. (2001). The effect of autoclaving on the dispersibility and stability of three neutral polysaccharides in dilute aqueous solutions. *Carbohydrate Polymers, 45*, 355–362.

Wenger, L.V.G., Osterhaus, E.J., Smith, B.O., 1976. Method of preparing dense, uniformly layered vegetable protein meat analogue. United States Patent, 3,970,761.

Wolever, T. M. S., Tosh, S. M., Gibbs, A. L., Brand-Miller, J., Duncan, A. M., Hart, V., et al. (2010). Physicochemical properties of oat β-glucan influence its ability to reduce serum LDL cholesterol in humans: A randomized clinical trial. *American Journal of Clinical Nutrition, 92*, 723–732.

Wood, P. J. (2002). Relationships between solution properties of cereal β-glucans and physiological effects — a review. *Trends in Food Science & Technology, 13*, 313–320.

Wood, P. J. (2010). Oat and rye β-glucan: Properties and function. *Cereal Chemistry, 87*(4), 315–330.

Wood, P. J., Siddiqui, I. R., & Paton, D. (1978). Extraction of high-viscosity gums from oats. *Cereal Chemistry, 55*(6), 1038–1049.

Yiu, S. H., Wood, P. J., & Weisz, J. (1987). Effects of cooking on starch and β-glucan of rolled oats. *Cereal Chemistry, 64*(6), 373–379.