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Dissertation
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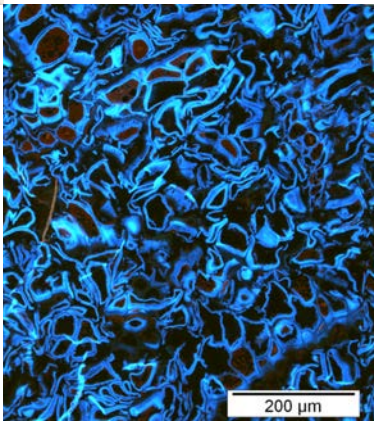
Processing of oat dietary fibre for improved functionality as a food ingredient

Juhani Sibakov



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Processing of oat dietary fibre for improved functionality as a food ingredient

Juhani Sibakov

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Preface

This study was carried out at VTT Technical Research Centre of Finland during the years 2008–2014. It included a 3-months research visit at the French National Institute for Agricultural Research (INRA, Montpellier) in 2011 (Publication II). The study was partly carried out in collaboration with MTT Agrifood Research Finland (Publication I), the University of Helsinki and the University of Eastern Finland (Publication IV). The research was funded by VTT, the Academy of Finland and the Finnish Graduate School of Applied Bioscience: Bioengineering, Food & Nutrition, Environment (ABS); their financial support is greatly appreciated. At VTT I thank Vice President, Professor Anu Kaukovirta-Norja and Vice President, Dr. Johanna Buchert, as well as Head of Research Area, Dr. Raija Lantto and Dr. Tuulamari Helaja for providing me with good facilities to carry out this work.

My most sincere gratitude goes to my thesis advisor, Academy Professor Kaisa Poutanen for giving excellent guidance, support and encouragement throughout the study. In addition to my PhD, I thank Kaisa for trusting me the administration of VTT's Nutritech website (2008–2011) and later on the duties of the secretary of the HealthGrain Forum (2011→). With the help of Kaisa, I have presented my work in several international conferences and acquired a broad network of partners from academia and food industry around the world. It has been a privilege to share your passion and enthusiasm. I am also thankful for Kaisa and Prof. Valérie Micard for arranging the research visits (mine and Natalia's) between Finland and France. That exchange led to results much larger than just a PhD thesis ☺

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I extend my heartfelt thanks to my family, relatives and friends. I warmly thank my dear parents Kirsti (in memoriam) and Mikko, my sisters Kristiina and Katriina, and my nephews Kristian, Sebastian, Joel and niece Helena for their love and support throughout my life. Enormous thanks go to my friends Miiro, Antero, Tuomas, Anssi, Johannes, Juha, Altti, Marko, Otto and Emna.

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Espoo, October 2014,

Juhani Sibakov

Academic dissertation

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Supervising professor	Professor Simo Laakso Department of Biotechnology and Chemical Technology Aalto University Espoo, Finland

List of publications

This thesis is based on the following original publications, which are referred to in the text as I–IV. The publications are reproduced with kind permission from the publishers.

I Sibakov, J., Myllymäki, O., Holopainen, U., Kaukovirta-Norja, A., Hietaniemi, V., Pihlava, J.-M., Poutanen, K., Lehtinen, P., 2011. Lipid removal enhances separation of oat grain cell wall material from starch and protein. *Journal of Cereal Science*, *54*, 104–109.

II Sibakov, J., Abecassis, J., Barron, C., Poutanen, K., 2014. Electrostatic separation combined with ultra-fine grinding to produce β-glucan enriched ingredients from oat bran. *Innovative Food Science and Emerging Technologies*, In Press.

III Sibakov, J., Myllymäki, O., Suortti, T., Kaukovirta-Norja, A., Lehtinen, P., Poutanen, K., 2013. Comparison of acid and enzymatic hydrolyses of oat bran β-glucan at low water content. *Food Research International*, *52*, 99–108.

IV Sibakov, J., Kirjoranta, S., Alam, S.A., Jurvelin, J., Kokkonen, H., Jouppila, K., Poutanen, K., Sozer, N., 2014. Effect of oat bran fractions on extrudates made of defatted oats. *Food and Bioprocess Technology*, In Press.

Author's contributions

I. The author was responsible for planning the work, interpretation of the results and writing the publication under the supervision of Dr. Pekka Lehtinen, Dr. Anu Kaukovirta-Norja and Dr. Kaisa Poutanen. The author carried out the pilot-scale grinding and fractionation trials as well as the chemical analyses. Dr. Veli Hietaniemi and Dr. Juha-Matti Pihlava were responsible for the defatting experiments in pilot-scale. MSc Olavi Myllymäki and the personnel from Hosokawa Alpine AG (Alfred Schorer and Michael Kuhnen) assisted in the industrial scale trials. MSc Ulla Holopainen-Mantila had the main responsibility for the microscopy analyses.

II. The author had the main responsibility for planning the work, interpretation of the results and writing the publication under the supervision of Dr. Joël Abecassis, Dr. Cécile Barron and Dr. Kaisa Poutanen. The author carried out the grinding and electrostatic separation as well as chemical analyses. MSc Ulla Holopainen-Mantila had the main responsibility for the microscopy analyses.

III. The author had the main responsibility for planning the work, interpretation of the results and writing the publication under the supervision of Dr. Pekka Lehtinen, Dr. Anu Kaukovirta-Norja and Dr. Kaisa Poutanen. The author carried out the laboratory work. Dr. Tapani Suortti had the main responsibility for the HP-SEC analysis of β-glucan. MSc Olavi Myllymäki assisted in the experimental work, as the work was based on two patents developed by him. MSc Ulla Holopainen-Mantila had the main responsibility for the microscopy analyses.

IV. The author was responsible for planning the work, interpretation of the results and writing the publication together with MSc Satu Kirjoranta (equal authorship). The work was supervised by Dr. Kaisa Poutanen, Dr. Kirsi Jouppila and Dr. Nesli Sözer. The experimental work was performed by the author and MSc Satu Kirjoranta with the assitance of MSc Ariful Alam. Dr. Jukka Jurvelin and Dr. Harri Kokkonen provided the X-ray tomographic imaging facilities. MSc Ulla Holopainen had the main responsibility for the microscopy analyses.

Contents

List of symbols

AX	Arabinoxylan
DF	Dietary fibre
DP	Degree of polymerisation
DP3/DP4	Cellotriose/cellotetraose ratio
d.w.	Dry weight
EF	Defatted endosperm oat flour
FA	Ferulic acid
HM_w	High molecular weight
IDF	Insoluble dietary fibre
LM_w	Low molecular weight
M_n	Number average molecular weight
M_w	Weight average molecular weight
M_w/M_n	Polydispersity
OBC	Oat bran concentrate
SC-CO_2	Supercritical carbon dioxide
SC-CO_2-OBC	Oat bran concentrate defatted by supercritical carbon dioxide
SDF	Soluble dietary fibre
WF	Defatted wholegrain oat flour
WIS-OBC	Water insoluble oat bran preparation
WS-OBC	Water soluble oat bran preparation

1. Introduction

1.1 Oat dietary fibre as a raw material for healthy foods

Oats (*Avena sativa* L.) are an important crop world-wide with a global production of about 21 million tons per year. The main producers of oats in 2012 were Russia, Canada, Poland, Australia, Finland and the USA. Finland accounted for 5.1% of the world production (FAOSTAT, 2013). Oats are the second largest crop in Finland after barley. According to TIKE (2013), the annual production has been around 1.2 billion kg for several decades. However, only 6% of the oats grown in Finland is used for food purposes.

Oats contain many health-promoting components, such as dietary fibres, proteins and minerals (Butt et al., 2008). The health benefits linked to oats have increased consumer awareness of this cereal, and the health claims approved both by FDA (1997; 2003) and by EFSA (2011a;b) further encourage the consumption of healthy oat foods. The health claims in the European Union allow food producers to market products containing 1 g β-glucan/portion with claims to reduce blood cholesterol concentrations and to attenuate post-prandial glyceamic response (EFSA 2011a). From the consumer's point of view, it can be challenging to obtain the recommended intake of β-glucan (at least 3 g/day). For example, a regular portion of oat porridge, which contains 1 dl of oat flakes, delivers around 1.5 g of β-glucan. Thus, technologies for enrichment of β-glucan ingredients as well as new product concepts are needed to support the adequate intake of β-glucan to reach the recommended intake.

In addition to the claims linked to cholesterol reduction and glyceamic response, the intake of oat and barley grain fibre is linked to the increase in faecal bulk (EFSA, 2011b). The authorised and non-authorised health claims related to β-glucan and oat grain fibre based on the decision of the scientific panel of EFSA are summarised in Table 1. Oats may also have a role in the prevention of metabolic syndrome (Cloetens et al., 2012), but their influence on appetite control and gut microbiota are still insufficiently characterised, and additional studies are needed in this field. Recently, Ahmad et al. (2012) and Singh et al. (2013) reviewed the therapeutic potential of oats, such as antioxidant, anti-inflammatory, wound healing, immunomodulatory, antidiabetic, and anticholesterolaemic activities, but there are currently no reliable data to support these claims.

Table 1. Authorised and non-authorised health claims related to oat β-glucan and grain fibre in EU (EC, 2013).

Claim type	Nutrient or substance	Claim	Conditions of use of the claim / Restrictions of use / Reasons for non-authorisation	Reference	Status (Dec 2013)
Art.13(1)	β-Glucans	β-Glucans contribute to the maintenance of normal blood cholesterol levels	The claim may be used only for food which contains at least 1 g of β-glucans from oats, oat bran, barley, barley bran, or from mixtures of these sources per quantified portion. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 3 g of β-glucans from oats, oat bran, barley, barley bran, or from mixtures of these β-glucans.	EFSA, 2009, 2011a	Authorised
Art.13(1)	β-Glucans from oats and barley	Consumption of β-glucans from oats or barley as part of a meal contributes to the reduction of the blood glucose rise after that meal	The claim may be used only for food which contains at least 4 g of β-glucans from oats or barley for each 30 g of available carbohydrates in a quantified portion as part of the meal. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained by consuming the β-glucans from oats or barley as part of the meal.	EFSA, 2011a	Authorised
Art.13(1)	Oat grain fibre	Oat grain fibre contributes to an increase in faecal bulk	The claim may be used only for food which is high in that fibre as referred to in the claim HIGH FIBRE* as listed in the Annex to Regulation (EC) No 1924/2006.	EFSA, 2011b	Authorised

Art.14(1)(a)	Oat β-glucan	Oat β-glucan has been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease	Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 3 g of oat β-glucan. The claim can be used for foods which provide at least 1 g of oat β-glucan per quantified portion.	EFSA, 2010	Authorised
Art.13(1)	Oat β-glucan	Consuming oat β-glucan increases satiety. Consuming oat β-glucan prolongs the feeling of satiety.	Non-compliance with the Regulation because on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	EFSA, 2011a	Non-authorised
Art.13(1)	Oat β-glucan	Consuming β-glucan promotes digestion, improves digestive function.	Non-compliance with the Regulation because on the basis of the scientific evidence assessed, this claimed effect for this food is not sufficiently defined to be able to be assessed and the claim could not therefore be substantiated.	EFSA, 2011a	Non-authorised
Art.13(1)	Oat grain fibre	Helps with weight control. For long-lasting sense of satiety. Frees energy slowly.	Non-compliance with the Regulation because on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	EFSA, 2009	Non-authorised

*HIGH FIBRE = A claim that a food is high in fibre, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 6 g of fibre per 100 g or at least 3 g of fibre per 100 kcal.

1.1.1 Oat kernel structure and composition

Hull

The outmost layer of oat kernel is called the hull. It usually accounts for 25–33% of the total weight of a whole oat kernel (Hutchinson, 1953; Salo and Kotilainen, 1970; Welch et al., 1983; Welch, 1995). The hull is mainly composed of cellulose and hemicellulose (Welch et al., 1983; Welch, 1995), with lesser amounts of lignin and phenolic compounds (Emmons and Peterson, 1999). Traditionally, hulls have not been used in food products, but there are several commercial prosesses to enrich the insoluble cellulosic fibre from oat hulls for food applications (Stevenson and Inglett, 2011).

Bran

Oat bran is a technical term for a milling fraction containing the outer parts of oat kernel. According to AACCI (Anon., 1989), the oat bran fraction is "not more than 50% of the original starting material and has a total β-glucan content of at least 5.5% (d.m.) and a total dietary fibre content at least 16.0% (d.m.), such that at least one-third of the total dietary fibre is soluble fibre". Ganssmann and Vorwerck (1995) stated that high-quality oat bran should contain at least 18–20% dietary fibre (of which 8–10% soluble) and 6–8% β-glucan, and thus the bran yield is limited to around 30–40% of the starting material.

Oat bran contains similar layers to those found in cereal brans, such as wheat bran (Surget and Barron, 2005), but the thickness and chemical composition of the layers are different. The layers of oat bran (starting from the outer surface) are pericarp, testa (seed coat), nucellum, aleurone, subaleurone and starchy endosperm (Fig. 1). Botanically subaleurone is not a separate layer, but a part of the starchy endosperm. Oat bran contains a larger portion of subaleurone starchy endosperm than wheat bran, because the aleurone layer of oats does not separate as cleanly from the endosperm as it does in wheat (Miller and Fulcher, 2011).

The aleurone layer contains typically one cell layer, has a thickness of 50–150 µm (Miller and Fulcher, 2011), and is quite resistant to digestion (Wood et al., 2002). Aleurone cells consist mainly of lipids and protein bodies, which are captured within the cell wall matrix (Bechtel and Pomeranz, 1981; Peterson et al., 1985). The aleurone protein bodies contain phytic acid and protease activity, which are absent in starchy endosperm protein bodies (Donhowe and Peterson, 1983).

The inner layer of the aleurone also contains some mixed-linkage (1→3),(1→4)-β-D-glucan, although much less than in the starchy endosperm, i.e. β-D-glucan is mainly located in the subaleurone region (Wood and Fulcher, 1978a). In cultivars with high β-glucan content, the polymer is more evenly distributed throughout the starchy endosperm, whereas in low β-glucan cultivars the polymer is concentrated in the subaleurone region (Fulcher and Miller 1993; Miller and Fulcher 1994).

Starchy endosperm

The largest tissue in oat grains is starchy endosperm, which depending on the variety, may constitute 55–70% of the dehulled oat groat (Youngs, 1972). The endosperm is composed of cells of only one type, each of which consists of starch, protein and lipids (Figure 1). Starch is the major single component in oat endosperm as well as in whole groats. It occurs as aggregates composed of several starch granules (Bechtel and Pomeranz, 1981). The diameter of the aggregates ranges from 20 to 150 µm, and the size of the individual granules is 2–15 µm across (Hoover and Vasanthan, 1992; Hartunian-Sowa and White, 1992). Starch content usually ranges between 43 and 64% of the groat (Paton, 1977; Lim et al., 1992). Starch content was shown by MacArthur and D'Appolonia (1979) to correlate inversely with the protein content. In addition, the authors showed that the lipid content was higher in a low starch and high protein variety, whereas a high starch and low protein variety contained significantly less lipids. The distribution of starch, protein and lipids is however strongly dependent on the oat variety, and thus it is difficult to generalise these results.

The second most abundant component in oats is protein. Typically protein concentration increases from the interior to the periphery of the kernel, whereas starch concentration increases from the subaleurone region towards the centre of the kernel (Miller and Fulcher, 2011). Protein comprises around 15–20% of dehulled groats (Brown et al., 1966; Youngs and Senturia, 1976). Oats lack the matrix type of storage protein typically found in wheat (Adams et al., 1976) and barley (Bechtel and Pomeranz, 1979). The protein bodies in starchy endosperm and aleurone have rather similar structure (both contain mainly globulin and prolamin). However, more than 55% of endosperm protein consists of globulin (Peterson and Smith, 1976). The protein in the middle of endosperm occurs as individual protein bodies, which are fused with lipids (Heneen et al., 2009). The diameter of protein bodies can range from 0.3 to 5 µm (Bechtel and Pomeranz, 1981). The larger protein bodies are typically concentrated in the subaleurone, whereas the smaller bodies are distributed throughout the central endosperm, where the concentration of starch granules is higher (Miller and Fulcher, 2011).

The cell walls of endosperm are relatively thin in the middle or inner endosperm, whereas the cell walls immediately adjacent to the aleurone layer (the subaleurone layer) can be up to four or five times thicker (Miller and Fulcher, 2011). The endosperm cell walls are rich in β-glucan, with smaller amounts of arabinoxylan, cellulose, and glucomannan (Miller and Fulcher, 1995; Miller et al., 1995).

The majority (up to 90%) of lipids in oats is found in the endosperm. Most of the lipids are neutral lipids (di- and triacylglycerols), with small amounts of glyco- and phospholipids (Youngs et al., 1977; Youngs, 1978; Price and Parsons, 1979). Banás et al. (2007) showed that the oil bodies in the aleurone layer and in the embryo occur as individual entities, whereas in the endosperm the oil bodies tend to fuse together during the development of the kernel and thus oil, starch and protein are all attached to each other in the endosperm of a mature oat kernel.

Germ

Germ is a viable structure, capable of metabolic activity, from which a new plant starts to sprout. The germ contains high amounts of protein and lipid but very little starch (Miller and Fulcher, 2011). The protein content in germ tissues can be as high as 29–38%, as compared to around 23% in the bran and 12% in the starchy endosperm (Youngs, 1972). The protein bodies in the germ are surrounded by lipid bodies similar to those in aleurone cells (White et al., 2006). The germ has the highest lipid concentration (15–24%) of any of the groat tissues (Youngs et al., 1977).

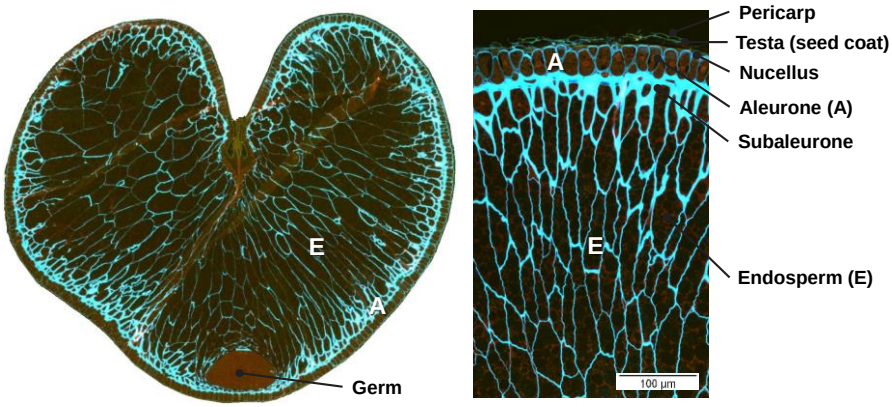

Figure 1. Cross sections of whole oat grain and the outer layers of oat grain stained with Calcofluor and Acid Fuchsin. Endosperm cell walls rich in β-glucan appear as blue and protein as brownish red (Courtesy of MSc. Ulla Holopainen-Mantila, VTT).

1.1.2 Oat dietary fibre and β-glucan

The main dietary fibre (DF) components of oats are mixed-linkage (1→3), (1→4)-β-D-glucan (β-glucan) and arabinoxylan (AX) (Wood et al., 1994). Both β-glucan and AX are concentrated more in the bran fraction than in the starch-rich endosperm area. Approximately one-third of the insoluble fibre of oats consists of β-glucan, whereas the majority of the soluble fibre is β-glucan (Manthey et al., 1999). The β-glucan concentration in whole grain oats varies typically between 2 and 8.5% (Wood, 1986) and in oat bran between 6 and 9% (Marlett, 1993; Shewry et al., 2008).

Oat β-glucan is a linear polysaccharide, composed of β-D-glucopyranose units linked by (1→4) and (1→3) linkages (Parrish et al., 1960). The ratio of (1→4) and (1→3) linkages is around 70:30, and about 90% of (1→4) linked β-D-glucopyranoses exist in groups of three (cellotriose) or four (cellotetraose) units separated by one (1→3) linkage (Wood et al., 1994; Doublier and Wood, 1995). The β-(1→3)-bonds increase the flexiblility of the chain preventing the close packing

characteristic of cellulose molecules, and enhance β-glucan's solubility in water (Buliga et al., 1986). Böhm and Kulicke (1999) proposed that a higher ratio of cellotriose/cellotetraose (DP3/DP4) would reduce the solubility of β-glucan. Thus, oat β-glucan with a DP3/DP4 ratio of 1.4–2.4 (Wood et al., 1991; Lazaridou and Biliaderis, 2004; Papageorgiou et al., 2005) is more soluble in water than β-glucan of barley (ratio 2.8–3.0) (Lazaridou and Biliaderis, 2004; Lazaridou et al., 2004) and wheat (ratio 3.0–4.8) (Miller and Fulcher, 1995; Lazaridou and Biliaderis, 2004; Tosh et al., 2004). At high DP3/DP4 molar ratio values, β-glucan molecules self-assemble faster and more closely and firmly packed domains are generated. β-Glucans with low DP3/DP4 molar ratio have fewer association points and thus the self-assembly process is prolonged since greater rearrangements and reorientation of the polysaccharide molecules are required. As a result, β-glucan molecules with low DP3/DP4 gather together and larger open-structure clusters are formed before the structural entities impinge on with each other (Moschakis et al., 2014).

The 4-linked units predominately (ca. 85–90%) occur in contiguous groups of two or three separated by 3-linked units that occur singly. This gives a structure of β-(1→3)-linked cellotriosyl (DP3) and β-(1→3)-linked cellotetraosyl (DP4) units. About 9–15% of oat β-glucan molecules contain cellulose-like sections where there are four or more consecutive 4-linked units (Wood, 2011). Enzymatic hydrolysis by lichenase has shown that released oligosaccharides of β-glucan are water-soluble until DP 9, but become increasingly insoluble at higher DP (Doublier and Wood, 1995).

The molecular weight (M_w) of oat β-glucan is typically between 1000 and 3100 kDa (Wood et al., 1991; Beer et al., 1997a; Roubroeks et al., 2000; Rimsten et al., 2003; Åman et al., 2004; Lazaridou and Biliaderis, 2007; Sikora et al., 2013). Differences in the Mw results are mainly derived from analysis and extraction methods, and less from the oat fraction or cultivar. The most commonly used analysis method for Mw distribution is high-performance size-exclusion chromatography (HPSEC). However, size-exclusion depends on molecular size, not weight, and is thus dependent on the structure of the molecule. The values obtained by HPSEC also depend on the detector in the analysis. For example, low-angle laser light scattering (LALLS) can yield over-estimated values in comparison to multi-angle (MALLS) or right-angle (RALLS) scattering (Wood et al., 1991; Beer et al., 1997a; Wood, 2011). HM_w β-glucan may sometimes occur in an aggregated form. The M_w of β-glucan can easily be reduced in the different steps of the agricultural chain and during food processing (Åman et al., 2004; Tiwari and Cummins, 2009; Hager et al., 2011). Extractability of β-glucan is also enhanced when its M_w is decreased. For example, Tosh et al. (2010) reported a significant increase in the extractability (from 40 to 100%) when the M_w of an extruded oat product was decreased from 2200 to 210 kDa.

The total amount of arabinoxylan (AX) in whole grain oat flour has been reported to be 2.0–4.5%, but only around 0.2–0.4% of this is water-extractable (Bhatty, 1992; Westerlund et al., 1993; Gebruers et al., 2008; Shewry et al., 2008). However, Westerlund et al. (1993) showed that the AX from oat endosperm is more soluble than that from oat bran. In oat bran, the AX concentration has been reported to vary between 4 and 13%, depending on the cultivar and milling process

(Westerlund et al., 1993; Jaskari et al., 1995; Shewry et al., 2008). The outer layers of oat bran, especially pericarp, contain more AX than the inner bran layers and the cell walls of endosperm (Miller and Fulcher, 1995; Miller et al., 1995; Dornez et al., 2011).

Cereal AXs have a basic backbone chain of β-D-xylopyranosyl residues linked by (1→4)-glycosidic linkages (Colleoni-Sirghie et al., 2004). In common cereals, ferulic acid (FA) is linked to arabinosyl units attached to the xylan backbone (Smith and Hartley, 1983). Miller et al. (1995) revealed the presence of FA and *p*-coumaric acid in the isolated endosperm cell walls of oats. Nevertheless, there is currently no detailed information about ferulated oat grain polysaccharides (Collins, 2011), although a link between AX and FA can also occur in oats, as an association of FA with the water-insoluble AX has been discovered in isolated endosperm cell walls of barley (Ahluwalia and Fry, 1986). Evidence for the FA–AX association in oats could also be derived from the higher FA concentration in oat bran layers (330–409 µg/g) (Mattila et al., 2005; Alrahmany et al., 2013) compared to whole oat flakes (189–250 µg/g) (Mattila et al., 2005; Xu, 2012) or to debranned oat flour (66 µg/g) (Sosulski et al., 1982), because AX is normally concentrated in the bran layers (Dornez et al., 2011). However, it is important to bear in mind that the FA concentration of oat bran is only about 10% of that in rye bran (2800 µm/g) and wheat bran (3000 µm/g) (Mattila et al., 2005).

1.1.3 Other dietary fibre-associated compounds in oats

In addition to dietary fibre, whole grain oats and oat bran fractions contain so called co-passengers, such as amino acids and peptides, minerals, lipids, vitamins, phytosterols and stanols, betaine, choline, lignans, and avenanthramides, which all may contribute to the general health benefits of oat dietary fibre (Lærke and Bach Knudsen, 2011). For example, avenanthramides are low molecular weight, soluble phenolic compounds, which are not present in other cereal grains than oats (Bratt et al., 2003; Collins, 1989; Shewry et al., 2008). They have strong antioxidant activity and may also exhibit anti-inflammatory, antiproliferative, and anti-itching activity, and provide additional protection against coronary heart disease, colon cancer, and skin irritation (Meydani, 2009). The amount of aventhramides (around 20 µg/g in whole grain oats) has been shown to be higher in a bran-enriched oat fraction (63 µg/g), and especially in the sieve-separated endosperm cells walls (110 µg/g), where as a fraction rich in oat proteins contained only a low amount of avenanthramides (7 µg/g) (Sibakov et al., 2010). According to Bryngelsson et al. (2002), processing (especially drum drying of steamed rolled oats) can result in significant losses of tocopherols, tocotrienols, cinnamic acids and avenanthramides.

1.2 Processing of oats for high-fibre ingredients

Oats are a challenging raw material for food processes, as they contain high amounts of lipids (most commonly 5–9% d.m.) (Brown et al., 1966; Brown and Craddock, 1972). Lipids complicate the milling of oats as the material easily sticks on the surfaces of processing equipment. Lipids may also cause structural and sensory problems in the final food products. The structural problems can be encountered for example in expanded products or beverages and liquid foods, where fat can appear as a floating layer on the top of the product. The sensory problems are usually linked to the enzymatic hydrolysis of acylglycerols, and non-enzymatic oxidation of unsaturated fatty acid moieties acylated to polar lipids (Lehtinen 2003). These reactions easily lead to rancid off-flavour of oat products. The rancidity can appear already within a couple of weeks after the oat grain has been crushed, and the reactions can also occur at low water activities (Deane and Commers, 1986).

1.2.1 Dehulling, kilning and flaking

In commercial oat mills, hull is removed prior to other processes (Deane and Commers, 1986). Dehulling is based on a combination of impact and abrasion forces. The miller tries to maximise hulling efficiency while minimising groat breakage. Moisture content, groat/hull percentage and kernel weight are the most important kernel characteristics influencing hulling efficiency. For example, a kernel moisture content of 12–13% is generally recommended. The stream exiting the dehuller contains a mixture of groats, hulls, unhulled oats, broken groats, groat chips and fines. The groats (i.e. hulled grains) and unhulled oats are first separated from this mixture by aspirators. Then, the groats are separated from the remaining oats by table or paddy separators, in which the grains of equal size and shape can be separated based on the differences in specific gravity and the smoothness of the grain. The unhulled oats are recycled back to the dehullers (Girardet and Webster, 2011).

A traditional way to avoid the development of rancidity in dehulled groats is to apply a heat treatment with steam, a process called kilning. The primary aim of kilning is to inactivate lipid hydrolysing enzymes: lipases and peroxidases (Gates 2007). Peroxidase is the most stable of oat lipid-hydrolytic enzymes, and its removal represents the gold standard for assuring optimum product stability. Proper control of the time-temperature-moisture profile is important for the flavour and nutritional quality of final oat products. The intensity of the flavour components is dependent on the temperature and duration of the kilning, but too high temperature or too long treatment time can decrease the content of heat-labile components, such as vitamin B_1 (Girardet and Webster, 2011) and initiate thermal oxidation reactions (Faure et al., 2014; Kivelä et al., 2011).

The kilning times and temperatures can be very different, depending on the process applied. For example, in Germany the typical retention time is about 90–

120 min and temperature decreases from 102 to 69 °C in the heating section (Ganssmann and Vorwerck, 1995). An Australian study described typical conditions to be steaming for 9 minutes, kilning at 100°C for 45 minutes, holding at 65°C for 15 minutes and cooling to room temperature (Zhou et al., 2000). A Finnish process uses steaming for 2–3 min at 100°C (moisture content is increased from 12–13% to 16–17%), and retention at >95°C for 70 min. The groats are then dried to 13% moisture for 30 min (Salovaara, 1993). According to Gates (2007), even 20 s of steaming can be sufficient to inactivate the lipid-hydrolysing enzymes, providing that the oats are tempered at 100 °C for 30 min. However, the kilning process in industrial scale requires much longer tempering times, because a large volume of grains is difficult to mix to an even temperature and moisture distribution. The groat moisture content after kilning is generally 9–12%, and groats are relatively fragile (Girardet and Webster, 2011).

In Finland, the most popular way of using oats is in the form of flakes, mainly to prepare porridge (Finnish Oat Association, 2014). Oats can be flaked immediately after the drying phase in the kilning process, if the steaming period in the kilning has been long enough to inactive the lipid hydrolysing enzymes. More commonly, an additional steaming is applied to the dried groats after kilning in order to plasticise the groats. Steam softens the bran, and gelatinises starch near the surface of the groat, which helps the bran to adhere to the surface of the flake. This protects the flake from attrition and results in less disintegration. Typically, 3–5% moisture is added in the form of steam and the moisture of the groats is allowed to equilibrate in a tempering chamber for 20–30 min. During tempering, groat temperature is increased to 95–102 °C.The flaking itself is performed by passing the tempered groat through a pair of flaking rolls.Typical flake designations and sizes are: jumbo flakes 0.7–1.2 mm, small flakes 0.4–0.5 mm and quick-cooking flakes 0.3–0.4 mm. The flakes exiting the flaking rolls are cooled to 5–10 °C above ambient temperature and dried to 10–12% moisture content (Ganssmann and Vorwerck, 1995; Girardet and Webster, 2011).

1.2.2 Oat flour milling and bran separation

Oat flour can be milled from stabilised groats or flakes. Grinding is usually done in a pin disc, hammer or roller mill. The high fat content of the groats makes it necessary to use a large volume of exhaust air drawn through the mills to keep the screen perforations open, to prevent fine product from sticking inside the screen and on the beaters, and in order to prevent over-heating of the machine. If the milling is performed solely by a roller mill, only a very gradual reduction of the groats is carried out at each passage to prevent the oat material from sticking in the roll corrugations (Deane and Commers, 1986). Oat bran can be separated from the flour in one or several grinding and sieving operations (Gould et al., 1980). The coarse bran fraction contains the major part of the cell walls, whereas the fine fraction (endosperm flour) has most of the starch and protein of the original kernel (Gould et al., 1980; Paton and Lenz, 1993).

Steaming of the groats or flakes can improve the separation of bran by milling, as the bran layers of oat kernel will remain more intact, i.e. they are easier to separate from the starch-rich fine particles of the endosperm (Lehtomäki and Myllymäki, 2010). However, the disadvantage of steaming in terms of β-glucan separation is that the cell walls can agglomerate with the partly gelatinised starch on the surface of the bran particles, thus making further separation of cells walls from starch more challenging. Thus, Doehlert and Moore (1997) proposed 12% moisture content for optimal separation of bran from the adhering starchy endosperm.

1.2.3 Dry fractionation

Dry and wet fractionation methods have been developed to separate fibre-rich cell walls, starch or protein from each other. Most of the fractionation schemes have been developed to enrich cell walls rich in β-glucan. Other components, such as starch, protein and lipids can also be collected as partially purified fractions at the same time. However, the separation of a purified starch fraction is difficult, because the starch granules in oats are significantly smaller than those in wheat, rye and barley, and form aggregates (Hartunian-Sowa and White, 1992; Hoover and Vasanthan, 1992).

Dry fractionation methods are often superior to wet extractions when developing ingredients for food products. It is usually more economical to use dry methods, as they avoid the need for energy-intensive drying steps. In addition, the mass yields of the fractions are often much higher than in the case of wet extracted products. Dry fractionation leads to partial purification or enrichment, which is usually sufficient for food products. However, when aiming at substantially more purified fractions, a dry fractionation step can be used as a pre-processing method prior to the final purification step as described by Redmond and Fielder (2004). In addition, dry processes are usually more feasible, as all the residual fractions (such as starchy endosperm) are utilisable without extensive drying and the microbiological risks related to the storage of wet material after fractionation.

Patented dry fractionation methods of oats are presented in Table 2. For example, Mälkki et al. (2001) developed a dry fractionation process based on two or more subsequent millings and air classifications. The final product is characterized by having a β-glucan concentration of 11–25% (e.g. 16.9% β-glucan with a yield of 21.3% calculated from the dehulled oats). Similar concentrations have been obtained by Knuckles et al. (1992) (e.g. 27.2% β-glucan with a yield of 9–10%), Lehtomäki and Myllymäki (2010) (e.g. 20.5% β-glucan with a yield of 11–12%), Wu and Doehlert (2002) (e.g. 20.3% β-glucan with a yield of 15–16%) and Wu and Stringfellow (1995) (e.g. 17.7% β-glucan with a yield of 28%). The patent of Hellweg et al. (2009) reported only 7–10% β-glucan concentrations, but the yield of the coarse fraction (around 40%) was higher than in other methods, and closer to that of regular oat bran.

Heneen et al. (2009) demonstrated that the lipids in oat endosperm are linked to protein and starch. Thus, the removal of lipids can enable more efficient separation

of these components by dry fractionation, as shown by the higher β-glucan concentrations (Knuckles et al., 1992; Wu and Doehlert, 2002; Wu and Stringfellow, 1995) compared to methods without lipid removal (Lehtomäki and Myllymäki, 2010; Mälkki et al., 2001). Commercial Oatwell® oat bran concentrates from Swedish Oat Fibre AB contain 14–28% β-glucan. The production of Oatwell® is based on lipid removal by ethanol extraction, dry milling and air classification (Girardet and Webster, 2011; Weightman et al., 2002). The process developed by Kaukovirta-Norja et al. (2008) utilises super-critical carbon dioxide (SC-CO_2) to remove lipids from non-heat-treated oats. In this way, oats can be separated into fractions enriched with starch, protein and β-glucan, the β-glucan concentration of the latter fraction being as high as 35%, with a yield of 8–9%. After enrichment with dry fractionation, the M_w of β-glucan usually stays close to its natural form. However, none of the patents in Table 2 has reported the M_w-values. The M_w of β-glucan in the bran fraction described by Kaukovirta-Norja et al. 2008 was characterised in Publications II–IV (780 kDa). The M_w in OatWell® bran concentrate was reported to be 1930 kDa by Tosh et al. (2010).

Table 2. Patented dry fractionation technologies for enrichment of oat β-glucan.

Description of the β-glucan fractionation	BG content (%), as is basis	Reference
Oat bran or oat bran concentrate is milled and fractioned by sieving or air classification to enrich β-glucan in the coarse fraction. The process can consist of one or several steps.	15–25	Lehtomäki and Myllymäki, 2010
Oat material is milled and sieved without defatting. The first coarse fraction is flaked and further milled and sieved to enrich β-glucan.	7–10	Hellweg et al., 2009
Non-heat-treated or minimally heat-treated oat material is subjected to lipid removal by super-critical CO_2. Defatted material is milled and air classified to separate coarse bran fraction and fine endosperm fraction. β-Glucan is concentrated by repeating the milling and classification to remove fines from the coarse bran material.	30–40	Kaukovirta-Norja et al., 2008
Oat material is milled and air classified without defatting to separate coarse bran fraction and fine endosperm fraction. β-Glucan is concentrated by repeating the milling and classification to remove fines from the coarse bran material.	11–25	Mälkki et al., 2001

1.2.4 Wet fractionation

Fractions with high purity of certain compounds, e.g. β-glucan, can be produced by wet fractionation methods. Several factors, such as particle size, pH and temperature, affect the yield of extracted β-glucan in aqueous systems (Wood et al., 1978b; Dawkins and Nnanna, 1993). Wet processes are often limited by the high viscosity of the aqueous extracts even at low β-glucan concentrations. Thus, large volumes of liquid are needed, which results in high costs related to the drying and/or solvent recovery steps. The high water content is also challenging in terms of microbiological quality, stability of oat lipids and utilisation of the residual side streams.

The purification of β-glucan from oats by wet fractionation is usually based on the following technologies: enzymatic hydrolysis of non-β-glucan components, acid, base or solvent precipitation, and wet sieving of the water insoluble materials. The purity of β-glucan in liquid extracted fractions can be as high as 95% (Table 3). For example, in a process based on α-amylase-assisted hot water extraction, the solid particles containing β-glucan are separated and dried after the extraction (Inglett, 1991). The product can be further modified by separating the soluble fibre into a specific product by mechanical shearing of solids containing β-glucan in hot water. From this, non-soluble fibres are removed by filtration or centrifugation, and the liquid is dried to produce the soluble β-glucan concentrate (Inglett, 1997).

Another possibility is to use aqueous ethanol extraction in combination with enzymatic treatment, which permits hydrolysis of starch and protein but prevents solubilisation of β-glucan (Vasanthan and Temelli, 2002). The β-glucan concentrate can be recovered from the slurry by screening or filtering. By the method of Vasanthan and Temelli (2002), 3.6% β-glucan in one oat flour could be concentrated up to 24.8–32.9% (yield 12.4–11.7%) and 7.3% β-glucan in another oat flour up to 30.2–44.2% (yield 20.7–14.0%).

Kvist et al. (2002) and Kvist and Lawther (2005) developed a β-glucan enrichment method based on xylanase and/or β-glucanase treatment and wet milling, followed by sequential centrifugation and ultrafiltration. The enzymatic treatment is to facilitate the release of β-glucan from the matrix. The β-glucan (initially around 14% on a dry matter basis) can be further concentrated by freezing / thawing and precipitation, resulting in a β-glucan content up to 34–57%. The M_w of the product ranges between 800 and 3000 kDa, but no information on the yield was presented. In the method of Potter et al. (2002a;b), milled oat bran was slurried with cold water and screened to remove starch. The material, which did not pass through the screen, was extracted with alkaline solution to solubilize β-glucan. Proteins could be precipitated from the solution by acidification. The remaining solution could either be evaporated or micro-filtered to collect the β-glucan concentrate. The concentration of β-glucan was up to 50–95% and the M_w was 50–2400 kDa.

In the process of Redmond and Fielder (2004), oat bran was purified by air-classification or sieving, and then extracted in alkaline conditions (pH 9–10). The

solids were removed by centrifugation and a flocculant or coagulant was added to the solution to precipitate proteinaceous material. Amzylolytic enzymes could be used to hydrolyze starch. Finally, β-glucan was recovered from the solution by ethanol precipitation followed by centrifugation. The concentration of β-glucan could be up to 75–92% (yield 1.2–1.6%), with a M_w range of 1000–2000 kDa.

Table 3. Patented wet fractionation technologies for enrichment of oat β-glucan.

Description of the β-glucan fractionation	BG content (%), as is basis	Mw of BG (kDa)	Reference
Aqueous low β-glucan product is subjected to a concentration process by deep freezing and subsequent thawing. Thus, material containing β-glucan can be separated as a precipitate on top of the aqueous solution.	30–58	800–3 000	Kvist and Lawther, 2005
Milled grains are extracted with alkaline solution (pH 9–10). Starch is enzymatically digested after neutralisation. Purified β-glucan is obtained by alcohol precipitation.	85–100	n.d.	Redmond and Fielder, 2004
Oat flour is slurried with 40–50% ethanol and filtered using a 40–75 µm screen. The residue is treated with a combination of sonication, protease and α-amylase. Thus, oat starch and protein are washed away, and alcohol-insoluble β-glucan remains intact within the cell walls.	24–41	n.d.	Vasanthan and Temelli, 2002
Bran is subjected to a combination of enzyme treatment and wet milling. Enzyme is inactivated by heat treatment and insoluble phase containing a cleaned bran is separated by centrifugal forces.	n.d.	n.d.	Kvist et al., 2002
β-Glucan source is extracted with alkaline solution (pH 10). A flocculate is formed after neutralisation, heating and cooling of the extract. The flocculate is removed by centrifugation. The supernatant can be dried as such (30–45% β-glucan concentration), or further concentrated by ultrafiltration or by collecting the spontaneously formed β-glucan films after heating the solution in an evaporator.	30–95	400–1 000	Potter et al., 2002a; 2002b
Thermostable α-amylase is used to convert the gelatinised starch in oat flour or bran to maltodextrins. After inactivation of the enzyme, solubilised portion (mainly β-glucan and maltodextrin) is recoved by centrifugation.	n.d.	98 % of molecules >DP 9	Inglett, 2000; 1997; 1991

β-Glucanase activity of oat flour is first inactivated. The flour is treated with proteolytic enzyme, such as trypsin, to enrich β-glucan due to the degradation of protein. β-glucan is then extracted with hot water, precipitated from the solution by an organic solvent and dried.	10–36	n.a.	Mälkki and Myllymäki, 1998
A β-glucan-containing bran is extracted with alkaline solution (pH > 10). Amylolytic agent is added to degrade the contaminating starch. Degraded starch is removed and alcohol is added to precipitate β-glucan from the solution.	n.d.*	n.d.	Bhatty, 1996
Oat or barley grains are slurried in cold water, which may contain an organic solvent. The slurry is homogenized and screened to obtain a β-glucan-enriched fraction from the screens. The obtained moist β-glucan fraction is dried as rapidly as possible.	15–40	n.d.	Lehtomäki et al., 1990

n.d. = not described

n.d.* = not described, but it is mentioned that 50–80% of β-glucan in the initial raw material can be recovered by this method

1.3 Functionality and modification of oat dietary fibre

Studies on physiological responses related to oat β-glucan have mainly been conducted with oat brans or oat bran concentrates in different food products. Only a small amount of research has been carried out on purified oat dietary fibre components other than β-glucan. Thus, this section concentrates mainly on the physiological effects and biochemical properties of oat β-glucan. However, the functionality of oat dietary fibre ingredients is not only dependent on β-glucan, but also on other components, such as arabinoxylans and fibre-associated compounds. The composition and structure of dietary fibres in oat bran or oat bran concentrates can have a significant effect on the properties of the food product as well as on the physiological responses.

1.3.1 Physiolocial effects of oat β-glucan in humans

Several studies and meta-analyses have shown that oat β-glucan can reduce LDL cholesterol (low-density lipoprotein) in hypercolesterolemic subjects (Ripsin et al., 1992; Othman et al., 2011). A review of Othman et al. (2011) came to the conclusion that intake of oat β-glucan (at least 3 g per day) may reduce blood total and LDL cholesterol levels by 5–10% in normo- or hypercholesterolemic subjects. However, the effect appears to be negligible in young healthy adults (Ibrügger et al., 2013). The mechanisms of oat β-glucan's cholesterol-lowering effect are still not fully understood.

One of the most potential explanations is that the viscous oat β-glucan encapsulates bile acids, resulting in their excretion in the faeces. Bile acids are recycled, taken up in the lower part of the intestine and used again. Through excretion in faeces, the body loses bile acids and has to synthesise new ones in liver. Cholesterol is a building block for bile acids, and liver extracts it from the blood, leading to a decreased cholesterol level in the blood (Lund et al., 1989; Andersson et al., 2010; Bae et al., 2010). Another theory is that oat β-glucan interferes with the absorption of lipids, as well as decreasing the absorption of intestinal cholesterol (Mälkki et al., 1992; Marlett et al., 1994; Lia et al., 1995; Naumann et al., 2006; Poppitt, 2007). Several studies have also shown that soluble fibres are fermented in the colon, giving rise to short-chain fatty acids that can be absorbed and may inhibit hepatic cholesterol synthesis (Marlett, 1997; Andersson et al., 2002).

The postprandial rises in blood glucose and insulin have been shown to be reduced by viscous solutions of oat β-glucan (Jenkins et al., 1978; Braaten et al., 1991; Wood et al., 1994; Hallfrisch et al., 1995; Biörklund et al., 2005; Tapola et al., 2005; Panahi et al., 2007). The viscous solution absorbs fluids and results in an extended digestion period. When digestion is delayed, blood sugar increases more slowly, causing a low insulin response. This effect was confirmed by Battilana et al. (2001) and Jenkins et al. (2002), but the course of events leading to the effect is not fully understood. One hypothesis is that food in the intestine is 'incorporated' in the viscous oat β-glucan solution, making it more difficult for enzymes to degrade the food components and causing digestion to take longer. Another hypothesis is that oat β-glucans form a protective layer along the intestinal wall that acts as a viscous barrier, slowing food uptake from the intestine (Duss and Nyberg, 2004).

The thick and viscous fluid formed by oat β-glucan in the stomach and small intestine can also stimulate the sensation of satiety and help to limit appetite. As described above, oat β-glucan can extend the period of digestion, and thus nutrients are utilised by the body for longer periods. This may contribute to an increased sensation of satiety and provide advantages in weight management (Lugwig, 2000; Juvonen et al., 2009; Lyly et al., 2009; Pentikäinen et al., 2014).

1.3.2 Rheological behaviour of oat β-glucan

Molecular weight (M_w) distribution is an important characteristic of oat β-glucan, since very different rheological behaviour may be observed depending on the M_w. At low concentrations, very little interaction occurs between the isolated β-glucan polymer domains, and viscosity is close to Newtonian. The shear viscosity diagrams usually show three different linear regions for dilute, semi-dilute and concentrated solutions, and the points separating them are called critical concentrations c* and c** (Doublier and Wood, 1995; Lazaridou et al., 2003; Skendi et al., 2003). The first critical concentration c* between dilute and semi-dilute solutions represents the state when the molecules begin to interact with each other in the solution. The second critical concentration c** between the semi-dilute and concentrated region

represents the beginning of entanglements. In the concentrated region, the viscosity increases more intensively with increasing concentration.

The critical concentrations for HM_w oat β-glucan ($M_w \approx 900$ kDa) were reported by Doublier and Wood (1995) to be $c^* \approx 0.1\%$ and $c^{**} \approx 0.3\%$. However, Ren et al. (2003) analysed the same samples and found only two regions and one critical concentration with 0.2–0.4% of β-glucan. In the concentrated region, the shear viscosity of oat β-glucan is shear thinning, and in the dilute region it is Newtonian. The shear thinning behaviour is due to decline in the entanglements resulting from the orientation of the chains in the flow. This is supported by the observations of Grimm et al. (1995), Böhm and Kulicke (1999) and Tosh et al. (2004), who showed that in the diluted region, oat β-glucan occurs as fringed micelle-type aggregates, which grow side-to-side via hydrogen bonding of the cellotriose-sequences. In addition, Vårum et al. (1992) observed that only a fraction of the molecules was involved in association to form large stable aggregates.

Agbenorhevi et al. (2011) showed that the solution viscosity was higher for HM_w samples when oat β-glucan molecules with different M_ws (142–2800 kDa) were compared at the same concentrations (0.01–8.0% w/v). Increased critical concentration (c^*=0.25–1.10%) with decreased M_w of β-glucan (from 2800 to 142 kDa) showed that coil overlap occurred at lower concentrations in the case of HM_w samples. A similar trend in critical concentrations (c^*=0.5–2.0%) was also observed by Böhm and Kulicke (1999) for hydrolysed barley β-glucans (M_w range between 375 and 40 kDa, respectively).

Partially depolymerised oat β-glucan has been shown to form gels (Doublier and Wood, 1995). The loss modulus (G'') decribes the liquid flow component, in which the deformation energy from applied stress is dissipated as frictional heat, and the storage modulus (G') represents the solid or gel-like component, in which energy is stored in elastic deformation (Wood, 2011). The change in G' observed at different frequencies of measurement indicates the type of intermolecular interactions present; G' is relatively constant across different frequencies (frequency-independence) when gel networks are present but G' changes at different frequencies (frequency-dependence) when molecular entanglements are present. G'' measures the ability of a substance to flow, and a high G'' value is observed for solutions with high viscosity. The frequency-independence of G'' under dynamic shear is observed for solutions of high concentration and a low tendency to flow. At a given frequency, substances with low ratio of G''/G' are said to be more solid-like than liquid-like (Kwong et al., 2013a).

The crossover point of G'' and G' is at a frequency that is lower for higher concentrations and/or Mw of β-glucan molecules in freshly prepared solutions (Ren et al., 2003; Lazaridou et al., 2003). When 8% (w/v) aqueous solution of around 100 kDa oat β-glucan was studied in a rheometer, it was noticed that a typical liquid-like mechanical spectrum (G'' > G') was observed at all frequencies until 15 h. Between 15 and 40 h, G' (at 1 Hz) increased much more rapidly than G'', until it exceeded G'' and became much less frequency-sensitive, showing typical characteristics of a gel system (Lazaridou et al., 2003).

Lazaridou et al. (2003), Skendi et al. (2003) and Tosh et al. (2003) concluded that the rate of gelation increased when the M_w of β-glucan decreased. They showed a rapid increase in the gelation rate when the M_w fell below 60 kDa, whereas molecules with M_w > 250 kDa did not form any kind of gel during 200 h of storage. The link between low M_w fractions and higher aggregations has been proposed to be due to greater mobility of low M_w fractions increasing the probability of interactions between the β-glucan chains (Doublier and Wood, 1995; Lazaridou et al., 2003; Skendi et al., 2003; Tosh et al., 2004). An exponential correlation between the gelation rate and concentration has been found, and the critical gelling concentration (below which gel formation is not observed) was found at 3.5–4% for oat β-glucans of M_w 35 and 110 kDa (Lazaridou et al., 2003). However, the gelling was temperature-dependent, for example the gels formed at 45 °C were much weaker than those formed at 25 °C. Lazaridou et al. (2003) and Tosh et al. (2003) showed that β-glucan gels melt over a temperature range, rather than with a sharp transition. In addition, the melting temperature increased as the age of the gel increased, but the gels of lower M_w β-glucans generally had lower melting temperatures.

1.3.3 Thermal and mechanical modification of oat dietary fibre

Most of the studies aiming towards modification of oat dietary fibres have concentrated on the content and M_w of β-glucan before and after the modification. Thus, there are not many results on the effect of the modification on arabinoxylan and other fibre-linked compounds. Thermal or thermomechanical modification of dietary fibres often occurs during baking and extrusion processes. For example, the M_w of β-glucan in oat-based breakfast cereals can be reduced by extrusion processing. Tosh et al. (2010) produced cereals with 1930–251 kDa β-glucan by varying the temperatures (181–237 °C) and water contents (7.0–18.7%). In the reduced molecular weight breakfast cereals (251–950 kDa), temperature and pressure in the extruder were higher than those used to produce 1930 kDa cereals. The increasing energy input in the extruder caused disruption of the cell walls and protein bodies as well as solubilization of the starch and β-glucan (Tosh et al., 2010).

The properties of oat dietary fibre have also been modified by a microfluidization process as described by Chen et al. (2013). They showed that microfluidization increased the solubility of oat insoluble fibre (IDF) preparation (Herbacel Classic Plus HF 06, Werder, Germany) from 10.0 to 36.5%, and improved the water holding (from 3.0 to 6.2 ml g^{-1}) and oil holding (from 1.8 to 6.7 ml g^{-1}) capacities of the IDF preparation. The study of Chen et al. (2013) was based on an IDF ingredient manufactured from oat hull, and cannot be compared to oat bran-based dietary fibres. However, the greatly reduced particle size due to high-pressure microfluidization which leads to an increased surface area, could also contribute to the improved water holding capacity of bran-based fibres. Microfluidization could also increase the porosity and capillary attraction of hull- or bran-based fibres, and consequently enhance the physical entrapment of oil and increase the oil holding capacity.

Zhang et al. (2009) compared the effect of steam heating, extrusion and superfine grinding on the solubilisation of oat bran fibre. The yields of oat soluble fibre fraction (SDF) were 5.9% from untreated, 7.2% from steam heated, 12.3% from extruded and 16.6% from super-fine ground oat bran. The total dietary fibre contents of SDF varied from 81.4% to 83.9%. The solubility of the SDF-fractions varied between 47.2 and 87.8%, being highest after super-fine grinding and lowest after extrusion. The proportions of the fraction with a M_w higher than 500 kDa in the SDF were 31.3% in untreated, 30.3% in steam heated, 97.4% in extruded and 37.3% in superfine ground oat bran. The results showed that the superfine grinding and extrusion processes induced the effective extraction of HM_w SDF from oat bran, but in the steam heating the proportion of the fraction with molecular weight of more than 500 kDa was decreased. Similarly, Sharma and Gujral (2013) showed with eight different barley varieties that the ratio of soluble to insoluble β-glucan (originally 0.7–1.5, depending on the variety) was changed to 1.2–3.1 after extrusion at 150 °C and 15% moisture content.

Gaosong and Vasanthan (2000) presented even higher increases in the water solubility of barley β-glucan. The solubility of β-glucan was increased from 27 to 41% with a regular barley variety (with 3.9% total β-glucan) and from 42 to 90% with a waxy barley variery (with 6.4% total β-glucan) when using 140 °C extrusion temperature and 50% moisture content for barley flours. These results were supported by the observations of Huth et al. (2000) that β-glucan solubility from extrudates prepared from barley is mainly affected by the moisture of feed rather than by the temperature of extrusion cooking.

Another form of dietary fibre made from oats is amylose-lipid complex which is only partly digested in the small intestine (Lehtinen et al., 2004). The formation of this complex is based on extrusion with limited water-content (21–26%) to form a plastic mass, which leads to an increased concentration of damaged starch, as described by Case et al. (1992). According to Lehtinen et al. (2004), the damaged starch acts as a binding agent for the naturally occurring or added lipid, such as rape seed oil. The lipid is located inside the α-helix of the amylose molecule, as investigated by Neszmélyi et al. (1987) and Karkalas et al. (1995). Amylose-lipid complex has been proposed as resistant starch type 5, because of its resistance to enzyme hydrolysis. The resistance depends on the molecular structure of the lipid and the crystalline structure of the single helices. The increased resistance is due to the restricted swelling of starch granules during cooking (Hasjim et al., 2013). In addition to potential health benefits, the amylose-lipid complex can provide an advantage in baking applications, such as improving the water retention of wheat flour (Lehtinen et al., 2004).

1.3.4 Chemical and enzymatic modification of oat dietary fibre

The glycosidic linkages of β-glucan have proved to be stable at low pH (1.5) at 25–37 °C (Bhatty, 1992, Johansen et al., 1993). Johansson et al. (2006) reported that at 37 °C no degradation of β-glucan was observed with 0.1 M HCl over a 12 h

period. By contrast, at 120 °C total hydrolysis to D-glucose occurred with 3 M HCl already after 1 h hydrolysis. Hydrolysis with 0.1 M HCl at 120 °C for 1 h produced a range of products, but the majority of the resulting oligosaccharides had very low M_w (DP 1–7). Bhatty (1992) extracted β-glucan from several oat cultivars at 25 °C in acid, neutral and alkali conditions. The extracted amounts of β-glucan were 18–23% (pH 1.5), 28–55% (pH 6.0) and 17–44% (pH 10). Thus, it could be concluded that neutral pH gave the best yield. However, the viscosity of the extract was much lower at pH 6.0 (4 cSt) compared to acid (18 cSt) or alkali extracts (14 cSt), probably indicating enzyme action during the extraction at pH 6.0. Unfortunately, this study did not report the M_w values of extracted β-glucan. Tosh et al. (2004) investigated the ability of acid- (HCl) and enzyme-catalysed (lichenase and cellulase) hydrolyses to produce LM_w β-glucan molecules (31–237 kDa). Independently of the hydrolysis method, all 6% oat β-glucan solutions with M_w < 150 kDa formed gels at 5 °C in less than one week. The authors also demonstrated that the time required for a gel to form became shorter in relation to the reduction in Mw.

Oat β-glucan can also be depolymerised by an oxidative-reductive mechanism. Kivelä et al. (2009a; 2011) showed that ascorbic acid and H_2O_2 in the presence of ferrous ions (Fe^{2+}) can induce oxidative-reductive depolymerisation of β-glucan. This was explained by the Fenton reaction, which generates $^{\bullet}OH$ radicals capable of oxidising β-glucan. More recently, Faure et al. (2013) demonstrated that Fe^{2+} ions in aqueous solution of β-glucan can alone cause depolymerisation of β-glucan when heating the solution to 100 °C. At lower temperatures the radical-catalysed reactions were significantly slower, but an addition of ascorbic acid or H_2O_2 clearly enhanced the degradation of β-glucan (Faure et al. 2013; 2014). For example, the M_w of 1 wt.% β-glucan solution was degraded from 538 kDa to molecules smaller than the detection limit of the light scattering analysis already after 2 h of storage at 85 °C when 100 mM H_2O_2 was added to the solution. Without H_2O_2 addition, the M_w remained 513 kDa after 2 h and 318 kDa after 1 week of storage at 85 °C (Faure et al., 2014).

If the aim is to keep the M_w of β-glucan as intact as possible, the unwanted β-glucan-depolymerising enzymes can be denatured at high temperature. However, the heat-based denaturation is not an instantaneous process, and the enzymatic reactions are easily accelerated at elevated temperatures. Thus, the denaturation temperature should be reached as rapidly as possible (Wood, 2011). The same is true for alkaline conditions: although β-glucanase activity is suppressed at high pH, the treatment does not necessarily fully denature and deactivate the enzymes. Even a small amount of residual activity may still exert a significant effect over long periods of storage, especially in aqueous conditions (Wood et al., 1978b; 1989).

Henriksson et al. (1995) reported that a purified cellobiohydrolase II preparation (i.e. a cellulase enzyme) hydrolysed only (1→4)-linkages of barley β-glucan at 37 °C in high water content. No high molecular weight hydrolysis products could be detected after 2.5 h, i.e. the Mw had decreased to below 10 kDa. They found a rapid increase in the amount of reducing sugars during the first 24 h of hydrolysis.

After this, the hydrolysis slowed down. The dominant hydrolysis end products were di-, tri- and tetrasaccharides, with an average oligosaccharide length of 3.1.

In processes aiming at partial depolymerisation of β-glucan, different kinds of depolymerising enzymes can be utilised, e.g. β-glucanases, which are usually specific for (1→4)-β-D-linkages (McCleary and Matheson, 1987), or lichenases, which are known to cleave only the (1→4)-β-D-linkages adjacent to (1→3)-β-D-linkages of β-glucan, resulting in a mixture of mostly linear (1→4)-β-D-linked units in the dispersion (McCleary, 1988; McCleary and Codd, 1991). High amounts of linear (1→4)-β-D-linked units can enhance the gelling behaviour of the extracted β-glucan molecules (Tosh et al., 2004). Extrusion technology at low water content can also be utilized in combination with enzyme (Lehtomäki and Myllymäki, 2009) or acid-catalyzed hydrolysis (Kaukovirta-Norja et al., 2009) to solubilize and partially depolymerise oat dietary fibres (further discussed in Section '1.4.3. Liquid food products').

1.4 Use of oat bran and β-glucan for fibre-fortification

Oat bran is a widely used source of dietary fibres in various food products, such as porridges, breads and biscuits. However, problems related to the texture and shelf life restrict the quantity of oats in most of these products. This section reviews the effects of oats in several different food matrices, with special attention to the properties of dietary fibres before and after processing.

1.4.1 Baked products

The most common way of using oats in baking is to combine oats and wheat, even though oats usually reduce the baking quality due to the lack of gluten proteins and the high content of dietary fibres (Oomah, 1983). If oat bread is intended to meet the requirements of the β-glucan content needed for a cholesterol-lowering claim (1 g/portion and 3 g/day) (EFSA, 2011a) or the claim for the reduced risk of heart disease (0.75 g/portion and 3 g/day) (FDA, 1997), addition of at least 50% whole grain oat flour of the weight of the bread is needed. Another option is to use oat bran or oat bran concentrate to reach the required level of β-glucan. However, when aiming at high volume oat-based breads, addition of gluten (e.g. 13% gluten + 87% oat flour) is usually needed (Londono et al., 2014).

The high β-glucan content of oats is responsible for the increased water absorption and mixing requirements when compared to wheat dough (Zhang et al., 1998). Although Krishnan et al. (1987) reported that 10% addition of oat bran gave better stability than that of 100% wheat-based dough, it is generally agreed that increasing the supplementation level of oat bran, flakes or flour reduces the specific volume of breads (Flander, 2012). The specific volume of oat breads has been reported to range from around 1.0 (70-100% oats of the flour weight) (Kim and Yokoyama, 2011, Tiwari et al., 2013) to 5.8 ml/g bread (10–20% oats of the flour weight) (Zhang et al., 1998).

Tiwari et al. (2013) studied the substitution of wheat flour by 0–70% of oat flour or oat bran in bread. The specific volume was observed to decrease in line with the amount of wheat flour substituted by oats. Crumb hardness was observed to be negatively correlated with the specific volume of oat-supplemented breads. β-Glucan content was found to increase from 0.1 to 1.4 or 3.6% (d.w.) when 70% of wheat flour was substituted with oat flour or oat bran, respectively. The results showed that a substitution level of 50% for oat flour and 30% for oat bran still enabled the preparation of bread with acceptable quality. Similar results were obtained by Flander et al. (2007) (2.4% d.w. β-glucan in the final bread) when replacing 51% of wheat flour with whole grain oat flour. They reported a decrease in the M_w distribution of β-glucan in bread (30% of high M_w > 1 000 kDa; 30% of medium M_w = 200–1 000 kDa; 40% of low M_w < 200 kDa) as compared with the original β-glucan of whole oat flour (60% of high M_w, 30% of medium M_w, and 10% of low M_w). The level of β-glucan in the final bread was 25% lower than in the flour mixture. Tiwari et al. (2013) reported even higher reduction in the level of β-glucan (by 38–43%) in dough during proofing. This most probably indicated a higher activity of endogenous enzymes (probably originating from wheat flour) in the study of Tiwari et al. (2013), because the proofing time (45 min) was shorter and temperature (35 °C) lower than in the study of Flander et al. (2007) (65 min and 39 °C, respectively).

As described above, raw materials and their endogenous β-glucanase activities as well as processing and storage conditions can affect the amount and M_w of β-glucan in baked products. This can weaken the cholesterol-lowering effect of β-glucan (Kerckhoffs et al., 2003; Törrönen et al., 1992) as well as the ability to lower postprandial glycemic response (Lan-Pidhainy et al., 2007, Tosh et al., 2008, Regand et al., 2009). Understanding the influence of processing on β-glucan and the means to control its integrity are extremely important for the functionality of oats in food applications. The M_w values of β-glucan in final products of oat suplemented breads and muffins have been reported to vary between 100 and 2800 kDa (Törrönen et al., 1992; Beer et al., 1997b; Åman et al., 2004; Lan-Pidhainy et al., 2007; Tosh et al., 2008). Cleary et al. (2007) reported that HM_w β-glucan from barley degraded much more during baking (from 640 to 310 kDa) than the LM_w β-glucan molecules (from 210 to 200 kDa). They did not provide a thorough explanation for this, but it appears that higher M_w of barley β-glucan is more susceptible to degradation during bread processing than lower M_w β-glucan.

The texture and flavour of oat breads can be improved by sourdough baking or by using germinated ingredients. Flander et al. (2011) and Rieder et al. (2012) studied the effects of sourdough baking on oat bran-supplemented breads, showing improved bread volume and reduced crumb firmness when using sourdough. Flander et al. (2011) concluded that wheat sourdough did not affect the content of oat β-glucan in the bread. Both straight dough and sourdough bread contained 2.4–2.7% (d.w.) β-glucan. The average M_w of β-glucan was 550 kDa in both types of bread, whereas that of oat flour was 1 000 kDa. This indicated a slight degradation of β-glucan during proofing and baking. Similarly, Rieder et al. (2012) reported

that the M_w of β-glucan in oat bran was reduced during the 18 h fermentation from 630 to 488 kDa, but did not differ much from the non-fermented oat bran after baking in the oven (266 and 308 kDa, respectively).

Germination usually improves the texture and flavour of cereals, but it also generally causes breakdown of β-glucans. The endo-β-glucanases, generated by germination, can depolymerise the β-glucan chain already during malting of oats, and care must therefore be taken when choosing the malting parameters. For example, after six days of germination at 15 °C, the M_w of β-glucan decreased from 2400 kDa to 1500 kDa, after which no β-glucan could be detected with HPLC-SEC. It was interesting that the M_w of β-glucan remained higher than 1500 kDa until the β-glucan was completely degraded. Apparently, the smaller β-glucan molecules (M_w <1500 kDa) were degraded soon after they were formed. As the products of initial degradation are more soluble than the original HM_w β-glucan they may also be more easily degradable (Wilhelmson et al., 2001). In baking applications, it is also common to add barley or wheat malt to bring more flavour to oat-wheat breads, but the endogenous β-glucanases of the malt can reduce the viscosity and M_w of oat β-glucan if the enzymes in the malt ingredient are not properly inactivated prior to baking (Åman et al., 2004).

1.4.2 Extruded products

Extruded products can be divided into two different categories: expanded products (i.e. snacks and breakfast cereals) made by a high-temperature and short-time process, and pasta products made by a low-temperature process. In expanded products, starch is the most important ingredient, ensuring good expansion characteristics and gas-holding properties (Guy, 2001). However, extrusion of oats is challenging due to their high lipid (4–9%) and dietary fibre content (6–9%), and relatively low starch content (45–63%) (Chang and Sosulski, 1985; Wood, 1986; Fornal et al., 1995; Peterson and Wood, 1997; Liu et al., 2000; Vicidi et al., 2004; Yao et al., 2006; Núñez et al., 2010). The high lipid content can decrease the conversion of starch through lubrication, reduced degree of gelatinization, and prevented mechanical breakdown of starch, which can all lead to reduced expansion (Riaz, 2006). Oats have also been shown to have poor gas-holding capacity (Yao et al., 2006). Thus, most of the studies have reported poor expansion, hard texture and high bulk density when using oats in extrusion (Table 4).

Dietary fibres (DF) usually interfere with the expansion of the starch-based matrix. The effect of DF on the texture and structure of extrudates depends mainly on its interactions with starch and on the type and quantity of DF (Sozer and Poutanen, 2013). DF can bind water present in the matrix and reduce its availability for expansion (Moraru and Kokini, 2003). Due to the incompatibility of cereal bran particles with other raw materials, they act as fillers, and influence the mechanical and physical properties, particle size distribution, and orientation of polymers within the food matrix (Robin et al., 2011). If the bran particles are coarse, they can interrupt the matrix, disrupt the bubble wall film and result in bursting of gas cells before

expansion (Guy and Horne, 1988). Thus, fine grinding has been proposed to improve the compatibility of the cereal bran particles and starch to yield better expansion (Alam et al., 2014).

Addition of oat bran was shown to result in poor expansion because the bran particles ruptured the cell wall and made holes in the cells (Guy and Horne, 1988). The holes assisted evaporation of water, which resulted in collapsed expansion and dense extrudates. Addition of oat bran has also been shown to have a negative impact on the crispiness of extrudates (Chassagne-Berces et al., 2011). Nevertheless, up to 18% oat bran can give expanded, porous and low density extrudates when a high-starch ingredient, such as maize semolina, is used as the bulk carrier matrix (Rzedzicki, 1999; Rzedzicki et al., 2000). However, Rzedzicki et al. (2000) recommended that 9–12% oat bran of solids would be the practical application limit in highly expanded products. Rzedzicki and Blaszczak (2005) reported that incorporation of more than 30% of oat bran requires a higher temperature (180–220 °C), although the final product was found to be more compact due to the presence of a high content of DF. Recently, Lobato et al. (2011) suggested that even 37% of oat bran could be blended with other ingredients to obtain good quality extrudates.

Table 4. Effects of adding oats (flour or bran) into extruded products.

Addition level	Other ingredients	Reported effects (when adding the proportion of oats)	Reference
Oat flour			
70%	Rice flour, Sugar, Malt extract	Instable processing due to the polymer stick-slip transition, which was caused by the high lipid content	Núñez et al., 2010
50–100%	Maize bran	Samples with high level of maize bran presented a high breaking strength, due to the smaller expansion	Holguín-Acuña et al., 2008
66%	Wheat starch, Sugar, Salt, Sodium bicarbonate	Bile acid binding of oat containing extrudates ↑	Yao et al., 2006
55–100%	Maize flour	Hardness and Density ↑ Radial expansion ↓	Liu et al., 2000
100%	-	Expansion (at low temperature) ↓ Expansion (at increased moisture and high temperature) ↑ Die pressure (compared to wholegrain wheat flour) ↑ Torque and Specifc Mechanical Energy (compared to wheat) ↓	Singh and Smith, 1997

Oat bran			
20% OBC*	Wholegrain wheat flour	Hardness ↑ Number of peaks and Crispiness ↓	Chassagne-Berces et al., 2011
20–50%	Maize starch, soy flour, inulin	Hardness ↑ (acceptable until 37% addition) Radial expansion ↓	Lobato et al., 2011
10%	Maize flour	Hardness and density ↑	Zarzycki et al., 2010
20–80%	Maize semolina	Hardness ↑ (acceptable until 20% addition) Radial expansion ↓	Rzedzicki and Blaszczak, 2005
9–18%	Maize semolina	Hardness and Density ↑ (acceptable until 12% addition) Radial expansion ↓	Rzedzicki, 1999; Rzedzicki et al., 2000
100%	-	Soluble dietary fibre ↑ Insoluble dietary fibre ↓	Gualberto et al., 1997

* OBC = Oat bran concentrate

Gualberto et al. (1997) investigated the effect of extrusion on the insoluble (IDF) and soluble dietary fibre (SDF) contents of oat bran (originally 8.7 and 3.5%, respectively). In the extruded samples, IDF content ranged from 7.1 to 7.5% and SDF from 4.6 to 5.5%. They explained that extrusion might have led to an increase in the SDF values due to the depolymerisation of the IDF into smaller fibre molecules. There was a decrease in SDF content as the screw speed increased, which may have led to the degradation of SDF due to a breakdown of chemical bonds and production of smaller molecules (Repo-Carrasco-Valencia et al., 2009). Furthermore, extrusion cooking may alter the molecular weight distribution and the ratio of (1→3) and (1→4) linkages of β-glucan which may lead to increased SDF (Zhang et al., 2011).

Yao et al. (2006) produced extruded breakfast cereals (EBC), with 66% oat flour + 30% wheat starch, from two different oat lines with 8.1 and 4.8% β-glucan concent. The EBCs from high and regular β-glucan oats contained 5.3–6.0% and 3.4–3.9% β-glucan, respectively. Changing the extrusion temperature (165–180 °C) or moisture content (16–25%) did not affect the β-glucan concentration of the extrudates. EBCs produced at 165 °C and 16% moisture content with the flour from the high β-glucan oat variety showed greater bile acid binding than those made in other conditions. The greater bile acid binding capacity may have been caused by both a greater amount and a greater solubility of β-glucan (Yao et al., 2006). The original M_ws of β-glucan in these oat varieties were 3 240 and 2 730 kDa, respectively, but unfortunately the authors did not report the M_w values after the extrusion.

In pasta manufacture, oat bran can be added to durum wheat, as long as the substitution does not exceed 5 g/100 g (Bustos et al., 2011a;b). A higher addition of oat bran generates a disruption of the protein-starch matrix so that starch granules became more accessible. The cooking loss of oat bran-enriched pasta was similar to or lower than that in the control pasta when 2.5–5.0% of oat bran was added, but increased significantly at 7.5–10.0% supplementation level. It was hypothesised that at low oat bran concentrations, the fibres may be dispersed and incorporated into the protein-starch matrix. On the other hand, at higher degrees of substitution, disruptions in the protein matrix by oat bran particles became more important, promoting water absorption, and facilitating starch granule swelling and rupture. At 10% substitution, protein losses increased dramatically because the oat bran fibres generated a disruption in the protein network. Despite the observed cooking losses, DF was not significantly lost during cooking (Bustos et al., 2011a;b). Similar findings were observed by adding barley β-glucan concentrate (26.5% β-glucan) into durum wheat pasta. When 7.5% of β-glucan concentrate was added, there was no or minimal changes in the cooking loss, stickiness, water absorption, aroma, and sensory texture compared to the durum wheat control. At higher doses, pasta became browner, firmer, of inferior aroma, more rubbery and chewy (Aravind et al., 2012).

Kaur et al. (2012) investigated the addition of oat bran into wheat-based pasta up to a level of 25%. However, they found that 15% was the maximal substitution possible without adversely affecting the physicochemical, cooking and sensory quality. Padalino et al. (2011) produced gluten-free pasta from heat-treated maize flour and oat bran concentrate with 22% β-glucan content. The oat bran amount added to spaghetti was continuously increased until the overall sensory quality of pasta reached the sensory threshold, i.e. until the concentration of oat bran concentrate was 20%. They attempted to improve the sensory quality of oat bran supplemented spaghetti by the use of structuring agents, such as hydrocolloids and egg white. Most of the structuring agents improved the sensory characteristics of the spaghetti samples, and showed good elasticity and firmness as well as low adhesiveness and bulkiness. Moreover, the structuring agents did not alter the odour and taste of the samples, which remained pleasant despite the high percentage of oat bran. The best overall quality for both fresh and dry spaghetti was obtained by the addition of carboxymethylcellulose and chitosan at a concentration of 2%.

There are not many studies reporting the state of β-glucan in pasta products after processing and cooking. However, Åman et al. (2004) reported the M_w values from experimental macaroni (1880 kDa) and fresh pasta (570 kDa) products. The M_w distribution of β-glucan in fresh pasta showed significant depolymerisation due to the cooking, because 50% of the β-glucan molecules were below 230 kDa, whereas the corresponding value for macaroni was 1770 kDa.

1.4.3 Liquid food products

Oats have been utilized in many types of liquid food products. The best known product is ‘oat milk’, which can be used as a substitute for milk- and soy-based beverages. However, the content of dietary fibre (DF) in oat milks is usually low. For example, one of the leading oat milk products in Scandinavia, Oatly®, contains only 0.8 g DF per 100 ml. β-Glucan fibres can increase the viscosity and change the structure of the product, and therefore the major part of fibres is depolymerised and/or filtered away from most oat milk products (Lindahl et al., 1995; Patsioura et al., 2011).

The production of oat milk is typically based on treating oat flakes or flour in water suspension (10–20% of the dry oat material) with an enzyme preparation, which has the ability to hydrolyse starch but not proteins. Oat starch is hydrolyzed to dextrins, i.e. maltose or glucose, by the action of β- and α-amylases (Lindahl et al., 1995; Öste, 2000; 2002), by α- and β-amylases (Smith, 1995) or by α-amylase and glucoamylases/amyloglucosidases (Lewen et al., 2000; Alho-Lehto and Kuusisto, 2010). The enzymes hydrolyse starch in different manners: α-amylase randomly hydrolyses the (1→4)-α-D-glucosidic linkages in amylose and amylopectin of starch, whereas β-amylase hydrolyses (1→4)-α-D-glucosidic linkages removing successive maltose units from the non-reducing ends of the starch polymer chain, and glucoamylases/amyloglucosidases hydrolyse both 1,4- and 1,6-α-glucosidic linkages in starch and removes glucose units in a stepwise manner for the non-reducing end of the starch molecule (Lewen et al., 2000). In addition to α- and β-amylases, β-glucanase or xylanase can be used to partly depolymerise the viscosity inducing non-starch carbohydrates (Smith, 1995; Nilsson et al., 2003). However, care must be taken so that the β-glucanase is not allowed to catalyse the hydrolysis of β-glucan molecules to too great extent, if the aim is to retain β-glucan in the solution. That is why Smith (1995) recommended to use weight ratio of β-glucanase to oat flour to be in a range of about 0.0004:1–0.004:1 (β-glucanase:oat flour), and a substantially short incubation time (30–90 min).

Salovaara and Kurka (1991) described the production of an oat-based fermented liquid food product by fermenting oat bran. The process includes a heat-treatment of oat bran in water suspension (to gelatinise starch and pasteurise the product) and a subsequent fermentation with lactic acid, bifido- or propionic acid bacteria, or by a mixture of these. Löv et al. (2000) further developed the process, by first heating oat material to 89–95 °C and then homogenising it at a pressure of 150–170 bar. After homogenisation, the product is pasteurised or UHT-treated, and fermented as described by Salovaara and Kurka (1991). However, they were not able to prepare products with high dietary fibre content (i.e. TDF concentration was below 1%), because the viscosity of the product became too high.

According to Alho-Lehto and Kuusisto (2010), a fermented non-dairy beverage product with 25–60% glucose, 0.3–5% maltose and 5:1–10:1 ratio of glucose to maltose would provide a suspension with a balanced sweetness appealing to consumers. However, in their process the viscosity-related problem was solved by

reducing the amount of β-glucan to less than 0.5% in the final product. It was mentioned that the viscosity of the beverage should be at most 100 mPas, but the patent of Alho-Lehto and Kuusisto (2010) also covers spoonable yoghurts and ice cream-like products, which can have a higher viscosity. It is also possible to fortify the beverage product with SDF after the enzymes have been inactivated and the insoluble fraction of the oat raw material has been removed. The suitable SDF can be a 'purified β-glucan', for example, polydextrose, inulin or other fructo-olicosaccharides. The term 'purified β-glucan' refers to soluble β-glucan preparations that do not cause off-flavours or cereal taste in the oat suspension. The molecular weight of the 'purified β-glucan' must be low, preferably at most 200 kDa, to avoid increase in viscosity. One example presented by Alho-Lehto and Kuusisto (2010) described a product containing 78% glucose, 1% maltose, 9% protein and 4% added β-glucan of the dry matter. However, according to the sensory panel, the product was considered too sweet to be consumed as non-dairy milk, and it had a slimy mouth feel which could be suitable for preparing desserts, but not for drinks.

Laakso and Lehtinen (2006) developed a process specifically for oat bran concentrates (OBC) with higher than 15% β-glucan concentration. The OBC-fractions contain only a small amount of starch (5–30%), and thus the effect of starch in causing the viscosity is only minor (keeping in mind that the amount of bran is quite low in beverage applications), and starch degrading enzymes do not have such a crucial role as in the case of liquid products with higher amounts of oat starch. In the process the OBC material is suspended in water at room temperature and homogenised once or several times at a pressure of 300–600 bar. Subsequent homogenisations significantly reduced the viscosity of the water-OBC suspensions. However, the viscosity of the suspensions remained rather high (>300 mPas) when their β-glucan concentration was between 0.9 and 1.2%. When homogenising extruded oat bran concentrate four times at a pressure of 150 bar, Laakso and Lehtinen (2006) obtained a relatively stable water suspension with 0.9% β-glucan with a viscosity of around 100–120 mPas. However, they only reported the viscosity directly after preparation, not the long-term stability of the suspension. Kivelä et al. (2010) also reported that homogenisation can be used to increase the solution stability of oat β-glucan. They showed that a 0.3% β-glucan solution remained stable for several weeks. They explained the increased solution stability by the reduced Mw of β-glucan (initially 1440 kDa, after 1000 bar, 10 min homogenisation: 130 kDa) and by the more rounded β-glucan aggregates in the solution which was homogenised at high-pressure compared to the lower pressure homogenisation (e.g. 300 bar).

Lewen et al. (2000) developed a low viscosity (25–150 mPas) oat-based beverage containing 0.75–1.0 g β-glucan in a serving of 250 ml. Nilsson et al. (2003) descriped process to yield an even higher concentration (3.5–4.8%) oat β-glucan in a water suspension, but they did not provide information about the molecular weight β-glucan, or about the stability of the suspensions. Lyly et al. (2009) studied the satiating effect of a beverage enriched with an oat bran preparation. They added 5 g of β-glucan into a portion of 400 ml. However, due to the high molecular

weight of β-glucan, the oat bran ingredient was only suitable for instant drink powders, in which β-glucan is consumed immediately after mixing with water.

Kivelä et al. (2009b) reported that the addition of ascorbic acid (2 mM) into a water extract of β-glucan (0.15% w/v) led to a clear loss in the viscosity (from 145 to 6 mPas) and reduced the M_w of β-glucan (from 1400 to 50 kDa) already after one day of storage at +6 °C. They concluded that the degradation of β-glucan molecules was induced by metal-catalysed hydroxyl radicals. The adjustment of pH showed that ascorbic acid had a similar effect in the pH range 3.5–6.5. At each pH, the viscosity was lost within one week, and it was concluded that pH adjustment was not a suitable method to protect β-glucan from oxidative cleavage in beverages. The results indicated that hydroxyl radical-driven oxidative cleavage was the major factor causing β-glucan degradation in beverages, and that acid-induced hydrolysis may play only a minor role in the degradation of β-glucan molecules by ascorbic acid. Faure et al. (2012) showed that the ascorbate-induced degradation of β-glucan could be inhibited by adding various antioxidants. Catalase was the only antioxidant, which could completely stop the •OH formation. However, sucrose, phenylalanine and 4-hydroxybenzoic acid also showed high radical scavenging capacity, and slowed down the β-glucan degradation.

Controlled hydrolysis of oat β-glucan by acid hydrolysis at 45–50% water content in a twin-screw extruder (2–3 min reaction time) was reported in a patent of Kaukovirta-Norja et al. (2009). The M_w of β-glucan after acid hydrolysis of different oat bran preparations at different temperatures and acid concentrations was in the range of 5–360 kDa. For example, when 8% phosphoric acid was used at 110–130 °C, the Mw of β-glucan was between 37 and 135 kDa (oat bran with 22% β-glucan) or between 28 and 105 kDa (oat bran with 33% β-glucan). The patent claimed that with a reduced M_w, oat β-glucan could be used in aqueous solutions at a desired concentration (>1 g/100 ml) without excessive increase in viscosity reaching at about 100–150 mPas, which would complicate the drinking of the beverage.

Similarly, enzymatic hydrolysis at low water content has been shown to degrade the molecular weight of β-glucan in a more controlled way than at high water content (Lehtomäki and Myllymäki, 2009). Oat bran concentrates were hydrolysed enzymatically at 45–55% water content, using commercial cellulase and α-amylase preparations in a twin-screw extruder (0.5–2 min at 65 °C). The hydrolysis of β-glucan was controlled by the dosage of enzymes (17–17,000 nkat β-glucanase activity/g oat bran). The enzymes were inactivated after the hydrolysis by repeating the extrusion at 95 °C. Unfortunately, the patent of Lehtomäki and Myllymäki (2009) did not report the Mw values of the enzyme-hydrolysed oat bran preparations. However, they stated that a solution of 0.75 g / 100 ml of hydrolysed β-glucan should have a viscosity below 150 mPas, indicating a drinkable product.

1.4.4 Effect of storage and freezing on oat dietary fibre

For many food products, freezing is the only option to keep the product in a sensorial and microbiologically acceptable form for several months. The characteristics of oat dietary fibres and β-glucan can also change during the storage. Gamel et al. (2013) reported that the M_w, solubility and viscosity of β-glucan in oat bread stored at room temperature were relatively unchanged for 3 days, but a gradual decline in these parameters was observed after this. The M_w of β-glucan extracted from fresh bread (originally 610–753 kDa) had decreased to 140–233 kDa after 6 days of storage. The proportion of LM_w β-glucan fragments (<100 kDa, 9% in fresh bread) was increased to 15, 23 and 43% after 3, 4 and 5 days of storage, respectively. The solubility of oat β-glucan also decreased 38.8–47.6% after 6 days of storage. Similar results (>50% reduction in solubility) were obtained after 4 days of storage at room temperature for breads fortified with barley β-glucan concentrate (Moriartey et al., 2011).

Frozen storage has been reported to decrease the extractability, but not to lead to remarkable changes in the M_w of β-glucan. Beer et al. (1997b) found that under frozen storage (at –20 °C for 5 months) the extractable β-glucan decreased by more than 50% in oat bran muffins, whereas no change in the M_w (originally 1400–1800 kDa) was detected. Lan-Pidhainy et al. (2007) showed that freeze-thaw-cycling of oat bran muffins reduced the solubility of β-glucan (from 27–40% to 15–18%) and also slightly its M_w (from 2700–2800 kDa to 1800–2000 kDa). Gamel et al. (2013) also concluded that freezing (at –18 or –80 °C) and subsequent freeze drying did not dramatically affect the M_w of β-glucan (600–900 kDa in bread and ~2900 kDa in porridge). However, they did not report any reduction in the solubility of β-glucan in bread (43–46%) or in porridge (22–24%) due to freezing or freeze drying. This might be due to the significantly shorter storage time (2–3 days) in their study compared to 5 months in that of Beer et al. (1997b). These observations suggest that the solubility of oat β-glucan in products with high moisture content can be retained for several days during storage at –18 °C. Nevertheless, both freezing and freeze drying can decrease the final extract viscosity of β-glucan in bread or porridge, and liquid nitrogen appears to be the only option to retain the extraction viscosity (Gamel et al., 2013).

1.5 Aims of the study

Oat DF offers several nutritional benefits, but its application in food matrices is challenging due to its high viscosity-enhancing capacity. In addition, regular oat bran ingredients contain only a relatively low concentration of DF, which limits their availability for food products. The content and quality of oat DF, particularly β-glucan M_w and solubility, can be enhanced by new processing methods. The aim of this work was to develop technologies for processing of oat DF for efficient enrichment of β-glucan and thus better applicability in food products.

The main objectives were:

- Development of dry fractionation technologies to yield oat bran fractions enriched in DF, especially β-glucan
- Modification of DF for improved usability in aqueous food matrices
- Utilisation of developed oat fractions and modified DF-enriched ingredients for dry extrudates.

2. Materials and methods

2.1 Raw materials

The chemical compositions of the raw materials utilised in this work are summarised in Table 5. The oat bran concentrate (OBC) obtained in Publication I is shown as a reference to other OBC-fractions, because the compositions of (untreated) OBC-fractions were slightly different in Publications II, III and IV. Different oat bran preparations (non-defatted Elovena Plus and OBC-20NEF, as well as defatted SC-CO_2-OBC-1 and SC-CO_2-OBC-2) were used as a raw material in Publication II. The non-defatted oat bran preparations were kindly provided by Ravintoraisio Oyj (Raisio, Finland). Production of the defatted OBC-fractions is presented in Figure 2.

Defatted whole grain oat flour (WF), endosperm oat flour (EF) and differently treated OBCs were studied in Publication IV in order to produce oat-based extrudates. The same (untreated) OBC-fraction was also used as raw material for liquid food products (Publication III). This OBC-fraction was converted into four different forms: UF-OBC (ultra-fine ground), EH-OBC (enzyme-hydrolysed), WIS-OBC (water-insoluble) and WS-OBC (water-soluble) (Publication IV). More information about the manufacture of these OBC-fractions is provided in Figure 4.

The OBC-fraction produced in Publication I had a higher β-glucan concentration (33.9%) than that used in Publications III and IV (28.5%), because the fractions were produced in different batches (the industrial scale fractionation was performed during two separate years). The differently modified oat bran concentrates (untreated OBC, UF-OBC and EH-OBC) had almost identical β-glucan contents (28.1–28.5%), but the content of TDF varied between 33.2 and 50.4%, being the lowest in EH-OBC (Table 5). The WS-OBC had much higher β-glucan (52.2%) and TDF contents (53.5%) than WIS-OBC (11.6 and 31.8%, respectively). The DF in WS-OBC was 100% soluble, whereas 75% of the DF in WIS-OBC was insoluble.

WF had significantly higher β-glucan (3.2%) and total DF contents (6.0%) than EF (1.3% and 2.5%, respectively) (Table 5). The proportion of IDF compared to SDF was significantly higher in WF (60:40%) than in EF (52:48%). In turn, the starch content was lower in WF (65.6% of dry matter) than in EF (70.6%).

Table 5. The chemical composition and particle size of raw materials utilised in Publications II–IV. The process described in Publication I was applied to produce the defatted oat bran concentrates (untreated OBC, SC-CO_2-OBC-1 and SC-CO_2-OBC-2). The original OBC (2^{nd} coarse fraction from Publication I) is shown as a reference for the other OBC-fractions. Oat bran (ElovenaPlus) and oat bran concentrate (OBC-20NEF) were commercial samples provided by Ravintoraisio Oyj, Finland. The process described in Publication III was used to produce fractions EH-OBC (enzyme hydrolysed), WIS-OBC (water-insoluble) and WS-OBC (water-insoluble). The defatted whole grain (WF) and endosperm oat flour (EF) were obtained from the same fractionation process as described in Publication I. The differently treated OBC-fractions (untreated, EH-, WIS- and WIS-OBC) and oat flours (WF and EF) were utilised in Publication IV.

	Whole grain oats	**OBC (2^{nd} coarse fraction)**	**Oat bran (ElovenaPlus)**	**OBC-20NEF**	**SC-CO_2-OBC-1**	**SC-CO_2-OBC-2**
	Non-defatted	Defatted	Non-defatted	Non-defatted	Defatted	Defatted
Publication no.	I	I	II	II	II	II
β-Glucan (%)	3.1	33.9	5.8	19.6	21.3	35.0
Arabinoxylan (%)	1.0	9.9	n.a.	9.2	n.a.	15.2
Starch (%)	9.2	9.2	49.4	17.8	28.0	7.8
Protein (%)	16.4	23.0	16.9	26.3	18.6	18.9
Fat (%)	5.7	4.2	8.5	10.1	2.9	4.4
Particle size; D_{50}/D_{90} (μm)	n.a.	197/323	35/113	39/105	33/96	60/141

	OBC (untreated)	UF-OBC	EH-OBC	WIS-OBC	WS-OBC	WF	EF
	Defatted	Defatted	Defatted	Defatted	Defatted	Defatted	Defatted
Publication no.	III and IV	IV	IV	IV	IV	IV	IV
β-Glucan (%)	28.5	28.1	28.3	11.6	52.2	3.2	1.3
Total dietary fibre (%)	48.1	50.4	33.2	31.8	53.5	6.0	2.5
Starch (%)	9.7	12.4	11.1	13.7	6.7	65.6	70.6
Protein (%)	23.2	22.9	23.2	35.6	8.9	17.2	16.7
Fat (%)	4.0	n.a.	n.a.	n.a.	n.a.	2.0	1.2
Particle size; D_{50}/D_{90} (µm)	213/404	32/81	111/262	70/150	37/79	37/269	14/175

n.a. = not analysed

2.2 Processing methods

2.2.1 Defatting

In pilot scale, non-heat treated dehulled flaked oats were defatted by supercritical carbon dioxide (SC-CO_2) in a Multi-Use SFE Plant with a pressure vessel of 10 l (Chematur Ecoplanning, Rauma, Finland). The extraction method of oat flakes was based on the work described earlier by Aro et al. (2007). The extraction was performed either with SC-CO_2 alone or with SC-CO_2 followed by SC-CO_2 and 10% ethanol extraction. In the industrial scale trials, a pressure vessel of 250 l (NATECO2 GmbH & Co, Wolnzach, Germany) was used. The industrial scale extraction was performed only with SC-CO_2. The process parameters are presented in Table 1 of Publication I.

2.2.2 Milling and air classification

In the pilot scale trials the defatted flaked oats were first ground twice at a rotor speed of 17 800 rpm (tip speed 180 m s^{-1}) and a feed rate of 10 kg h^{-1}, using a Hosokawa Alpine 100 UPZ-lb Fine impact mill with pin disc grinders (Hosokawa Alpine AG, Augsburg, Germany). The ground material was then air classified using a Minisplit Classifier (British Rema Manufacturing Company Ltd., UK). Classification was performed with an air flow of 220m^3 h^{-1} and a feed rate of 5 kg h^{-1}. During the classification, the rotor speed was varied between 3000 and 7000 rpm in order to alter the mass balance between fine and coarse fractions. The coarse cell wall fraction from the first air classification step was further ground twice, using the same parameters as previously, and subsequently air classified with the same air flow and feed rate but altering the classifier rotor speed between 2500 and 4000 rpm.

In the industrial scale trials, the defatted oat grits were first ground in a Hosokawa Alpine Contraplex 250 CW mill. The rotation speeds of the mill discs were 11200 and 5600 rpm for the discs rotating in opposite directions (tip speed 250 m s^{-1}). The feed rate was 250 kg h^{-1}. The ground flour was subsequently air classified in a Hosokawa Alpine 315 ATP classifier, using an air flow of 1200/1200 m^3 h^{-1} and a rotor speed of 2200 rpm. The first coarse cell wall fraction, separated by air classification, was milled and air classified again with the same parameters to yield an oat bran concentrate (OBC) enriched in β-glucan and endosperm flour rich in starch. The separation of the protein-enriched fraction from the first fine fraction was made only for the industrial scale trial, using a Hosokawa Alpine 200 ATP-NG air classifier with air flow 400/400 m^3 h^{-1}, feed rate 100 kg h^{-1} and rotor speed 6600 rpm. The overall fractionation diagram is presented in Figure 2.

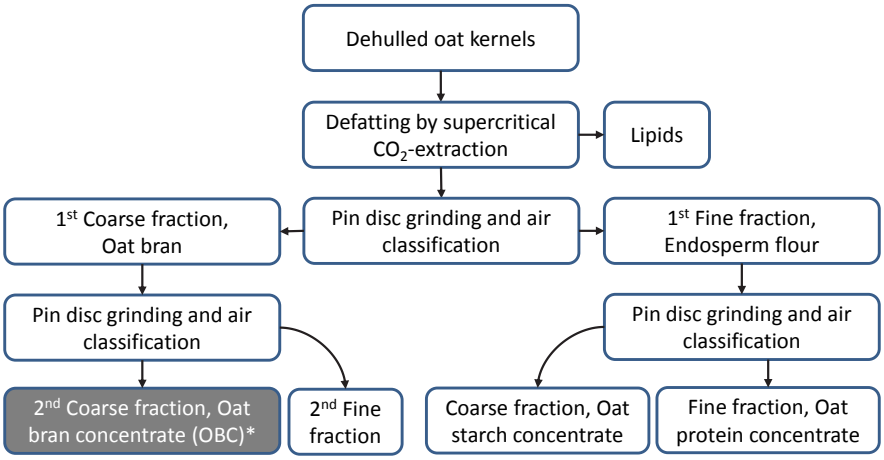

Figure 2. Fractionation of oats based on defatting with SC-CO_2 extraction, pin disc grinding and air classification. *The highlighted oat bran concentrate (OBC) was used as a raw material for later steps of this work.

2.2.3 Ultra-fine grinding and electrostatic separation

In order to fractionate the oat bran preparations (Elovena Plus, OBC-20NEF, and SC-CO_2-OBC-1 and SC-CO_2-OBC-2) by the electrostatic forces, they all were fine-ground at ambient temperature in an ultra-fine milling equipment Turborotor G-55 (Görgens Mahltechnik GmbH, Dormagen, Germany) with a rotor speed of 1 800 rpm. In addition, 'SC-CO_2-OBC-2' fraction (being identical to the 'OBC' fraction highlighted in Figure 2) was ultra-fine ground both at ambient temperature and in cryogenic conditions. The ambient griding was performed as described above for the other oat bran preparations. A fine impact mill 100 UPZ (Hosokawa-Alpine AG, Augsburg, Germany) was used for the cryogenic grinding with a 0.3 mm grid and a rotor speed 18 000 rpm. To reach –100 °C temperature, the mill and oat bran material were cooled with liquid nitrogen using a special screw feeder (Micronis, Agen, France).

A pilot-scale electrostatic separator (TEP System, Tribo Flow Separations, Lexington, USA) was used for the production of various fractions, using ultra-fine oat bran preparations as starting materials. Starting from the raw material ('F0'), fractions 'F1A-' and 'F1B+' were separated by the negative and positive electrodes, respectively. Fractions 'F2AA-' and 'F2AB+' were obtained by repeating the separation starting from fraction 'F1A-', whereas fractions 'F2BA-' and 'F2BB+' were obtained starting from fraction 'F1B+'.

The fraction 'F2BB+' (from SC-CO_2-OBC-2), which contained the highest concentration of β-glucan after the electrostatic separation, was further fractionated by a 100AFG Multi-Processing System (Hosokawa-Alpine AG) by combining jet-milling and air classification. The particles were forced to collide with each other

under 6 bar pressure, and an air classifier wheel with 10 000 rpm rotor speed was used to allow only the finest particles to pass through the classifier wheel. The coarse fraction, which could not exit from the milling chamber, was collected as a separate (β-glucan-enriched) fraction. The flow chart related to electrostatic separation and jet-milling is visualised in Figure 3.

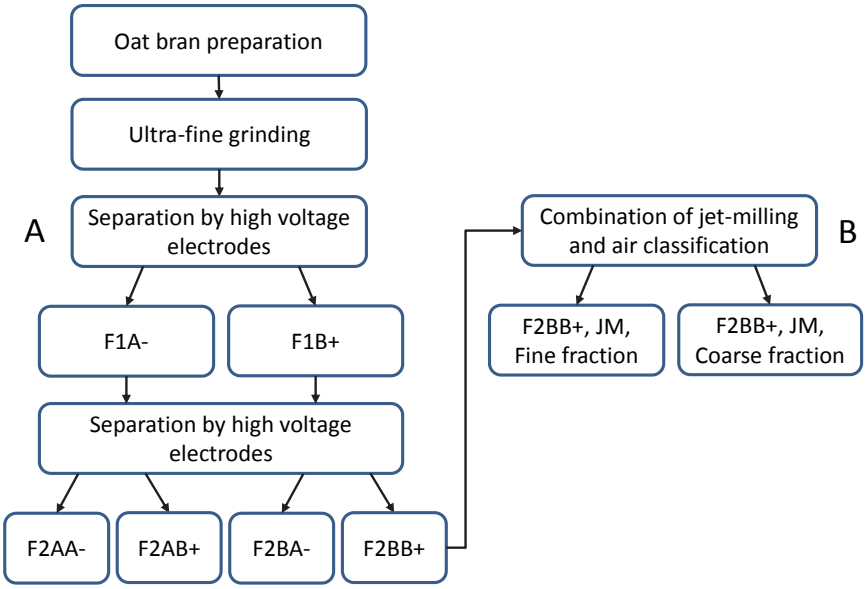

Figure 3. Fractionation of oat bran preparations based on electrostatic forces between high voltage electrodes (A), and by jet-milling and air classification (B).

2.2.4 Acid and enzymatic hydrolysis to produce water-soluble and -insoluble oat bran preparations

The β-glucan-enriched oat bran fraction (OBC) was first preconditioned by mixing it with 30% water. The acid-catalysed hydrolysis was performed with ortho-phosphoric acid (Merck KGaA, Darmstadt, Germany) and enzyme-catalysed hydrolysis with a commercial enzyme preparation (Depol 740L, Biocatalyst Ltd., Wales, UK). The hydrolysis was carried out using an APV MPF 19/25 twin-screw extruder (Baker Perkins Group Ltd., Peterborough, U.K.). The feed rate of the preconditioned oat bran into the extruder was 24 g min^{-1}, speed of the twin-screws was 75 rpm, and residence time inside the barrel about 3 min. The temperature inside the extrusion barrel was set to 110–130 °C for acid hydrolysis and to 50 °C for enzymatic hydrolysis. An aqueous solution of phosphoric acid (8% w/v) or Depol 740L enzyme preparation (50 nkat β-glucanase activity/g oat bran) was fed into the extruder at a rate of 12 ml min^{-1}, resulting in a final water content of 50%. After the extrusion, the dough-like mass was either ready for subsequent pro-

cessing (acid hydrolysis) or was incubated in sealed containers at 50 °C for 1–4 h (enzyme hydrolysis). After incubation with the enzyme, the dough-like mass was manually fed into the extruder again to inactivate the enzyme. The inactivation was performed at 110 °C using 75 rpm speed for the twin-screws, resulting in a residence time of 3–4 min. After the extrusion, the moist, hydrolysed material was dried overnight in an oven with recirculation air at 65 °C. The dried material was first ground in a Wiley cutting mill (Arthur H. Thomas Company, Philadelphia, U.S.A.) and subsequently in a Hosokawa Alpine 100 UPZ-lb Fine impact mill with pin discs (Hosokawa Alpine AG).

For the hot water extraction (Publication III), 66.7 or 83.3 g (d.w.) of dried and milled oat bran material was mixed with 1 l of distilled water at 70 °C. The mixture was stirred for 2 min with a hand-held homogeniser (Heidolph Diax 900 Ultra Turrax, Gemini BV, Apeldoorn, the Netherlands), using 12,000 rpm speed. The insoluble residue was separated with a centrifuge (Sorvall RC-12BP, DuPont, U.S.A.) at 4000 rpm (ca. 4000×g) for 15 min. The centrifugation was performed at room temperature without cooling to avoid gelling of the water-soluble fraction. The supernatant of acid hydrolysed oat bran was neutralised from pH 2.1–2.2 to pH 5.0–5.2 with calcium hydroxide (Merck KGaA, Darmstadt, Germany), and the $Ca_3(PO_4)_2$ precipitate formed was separated by centrifugation as described above. Neutralisation was not needed for the enzyme-hydrolysed material, which had a pH of 5.6–5.8 (Figure 4).

A similar approach was used to produce raw materials for studying the effects of differently treated oat bran preparations in expanded snack products (Publication IV). Fraction 'EH-OBC' was obtained after 4 h enzymatic hydrolysis and inactiviation, oven drying and fine-grinding. Fractions 'WIS-OBC' and 'WS-OBC' were manufactured by separating the water-insoluble and -soluble fractions from 'EH-OBC', first by hot-water extraction and centrifugation and subsequently by freeze drying these fractions (Figure 4).

2.2.5 Production of expanded food products with defatted oat fractions

Defatted endosperm oat flour (EF) was used as the reference and main carrier matrix during extrusion processing. In addition, SC-CO_2 defatted wholegrain oat flour (WF) was used as another reference. EF and WF were similar to the endosperm fraction (first fine fraction) and defatted whole grain oats of Publication I (see Figure 2). Defatted oat bran concentrate (OBC) was used as a source of DF in untreated and modified form. In order to study the effect of particle size, the OBC with an original particle size of around D_{50}=200 µm was ground into ultra-fine powder (D_{50}=30 µm) in a Turborotor G-55 mill (Mahltechnik Görgens GmbH) using 60 Hz rotor speed and an average feed rate of 30 kg h^{-1}. This fraction was designated as 'UF-OBC'. The manufacture of other raw materials (EH-OBC, WIS-OBC and WS-OBC) is described above and in Figure 4.

Extrusion trials were conducted in a co-rotating twin-screw extruder (Poly Lab System, Thermo Prism PTW24, Thermo Haake, Germany) with a barrel length (L)

of 672 mm and diameter (D) 24 mm, and L/D ratio 28:1. The extruder consisted of seven sections with a die exit of 5 mm diameter. The screw speed was 500 rpm and the total feed rate (including both solids and water) was 76 g/min. The temperature profile in the barrel was: 40, 70, 70, 100, 110, 130 and 130 °C (sections 1–6 and the die, respectively), and cooling water circulation was utilized to keep the temperatures constant. The torque of the extruder (38–48 Nm) and the pressure at the die exit (39–58 bar) varied only slightly during the trials and there were no clear correlations between torque, pressure and the raw materials used.

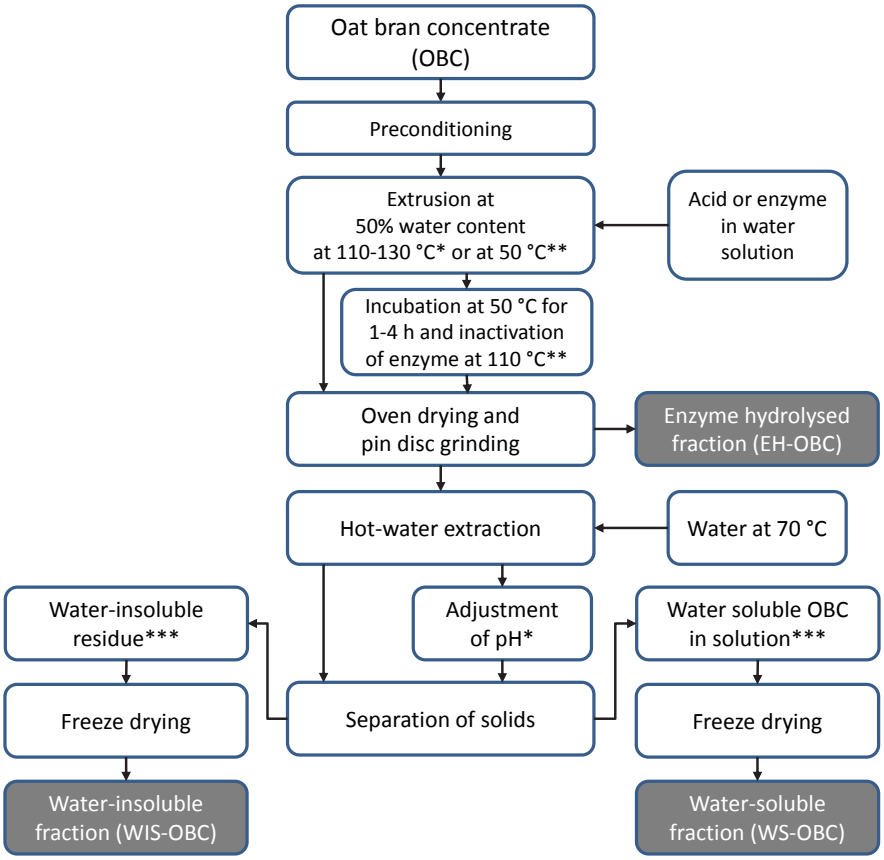

Figure 4. Flow chart describing the acid and enzymatic hydrolysis of OBC at 50% water content using an extruder as a bioreactor. The hydrolysis was performed at 110–130 °C (*acid hydrolysis) or at 50 °C with subsequent inactivation at 110 °C (**enzyme hydrolysis). Water-soluble OBC and water-insoluble residues (***) were investigated in liquid model foods (Publication III). The highlighted fractions (EH-OBC, WIS-OBC and WS-OBC) were used as raw materials for expanded model foods (Publication IV).

2.3 Biochemical analyses

The β-glucan content was determined by a standard method 32-23.01 (AACC, 2000) using the Megazyme β-glucan mixed-linkage assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The molecular weight (M_w) of β-glucan was analysed by HPLC-SEC. Two different methods were applied. The M_w of β-glucan in the enriched oat bran fractions as well as in extruded snacks (Publications II and IV) was analysed after stirring 1 g of the sample overnight with a magnetic stirrer in 1 litre of 0.1 N NaOH containing 0.1% $NaBH_4$. The samples were analysed by HPLC-SEC with Calcofluor staining using right-angle laser light scattering for detection, according to Suortti (1993). The linear size-exclusion calibration curve was constructed on the basis of β-glucan standards ranging from 33.6 to 667 kDa. The M_w of β-glucan in liquid model food products (Publication III) was analysed by dissolving the samples at concentration levels of 12 and 8 g/l in 0.2% H_3PO_4 and 200 ppm Na-azide at room temperature overnight with magnetic stirring. The samples were analysed by HPLC-SEC using a refractive index detector. The linear size-exclusion calibration curve was constructed on the basis of pullulan standards ranging from 788 to 5.9 kDa and malto-oligomers ranging from maltoheptaose to maltose. The weight average molecular weight (M_w) and the number average molecular weight (M_n) were calculated over the whole β-glucan peak. Polydispersity, a measure of the distribution of individual molecular masses, was calculated as a ratio between M_w and M_n.

The neutral sugars were quantified as anhydro-sugars, after hydrolysis of the samples by sulphuric acid (1 M, 2 h, 100 °C) and conversion into alditol acetates (Blakeney et al., 1983). Anhydrosugar determination was performed by GC-LC. The content of arabinoxylan (AX) was calculated as the sum of arabinose and xylose. Nitrogen was analysed using a Kjeldahl autoanalyzer (Foss Tecator Ab, Höganäs, Sweden), and protein concentration was calculated as N x 6.25 according to method 46-11A (AACC, 2000). Starch was quantified using the Megazyme total starch assay kit according to method 76-13.01, and the total fat content using a Soxhlet extraction by heptane according to method 30-25.01 (AACC, 2000).

2.4 Particle size measurement

The D_{50} and D_{90} values of the raw materials, indicating that 50 or 90% of the particles, respectively, have a diameter below a certain level (μm), were analyzed by a a laser diffraction particle size analyser. Beckman Coulter LS 230 (Beckman Coulter Inc., CA, U.S.A.) was used with the liquid module and distilled water as a carrier for Publications I, III and IV. Mastersizer 2000 S (Malvern Instruments Ltd., United Kingdom) was used with the liquid module and ethanol as a carrier for Publication II. The suspensions were analysed when the obscuration was between 10 and 20%, using continuous sonication.

2.5 Textural and structural analyses

The expansion rate was calculated by the equation (1) according to Kumagai et al. (1987). The diameters of the extrudates were measured by a vernier caliper using an average of 20 replicates.

$$\text{Expansion rate } (\%) = \frac{D_e}{D_d} \text{x } 100\%, \text{ in which} \quad (1)$$

D_e = Average diameter measured at three different points of the extrudate sample (mm)
D_d = Diameter of the die (5 mm).

The samples were cut into 10 mm long pieces with a band saw (Power ST-WBS800, Taiwan Sheng Tsai Industrial Co. Ltd., Taiwan), and the hardness and crispiness of the extrudates were determined by a TA.XT2i TextureAnalyzer (Stable Micro Systems Ltd., Godalming, U.K.) equipped with a 30 kg load cell (cross-head speed 1.0 mm/s) and a cylindrical 36 mm aluminium probe (20 replicates). The compression curve was linearized and its length was calculated to describe the crispiness of the material. Crispiness index (C_i) was calculated with equation (2) (Heidenreich et al., 2004). High crispiness was accompanied by a high C_i-value, whereas low crispiness corresponded to a low C_i-value.

$$C_i = \frac{L_N}{A \times F_{mean}}, \text{ in which} \quad (2)$$

L_N = Normalized curve length (Length of actual curve / F_{max}), and F_{max} = hardness of extrudates
A = Area under the force/deformation curve = Toughness (N s)
F_{mean} = Sum of the actual force values divided by the number of peaks (N)

X-ray microtomography was used to investigate and quantify the porous structures of extrudates. Triplicate samples (10 mm long pieces of each extrudates) were scanned with a SkyScan 1172 microtomograph (Bruker-microCT Ltd., Kontich, Belgium). The instrument was operated at 40 kV / 250 µA. The pixel size was 11.65 µm, exposure time 0.079 s and the total scanning time 18 min. After scanning, binarized projection images from each sample were reconstructed into a 3D object by NRecon reconstruction software (Skyscan, Belgium) and further analysed for porosity (%), cell wall thickness (mm) and pore diameter (mm) by Ctan image analysis software (Skyscan, Belgium). In addition, the image analysis software provided the distributions of pore size and cell wall thickness (Publication IV).

2.6 Microscopic analyses

The samples were embedded into 2% agar and then fixed in 3.0% (w/v) paraformaldehyde and 1.0% (v/v) glutaraldehyde in 0.1 M Na-K phosphate buffer (pH 7.0),

dehydrated in graded ethanol series, and embedded in hydroxyethyl methylacrylate resin (Leica Historesin embedding kit, Heidelberg, Germany) prior to microscopic analyses. The embedded samples were sectioned (2 µm) in a rotary microtome HM 355 (Microm Laborgeräte GmbH, Walldorf, Germany) using a steel knife. The sections were transferred onto glass slides and stained separately: 1) with aqueous 0.1% (w/v) Light Green (BDH Chemicals Ltd, Poole, Dorset, UK) and 1:10 diluted Lugol's iodine solution (I_2 0.33%, w/v and KI 0.67%, w/v) when observed in brightfield, 2) with aqueous 0.1% (w/v) Acid Fuchsin (BDH Chemicals Ltd., Poole, Dorset UK)) in 1.0% acetic acid and aqueous 0.01% (w/v) Calcofluor White (Fluorescent Brightener, Aldrich, Germany), or 3) with 0.01% (w/v) Nile Blue (Gurr Products, Romford, Essex, UK) for epifluorescence observation. Light Green stains protein green/yellow, whereas Lugol's iodine solution stains starch dark blue. Acid Fuchsin and Calcofluor White were used for staining protein red and β-glucan-rich cell walls light blue, respectively (excitation at 400–410 nm and fluorescence at >455 nm). Nile Blue showed lipids in yellow (excitation at 420–480 nm and fluorescence at >515 nm). The samples were then examined under a BX-50 microscope (Olympus Corp., Tokyo, Japan). Micrographs were obtained using a PCO SensiCam CCD colour camera (PCO AG, Kelheim, Germany) and the Cell^P imaging software (Olympus). At least five images were acquired from the sections cut from three blocks per sample. The most representative micrographs were selected for the comparison of the different samples (Publications I–IV).

2.7 Statistical analyses

Statistical analyses were performed by analysis of variance using IBM SPSS Statistics 20 (IBM Corporation, Somers, NY, U.S.A.), and significant differences ($P < 0.05$) between individual means were identified by the Tukey's test (Publications II–IV). All analyses were performed in triplicates unless otherwise stated.

3. Results

3.1 Dry fractionation (Publications I and II)

Dry fractionation of different oat components was studied by integrating defatting, grinding and air classification technologies (Figure 2). In addition, electrostatic separation and jet-milling in combination with air classification were used as optional or additional steps in the dry fractionation process (Figure 3).

3.1.1 Effects of defatting, particle size reduction and air classification on the separation of oat grain components

The SC-CO_2 extraction of flaked oats showed that the efficiency of the lipid extraction varied according to the lipid class. The extraction removed the majority (85%) of neutral triacylglycerols, but less than 2% of polar lipids. Free fatty acids were poorly extracted with SC-CO_2 (Table 2 of Publication I). Grinding of non-defatted whole grain oats (5.7% fat) in a pin disc mill was difficult due to the formation of lumps and adhesion of flour to the milling chamber. Thus, grinding was possible only in small batches. When defatted oats (2.0% fat) were used as a raw material, these problems were not encountered and the mill could be run continuously. Both non-defatted and defatted oats were ground twice in the pin disc mill in order to ensure good dissociation of oat particles for further fractionation.

Flours from both non-defatted and defatted oats were subjected to air classification and fractionated into coarse and fine fractions (Figure 2). The efficiency of the fractionation depended on the yield of the coarse fraction and on its β-glucan concentration. After the first round of grinding and air classification in pilot scale, the most efficient fractionation of non-defatted oats was obtained with 16.8% yield (13.4% β-glucan) of the coarse fraction. The fractionation of defatted oats was significantly more efficient (15.3% yield with 20.8% β-glucan concentration) (Table 6). The coarse fractions of the non-defatted material had notably larger particle size (D_{50}/D_{90} = 651/1016 µm) than the defatted material (D_{50}/D_{90} = 392/667 µm). In addition, a lower rotor speed was required for non-defatted than for defatted oats (Table 3 of Publication I).

Lipid removal had a remarkable effect on the β-glucan concentration in the coarse bran fractions. In pilot scale, after the second round of grinding and air classification, the highest concentration of β-glucan obtained from non-defatted oats was 17.1% (8.7% yield), whereas the corresponding concentration from defatted oats was 31.2% (8.8% yield) (Table 6). When the yield of coarse fraction was reduced below 9%, no further enrichment of β-glucan was observed with either non-defatted or defatted oat material (Table 3 of Publication I).

Table 6. The yields and β-glucan concentrations of non-defatted and defatted oats after pin disc grinding and air classification in pilot scale (A and B), and the chemical composition and particle size of defatted oats after the industrial scale fractionation (C).

	Raw material	**After grinding and 1st air classification**		**After grinding and 2nd air classification**	
A. Pilot scale	**Non-defatted oat flour**	**Fines**	**Coarse**	**Fines**	**Coarse**
Yield (%)	100.0	83.2	16.8	8.1	8.7
β-Glucan (%)	3.9 ± 0.1	2.0 ± 0.1	13.4 ± 0.2	9.4 ± 0.3	17.1 ± 0.1
B. Pilot scale	**Defatted oat flour**	**Fines**	**Coarse**	**Fines**	**Coarse**
Yield (%)	96.3	84.7	15.3	6.5	8.8
β-Glucan (%)	4.0 ± 0.2	1.1 ± 0.2	20.8 ± 0.1	6.7 ± 0.1	31.2 ± 0.6
C. Industrial scale	**Defatted oat flour**	**Fines**	**Coarse**	**Fines**	**Coarse**
Yield (%)	95.3	81.0	14.3	6.5	7.8
β-Glucan (%)	3.2 ± 0.3	1.3 ± 0.1	21.3 ± 0.5	11.4 ± 0.1	33.9 ± 0.2
Arabinoxylan (%)	1.0 ± 0.1	0.4 ± 0.1	8.3 ± 0.1	5.5 ± 0.1	9.9 ± 0.5
Protein (%)	17.2 ± 0.1	16.7 ± 0.1	23.9 ± 0.1	23.2 ± 0.1	23.0 ± 0.2
Starch (%)	65.6 ± 0.8	69.8 ± 0.3	17.5 ± 0.1	31.2 ± 0.2	9.2 ± 0.1
Particle size D_{50} / D_{90} (μm)	12 / 211	10 / 142	250 / 381	30 / 119	197 / 323

The industrial scale fractionation was performed only with the defatted oat material. In the first round of fractionation, the yield of coarse fraction was adjusted to 14.3% (21.3% β-glucan). This was further ground and air classified into another coarse fraction with 7.8% yield (33.9% β-glucan). A protein-enriched fraction with 5.0% yield (73.0% protein) was separated by re-classifying the fine fraction (i.e. endosperm flour) obtained after the first air classification step into protein- and starch-enriched fractions. Protein enrichment was possible only for the defatted sample,

and a very precise particle size cut-off at around 5 µm was required for the separation of protein fraction from starchy endosperm flour. The cut-off for β-glucan separation was at around 200 µm (Table 4 of Publication I). The microscopic pictures (Figure 2 of Publication I) showed that β-glucan (blue colour) was significantly concentrated in the 2nd coarse fraction and that this fraction contained mainly cells from the subaleurone layer. In addition, the amount of starch was significantly reduced compared to the whole grain oats and the 1st coarse fractions (visualised as low amount of spherical dark blue objects in row B of Figure 2 of Publication I).

The localisation of lipids in non-defatted and SC-CO_2-extracted whole grain oats, as well as in defatted oat fractions, is presented in Figure 5. The lipid contents were not reported in Publication I, but they were analysed later. In non-defatted whole grain oats, the lipids were mainly concentrated in the aleurone and subaleurone regions (picture A, 5.7% lipids). Most of the lipids were extracted by SC-CO_2, as shown by reduced yellow colour in picture B (2.0% lipids). The defatted endosperm oat flour (picture C) contained only 1.2% lipids, whereas the oat bran concentrate (2nd coarse fraction; picture D) and protein concentrate (picture E) had higher lipid concentrations (4.3 and 6.4%, respectively). Lipids were more concentrated towards the outer layers of the bran preparation, but were distributed throughout the matrix in the protein concentrate.

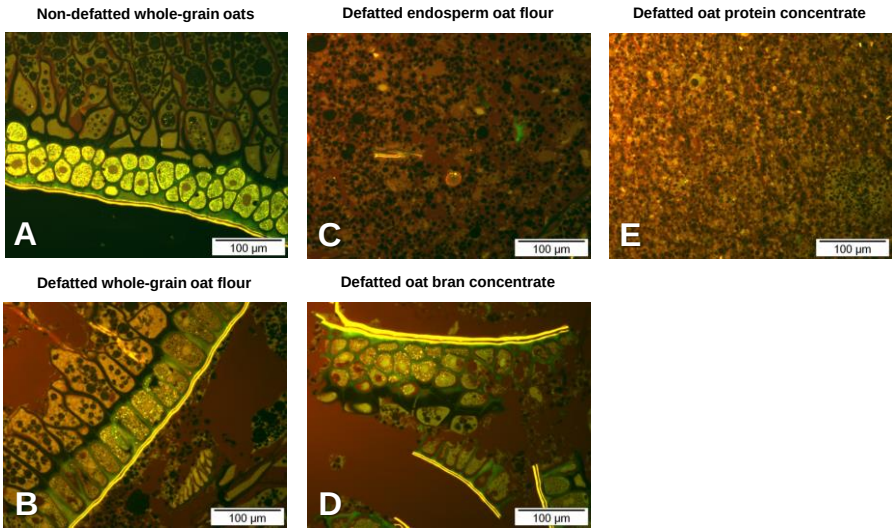

Figure 5. Microscopic pictures of non-defatted and defatted (SC-CO_2) oat fractions obtained by pin disc grinding and air classification. Samples were stained by Nile blue, showing lipids in yellow. Sample A: Non-defatted whole grain oats, B: Defatted whole grain oat flour, C: Defatted oat endosperm flour (1st fine fraction), D: Defatted oat bran concentrate (2nd coarse fraction), and E: Defatted oat protein concentrate. These pictures were not previously published elsewhere.

3.1.2 Ultra-fine grinding, electrostatic separation and jet-milling

In order to obtain higher β-glucan concentrations, non-defatted (Elovena Plus or OBC-20NEF) and defatted (SC-CO_2-OBC-1 or SC-CO_2-OBC-2) oat bran preparations were fractionated by electrostatic separation. Ultra-fine grinding was needed to provide a sufficient level of dissociation of different oat bran components. The concentration of β-glucan increased only slightly when non-defatted oat bran preparations were separated in the electric field (Table 1 of Publication II). After two consecutive steps of electrostatic separation, the maximal increases in β-glucan content were from initial 5.8 to 8.7% for Elovena Plus and from initial 19.6 to 25.0% for OBC-20NEF. With both non-defatted oat bran preparations, β-glucan was concentrated in the 'middle fractions' (i.e. 'F2AB+' and 'F2BA-').

The microscope pictures of non-defatted OBC-20NEF showed that more starch granules were present in the positive fractions compared to the negative fractions (Figure 2 of Publication II). In accordance with this, chemical characterisation showed that fraction 'F2BB+' contained more starch than fraction 'F2AA-'. Fraction 'F2AA-' had the lowest amount of β-glucan (19.6%), whereas the AX content was the highest (17.6%) in this fraction (Figure 6).

Remarkably higher β-glucan concentrations were achieved when defatted oat bran concentrates (SC-CO_2-OBC-1 with 21.3% or SC-CO_2-OBC-2 with 35.0% β-glucan) were used as starting material. The greatest enrichment of β-glucan was in the fraction 'F2BB+', which after two consecutive electrostatic separation steps contained 31.2% (OBC-1) or 48.4% (OBC-2) of β-glucan. The lowest β-glucan concentration (13.5% with OBC-1 and 28.0% with OBC-2) was observed in fraction 'F2AA-' (Figure 6). Starch concentration was highest in fraction 'F2BB+', and lowest in fraction 'F2AA-' for both defatted samples. The AX of defatted oat bran (OBC-2) was enriched in fraction 'F2AA-' (from initial 15.2 to 22.7%). Electrostatic separation had only a minimal effect on the protein concentration, which was between 16.8 and 19.5% in all defatted fractions (Table 1 of Publication II).

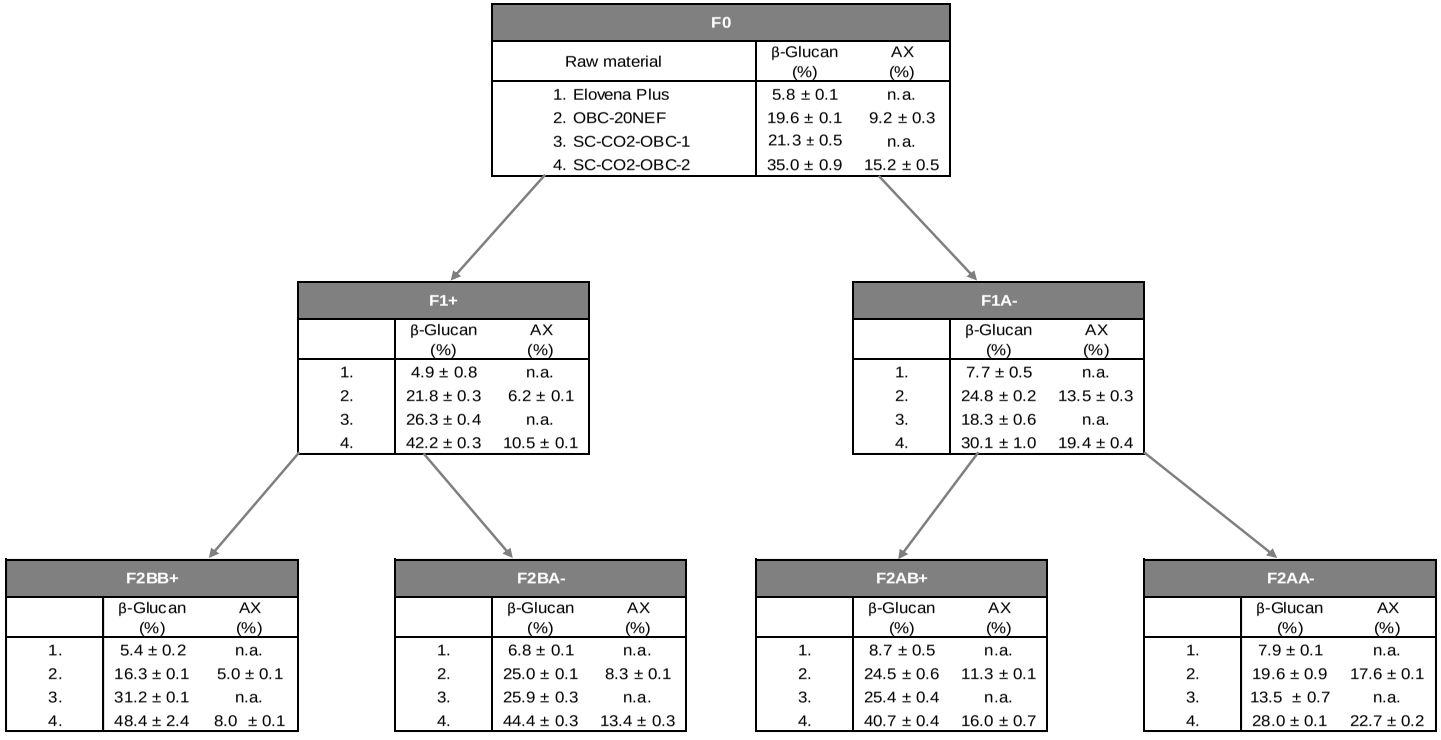

Figure 6. The β-glucan (BG) and arabinoxylan (AX) contents of fractions made by electrostatic separation from non-defatted (Elovena Plus and OBC-20NBEF) and defatted (SC-CO2-OBC-1 and 2) oat brans. The values are expressed as (%) per dry weight. n.a. = not analysed.

Microscopic pictures confirmed that fraction ‘F2AA-’ of SC-CO_2-OBC-2 was enriched in brown fibrous particles and less enriched in blue-coloured β-glucan and black starch granules as compared to other fractions (Figure 3 of Publication II). This indicated that fraction ‘F2AA-’ consisted mainly of pericarp particles, rich in AX and other non-β-glucan fibres. By contrast, fraction ‘F2BB+’ contained cell walls released from degradation of aleurone and endosperm cells. Starch granules were also enriched in this fraction, showing that part of the endosperm was still present. Fractionation in electrostatic separation was similar when SC-CO_2-OBC-2 was ground at ambient temperature or in cryogenic conditions. For example, 48.4 or 46.0% of β-glucan was detected in fraction ‘F2BB+’ when the electrostatic separation was performed after ambient or cryogenic grinding, respectively.

Fraction ‘F2BB+’ (with 48.4% of β-glucan) was further fractionated by a combination of jet-milling and air classification. The jet-milling and air classification separated fine starch and protein particles from coarse cell wall particles. The coarse cell wall fraction contained 56.2% β-glucan, 3.3% starch and 12.9% protein, whereas the fine particles contained a lower amount of β-glucan (34.2%), and significantly higher amounts of starch (19.0%) and protein (18.4%) (Table 1 of Publication II). The microscopic pictures showed that the starch granules surrounded by the cell wall structures were released by jet-milling, and concentrated in the fine fraction, whereas the endosperm and subaleurone cell walls were recovered in the coarse fraction (Figure 4 of Publication II). The recovery of β-glucan after pin disc grinding, air classification and electrostatic separation is shown in Table 7. Air classification showed higher recovery yields, whereas electrostatic separation enabled higher concentration of β-glucan.

Table 7. The recovery of β-glucan (BG) after pin disc milling and air classification (Part 1) and after electrostatic separation (Part 2).

Part 1: Pin disc milling and air classification

	First coarse fraction			Second coarse fraction		
	BG (%)	Yield (%)	BG recovery (%)	BG (%)	Yield (%)	BG recovery (%)
Non-defatted (pilot scale)	13.4 ± 0.1	16.8	57.7	17.1 ± 0.1	8.7	38.1
Defatted (pilot scale)	20.8 ± 0.1	15.3	81.6	31.2 ± 0.6	8.8	70.4
Defatted (industrial scale)	21.3 ± 0.5	14.3	99.1	33.9 ± 0.2	7.8	88.1

Part 2: Ultra-fine grinding and electrostatic separation

	Non-defatted oat bran preparation (OBC-20NEF)			Defatted oat bran preparation (SC-CO_2-OBC-2)		
	BG (%)	Yield (%)	BG recovery (%)	BG (%)	Yield (%)	BG recovery (%)
F0 (starting material)	19.6 ± 0.1	15.0*	75.4	35.0 ± 0.9	7.8	88.1
F1B+	21.8 ± 0.2	8.3	46.2	42.2 ± 0.3	3.6	39.0
F1A-	24.8 ± 0.1	6.8	42.9	30.1 ± 1.0	4.2	32.4
F2BB+	19.6 ± 0.9	4.7	19.4	48.4 ± 2.4	1.8	22.3
F2BA-	24.5 ± 0.6	3.6	23.1	44.4 ± 0.3	1.8	20.5
F2AB+	25.0 ± 0.1	3.9	24.5	40.7 ± 0.4	2.1	21.9
F2AA-	16.3 ± 0.1	2.9	14.3	28.0 ± 0.1	2.1	15.1

*The initial yield of OBC-20NEF is only an estimate, as it is a commercially produced ingredient.

3.2 Depolymerisation of oat β-glucan (Publication III)

The dietary fibre complex in defatted OBC (containing 28.5 β-glucan, 11.4% AX and 48.1% TDF, Table 5) was modified to improve its suitability for aqueous food matrices. Acid and enzymatic hydrolyses at low water content (50% water) in an extruder enabled a controllable depolymerisation of β-glucan.

3.2.1 Acid-catalysed depolymerisation of β-glucan

Acid-catalysed hydrolysis had a significant effect on the cell wall structures of defatted OBC. The cell walls were almost completely destroyed as compared to the initial OBC (Figure 2 of Publication III). After the hydrolysis at 100, 120 or 130 °C and subsequent hot water extraction, 48.2–52.9% of the OBC material was

solubilised and 61.6–68.9% of the total β-glucan was extracted in aqueous solution. The degree of OBC solubilisation did not differ significantly between the hydrolysis temperatures (100–130 °C) and the concentration of OBC during hot water extraction (66.7 or 83.3 g/l). The highest solubilisation of β-glucan (68.9%) was obtained when OBC was hydrolysed at 120 °C. The solubilisation level decreased to 63.4–64.3% when OBC was hydrolysed at 130 °C (Table 8).

The β-glucan concentration of the hot water extracts was highest (45.7%) after the hydrolysis at 120 °C and decreased slightly (to 41.6%) when the hydrolysis was performed at 130 °C (Table 8). AX and starch concentrations of hot-water extracts increased (from 11.4 to 12.6% and from 6.9 to 8.9%, respectively) when the hydrolysis temperature was increased. The insoluble residues, after hot water extraction and centrifugation, contained 6.1–6.5% β-glucan, 30.2–35.0% protein, 7.6–11.1% starch and 7.4–10.4% AX. The differences in β-glucan concentrations were not statistically significant. Protein concentration increased and starch and AX concentrations decreased with hydrolysis temperature (Table 8).

The depolymerisation of β-glucan was highly dependent on the temperature during acid hydrolysis. The average M_w of β-glucan in the hot water extracts was 110, 86 and 34 kDa after hydrolysis at 100, 120 and 130 °C, respectively. The corresponding polydispersity (M_w/M_n) values of the β-glucan molecules were 4.0, 4.3 and 6.7. The low polydispersity values were detectable as relatively sharp peaks in the molecular weight distributions (Figure 3 of Publication III).

Table 8. Solubilisation of oat bran and β-glucan into liquid phase after acid and enzymatic hydrolyses and hot water extraction at 70 °C (A). The composition of hot-water extract (B) and insoluble residue (C) were analysed after separation of solids by centrifugation. The OBC concentration during hot water extraction was a = 66.7 g / l or b = 83.3 g / l.

A. Hydrolysis and hot-water extraction	**Solubilisation of oat bran (%)**		**Solubilisation β-glucan (%)**	
	a	**b**	**a**	**b**
Acid, 100 °C	50.5 ± 0.8	48.2 ± 1.1	63.8 ± 0.3	61.7 ± 0.7
Acid, 120 °C	51.3 ± 0.9	51.6 ± 1.1	68.9 ± 0.2	68.9 ± 0.7
Acid, 130 °C	52.0 ± 0.7	52.9 ± 0.4	63.6 ± 0.3	64.3 ± 0.5
Enzymatic, 1 h	41.3 ± 0.9	29.0 ± 1.7	71.1 ± 0.7	44.3 ± 0.8
Enzymatic, 3 h	47.2 ± 0.6	43.9 ± 0.9	77.9 ± 0.4	70.5 ± 0.3
Enzymatic, 4 h	45.7 ± 0.8	44.1 ± 0.5	70.2 ± 0.3	67.7 ± 0.4
B. Composition of hot-water extract	**BG (%)**	**AX (%)**	**Starch (%)**	**Protein (%)**
Acid, 100 °C	42.9 ± 0.6	11.4 ± 0.1	6.9 ± 0.1	8.5 ± 0.1
Acid, 120 °C	45.7 ± 0.6	11.4 ± 0.1	7.8 ± 0.1	7.9 ± 0.1
Acid, 130 °C	41.6 ± 0.3	12.6 ± 0.3	8.9 ± 0.3	8.5 ± 0.2

Enzymatic, 1 h	58.6 ± 0.6	10.8 ± 0.3	4.1 ± 0.1	5.0 ± 0.1
Enzymatic, 3 h	56.2 ± 0.2	13.6 ± 0.5	4.4 ± 0.1	8.6 ± 0.1
Enzymatic, 4 h	52.2 ± 1.1	13.3 ± 0.2	6.7 ± 0.2	8.9 ± 0.1

C. Composition of insoluble residue	**BG (%)**	**AX (%)**	**Starch (%)**	**Protein (%)**
Acid, 100 °C	6.1 ± 0.1	10.4 ± 1.3	11.1 ± 0.1	30.2 ± 0.2
Acid, 120 °C	6.5 ± 0.1	6.8 ± 0.3	10.8 ± 0.2	34.5 ± 0.3
Acid, 130 °C	6.1 ± 0.1	7.4 ± 0.5	7.6 ± 0.1	35.0 ± 0.1
Enzymatic, 1 h	16.4 ± 0.2	12.0 ± 0.2	12.2 ± 0.1	35.1 ± 0.1
Enzymatic, 3 h	11.8 ± 0.1	10.0 ± 0.5	14.2 ± 0.4	35.4 ± 0.1
Enzymatic, 4 h	11.6 ± 0.1	8.9 ± 0.1	13.6 ± 0.5	35.6 ± 0.2

3.2.2 Enzyme-catalysed depolymerisation of β-glucan

Enzyme-catalysed hydrolysis destroyed most of the cell wall structures, although less than acid hydrolysis. The microscopic analysis showed that some of the cell wall structures still remained intact after 1 h enzymatic hydrolysis, but that they were mostly destroyed after 4 h (Figure 2 of Publication III). In contrast to acid-catalysed hydrolysis, starch granules were still detectable after 1 h enzymatic hydrolysis.

In the subsequent hot water extraction, 29.0–47.1% of the enzymatically hydrolysed OBC was solubilised and 44.3–77.9% of the total β-glucan in OBC was extracted in water. The degree of OBC solubilisation did not differ significantly after hydrolysis times of 2–4 h. However, 1 h hydrolysis gave significantly lower solubilisation of OBC. The OBC concentration during hot water extraction also affected solubilisation, which was higher with the lower (66.7 g/l) than with the higher concentration (83.3 g/l). With higher concentration of OBC, the solubilisation of β-glucan improved significantly (from 44.3 to 70.5%) when the hydrolysis time was increased from 1 to 3 h (Table 8).

The hot water extracts of enzymatically hydrolysed OBC contained 52.2–58.6% β-glucan, 10.8–13.4% AX, 5.0–8.9% protein, and 4.1–6.7% starch. β-Glucan concentration decreased with hydrolysis time, but AX, protein and starch concentrations increased. Insoluble residues after the hot water extraction contained 11.6–16.4% β-glucan, 8.9–12.0% AX, 35.1–35.6% protein and 12.2–13.6% starch. The clearest difference in the chemical composition of the insoluble residues was in the concentration of β-glucan, which decreased when hydrolysis time was prolonged from 1 to 4 h. Along with the longer hydrolysis time, there was a decrease in the concentration of AX in the insoluble residues (Table 8).

The average M_w of β-glucan in the hot water extracts was 218, 93, 71 and 49 kDa after 10 min, 1 h, 3 h and 4 h incubation times, respectively. Compared to acid hydrolysis, the enzyme-catalysed hydrolysis resulted in wider M_w distribu-

tions. The polydispersity value (M_w/M_n) varied between 19.0 and 24.2, being lowest with 10 min incubation time of the enzyme. The 'tails' at the ends of M_w profiles of enzyme-hydrolysed oat bran (Figure 3 of Publication III) indicated that part of the β-glucan was depolymerised into shorter oligosaccharides, but their concentrations were not quantified in this work.

3.2.3 Stability of depolymerised oat β-glucan dispersions

The hot water extracts prepared from OBC after 4 h enzymatic hydrolysis (M_w= 49 kDa) retained their low viscosity at 5 °C only for 2 weeks at 1.9% β-glucan concentration but as long as for 12 weeks at 1.6% β-glucan concentration. Acid hydrolysis at 130 °C (M_w = 34 kDa), in turn, resulted in elevated viscosities after 3 or 7 weeks at 1.8 or 1.4% β-glucan concentrations, respectively (Figure 7).

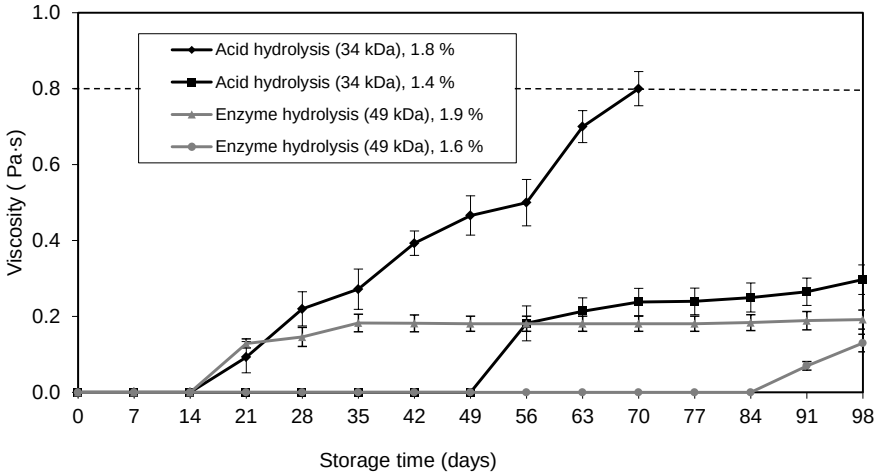

Figure 7. The viscosity development of acid- (34 kDa) and enzyme-hydrolysed (49 kDa) oat bran extracts with time at 5 °C. The viscosity profiles were measured from solutions with 1.4–1.9% β-glucan (w/v). The viscosity values were measured at 24 s^{-1} shear rate in the viscometer. The dashed line (at 0.8 Pa s) shows the measurement limit of the viscometer.

3.3 Oat dietary fibre in extrusion processing (Publication IV)

3.3.1 Extrusion with untreated, ultra-fine and enzymatically hydrolysed oat bran preparations

The behaviour of differently treated OBC-preparations: OBC (untreated), UF-OBC (ultra-fine milled) and EH-OBC (enzymatically hydrolysed) were studied in a recipe based on EF (defatted oat endosperm flour). The starch (71.4–73.4%), protein

(14.0–14.4%), TDF (8.1–9.4%), and β-glucan (4.6–4.9%) contents of extrudates made of 10% OBC, UF-OBC or EH-OBC and 90% EF did not differ remarkably from each other. In addition, the ratio between IDF and SDF was around 50:50 in all these extrudates. Interestingly, the enzymatic hydrolysis did not improve the solubility of DF although the M_w of β-glucan was reduced (from 780 to 455 kDa; Table 1 of Publication IV). The expansion properties of these three extrudate samples were also similar to each other (171–176%) (Table 9).

The hardness of extrudates containing untreated OBC or UF-OBC was similar (258 and 265 N), whereas extrudates with EH-OBC were less hard (200 N), but the difference compared to the other OBC-extrudates was not statistically significant. Thus, the addition of any of these three oat bran preparation into EF-based extrudates increased the hardness (Table 9). The crispiness values of OBC-substituted extrudates (C_i = 1.6–3.7 x 10^{-4}) did not differ from each other, or from the WF-extrudates (made of defatted whole grain oat flour; C_i = 3.7 x 10^{-4}). The standard deviation of C_i for EF-extrudates was so high (C_i = 13.3 ± 7.8 x 10^{-4}) that they were statistically in the same group as WF- and OBC-substituted extrudates. The extrudates with UF-OBC and EH-OBC had slightly higher porosity (68.5 and 71.3%) compared to extrudates with untreated OBC (64.7%) (Table 9). The void area of pores in EF-based extrudates decreased, and cell wall thickness increased, when 10% of untreated OBC, UF-OBC or EH-OBC was added in the recipe (Table 5 and Figure 3 of Publication IV). The differences between the chemical compositions of OBC-supplemented EF-extrudes are presented in Table 9.

3.3.2 Extrusion with water-insoluble and -soluble oat bran preparations

The water-insoluble (WIS-OBC) and water-soluble (WS-OBC) oat bran preparations obtained after enzymatic hydrolysis and hot water extraction behaved differently in extrusion. When 10% of WIS-OBC was mixed with 90% EF, the starch content was reduced (from 73.2 to 68.5%) and the protein and β-glucan contents increased (from 14.5 to 17.1% and from 1.5 to 2.7%, respectively). However, the TDF content did not change significantly (only from 3.9 to 4.1%) (Table 9). The addition of 10 or 20% of WS-OBC resulted in even lower starch (66.6 or 59.6%), lower protein (14.1 or 13.7%), higher β-glucan (6.2 or 11.4%), and higher TDF (6.5 or 10.4%) contents (Table 9).

The expansion of EF-based extrudates clearly decreased (from 199 to 163%). with the 10% addition of WIS-OBC, even more compared to the 10% addition of untreated OBC (172%). By contrast, 10 or 20% addition of WS-OBC significantly increased the expansion of EF-based extrudates (from 199% to 218–226%). The hardness of 100% EF-based extrudates increased (from 156 to 311 N) when 10% WIS-OBC was added, and decreased (to 141–146 N) when 10 or 20% WS-OBC was added (Table 9). The crispiness of extrudates with 10% WIS-OBC (C_i = 4.2 x 10^{-4}) did not differ from WF- and OBC-substituted extrudates. However, the 10–20% addition of WS-OBC increased the crispiness (C_i = 12.8–21.3 x 10^{-4}), alt-

hough there was no clear correlation between the level of WS-OBC addition and the crispiness of extrudates (Table 9).

The porosity of the EF-based matrix was reduced (from 75 to 59%) when 10% of WIS-OBC was added, whereas approximately the same porosity (75%) as in 100% EF-extrudates was obtained with 10% addition of WS-OBC. Higher (20%) addition of WS-OBC resulted in even higher porosity (81%). A strong correlation (R^2 = 0.99) was observed between the porosity and hardness of EF-based extrudates enriched with different OBC-fractions (Figure 8).

The average cell wall thickness decreased (from 0.38 to 0.22 mm) when increasing the content of WS-OBC from 0 to 20% in EF-based extrudates (Table 5 of Publication IV). The proportion of very small pores was highest in the extrudates with 10% WIS-OBC, and significantly decreased in 10% WS-OBC and even more in 20% WS-OBC extrudates (Figure 4 of Publication IV). By contrast, the extrudate cell wall thickness shifted towards significantly thinner cell walls when comparing 10% WIS-OBC with 10 and 20% WS-OBC extrudate.

Table 9. The chemical composition and physical properties of EF-extrudates supplemented with differently treated OBC-fractions. Values within the same column followed by a common letter are not significantly different (P < 0.05).

	Starch (g/100g)	Protein (g/100g)	β-glucan (g/100g)	TDF (g/100g)	SDF (%)	IDF (%)
1. WF 100 %	63.3 ± 0.8 b	14.9 ± 0.1 f	5.2 ± 0.1 d	8.8 ± 0.6 b	40	60
2. EF 100 %	73.2 ± 0.2 f	14.5 ± 0.1 e	1.5 ± 0.2 a	3.9 ± 0.6 a	51	49
3. EF 90 % + OBC 10 %	71.8 ± 0.1 e	14.2 ± 0.1 c	4.6 ± 0.3 c	8.9 ± 0.5 b	52	48
4. EF 90 % + UF-OBC 10 %	73.4 ± 0.3 f	14.0 ± 0.1 b	4.9 ± 0.1 cd	9.4 ± 0.9 b	48	52
5. EF 90 % + EH-OBC 10 %	71.4 ± 0.4 e	14.4 ± 0.1 d	4.7 ± 0.1 c	8.1 ± 1.4 b	51	49
6. EF 90 % + WIS-OBC 10 %	68.5 ± 0.1 d	17.1 ± 0.1 g	2.7 ± 0.1 b	4.1 ± 0.5 a	26	74
7. EF 90 % + WS-OBC 10 %	66.6 ± 0.6 c	14.1 ± 0.1 b	6.2 ± 0.4 e	6.5 ± 0.7 a	96	4
8. EF 80 % + WS-OBC 20 %	59.6 ± 0.3 a	13.7 ± 0.2 a	11.4 ± 0.1 f	10.4 ± 0.6 b	99	1

	Expansion (%)	Hardness (N)	Crispiness (10^{-4})	Porosity (%)
1. WF 100 %	151 ± 4 a	399 ± 62 de	3.7 ± 1.7 a	48.2 ± 0.6 a
2. EF 100 %	199 ± 8 d	148 ± 37 ab	13.3 ± 7.8 ab	75.3 ± 1.8 e
3. EF 90 % + OBC 10 %	172 ± 5 bc	258 ± 37 c	1.6 ± 0.7 a	64.7 ± 2.8 c
4. EF 90 % + UF-OBC 10 %	176 ± 2 c	265 ± 49 c	1.6 ± 0.7 a	71.3 ± 2.2 de
5. EF 90 % + EH-OBC 10 %	171 ± 3 bc	200 ± 33 bc	3.7 ± 2.1 a	68.5 ± 0.4 cd
6. EF 90 % + WIS-OBC 10 %	163 ± 4 b	313 ± 59 cd	4.2 ± 2.5 a	58.8 ± 0.2 b
7. EF 90 % + WS-OBC 10 %	218 ± 7 e	141 ± 23 a	21.3 ± 9.1 b	74.6 ± 2.6 e
8. EF 80 % + WS-OBC 20 %	226 ± 10 e	146 ± 20 a	12.8 ± 3.5 b	81.3 ± 0.1 f

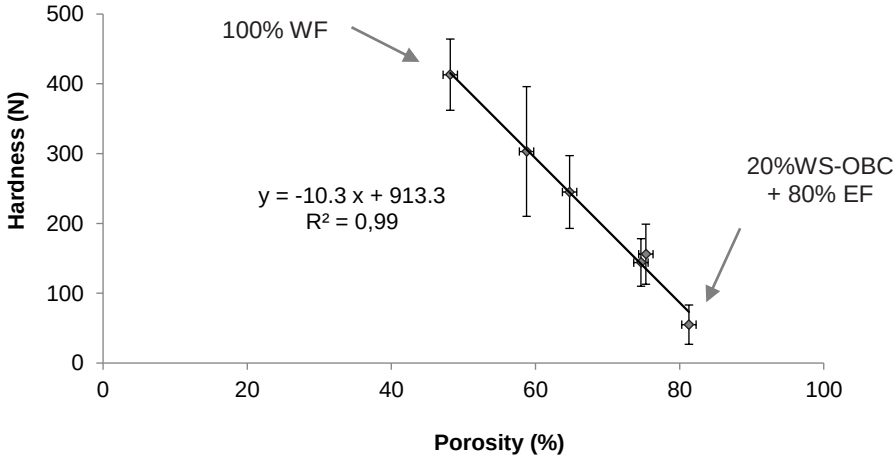

Figure 8. Correlation between porosity and hardness of extrudates made of different oat fractions. Porosity was lowest and hardness highest with extrudates made of 100% WF (defatted whole grain oat flour). Extrudates with 20% WS-OBC (water-soluble oat bran preparation) + 80% EF (defatted endosperm flour) showed the highest porosity and lowest hardness.

4. Discussion

4.1 Interactions of grain components and impact on dry fractionation

4.1.1 Matrix disintegration by grinding

Grinding is an essential part of dry fractionation, because it determines how well grain components can be separated from each other. However, grinding of oats is more challenging as compared to other cereals due to the high content of lipids, which tend to hold especially the bran components together (Miller and Fulcher, 2011). Further enrichment of DF components is easier when most of the starchy endosperm is first separated from the bran particles. This is mainly because bran particles will be subjected to more mechanical energy in the grinding equipment compared to whole grain flour, in which starch granules absorb part of the grinding energy (Mälkki et al., 2001).

The particle size of regular oat bran is around 500–2000 µm (Wang et al., 2007). These coarse particles need to be further ground in order to separate the relatively thin pericarp layer (<50 µm) from the aleurone cell layer (50–150 µm) and from the starch aggregates (20–150 µm) and protein bodies (1–10 µm) present in the starchy endosperm (Miller and Fulcher, 2011; Hoover and Vasanthan, 1992, Hartunian-Sowa and White, 1992). The dissociation of oat bran components is difficult, because the pericarp layer is very brittle, and the aleurone layer does not separate as cleanly from the endosperm in oats as it does in wheat (Antoine et al., 2004; Miller and Fulcher, 2011).

One of the most efficient disintegration methods for oats is pin disc milling, which has been utilised to dissociate subaleurone endosperm cells rich in β-glucan from other components of oat bran (Mälkki et al., 2001). The grinding effect of pin disc milling appears to be superior compared to most of the other grinding methods, firstly because the oily oat material does not easily stick to the pins, and secondly because grinding with pin discs tends to leave aleurone and subaleurone cell walls more intact while dissociating them from the surrounding starch and protein particles. This was the main reason why pin disc milling was also chosen as the first grinding method in the current study (Publication I).

When different oat bran preparations were ground in the ultra-fine grinding equipment at ambient temperature, it was demonstrated that the oat bran fraction enriched in β-glucan was not ground into as fine particles as the brans containing lower concentrations of β-glucan (Table 2 of Publication II). The main reason for this was higher starch concentrations in the latter fractions. Thus, the regular oat bran (ElovenaPlus, 49.4% starch), the non-defatted oat bran concentrate (OBC-20NEF, 17.8% starch) and the defatted oat bran preparation (SC-CO2-OBC-1, 28.0% starch) had smaller particle size than the highly enriched β-glucan fraction (SC-CO2-OBC-2, 7.8% starch) after they were ultra-fine ground in a similar way (Table 2 of Publication II).

The ultra-fine grinding of defatted oat bran enriched in β-glucan (SC-CO2-OBC-2) yielded almost identical particle size distributions at ambient temperature and in cryogenic conditions (D_{50} = 60 and 63 µm, respectively). It is interesting that these two milling methods resulted in such similar particle sizes. The limitation in the particle size reduction could be due to the efficiency of the grinding equipment. Hemery et al. (2011) showed a similar kind of restriction in the particle size reduction of wheat bran. They reported that the particle size of ultra-fine wheat bran was almost identical after ambient and cryogenic ultra-fine grinding (D_{50} = 51 and 55 µm, respectively). Nevertheless, three successive grinding passes were needed in ambient conditions, compared with only one in cryogenic grinding. The similar particle size range of ultra-fine ground oat bran and wheat bran can be due to the high proportion of aleurone cells, which are usually highly resistant towards breakage (Antoine et al., 2004; Miller and Fulcher, 2011).

There might also be some changes or degradation of DF components during very efficient grinding. For example, grinding can change the solubility of DF compounds, as was demonstrated in extensive ball milling of wheat and rye bran (Van Craeyveld et al., 2009). Similar observations were not made in the current study (Table 1 of Publication IV), most probably because the grinding time was very fast (less than 1 min) compared to the study of Van Craeyveld et al. (2009), in which the authors used ball milling for 24–120 h.

In the present study, the microscopic pictures of aleurone and subaleurone cells showed that they were partly broken due to the efficient milling (Figure 2 of Publication I and Figure 3 of Publication II). This was opposite to the behaviour of wheat aluerone cells, which have been shown to be more resistant towards fine grinding (Antoine et al., 2004; Rosa et al., 2013). However, the subaleurone cells of hard wheat varieties might behave similarly to oats. In hard wheat varieties, the aleurone layer forms a fairly complete shell around the inner endosperm. In soft wheats, the shell is often discontinuous. At the points of discontinuity, the inner endosperm cells extend out to the aleurone layer, and thus the subaluerone cells are much more difficult to separate from soft than from hard wheat varieties by air classification (Kent, 1966; Wu and Stringfellow, 1979).

The oat cultivar can also affect the dissociation of different grain components. MacArthur and D'Appolonia (1979) showed that in a low starch and high protein variety the lipid content was high, whereas in a high starch and low protein variety it was lower. According to Fulcher and Miller (1993) and Miller and Fulcher (1994),

β-glucan was more evenly distributed throughout the starchy endosperm in cultivars with high β-glucan content, and more concentrated in the subaleurone region in low β-glucan cultivars. Thus, the recovery of β-glucan from the low β-glucan cultivars might be more efficient than from the high β-glucan cultivars, as β-glucan is more located in the subaleurone region.

4.1.2 Molecular interactions of major grain components

As mentioned above, if the aim is to enrich DF components, it is important to dissociate particles rich in starch and protein from particles rich in cell walls. Oat grain is a complex material and it is composed of several different layers and cell types, which are surrounded by cell walls. The different layers of oat kernel (i.e. pericarp, testa, nucellum, aleurone, subaleurone and starchy endosperm) have different types of cell walls. The cell walls of aleurone are closely grouped to other intracellular compounds (especially proteins), thick and strong, whereas the subaleurone cell walls are loosely packed, thinner and easier to break as they surround the endosperm starch- and protein-rich particles (Kent, 1966; Miller and Fulcher, 2011).

The bran layer contains fibrous cell wall polymers, i.e. β-glucan, arabinoxylan, cellulose and lignin (Miller and Fulcher, 2011), whereas the endosperm contains mainly starch, protein and lipids (Paton, 1977; Lim et al., 1992). Oat lipids and proteins are usually bound to each other (Heneen et al., 2009). Thus, starch granules and protein bodies are difficult to separate from the endosperm flour. In addition, proteins and lipids inside cell wall matrices (especially inside aleurone) are protected by the rigid membranes and are located in separate compartments (Bechtel and Pomeranz, 1981; Peterson et al., 1985).

In the current study, it was shown with non-defatted oats that β-glucan could not be enriched to a higher concentration than 17.1%, even though the mass yield was reduced below 8.7% (Table 3 of Publication I). This was most probably due to the effect of lipids, which kept the bran components connected so that the air classifier could not separate them. In addition, it was noticed that the separation of protein-enriched fraction was not possible from the non-defatted oat endosperm flour, possibly due to the attachment of protein bodies into starch granules.

There are not many studies on the effects of oat starch and protein on the dry fractionation of oat grain components. Dijkink et al. (2007) studied protein and starch interactions and their influence on the dry fraction behaviour by fundamental models. They showed that the type of protein and type of starch (especially granule size and roughness) affected their dispersability. Increased starch content enhanced dispersability, but did not appear to affect the adhesion between starch and protein particles. According to Barlow et al. (1973), water-soluble proteins of wheat were confined to a position immediately surrounding starch granules. The water-soluble material appears to be a cementing substance between starch granules and storage protein (Stevens et al., 1963). Thus, it is not easy to separate high-protein fractions from wheat. Nevertheless, high-protein soft wheat varieties

have shown potential for protein shifting (e.g. from 16% protein content of whole grain flour up to 27% protein content) by air classification (Wu and Stringfellow, 1979).

The relatively high protein content (23% protein) of β-glucan-enriched oat bran fraction (Table 4 of Publication I) can be explained by the intracellular protein bodies surrounded by the cell wall matrix (Figure 2 of Publication I). Protein was so tightly enclosed by the cell walls that it did not leach out even during the extensive grinding. Histochemically, the β-glucan-enriched oat bran fraction appeared to consist mainly of aleurone and subaleurone endosperm cells. Similar results were reported by Kent (1966), who showed that a coarse subaleurone fraction enriched from hard red winter wheat by air classification possessed much higher protein content (45% protein) than the corresponding inner endosperm fraction (11% protein).

4.1.3 Lipid removal

Removal of lipids has been demonstrated to improve the fractionation of oats in several studies (Wu and Stringfellow, 1995; Wu and Doehlert, 2002; Liu, 2014). The majority (up to 90%) of lipids in oats is found in the endosperm, and is composed of non-polar lipids (Youngs et al., 1977; Youngs, 1978; Price and Parsons, 1979). Banás et al. (2007) showed that the oil bodies in the aleurone layer and in the embryo occur as individual entities, whereas in the endosperm the oil bodies tend to fuse together during the development of the kernel and thus oil, starch and protein are all attached to each other in the endosperm of matured oat kernel.

The results of Publication I showed that SC-CO_2 extraction removed mainly the lipids from starchy endosperm (see Figure 5), and to a lesser extent the lipids from outer grain layers. Similar to the proteins in aleurone (Kent, 1966), the lipids surrounded by the cell walls of outer grain layers had a physical barrier which prevented them from being leached out during the SC-CO_2 extraction. The removal of endosperm lipids significantly improved the dry fractionation of oat components, because endosperm lipids are not tightly surrounded by the cell walls and they can impede the fractionation by forming large aggregates with starch- and protein-rich particles after the kernel has been crushed by grinding. The defatted bran particles were easier to grind and fractionate, although they contained rather similar amounts of lipids (4.3%) as the non-defatted whole grain oats (5.7%) (Table 5). This was because the bran particles stayed free-flowing because the lipids did not leach out of the matrix and thus the bran particles did not stick on the surfaces of the fractionation equipment (as shown in Publications I and II).

The results obtained in pilot scale were in accordance with the lipid contents of the defatted fractions produced in the industrial scale fractionation process. The results of Publications I and II showed that the dry fractionation of non-heat-treated and defatted oat material was much more efficient compared to the heat-treated and non-defatted oat material. Defatting increased the separation efficiency, and much higher β-glucan content (31.2%) was reached with similar mass yield (8.8%) than with the non-defatted material (only 17.1% β-glucan content). In addition

to β-glucan, arabinoxylan was enriched into the fractions with coarse bran particles. A similar kind of AX-enrichment was reported by Johansson et al. (2004).

Addition of ethanol to SC-CO_2 extraction improved the removal of polar lipids (Table 2 of Publication I). The total lipid contents of non-defatted, SC-CO_2 extracted and SC-CO_2+EtOH extracted wholegrain oat flours were 5.7, 2.0 and 0.8%, respectively. The corresponding contents of polar lipids were 0.8, 0.8 and 0.4%, indicating that SC-CO_2 itself could not extract polar lipids. The distribution of non-polar and polar lipids was not studied in this research, but Youngs et al. (1977) showed that the distribution of different lipid classes is almost equal in the bran and endosperm fractions. Thus, the distribution of different lipid classes does not seem to be the reason for the differences in the extractability of lipids from different oat fractions. Instead, more intensive encapsulation of lipid bodies surrounded by the cell walls in the bran fractions could explain the easier extractability from the looser and porous endosperm fraction. The addition of ethanol during the pilot-scale SC-CO_2 extraction enhanced the separation of β-glucan (up to 33.2% content). Nevertheless, it was not considered economically feasible, because ethanol as co-solvent increases process costs and would require much thicker walls in the extraction vessels due to the risk of ethanol explosion under high pressure. SC-CO_2 alone is already a costly method due to the long processing times (several hours per batch). However, there are existing factories offering contract manufacturing in Europe, which can make the fractionation processs economically feasible, especially when all the fractions are utilised in high-value applications.

In the industrial scale, the two-step pin disc grinding and air classification of defatted oats yielded a bran fraction with 33.9% β-glucan (Table 4 of Publication I). A concentration up to 40.3% β-glucan was reached when ultra-fine grinding was used prior to a third air classification step (Publication II). These results were in line with those of Wu and Doehlert (2002), who showed that β-glucan was enriched more efficiently when the coarse bran fraction was intensively ground (3 x pin disc milling) prior to air classification. However, the results of Wu and Doehlert (2002) showed lower content (max. 20% of β-glucan) compared to the current study, mainly due to the heat-treatment of oat flakes prior to defatting as well as due to lower mechnanical energy of the grinding equipment used. Wu and Doehlert (2002) used a Hosokawa Alpine 160Z pin disc mill, which has only about two thirds of the tip speed (around 160 m s^{-1}) of the Hosokawa Alpine 250CW mill (250 m s^{-1}) used in the current study.

Wu and Stringfellow (1995) were possibly the first investigators to obtain very high protein concentrations (up to 81% protein) from defatted oats by air classification. However, the high protein concentration could only be collected from the ultra-fine fraction from the exhaust bag of the classifier, and the yield of the fraction was less than 1%. Cloutt et al. (1987) reported that protein–starch separation of legumes was most efficient when the cut-off was at around 10 µm, i.e. below the size of most starch granules. Similarly, in the current study (Table 4 of Publication I) a high concentration of oat protein (72%) was obtained with 5% yield using a precise cut-off of the air classifier allowing only particles below 5–10 µm to pass through the classifier wheel. The high protein content was reached mainly because oat

endosperm protein bodies occur between 0.3 and 5 µm (Bechtel and Pomeranz, 1981), whereas oat starch occurs as aggregates of 20–150 µm (Hoover and Vasanthan, 1992; Hartunian-Sowa and White, 1992). The pin disc grinding was unable to break the starch aggregates below 10 µm.

4.1.4 Electrostatic separation and jet-milling fractionation

Electrostatic separation was studied as a method to separate particles based on their charge, as further enrichment of β-glucan from ultra-fine oat bran was not possible by air classification. The ultra-fine ground defatted oat brans (ground at ambient temperature or in cryogenic conditions) were separated in the electrostatic field, and the obtained maximal β-glucan concentrations were close to each other (48.4 and 46.0%, respectively) (Table 1 of Publication II). According to Hemery et al. (2009), the lipidic non-polar compounds present in the intermediate layers of wheat bran affected the dissociation of particles, especially after cryogenic grinding. Thus, defatting might have removed the effect of lipidic intermediate layers in oat bran on the particle dissociation. However, Antoine et al. (2004) showed that wheat brans fractured more rapidly than isolated aleurone layers because of the presence of the friable pericarp and possible mechanical constraints due to tissues surrounding the aleurone layer.

In the present study, β-glucan and starch concentrated in the positive fractions during the electrostatic separation of defatted oat brans, whereas arabinoxylan concentrated in the negative fractions (Table 1 of Publication II). Arabinoxylan and β-glucan concentrated similarly to the current study after electrostatic separation of wheat bran (Hemery et al., 2011). However, Hemery et al. (2011) reported significantly different protein concentrations from the opposite fractions (19% in the most positive, and 6% in the most negative fraction), whereas in the current study the protein contents of defatted oat brans were similar in all fractions (around 18% protein). In addition, in wheat bran processing the A/X ratio varied much more between the fractions; being 0.4 in the most positive and 1.1 in the most negative fraction (Hemery et al., 2011). This was an opposite trend compared to the results obtained with defatted oat bran (0.8 in the most positive and 0.6 in the most negative fraction) (Table 1 of Publication II).

The relationship between the surface properties of the cereal particles and their tribo-charging behaviour is still largely unknown. The ability of a material's surface to donate or accept electrons when it comes into contact with another material determines the positive or negative surface charge density that it will acquire during tribo-charging (Mazumder et al., 2006). Different chemical groups and the type of cell wall polysaccharides (branched and cross-linked vs. linear) may be responsible for the acquired charges of the different types of particles (Hemery et al., 2011). Antoine et al. (2004) reported that the outer pericarp layer of wheat bran had high porosity and thus exhibited poor permittivity. By contrast, the aleurone layer showed a substantial permittivity, about sixfold higher than the pericarp. The high capacity of the aleurone layer was postulated to be due to the high amount of

linear polymers in aleurone. The proportion of pericarp layer in oat bran is lower than that in wheat bran (Dornez et al., 2011; Miller and Fulcher, 2011), which could explain the observed differences between the fractionation of oat and wheat bran samples.

Air-jet milling and simultaneous air classification showed a good potential to purify the subaleurone endosperm cells from starch and protein, either before or after the electrostatic separation. The highest β-glucan concentration of the coarse fraction obtained after jet-milling and air classification was 56.2% (Table 1 of Publication II). At the same time, the protein and starch concentrations were reduced significantly (12.9% protein and 3.3% starch) compared to the starting material (17.9% protein and 9.5% starch). Similar results were obtained by Wu and Norton (2001), who ground corn fibre into ultra-fine particles and removed protein and starch by air classification and sieving. They were able to enrich the TDF content up to 83.0% (76.4% in the initial raw material), while decreasing the protein (from 13.1 to 7.0%) and starch contents (from 15.4 to 4.7%). In addition, Létang et al. (2002) demonstrated how jet-milling could be utilised to purify wheat starch granules from attached proteins. This type of approach was especially suitable for hard wheat varieties, as proteins were less attached on the surface of hard wheat than on the surface of soft wheat starch granules.

4.2 Depolymerisation of β-glucan at low water content

Addition of oat DF and especially β-glucan to aqueous foods is challenging due to their high viscosity-enhancing capacity. Thus, strategies to tailor the molecular properties of β-glucan are needed. This section focuses on controlled depolymerisation of β-glucan in order to facilitate a better applicability of β-glucan for liquid food applications.

4.2.1 Comparison of acid and enzymatic hydrolysis

The depolymerisation of oat bran β-glucan was studied by comparing acid and enzymatic hydrolysis at low water content. In acid hydrolysis, the M_w of β-glucan was decreased down to 86 or 34 kDa when the hydrolysis was performed at 120 or 130 °C, respectively. The polydispersity (M_w/M_n) became higher (from 4.0 to 6.7) when increasing the temperature and lowering the M_w of β-glucan. In enzymatic hydrolysis, the M_w of β-glucan was degraded in line with the hydrolysis time (at 50 °C). The M_w of β-glucan was 71 or 49 kDa when the oat bran preparation was hydrolysed for 3 or 4 h, respectively. The polydispersity was much higher (19.0–24.2) compared to acid hydrolysis, and increased with the hydrolysis time (Publication III).

The extent of acid hydrolysis was strongly dependent on water content and temperature during the hydrolysis. Acid hydrolysis at 90% water content and at high temperature (120–130 °C) resulted in rapid breakdown of β-glucan into short oligosaccharides (Publication III). The concentration of acid also played a signifi-

cant role in the depolymerisation, although the effect of concentration was not studied in the current work. However, it was shown by Kaukovirta-Norja et al. (2009) that 8% phosphoric acid was optimal for the hydrolysis, as at this concentration the acid did not break the β-glucan molecules into too small fragments and the depolymerisation of β-glucan molecules was controllable by changing the temperature during hydrolysis in the extruder.

The effects of acid concentration, temperature and hydrolysis time were also demonstrated by Johansson et al. (2006). The authors showed that at relatively low temperature (37 °C), no degradation of β-glucan was observed with 0.1 M HCl over a 12 h period. By contrast, at high temperature (120 °C) total hydrolysis to D-glucose occurred with 3 M HCl already after 1 h hydrolysis. Hydrolysis with 0.1 M HCl at 120 °C for 1 h produced a range of products, but as the water content was high, the majority of the resulting oligosaccharides had a very low M_w (DP 1–7) compared to the molecules in the present study ($M_w \geq 34$ kDa, i.e. DP ≥ 190; Publication III).

In the current study, acid hydrolysis was much faster (3 min) than enzymatic hydrolysis (1–4 h), mainly because high temperatures (100–130 °C) accelerated the acid hydrolysis, whereas the enzymatic hydrolysis (at 50 °C) was less efficient and required longer time during the stationary incubation at low water content. Shear forces inside the extruder may also have contributed even more to the extent of depolymerisation during acid than enzymatic hydrolysis, because oat bran became more plasticized during the acid hydrolysis. The slower hydrolysis by enzymes was also observed from the consistency of the enzymatically hydrolysed oat bran, which became remarkably plasticized only after incubation times of 3 or 4 h. Efficient mixing and mass transfer are generally considered to be essential for the performance of enzymatic reactions, and in this respect the lack of mixing during the low water content incubation could be one of the reasons for the slower hydrolysis by the enzyme (Lavenson et al., 2012). Similarly, Viamajala et al. (2009) explained that the absence of continuous free water phase may cause the bulk to behave as a wet granular material when portions of the 'void' volume contain air rather than liquid, which is detrimental to enzymatic action.

The dosage of enzyme is a crucial parameter for the depolymerisation reactions. In general, enzymes need water for their catalytic activity, but water also influences the structure of enzyme proteins via non-covalent bonding, disrupts the hydrogen bonds, facilitates the reagent diffusion, and influences the reaction equilibrium (Hari Krishna, 2002). Too low water content generally reduces the activity of enzymes, but the minimum hydration level for the enzymatic activity varies considerably between different enzyme preparations (Hobbs and Thomas, 2007). The synergistic action of β-glucanase and xylanase in the enzyme preparation (Depol 740L) utilised in the current work can also be considered as beneficial in the degradation and solubilisation of oat bran DF, as reported by Petersson et al. (2013) for rye and wheat bran DF.

Lehtomäki and Myllymäki (2009) determined that 45–50% water content was necessary to obtain a plasticized mass and optimal depolymerisation of oat bran β-glucan. Similar results were reported in the current study at 50% water content

(Publication III) and by Santala et al. (2011), who showed that the enzymatic solubilisation of AX from wheat bran at 40% water content was as efficient as at 90% water content when the material was continuously mixed in a Farinograph. Further study with a short pre-mixing in an extruder combined with a stationary incubation (at 50 °C) showed that AX was solubilised at a similar level at 40% water content as compared to 40 or 90% water contents when continuous mixing was used (Santala et al., 2013). Roche et al. (2009) also reported that effective initial mixing can promote the distribution of enzyme and continue the hydrolysis without continuous mixing at high solids concentration. This can explain why the enzymatic hydrolysis progressed during the stationary incubation in the current study (Publication III), although the hydrolysis reactions were slower compared to acid hydrolysis.

The higher yields of the water extracts after acid hydrolyses of oat bran (48.2–52.6%) than after enzymatic hydrolyses (29.0–47.1%) (Table 8) could be explained by the rather unspecific hydrolysis of grain compounds by the acid as compared to the enzymatic treatment with Depol 740L enzyme preparation, which degraded mainly polysaccharides. The separation of insoluble residue from the hot water dispersions of enzymatically hydrolysed oat brans was more challenging than from acid hydrolysed material (at around 2% β-glucan concentration), due to the presence of β-glucan molecules (49–71 kDa with high polydispersity) with higher water absorption capacity compared to acid hydrolysed molecules (34–86 kDa with lower polydispersity).

The insoluble residues after hot water extraction and separation of solids contained high amounts of proteins. This was observed after both acid and enzymatic hydrolysis (30.2–35.0% and 35.1–35.6% of protein in the residue, respectively) (Table 8). The high protein concentrations can be explained by a strong protein–cell wall interaction, which kept the protein closely bound to the water-insoluble cell wall matrix. As the hydrolysis of cell walls by acid or enzyme and simultaneous mechanical mixing in the extruder did not liberate the cell wall-bound protein, it can be concluded that this protein was much more difficult to separate compared to the endosperm proteins (as demonstrated by air classification in Publication I).

4.2.2 Molecular properties of β-glucan after acid and enzymatic hydrolysis

Both acid and enzyme hydrolysis at low water content maintained the M_w of β-glucan higher than 34 kDa (Publication III). Similar results were obtained by Kaukovirta-Norja et al. (2009), who used acid hydrolysis of oat β-glucan at 45–50% water content. When 8% phosphoric acid was used at 110–130 °C, the M_w of β-glucan was between 37 and 135 kDa (OBC with 22% β-glucan) or between 28 and 105 kDa (OBC with 33% β-glucan). In the current study, the M_w of β-glucan was decreased from 110 to 34 kDa at temperatures of 100–130 °C.

The enzyme-catalysed reaction resulted in wider M_w distributions (Figure 3, Publication III) and higher polydispersity values (19.0–24.2) as compared to acid hydrolysis (4.0–6.7). The side activities of the enzyme preparation may also have affected the results, because the enzyme preparation had several polysaccharide-

hydrolysing activities. In earlier studies, for example, a very low endo-glucanase contamination in exo-acting cellobiohydrolase II preparation caused considerable changes in the hydrolysis products of β-glucan (Reinikainen et al., 1995). However, as Depol 740L contained only low β-glucosidase side activity (around 3.4 nkat/g OBC), it did not produce free D-glucose and all the β-glucans remained in polymeric or oligomeric form after enzymatic hydrolysis.

Similar enzymatic hydrolysis at low water content was reported by Lehtomäki and Myllymäki (2009), who investigated the enzymatic hydrolysis of oat bran at 45–55% water content with commercial cellulase and α-amylase preparations. They used a twin-screw extruder with a short reaction time (65 s) at an intermediate temperature (65 °C), and controlled the hydrolysis of β-glucan by the dosage of enzymes (17–17000 nkat β-glucanase/g oat bran). The enzymes were inactivated immediately after the short hydrolysis time by repeating the extrusion at 95 °C. Unfortunately, the authors did not report the M_w values of the enzyme-hydrolysed oat bran preparations.

4.3 Development of food matrices high in oat dietary fibre

4.3.1 Factors influencing the achievement of a high level of dietary fibre

In order to produce food products high in oat DF, a DF-rich ingredient is required. The recommended level of oat β-glucan to assist in cholesterol maintenance is 1 g/portion (EFSA, 2011a). To achieve this level, for example in a 50 g portion, it is necessary to add around 12.5 g of regular oat bran (with 8% β-glucan) or around 3.5 g of oat bran concentrate (OBC, with 29% β-glucan). As the required amount of regular oat bran would be relatively high (25% of product dry weight), it would also bring a high content of starch and other non-DF compounds into the food matrix, and could radically change the texture of the product. In the case of OBC, the required amount is significantly lower (7% of dry weight), as the TDF content of this ingredient can be around 48–50% (as shown in Table 2 of Publication IV). Thus, in the latter case it is easier to retain product texture closer to that with no added oat DF. Products which would mostly benefit from the DF-rich ingredients include, for example, yoghurts and meat products (Havrlentová et al., 2011), biscuits (Pentikäinen et al., 2014), soups (Lyly et al., 2004), beverages (Publication III) and extruded snack products (Publication IV).

Both the concentration and the Mw of β-glucan affect the texture of food products. HM_w β-glucans tend to form slimy or gummy textures, as they have very high water absorption capacity (Zhang et al., 1998). In baking application, oat DF has been reported to reduce the volume of breads (Kim and Yokoyama, 2011; Flander, 2012; Tiwari et al., 2013), but using sourdough fermentation it was possible to add a reasonable amount of β-glucan (e.g. 2.4–2.7g/100 g) into a bread containing oat bran and wheat flour and still obtain a high bread volume (Flander et al., 2007). After the sourdough fermentation, the M_w of β-glucan also remained relatively high (500 kDa) when compared to the original oat bran (1000 kDa). This observation

was consistent with most oat baking studies in which the activity of β-glucan-degrading enzymes was restricted at the dough-making stage. However, LM_w β-glucan (210 kDa) appeared to be less susceptible to further degradation during baking than higher M_w β-glucan (640 kDa) (Cleary et al., 2007).

Due to their high water absorption, HM_w oat β-glucans develop high viscosity already at <0.5% in aqueous solution (Doublier and Wood, 1995; Lazaridou et al., 2003; Skendi et al., 2003). This limits the use of HM_w oat β-glucans in beverages, but could be beneficial for other types of aqueous food matrices, such as jellies and puddings (Brummer et al., 2014). By lowering the M_w, higher concentration of β-glucans can be added into beverages without viscosity-related problems, as will be is discussed in the section below.

4.3.2 Oat dietary fibre in high moisture applications

Adding DF into liquid foods creates challenges for product texture and stability. β-Glucan appears to be the most important compound affecting the viscosity of beverages made of oat bran extracts, because β-glucan comprises the majority of water extractable DF in oat bran (Wood et al., 2011). Other important compounds affecting the viscosity of oat bran extracts are AX and starch. It can be hypothesised that the slimy texture originates from HM_w β-glucan and the sticky texture from AX, as the sticky texture of rye bread dough mainly originates from AX (Virtanen and Autio, 1993; Gräber, 1999). Starch can also increase the viscosity of oat bran extracts, but it has a much smaller effect compared to (HM_w) β-glucan (Kim and White, 2013). Jaskari et al. (1995) even showed that, at relatively high levels of β-glucan (17.4%) and AX (7.4%), the viscosity of oat bran slurries changed only slightly when starch was completely degraded to water-soluble oligosaccharides, but the viscosity clearly decreased when β-glucan was hydrolysed to lower M_w products (from 600 to 40 kDa). The effects of starch can also be minimised using oat bran ingredients, which are high in β-glucan and low in starch (Publication III).

In order to reach a sufficient amount of β-glucan in beverages (e.g. 1 g β-glucan in 100 mL portion) the β-glucan cannot be in HM_w form, otherwise the viscosity of beverage would become too high and it would not be drinkable (Lyly et al., 2003). The results of Publication III showed that acid or enzyme hydrolysis of OBC at low water content resulted in LM_w (<50 kDa) β-glucans, which remained stable in liquid suspension for several weeks at 1.4–1.6% β-glucan concentration. The concentration of β-glucan could be even lower and the M_w higher (than 50 kDa), to obtain products which could still fulfil the requirements of the EU-level health claim (EFSA, 2011a).

In acid hydrolysis, the highest solubilisation of oat bran and β-glucan was obtained when OBC was hydrolysed at 120 °C, and the solubilisation level of β-glucan was reduced from around 69 to 64% when OBC was hydrolysed at 130 °C. This was most probably due to the harsh hydrolysis conditions, in which a small proportion of the β-glucan molecules might have been cleaved down to

glucose or even its degradation products. Similarly, the solubilisation of β-glucan was highest after 3 h of enzymatic hydrolysis, whereas 4 h incubation resulted in slightly decreased solubilisation (Table 8). This was presumably because when the M_w or concentration of hydrolysed β-glucan molecules was increasing in the aqueous solution, the aggregation of β-glucan became faster (Figure 4 of Publication III). Moschakis et al. (2014) assumed that β-glucan molecules start to self-assemble due to inter- and intra-chain hydrogen bonding, which can cause phase separation into β-glucan rich regions and depleted regions.

In the present study, even though the M_w of β-glucan was lower after the acid (34 kDa) than the enzymatic hydrolysis (49 kDa), the acid hydrolysed β-glucan molecules resulted in faster and more intensive gel formation as compared to enzymatically hydrolysed molecules. The reason for this could be that acid hydrolysed polymer populations had a sharper M_w distribution (Figure 3 of Publication III) and more linear chains of β-glucan, which can easily form aggregates with each other (Doublier and Wood, 1995). This explanation was supported by the findings of McCleary and Matheson (1987) and Tosh et al. (2004), who showed that acid could cleave both (1→3)- and (1→4)-β-D-linkages, whereas the β-glucanase in the enzyme preparation used, Depol 740L, was specific only for (1→4)-β-D-linkages. Therefore the acid hydrolysed β-glucan molecules may have contained relatively more (1→4)-β-D-linkages, meaning that the polymers were more linear and enhanced the gelling behaviour of the extracted β-glucan molecules (Tosh et al., 2004).

The agglomeration phenomenon has also been explained by the intermolecular interactions resulting from the increased mobility of hydrolysed macromolecules (Böhm and Kulicke, 1999; Doublier and Wood, 1995). As the hydrolysed macromolecules are more mobile, they have greater probability to achieve proximity with other compounds containing regions required for aggregation (Doublier and Wood, 1995; Vaikousi et al., 2004). The HM_w oat β-glucans (>250 kDa) have been shown to form stronger gel networks, which consist of micro-aggregates with better organization than their LM_w (35–140 kDa) counterparts (Lazaridou et al., 2003). However, unhydrolysed HM_w oat β-glucan solutions (>1200 kDa) have not shown any tendency to form gel (Doublier and Wood, 1995). Earlier studies have reported relatively high critical concentrations for depolymerised β-glucan molecules, such as $c^* = 2.0\%$ for 40 kDa barley β-glucans (Böhm and Kulicke, 1999), but this only described the transformation of diluted region to semi-diluted when the solution was mixed, without giving any indication of the long-term stability of the molecules in solution.

According to Lazaridou et al. (2008), the gelation behaviour of β-glucan in cryogels (obtained by repeated freezing and thawing cycles) could be slowed down by adding di- and monosaccharides (sucrose, fructose, glucose, xylose) into the solution. Xylose and fructose had a stronger inhibitory effect on structure formation compared to sucrose and glucose, but for example sorbitol promoted the gelation of medium-M_w β-glucans (210 kDa). However, the inhibition of network formation was similar with the di- and monosaccharides as well as with sorbitol when β-glucan was in its LM_w form (70 or 140 kDa). Thus, it might be possible to im-

prove the solution stability of partially hydrolysed oat bran extracts by adding sugars or sorbitol to the solution, although the study of Lazaridou et al. (2008) was performed with barley β-glucan.

4.3.3 Oat dietary fibre in extruded products

The extrusion properties of defatted wholegrain oats (WF) were compared to defatted oat endosperm flour (EF) and to EF with addition of different oat bran preparations (OBC, EH-OBC, UF-OBC, WIS-OBC and WS-OBC) (Publication IV). The WF extrudates showed significantly poorer expansion (151%) and harder texture (399 N) than EF extrudates (199% and 148 N, respectively), mainly due to higher DF and protein content, which both have been demonstrated to decrease the expansion of starch-based extrudates (Robin et al., 2012). The 10% addition of oat bran preparations (OBC, EH-OBC or UF-OBC) into an EF-based recipe gave better expansion (171–176%) and less hard texture (200–265 N) compared to WF-extrudates, because they had higher starch and lower protein content, even though the TDF content was similar to that of WF-based extrudates. In addition, the content of SDF was higher in extrudates with 10% of oat bran preparation, which could be one of the reasons for better expansion when compared to WF extrudates (Tables 2 and 4 of Publication IV).

The 10% addition of water-insoluble oat bran preparation (WIS-OBC) into the EF-based recipe revealed the negative impact of IDF and protein on the expansion of starch-based matrix. The expansion of these extrudes was only 163% and they were almost as hard (313 N) as the WF extrudates. By contrast, the 10 or 20% addition of water-soluble oat bran preparation (WS-OBC) improved the expansion (up to 218–226%), which was even higher than seen with the 100% EF-based recipe. However, the hardness of the WS-OBC supplemented extrudates (141–146 N) did not statistically differ from the 100% EF-based recipe (Tables 2 and 4 of Publication IV).

In general, the addition of cereal brans tends to have a negative effect on the structure of extruded products. This is mainly because coarse and fibrous bran particles can cause early bursting of air bubbles and lead to smaller pores and higher density in the matrix compared to extrudates containing only starch (Robin et al., 2011). Usually, the physicochemical compatibility between DF and starch can be improved by reducing the size of the particles rich in DF, thus increasing the contact surface between DF and starch. For example, significant increases in the radial expansions were reported by Lue et al. (1991), Blake (2006) and Alam et al. (2014), when decreasing the average particle size of sugar beet fibre from 2000 to 74 µm, maize bran from 250 to 50 µm and rye bran from 750–1250 to 28 µm, respectively. However, in the present study ultra-fine milling or enzymatic hydrolysis of OBC did not lead to clear differences in the microstructure of the extrudates supplemented with 10% of OBC (Figures 3 and 5 of Publication IV). The reason for this might be the high proportion of SDF in oat bran (>50% of TDF) (Table 1 of Publication IV) compared to other cereal brans.

In the present study, the size of the pores was largest in samples containing 20% WS-OBC (Figure 3 of Publication IV), which could be due to the earlier attainment of the glassy state compared to 100% EF. The effect of SDF-containing ingredients on the expansion of starch-based products is still largely unknown, but the role of molecular weight in SDF preparations (for example with corn fibre gum, arabinogalactan and carboxymethylcellulose, CMC) has shown to play a significant role (Blake, 2006). The sectional expansion of extrudates containing 30% CMC with M_w of 210–250 kDa was significantly improved as compared to higher M_w CMC molecules. A branched versus linear polymer was also found to better facilitate increased extrusion expansion in high fibre products (Blake, 2006). A high proportion of SDF with reduced M_w might have decreased the shear viscosity to promote the bubble growth of starch melt inside the extruder, but the viscosity needed to be high enough to prevent bubble collapse after coming out from the die exit, as hypothesised by Pai et al. (2009).

Extrusion of oat bran DF has also been shown to increase the content of total and soluble β-glucan as well as the content of SDF (Gualberto et al., 1997; Zhang et al., 2011). Gualberto et al. (1997) reported that the insoluble part of oat bran DF was converted into a more soluble form during extrusion (the content of SDF increased from 3.5 to 5.5% and the content of IDF decreased from 8.7 to 7.1%) when the water content of the feed was around 22% and the extrusion temperature was 180 °C. The SDF content was lower (4.6%) and the IDF content higher (7.5%) when the water content of the feed was around 41% and the temperature 194 °C. Interestingly, they did not observe changes in the DF composition of wheat and rice bran under the same extrusion conditions. Zhang et al. (2011) also showed that the content of SDF in extruded oat bran was dependent on the water content of the feed. The content of SDF in untreated oat bran was originally 8.9%. The highest SDF content (14.2%) was obtained at 140 °C with 10% water content of the feed. The SDF content was reduced to 11.5% when the water content was 30%. In the current study, the SDF content of OBC-supplemented extrudates was slightly increased (from 3.3–3.7% to 4.1–4.6%) as compared to the SDF content in the ingredients (Table 9). However, the effect of feed moisture (16% for all extrudates) on the SDF content was not studied in this work, because the aim was to obtain well-expanded products and thus only a low amount of water was added to the extrusion mixture.

4.4 Health aspects of oat dietary fibre and β-glucan

As mentioned in the earlier sections, the DF of oats is mainly composed of β-glucan (e.g. around 60% of TDF was β-glucan in OBC, according to Table 5). The effects of β-glucan M_w on physiological responses will be discussed in this section. The M_w is an important factor with respect to the anticipated health effects of the β-glucan preparations and model food products produced in the current work.

4.4.1 Cholesterol lowering

All processing performed in this work influenced the M_w of β-glucan, which is known to be an important factor for cholesterol-lowering capacity (Theuwissen and Mensink, 2008; Wolever et al., 2010). The M_w of β-glucan was 766–799 kDa in the β-glucan-enriched oat bran fractions produced by air classification and electrostatic separation (Publications I and II). The M_w of β-glucan in extruded products (Publication IV) remained high (686–911 kDa), whereas in the liquid food products (Publication III) it was reduced (to 34–49 kDa) to obtain lower viscosity and enhanced solution stability.

Wolever et al. (2010) showed that 3 g of both high-M_w (2210 kDa) and medium-M_w (530 kDa) β-glucan lowered LDL cholesterol similarly, but the efficacy was decreased by 50% when using low-M_w (210 kDa) β-glucan in extruded breakfast cereals. According to the results of Wolever et al. (2010), the β-glucan-enriched fractions (Publications I and II) as well as the extruded products produced in the current study (Publication IV) could have a cholesterol-lowering potential. By contrast, the β-glucan in liquid food products (Publication III) might have a reduced cholesterol-lowering ability. However, the food matrix can significantly influence the cholesterol-lowering effect of β-glucan. In particular, the effect of LM_w β-glucan in liquid food matrices remains a controversial topic (Kerckhoffs et al., 2003; Biörklund et al., 2005; Naumann et al., 2006; Othman et al., 2011).

In a study of Biörklund et al. (2008), a soup enriched with 4 g of LM_w (80 kDa) oat β-glucan lowered the total- and LDL cholesterol levels in healthy hyperlipidemic subjects, but the reductions were not significantly different from those of the group that consumed a soup without β-glucan. This indicated that the soup itself might have been a healthy alternative, with a positive effect on serum lipids similar to that of soup supplemented with β-glucans. Nevertheless, Biörklund et al. (2005) and Naumann et al. (2006) demonstrated that LM_w β-glucan (70 or 80 kDa) decreased LDL cholesterol when consumed in beverages. Thus, the LM_w β-glucan molecules produced by acid or enzymatic hydrolysis in the current study (Publication III) could still show a cholesterol-lowering effect, but clinical studies would be needed to verify this.

The cholesterol lowering ability through binding of bile acid may also happen by mechanisms which do not depend on the viscosity of β-glucan, but rather on the interactions between the molecules. For example, de Moura et al. (2011) and Park et al. (2009) showed that the oxidised β-glucan could have a better bile acid binding capacity compared to native β-glucan when β-glucan molecules were treated with H_2O_2 or TEMPO (2,2,6,6-Tetramethyl-1-piperidine oxoammonium) ions.

4.4.2 Glycaemic response

The ability of β-glucan to decrease glycemic response appears to be quite consistently dependent on its M_w and induced viscosity in the gut (Wood et al., 2000; Juvonen et al., 2009; Wood, 2011; Brummer et al., 2012; Kwong et al., 2013a;b).

Thus, the HM_w β-glucan molecules in the β-glucan-enriched bran fractions (766–799 kDa; Publications I and II) as well as in the bran-supplemented extrudates (686–911 kDa; Publication IV) could attenuate the post-prandial glycaemic response. The depolymerisation of β-glucan and subsequent loss of viscosity in Publication III might have reduced its effect on glycaemic response. Wood (2011) suggested that the glycaemic response data is valid only for fixed-volume drinks. However, Kwong et al. (2013b) recently showed that the same amount and M_w of β-glucan fed to subjects in a greater or lesser volume of liquid had similar effects on the glycaemic response, i.e. the viscosity of the drink itself did not affect its glycaemic response.

Kwong et al. (2013a) also showed that *in vitro* results do not necessarily match the *in vivo* results. This appeared to be especially true for LM_w (145 kDa) oat β-glucan in a gel form. Whereas the gel form impeded glucose diffusion *in vitro*, it was unable to decrease blood glucose levels *in vivo*. However, similar LM_w oat β-glucan attenuated blood glucose levels when consumed as a totally dissolved beverage. It can be speculated that although the molecular size of the LM_w polymers is sufficiently large to lower the glycaemic response, the polymers lose their effect on blood glucose levels when they are in the form of a gel, due to the formation of a polymer network structure. Many other studies have also demonstrated that the solubility of β-glucan in aqueous matrices is important for its bioactivity, since β-glucan gels do not melt at physiological temperatures (Tosh et al., 2004; Lazaridou et al., 2004; Lan-Pidhainy et al., 2007; Tosh et al., 2008).

4.4.3 Satiety inducing properties

Like other viscous fibre, oat DF, especially β-glucan, can enhance the sensation of post-prandial satiety (Lugwig, 2000; Juvonen et al., 2009; Lyly et al., 2009; 2010; Pentikäinen et al., 2014). The results of Juvonen et al. (2009) with low viscosity (LM_w) β-glucan could support the satiating effect of the oat bran extracts studied in the present study, which had <200 mPas viscosity (Figure 4 of Publication III). Juvonen et al. (2009) compared two oat bran preparations with different M_w of β-glucan: HM_w (50% of molecules >1000 kDa and 15% of molecules <100 kDa) or LM_w (5% of molecules >1000 kDa and 85% of molecules <100 kDa). When 30 g of the HM_w and LM_w oat bran preparations were mixed into 300 mL of water, the viscosities were >3000 mPas and <250 mPas, respectively. Oat bran beverage with low viscosity induced a greater postprandial increase in satiety than the beverage with high viscosity.

In addition, the results of Pentikäinen et al. (2014) could support the satiety-enhancing effect of oat bran β-glucan in beverages. In the studied meal setting, oat bran enrichment (4 g of β-glucan) was more efficient in enhancing satiety when added in juice than in biscuits. The reason could be that β-glucan provided in liquid form has a higher rate of fibre hydration compared to fibres ingested as a part of solid food, and thus may have a stronger appetite-reducing effect. However, the perceived satiety may not be directly linked to the amount of β-glucan. For

example, Lyly et al. (2010) showed that beverages containing 2.5 and 5 g oat β-glucan increased the satiety compared to the control beverage without fibre, but the difference between two β-glucan dosages was not significant. Increasing the energy content from 700 to 1400 kJ did not affect the satiety-related perceptions. As a conclusion, the developed oat fractions and food products may help to increase postprandial satiety, but further studies would be needed.

4.5 Future prospect

This thesis studied different technologies for processing of oat DF. Although the enrichment and modification of β-glucan showed promising results, there are still possibilities to improve the fractionation and depolymerisation processes, and thus to facilitate the usability of β-glucan in various food applications.

The most efficient enrichment of β-glucan was obtained when defatting was performed for non-heat-treated oat material. However, the lipid extraction process had challenges, because oat groats needed to be flaked or pre-ground prior to SC-CO_2 extraction. If the storage time between flaking and SC-CO_2 extraction was too long, the lipids became rancid due to oxidation reactions. Thus, a minimal heat-treatment could be studied as an additional stabilisation step prior to lipid extraction.

The dry fractionation process studied in Publication I also produced a large quantity of defatted oat endosperm flour. This flour was further separated into protein- and starch-rich fractions. The protein-rich fraction can have several different food applications due to the essential amino acids present (Mohamed et al., 2009; Lapveteläinen and Aro, 1994) and the potential for using it in gluten-free diets (Tapsas et al., 2014; Pawłowska et al., 2012). Specific uses could include e.g. tailored products for sportsmen and elderly people. The defatted oat endosperm flour and further separated starch-rich fraction can open new possibilities for bread making (Kaukonen et al., 2011) and for high moisture food applications (Konak et al., 2014), due to the good foaming capacity of these flours.

Depolymerised β-glucan molecules showed good potential as novel sources of SDF for liquid food applications (Publication III). The M_w of β-glucan could be further optimised based on the final concentration of β-glucan in beverages. For example, if the β-glucan concentration would be around 1.0%, the M_w could be higher than 34–49 kDa, which was shown to be the limit for a stable suspension at 1.4–1.6% β-glucan concentration. If the end product is a β-glucan gel, the gel properties could be tailored by mixing HM_w and LM_w molecules (Brummer et al., 2014) or by adding sugars (Lazaridou et al., 2008).

The drying of depolymerised oat β-glucan extracts would enhance their usability in the food industry. However, thermal drying of β-glucan extracts easily leads to very high viscosity, makes them dark-coloured and decreases their solubility. Thus, novel types of drying methods should be investigated (e.g. foam mat drying; Ratti and Kudra, 2006 or refractance window dehydration; Nindo and Tang, 2007). Instead of complete drying, syrups of dehydrated β-glucan extracts could be a

practical way of providing material for the food and beverage industry. When dried, the β-glucan gels could also be utilised as delivery vehicles for nutraceuticals or pharmaceuticals (Comin et al., 2012).

In extrusion, the use of 100% oats is challenging and the addition of β-glucan-rich ingredients easily leads to poor expansion. The texture of extrudates could be improved by substituting some of the defatted oat endosperm flour with maize- or rice-based starch ingredients, which have better expansion potential compared to oats (Rzedzicki et al., 2000). The textural properties of oat-based extrudates could also be modified by adding processing aids which produce CO_2 during extrusion (such as calcium bicarbonate) or by feeding SC-CO_2 into the extruder, enabling well expanded and crispy products at lower processing temperatures compared to traditional cooking extrusion (Rizvi et al., 1995; Cho and Rizvi, 2010).

The addition of ingredients rich in SDF (e.g. WS-OBC in Publication IV) can improve the expansion and reduce the hardness of high-fibre oat extrudates. It might be possible to improve the texture even more by modifying the M_w of β-glucan in the SDF-rich ingredient. However, too extensive degradation of β-glucan can decrease the positive effect on the extrusion melt viscosity and lead to reduced expansion, as was detected with enzymatically solubilised AX in bread applications (Courtin and Delcour, 2002).

Processing parameters, such as acid/enzymatic hydrolysis, amount of water during hydrolysis and drying method, may also limit the suitability of SDF-rich ingredients for extrusion. For example, the improvement of expansion by WS-OBC fraction (Publication IV) might have been lost if the fraction had not been freeze dried. Freeze drying retains the rehydration quality and does not affect the colour of the product, but it is an expensive drying method. More feasible drying technologies, which retain material quality similarly to freeze drying, should be studied in order to utilise the promising results of this study in industrial scale extrusion processes.

5. Conclusions

Oats are a good source of many nutritionally valuable compounds, especially DF. However, they are still largely under-utilised in human consumption. New types of DF ingredients developed in the current study can hopefully increase the use of oats in food products, and decrease the gap between the actual intake of DF and the nutritional recommendations (i.e. 25–30 g/day).

This work showed that defatting had a significant effect on the enrichment of oat DF components, especially β-glucan. After SC-CO_2 extraction, oat β-glucan and protein were enriched into higher concentrations than in any existing commercial dry fractionation process. The β-glucan-enriched fractions can be utilised in functional foods and nutraceutical products with EU-approved health claims for cholesterol lowering. The most promising application areas for β-glucan-enriched fractions would be in food matrices in which regular oat bran would bring too much starch and other non-β-glucan compounds to the product.

Ultra-fine grinding and electrostatic separation showed good potential and selectivity in the enrichment of different DF compounds from oat bran. A limit of separation capacity at around 40% β-glucan content was detected by air classification of the finely ground oat bran material. Higher β-glucan concentrations were reached by electrostatic separation, because the separation was based on the acquired charge of the particles, not on their size and density as in air classification. Air-jet milling in combination with air classification revealed another interesting possibility to enrich β-glucan-rich cell walls from protein and starch particles.

The applicability of oat bran β-glucan in liquid food products was enhanced by controlled depolymerisation through acid or enzymatic hydrolysis at low water content. In acid hydrolysis, both (1→3)- and (1→4)-β-D-linkages were cleaved, whereas the enzyme appeared to act mainly on (1→4)-β-D-linkages. In acid hydrolysis the degradation was controlled by temperature. In enzymatic hydrolysis it was dependent on the duration of stationary incubation. The clearest changes in the M_w of β-glucan and in the subsequent dispersion stability occurred between 120 and 130 °C in acid hydrolysis and between 3 and 4 h incubation in enzymatic hydrolysis.

Enzymatic hydrolysis was evaluated as superior to acid hydrolysis because it required less harsh conditions, it did not produce inorganic side streams, and it resulted in more stable extracts. The stability of the acid and enzymatically hydro-

lysed oat bran extracts could also be affected by other bran components, such as protein, starch and AX, but this was not studied in the current work.

The defatted oat fractions were suitable ingredients for expanded snacks. Defatted oat endosperm flour (EF) was superior to defatted wholegrain oat flour (WF) in terms of textural properties, mainly because of the lower amount of DF and protein in EF-extrudates. A similar level of DF as in WF-extrudates was achieved when 10% of oat bran concentrate (OBC) was added in endosperm flour. Ultra-fine grinding and enzymatic hydrolysis of OBC as such did not result in any improvements in expansion or hardness. This was postulated to be due to the high SDF content and relatively small particle size already prior to ultra-fine grinding.

The detrimental role of IDF and protein on expansion and hardness was confirmed by adding 10% of water-insoluble fraction (WIS-OBC) to endosperm flour. By contrast, even higher expansion and lower hardness values than in 100% EF-extrudates were obtained when adding 10–20% of water-soluble fraction (WS-OBC) to endosperm flour. The M_w of β-glucan may also play an important role in the expansion characteristics. Based on the literature, it was hypothesised that a high proportion of SDF with reduced M_w can decrease the shear viscosity of starch melt inside the extruder and thus promote bubble growth. However, the viscosity needs to be high enough to prevent bubble collapse after coming out from the die exit.

All in all, the results from this study can assist the milling industry in providing new oat ingredients for several types of food products, and broaden the use of oats in human consumption. However, especially oat bran ingredients rich in DF will often need further modification in order to be suitable for food products. The developed processes for depolymerised β-glucan and utilisation of the resulting hot water extracts in liquid foods or extruded snacks would need economical calculations and a business study in order to assess the feasibility of the production.

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PUBLICATION I

Lipid removal enhances separation of oat grain cell wall material from starch and protein

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Lipid removal enhances separation of oat grain cell wall material from starch and protein

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ABSTRACT

Effects of lipid removal on the fine milling and air classification processing of oats were studied. Lipid removal by supercritical carbon dioxide (SC-CO_2) extraction enabled concentration of the main components of oats – starch, protein, lipids and cell walls – into specific fractions. Using defatted oats as raw material, the highest β-glucan concentration of the cell wall-enriched fraction was 33.9% as compared to 17.1% without lipid removal. This was probably due to more efficient milling yielding smaller particles, and release of starchy material from cellular structures during milling of defatted oats, resulting in better classification. The removal of lipids also enabled separation of an oat protein concentrate with a protein concentration of 73.0% and a mass yield of 5.0%. A trial with 2310 kg of oat groats showed that the process based on defatting and dry fractionation was also industrially applicable.

1. Introduction

Oats are known as a good source of β-glucan, but they also contain high amounts of lipids. The β-glucan concentration of oats varies typically between 2 and 8.5% and total lipids between 6 and 10% of the whole grain (Butt et al., 2008; Peterson, 2002; Wood, 1986). Oat β-glucans are located throughout the starchy endosperm, but they are concentrated in the cell walls of the aleurone and sub-aleurone layers (Wood, 1986). Lipid content is high in the embryonic cells, but due to their low mass proportion, the bran and endosperm contain the majority of oat lipids (Price and Parsons, 1979). In a sieving process, oat lipids are distributed so that around 35% are recovered in starchy fine flour and 65% in coarse flour and bran fractions (Doehlert and Moore, 1997).

Oat dietary fibre is characterized by a high concentration of mixed linked (1→3),(1→4)-β-D-glucan (Wood, 2007). This water-soluble dietary fibre has attracted significant nutritional interest during recent years, as several independent studies have shown that products containing oat β-glucan have a cholesterol-lowering effect (Brown et al., 1999; Ripsin et al., 1992; Truswell, 2002). In addition, oat β-glucan has been reported to attenuate glycaemic response (Butt et al., 2008). In the U.S.A., the Food and Drug Administration (FDA, 1997, 2003) has allowed a heart health claim for products containing oat or barley β-glucan. The European Food Safety Authority (EFSA, 2009) recently also accepted a claim that regular consumption of β-glucans contributes to maintenance of normal blood cholesterol levels.

The nutritional potential of oat bran or its components has motivated research into the development of oat fractionation processes for the production of various value added products (Lehtinen et al., 2009; Stevenson et al., 2008; Vasanthan and Temelli, 2008). Preliminary experiments have indicated that lipid removal enhances the separation of β-glucan (Lehtinen et al., 2009). The major advantage of the use of supercritical fluid technology, especially with carbon dioxide (SC-CO_2), compared to the more classical solvent extraction e.g. by hexane, in lipid removal is that no solvent residues remain in the solid material after the extraction process. Typical applications of the SC-CO_2 technique are speciality seed oils, e.g. sea buckthorn or black currant, and essential oils from various herbs. A review of the aspects of SC-CO_2 in processing of fats and oils has been published recently (Temelli, 2009). However, our approach differed from the more conventional applications of supercritical fluid extraction in that we were interested only in the remaining defatted solids.

Conventional dry processes are usually unable to yield highly concentrated β-glucan fractions. Instead, many known processes for the isolation of highly concentrated β-glucan are based on wet methods (Kvist and Lawther, 2005; Potter et al., 1999; Redmond

Abbreviations: DG, diacylglycerols; FFA, free fatty acids; PL, polar lipids; TG, triacylglycerols; SC-CO_2, supercritical carbon dioxide.

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and Fielder, 2004). Wet processes are typically limited by high viscosity of the aqueous extracts even at low β-glucan concentrations, which leads to large liquid volumes and high costs related to drying and solvent recovery steps.

The aim of this work was to study the effects of lipid removal on dry fractionation of oats and on the properties of the fractions obtained, especially in order to produce products with high β-glucan concentration. The fractionation process was also demonstrated on an industrial scale.

2. Experimental

2.1. Raw materials

The raw material in the pilot scale trial (2 kg) was non-heat-treated, dehulled and mechanically flattened to flakes in a local flour mill (Riihikosken Vehnämylly, Pöytyä, Finland) and contained 5.7% total lipids, 14.5% protein, 65.0% starch and 3.9% β-glucan. In the industrial scale trial, the raw material was obtained from Raisio Oyj (Kokemδki, Finland). This contained 6.3% total lipids, 16.4% protein, 62.5% starch and 3.0% β-glucan. The industrial scale trial was made using 2310 kg of raw material.

2.2. Overall description of the extraction and fractionation processes

For pilot scale studies (2 kg batch size), oat groats were first flaked to 0.2–0.3 mm thickness, whereas for the industrial scale trial (2310 kg batch size), they were milled to oat grits in a conventional roller mill. Lipids were then extracted by SC-CO_2 with or without ethanol as a polar modifier. The defatted oat materials were then fine milled with a pin disc mill and subsequently fractionated by an air classifier. After the first air classification, the coarse fraction was milled and air classified again to further concentrate the β-glucan fraction. The same process was also performed without lipid extraction (Fig. 1). A highly concentrated protein fraction was separated from defatted endosperm flour by re-classifying the fine fraction after the first air classification.

2.3. Lipid extraction with SC-CO_2

On a pilot scale, the SC-CO_2 extraction of lipids was performed in a Multi-Use SFE Plant with a pressure vessel of 10 l (Chematur Ecoplanning, Rauma, Finland). The extraction method of oat flakes was based on the work described earlier by Aro et al. (2007). The extraction was performed either with SC-CO_2 alone (one step) or with SC-CO_2 followed by SC-CO_2 and 10% ethanol extraction (two steps). On the industrial scale, a pressure vessel of 250 l (NATECO2 GmbH & Co, Wolnzach, Germany) was used. The industrial scale extraction was performed only with SC-CO_2. The process parameters are presented in Table 1.

2.4. Fine milling and air classification

On the pilot scale, non-defatted and defatted oat flakes were first milled twice at a rotor speed of 17 800 rpm (tip speed 180 m s^{-1}) and a feed rate of 10 kg h^{-1}, using a Hosokawa Alpine 100 UPZ-lb Fine impact mill with pin disc grinders (Hosokawa Alpine AG, Augsburg, Germany). The ground material was then air classified using a Minisplit Classifier (British Rema Manufacturing Company Ltd., UK). Classification was performed with an air flow of 220 m^3 h^{-1} and a feed rate of 5 kg h^{-1}. During the classification, the rotor speed was varied between 3000 and 7000 rpm in order to alter the mass balance between fine and coarse fractions. The coarse cell wall fraction from the first air classification step was

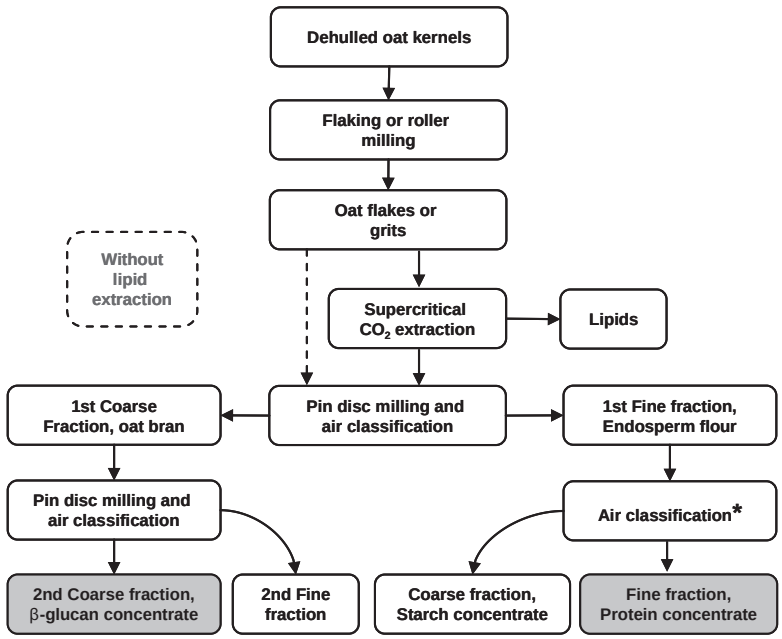

Fig. 1. Process flow chart of the industrial scale oat fractionation. *Protein separation was only performed in the case of defatted flour. The most valuable fractions are highlighted with grey colour.

Table 1
The process parameters in supercritical CO_2-extrations performed either with SC-CO_2 alone (one step) or with SC-CO_2 with added ethanol (two steps) in pilot and industrial scale.

	Pilot scale			Industrial scale
	One step extraction	Two step extraction		One step extraction
	SC-CO_2	1st with SC-CO_2	2nd with SC-CO_2 + EtOH	SC-CO_2
Pressure (bar)	450	450	400	290
Temperature (°C)	70	70	70	40
EtOH (%)	–	–	10	–
CO_2/kg flour	37.5	11	26.5	50
Extraction time (h)	5	1.5	4	13

further fine milled twice, using the same parameters as previously, and subsequently air classified with the same air flow and feed rate but altering the classifier rotor speed between 2500 and 4000 rpm.

On the industrial scale, the defatted oat grits were first milled in a Hosokawa Alpine Contraplex 250 CW mill. The rotation speeds of the mill discs were 11,200 and 5600 rpm for two stainless steel discs rotating in opposite directions (tip speed 250 m s^{-1}). The feed rate was 250 kg h^{-1}. The milled flour was subsequently air classified in a Hosokawa Alpine 315 ATP classifier, using an air flow of 1200 m^3 h^{-1} and rotor speed of 2200 rpm. The first coarse cell wall fraction, separated by air classification, was milled and air classified again with the same parameters to yield a cell wall concentrate enriched in β-glucan and endosperm flour rich in starch. The separation of the protein-enriched fraction from the first fine fraction was made only for the industrial scale trial, using a Hosokawa Alpine 200 ATP NG air classifier with air flow 400 m^3 h^{-1}, feed rate 100 kg h^{-1} and rotor speed 6600 rpm.

2.5. Biochemical analyses

The concentration of β-glucan was analyzed by the spectroscopic method 32-23 (AACC, 2000) using the Megazyme β-Glucan mixed linkage assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The concentration of arabinoxylan was analyzed by spectroscopic determination of pentoses according to Douglas (1981). Nitrogen was analyzed using a Kjeldahl autoanalyzer (Foss Tecator Ab, Höganäs, Sweden), and protein concentration was calculated as N × 6.25 according to method 46-11A (AACC, 2000). The lipid class composition was analysed by thin layer chromatography and subsequent gas chromatography (Lehtinen et al., 2003). Starch was quantified using the Megazyme total starch assay kit according to method 76–13.01 (AACC, 2000).

2.6. Particle size measurement

Different fractions, as well as D_{50} and D_{90} values indicating that 50 or 90% of the particles have a diameter under a certain level, were analyzed with a Beckman Coulter LS 230 (Beckman Coulter, Inc., CA, USA) using the dry powder module.

2.7. Microscopic analysis

Prior to light microscopy, the samples were embedded into agar according to the Historesin embedding kit (Leica instruments GmbH, Heidelberg, Germany). The embedded samples were sectioned (2 μm) in a rotary microtome HM 355 (Microm Laborgeräte GmbH, Walldorf, Germany) using a steel knife. The sections were stained either with Light Green (BDH Chemicals Ltd, Poole, Dorset, UK)/Lugol's iodine solution or with Acid Fuchsin (BDH Chemicals Ltd., Poole, Dorset UK)/Calcofluor White (Fluorescent Brightener, Aldrich, Germany). When imaged in bright field, Light Green stains protein green/yellow, whereas Lugol's iodine solution stains the amylose component of starch blue and amylopectin brown. Most starch appears dark blue because amylose masks the amylopectin. Acid Fuchsin and Calcofluor White were used for staining protein red and β-glucan rich cell walls light blue, respectively, and the samples were imaged using exciting light (epifluorescence at 400–410 nm and fluorescence at >455 nm). The samples were then examined under a BX-50 microscope (Olympus Corp., Tokyo, Japan). Micrographs were obtained using a PCO SensiCam CCD colour camera (PCO AG, Kelheim, Germany) and the Cell^P imaging software (Olympus).

3. Results

3.1. Lipid removal and dry fractionation in pilot scale

Preliminary data indicated that lipid extraction from whole groats was relatively difficult. On the pilot scale, approximately 65% of lipids was extracted from oat flakes by SC-CO_2. The extraction efficiency of oat lipids with SC-CO_2 varied according to the lipid class, so that ca. 85% of neutral triacylglycerols and less than 2% of polar lipids were extracted (Table 2). Free fatty acids, related to a perceived rancid flavour, were poorly extracted with SC-CO_2. Addition of 10% ethanol increased the extractability of both polar lipids and free fatty acids.

Milling of non-defatted oats in a high intensity pin disc mill was difficult due to the formation of several lumps and adhesion of flour to the milling chamber. Consequently, milling was possible only in small batches when the equipment was cooled and cleaned between batches. When defatted flakes were used as a raw material, these problems were not encountered and the mill could be run continuously.

Flours from both defatted and non-defatted oats were subjected to air classifications with varying rotor speeds in order to fractionate flour into coarse and fine fractions. The yield of the coarse fraction of both defatted and non-defatted oats varied from approximately 10–25%. In each case β-glucan concentrated into the coarse fraction. The coarse fractions of the non-defatted material had notably larger particle size (e.g. column C in Table 3: D_{50}/D_{90} = 651/1016 μm) than the defatted material (D_{50}/D_{90} = 392/667 μm). During the fractionation process, defatted and non-defatted oat flours behaved differently, so that in order to produce a similar yield of coarse fraction, a lower rotor speed was required for non-defatted than for defatted material. When the coarse fraction was subjected to a second milling and air classification, a similar behaviour was observed.

Lipid removal had a remarkable effect on the β-glucan concentration of the coarse fractions. Without lipid removal, the highest concentration of β-glucan obtained was 17.1%, whereas when using

Table 2
The concentrations of different lipid classes in non-defatted flour and after the defatting either by SC-CO_2 alone or by SC-CO_2 with added ethanol. PL = polar lipids, DG = diacylglycerols, FFA = free fatty acids and TG = triacylglycerols.

	Unextracted flour (mg/g)	SC-CO_2 – extracted flour (mg/g)	SC-CO_2+EtOH – extracted flour (mg/g)
PL	7.8 ± 0.1	7.7 ± 0.4	4.3 ± 0.7
DG	6.2 ± 0.04	1.7 ± 0.1	0.4 ± 0.07
FFA	10.6 ± 0.9	5.4 ± 0.5	0.2 ± 0.02
TG	32.0 ± 0.7	4.7 ± 0.07	2.7 ± 0.4
Total	56.7	19.5	7.7

Table 3
The effect of supercritical CO_2 extraction on the concentration of β-glucan in coarse oat fractions after first and second air classification steps: 1) oat flour without lipid extraction, 2) SC-CO_2 extracted oat flour. Columns A, B, C and D correspond to different mass yields, which were obtained by varying the classifier rotor speed.

1) Oat flour without lipid extraction		A	B	C	D
After milling and 1st classification:	classifier speed (rpm)	2500	2000	1900	1800
	particle size D_{50}/D_{90} (μm)	611 / 977	635 / 997	651 / 1016	679 / 1037
	mass yield (%)	26.9	20.3	**16.8**	12.1
	β-glucan (%)	12.9 ± 0.1	11.6 ± 0.4	**13.4 ± 0.1**	12.3 ± 1.4
After milling and 2nd classification:	classifier speed (rpm)	4000	3500	3000	2500
	particle size D_{50}/D_{90} (μm)	334 / 544	336 / 542	388 / 587	383 / 578
	mass yield (%)	9.4	**8.7**	7.1	5.0
	β-glucan (%)	14.9 ± 0.9	**17.1 ± 0.1**	16.8 ± 0.1	16.4 ± 0.5
2) SC-CO_2 extracted oat flour		A	B	C	D
After milling and 1st classification:	classifier speed (rpm)	7000	5500	4000	3000
	particle size D_{50}/D_{90} (μm)	236 / 550	343 / 625	392 / 667	435 / 694
	mass yield (%)	24.4	18.0	**15.3**	9.0
	β-glucan (%)	13.0 ± 0.3	14.3 ± 0.6	**20.8 ± 0.1**	23.3 ± 0.2
After milling and 2nd classification:	classifier speed (rpm)	4000	3000	2850	2500
	particle size D_{50}/D_{90} (μm)	300 / 470	273 / 441	254 / 411	274 / 456
	mass yield (%)	10.4	**8.8**	6.7	5.2
	β-glucan (%)	30.0 ± 0.3	**31.2 ± 0.6**	30.1 ± 0.3	27.7 ± 0.8

the SC-CO_2 extraction, the highest β-glucan concentration was 31.2% (Column B in Table 3). The highest concentration of β-glucan was reached when the total mass yield of bran-enriched fraction was about 9%, both with and without SC-CO_2 extraction (Table 3). When the yield of coarse fraction was reduced below 9%, no further enrichment of β-glucan was observed. Addition of ethanol as a co-solvent into SC-CO_2 extraction improved the separation process, yielding ca. 2% higher concentration of β-glucan. However, from the economical point of view ethanol as co-solvent increases process costs.

3.2. Demonstration on the industrial scale

The defatting and dry fractionation of oats were repeated using industrial scale equipment. In the industrial scale SC-CO_2 extraction, the lipid concentration of oats was reduced from 6.3 to 1.2%. Lipid class composition was similar to that for pilot scale: about 80% of the total oat lipids were extracted, of which over 90% were neutral triacylglycerols. Less than 2% of the polar lipids were extracted.

In the first air classification, the mass yield of the coarse fraction was adjusted to 14.3%. This fraction, containing 21.3% of β-glucan, was further milled and air classified to obtain a coarse fraction with 33.9% of β-glucan with ca. 7.8% mass yield. The required rotor speed in industrial scale air classification was lower than for the pilot scale; 2200 rpm in both of the separations. Particle size distributions were similar to those for the pilot scale, although the D_{50} and D_{90} values of the first and second coarse fractions were somewhat lower because of the more intensive grinding in the Contraplex 250 CW pin disc mill (e.g. for the second coarse fraction $D_{50}/D_{90} = 273/441$ μm in pilot scale vs. 197/323 μm for the industrial scale).

The protein-enriched fraction was separated by re-classifying the endosperm flour (first fine fraction) obtained after the first air classification. Protein enrichment was possible only for the defatted sample. The rotor speed of the classifier played the most significant role in the separation of protein particles from starch granules. By using a classifier speed of 6600 rpm, a 5.0% mass yield of very fine fraction with 73.0% of protein was obtained. Higher mass yields with lower protein concentration were obtained by lowering the rotor speed. For example, by using a rotor speed of 5600 rpm, a protein concentrate with 49.3% of protein was produced with a mass yield of 14.4%.

3.3. Characterization of fractions produced on the industrial scale

After the first milling and air classification, starch granules and protein were localised in the coarse fraction both inside large aleurone and sub-aleurone structures and as a loose material released from cells (Fig. 2). After the second milling and classification, the coarse fraction again contained large cellular entities of aleurone and sub-aleurone cells. However, only a very limited amount of starch or protein was visible outside the cells. In the most concentrated β-glucan fraction, the content of β-glucan was 33.9%, arabinoxylan 9.9% and total dietary fibre 51.7% (Table 4).

The most pure starch and protein fractions were obtained by re-classifying the first fine fraction. The protein was recovered in the fine fraction, whereas starch was concentrated into the coarse fraction. The separation was very efficient and resulted in starch- and protein-enriched fractions with 77.2% of starch (coarse) and 73.0% of protein (fine). The protein concentrate contained a few starch granules distributed within the protein matrix (Fig. 2.). A few cell wall fragments were also evident in the protein concentrate, but no intact cellular structures could be identified. A very precise particle size cut-off at 6–8 μm was reached during the separation of protein and starch fractions.

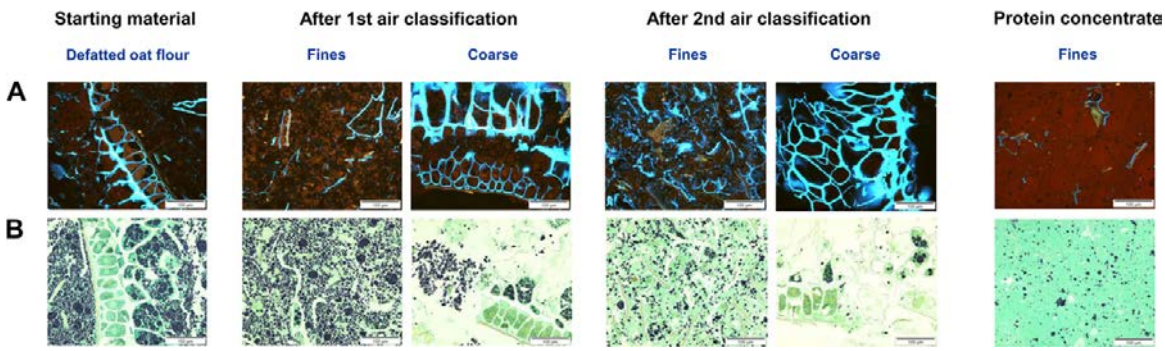

Fig. 2. Microscopic pictures of the oat fractions obtained from the industrial scale dry fractionation process (see Fig. 1). Row A: Acid Fuchsin/Calcofluor White staining, showing protein as red and β-glucan rich cell walls as light blue. Row B: Light Green/Lugol's iodine staining, showing protein as green and starch as spherical objects in blue or brown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Lipid extraction from the oat raw material prior to further fractionation made it possible to obtain a fraction with a β-glucan concentration of 33.9%, with a mass yield of 7.8%. Air classification processes without lipid removal have previously yielded fractions with only slightly over 20% of oat β-glucan (Lehtomäki et al., 1990; Mälkki et al., 2004; Wu and Doehlert, 2002; Wu and Stringfellow, 1995). Lipid removal in the current study affected both the particle size reduction and air classification steps in fractionation.

Oat grain flour is a complex material, where each particle, depending on from which part of the grain it originates and on the extent of size reduction, varies in its chemical composition (Vasanthan and Temelli, 2008). Based on the scanning electron microscope characterization, Stevenson et al. (2007) suggested that defatting of oat bran by SC-CO_2 extraction modifies its structure so that the particle exterior becomes smoother. This change could partially explain the effect of defatting in separation of different grain constituents compared to non-defatted oats reported in the present study. Defatting affects also the starch granules, so that they become less aggregated which consequently enhances the separation of starch and other flour constituents (Stevenson et al., 2008). In the present study, the defatted material could be milled to a much finer flour as compared to the non-defatted oats, resulting in a flour in which starch granules were mostly loose and not embedded in cells (Fig. 2, row B).

The composition of the coarse fraction was altered when the air classification process was adjusted by changing the rotor speed. Defatted and non-defatted materials behaved differently in this respect. When non-defatted oats were used, the coarse fractions had almost identical compositions regardless of the rotor speed. However, when defatted oats were used, the composition of the coarse fraction changed as a function of the rotor speed. This indicates that particles with different composition were produced during the milling of defatted oats, whereas milling of non-defatted oats produced large cellular structures with little variation in the composition of the particles.

Non-heat-treated oats were used in the current study, because in the heat treatment, lipids, starch and proteins formed tight agglomerates. The removal of lipids from heat-treated material would also be more difficult, due to lipid fusion with structural proteins (Heneen et al., 2009). The omittance of the heat treatment causes a risk of lipase-induced reduction of the sensory quality. However, the significantly reduced lipid content in defatted fractions might obviate the need for heat treatment in the first processing step.

SC-CO_2 is an effective solvent for oat lipids and is comparable to other non-polar solvents, such as n-hexane (Knuckles et al., 1992; Wu and Doehlert, 2002). Lipid removal and to some extent the performance during milling and air classification were improved when ethanol was used as a co-solvent. The effect of ethanol on extraction of oat lipids was also reported by Fors and Eriksson (1990), who observed that both ethanol and high pressure enhanced the removal of polar lipids.

Extraction of polar membrane lipids does not seem to be crucial for separation, as the fractionation process was efficient without the use of ethanol as a co-solvent during the supercritical extraction. However, the use of ethanol would likely improve the sensory properties of the end products, as ethanol extracted the free fatty acids inherently present in the raw material (Table 2). Storage stability could also be improved by extracting the polar lipids which are susceptible towards oxidation and formation of rancidity (Lehtinen et al., 2003). However, due to the high costs of using ethanol as a co-solvent, the use of SC-CO_2 alone appears to be the most promising approach for industrial scale production.

Table 4
Composition of oat fractions after industrial scale SC-CO_2 extraction and dry fractionations.

	CO_2-extracted oat flour	After milling and 1st air classification		After milling and 2nd air classification		After 3rd air classification, Protein separation	
		Fines	Coarse	Fines	Coarse	Fines	Coarse
Mass yield (%)	95.3	81.0	14.3	6.5	7.8	5.0	76.0
Particle size D_{50}/D_{90} (μm)	12/211	10/142	250/381	30/119	197/323	2/5	10/146
β-Glucan (%)	3.2 ± 0.3	1.3 ± 0.1	21.3 ± 0.5	11.4 ± 0.1	33.9 ± 0.2	n.a.	n.a.
Arabinoxylan (%)	1.0 ± 0.1	0.4 ± 0.1	8.3 ± 0.1	5.5 ± 0.1	9.9 ± 0.5	n.a.	n.a.
Protein (%)	17.2 ± 0.1	16.7 ± 0.1	23.9 ± 0.1	23.2 ± 0.1	23.0 ± 0.2	73.0 ± 0.1	10.7 ± 0.1
Starch (%)	65.6 ± 0.8	69.8 ± 0.3	17.5 ± 0.1	31.2 ± 0.2	9.2 ± 0.1	17.1 ± 0.9	77.2 ± 0.3

n.a. = not analysed.

Defatting, milling and air classification enabled concentration of the main components – starch, protein, lipids and β-glucan – into specific fractions by applying supercritical extraction technology, fine milling and air classification processes. For each of the fractions obtained, value added applications should be identified in order to make the process viable. Protein-enriched fractions could have versatile food applications, since oat proteins are considered nutritionally favourable, with high concentrations of essential amino acids such as lysine (Lapveteläinen and Aro, 1994; Mohamed et al., 2009). Oat protein concentrates with over 70% of protein have also been previously reported to be produced by wet milling processes (Wu et al., 1973) or by air classification from oat bran (Wu and Stringfellow, 1995). According to the current work the separation of protein concentrate with a similar protein content can also be achieved by dry processing from the currently very low value endosperm flour, as long as the oat material is defatted and an efficient classification system with precise cut-off is used.

In conclusion, we demonstrated that lipid removal with supercritical carbon dioxide enhanced the separation of oat β-glucan, starch and protein in distinct fractions. This was most probably due to the improved milling behaviour, smaller flour particle size and altered starch granule aggregation properties, enabling better classification. The oat bran concentrate obtained had higher β-glucan content than the existing products, produced with dry fractionation techniques. The high β-glucan content enables formulations for functional food products suitable for cholesterol lowering. The high purity protein concentrate could serve as a substitute for animal and soy proteins.

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PUBLICATION II

Electrostatic separation combined with ultra-fine grinding to produce β-glucan enriched ingredients from oat bran

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Electrostatic separation combined with ultra-fine grinding to produce β-glucan enriched ingredients from oat bran

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Keywords: oats, β-glucan, arabinoxylan, dietary fibre, electrostatic separation, ingredient

Abbreviations: AX, arabinoxylan; A/X, arabinose/xylose; DM, dry material; JM, jet-milling; M_w, molecular weight; SC-CO_2, super critical carbon dioxide

Abstract

Electrostatic separation was studied in a dry fractionation diagram to obtain β-glucan-enriched fractions from oat bran preparations. Ultra-fine grinding at ambient or cryogenic temperature was needed to achieve good dissociation between the macronutrients of oat bran particles. Particles rich in β-glucan and starch were by electrostatic separation separated from particles rich in arabinoxylan. β-Glucan was only little enriched in the non-defatted bran fractions from 19.6 to 25.0%. In contrast, preliminary defatting by super critical carbon dioxide significantly improved the dissociation of particles during grinding and allowed enrichment of β-glucan to 42.2 or 48.4% after one or two successive electrostatic separation steps. The two times separated positive fraction, containing 48.4% β-glucan, was further fractionated by a combination of jet-milling and air classification, yielding up to 56.2% β-glucan concentration. The results point out the good potential of using electrostatic separation as a bran-fractionation method to produce nutritionally interesting food ingredients.

Industrial relevance: Oat β-glucan has a widely accepted health claim related to cholesterol lowering, and has a great industrial applicability in functional foods and nutraceuticals. The high concentrations of β-glucan obtained with defatted material are of major interest from an industrial point of view, since this kind of ingredients can be used in applications where normal oat bran preparations cannot provide β-glucan at high enough concentration. In addition, the obtained β-glucan fractions retained their original molecular weight. The high β-glucan fractions had an appealing white colour and were well solubilised in hot water and in 0.1 M NaOH compared to their low β-glucan counter-fractions. Supercritical carbon dioxide extraction is a relatively costly method due to the long processing time (several hours per batch), but there are existing factories offering contract manufacturing. Lipid extraction and fractionation process can be economically feasible, especially when the fractions obtained are used in high-value applications. Ultra-fine grinding and electrostatic separation are energy intensive methods, but they enable enrichment of components without any liquids or solvents. These methods can be used to produce unique fractions which are not possible to obtain with conventional fractionation methods such as sieving and air classification.

1. Introduction

Oats are known as a good source of β-glucan, but they also contain high amounts of lipids. Typically whole grain oats contain 2–8% β-glucan and 6–10% lipids (Shewry et al., 2008; Wood, 1986). β-Glucans are located throughout the starchy endosperm, but they are concentrated in the cell walls of the aleurone and sub-aleurone layers (Wood, 1986). According to Price and Parsons (1979), the lipid content is highest in the embryonic cells (21.2% lipids), but due to their low proportion (only 2.4% of the whole grain), the outer layers and endosperm fractions contain the majority (84.7%) of oat lipids, even if the concentration of lipid in these fractions is much lower than in the embryo.

Oat β-glucan has attracted significant nutritional interest during recent years, as several independent studies have shown that products containing oat or barley β-glucan can lower the LDL-cholesterol levels (Brown, Rosner, Willett & Sacks, 1999; Ripsin et al., 1992; Truswell, 2002) and attenuate the glycaemic response (Butt, Tahir-Nadeem, Khan, Shabir & Butt, 2008). A health claim related to cholesterol lowering has been approved in the U.S.A. and in the European Union (FDA, 1997, 2003; EU, 2011). To guarantee the physiological effects, the diet should contain at least 3 g of β-glucan per day. The products bearing the health claim should contain at least 0.75 or 1.0 g β-glucan / portion, according to FDA and EU, respectively.

The nutritional potential of oat bran has motivated research into development of oat fractionation processes for the production of various value-added products (Lehtinen et al., 2009; Stevenson, Eller, Jane & Inglett, 2008; Vasanthan & Temelli, 2008). When used as an ingredient in food products, the oat bran fraction needs to have a high initial concentration of β-glucan to ensure that the final product will contain enough of β-glucan. For example, the addition of regular oat bran (with around 8% β-glucan) can lead to structural problems in the final product, because 12.5 g of oat bran would needed in a serving size of 50 g to obtain the recommended 1 g β-glucan per portion (EU, 2011). Oat bran concentrates are manufactured by maximising the concentration of β-glucan and minimising the concentration of starch. Many known processes for the isolation of highly concentrated β-glucan (up to 90% of β-glucan) are based on wet extractions (Kvist & Lawther, 2005; Potter, Fisher, Hash & Sr. Neidt, 2001; Redmond & Fielder, 2004). Wet processes are typically limited by the high viscosity of aqueous extracts even at low β-glucan concentrations, leading to large liquid volumes and high costs related to the drying of β-glucan-rich ingredients. In addition, low pH and endogenous enzymes can reduce the molecular weight (M_w) of the β-glucan when exposed to wet conditions (Åman, Rimsten, & Andersson, 2004). High M_w (>500 kDa) has been shown to better retain the cholesterol lowering ability of β-glucan compared to lower M_w molecules, at least in dry food matrices such as breakfast cereals (Wolever et al., 2010).

Conventional dry fractionation methods, such as milling, sieving and air classification, allow the enrichment of β-glucan up to 20–25% concentration (Mälkki, Myllymäki, Teinilä & Koponen, 2004; Lehtinen et al., 2009; Vasanthan & Temelli 2008; Wu & Doehlert, 2002). Lipid removal has been shown to improve the separation to yield as high as 34% β-glucan concentrations (Sibakov et al., 2011; Stevenson et al., 2008). One of the most promising approaches is the defatting with super critical carbon dioxide (SC-CO_2). Compared to traditional solvent extraction (e.g. by hexane), no solvent residues remain in the solid material after removing the lipids by SC-CO_2 extraction.

Fractionation diagram could be improved by including innovative separation processes based on the electrostatic separation to enhance the enrichment of β-glucan, as previously applied on wheat bran layers. Indeed, Stone and Minifie (1988), Behrens and Bohm (2004), Bohm and Kratzer (2005), and

Hemery et al. (2011) have successfully separated aleurone-rich fractions from medium-ground and ultra-fine wheat brans with such process.

The current work aimed to study the effects of ultra-fine grinding and electrostatic separation on the fractionation of oat bran preparations, in order to achieve fractions highly enriched in β-glucan. The effect of lipid removal was investigated by characterising the distribution of different grain components in the electrostatically separated fractions.

2. Materials and methods

2.1. Raw materials

Two commercial non-defatted, heat-treated oat bran preparations were studied: regular oat bran (Elovena Plus) and oat bran concentrate (OBC-20NEF) both provided by Raisio plc, (Raisio, Finland). In addition, two defatted and non-heat-treated oat bran concentrates (SC-CO_2-OBC-1 and SC-CO_2-OBC-2) were studied. The defatted samples were manufactured according to Sibakov et al. (2011). The whole grain oats were first defatted with supercritical carbon dioxide (SC-CO_2) extraction, and then milled and air classified. The first coarse bran fraction (SC-CO_2-OBC-1), which was separated by air classification, was milled and air classified again to yield oat bran fraction highly enriched in β-glucan (SC-CO_2-OBC-2).

2.2. Dry fractionation

Ultra-fine grinding was combined with electrostatic separation to enhance the separation of the grain compounds from each other (Fig. 1). In addition, the fractionation capacity of jet-milling and air classification was studied either before or after the electrostatic separation.

2.2.1. Ultra-fine grinding of oat brans

Prior to electrostatic separation, the particle size of oat bran preparations was reduced down to D_{50} = 30–60 µm either by an ultra-fine milling equipment Turborotor G-55 (Görgens Mahltechnik GmbH, Dormagen, Germany) at room temperature (ambient conditions) with a rotor speed of 1 800 rpm, or by a fine impact mill 100 UPZ (Hosokawa-Alpine AG, Augsburg, Germany) with 0.3 mm grid at –100 ºC (cryogenic conditions) with a rotor speed 18 000 rpm. Cryogenic grinding was carried out by combining a cryogenic screw feeder with liquid nitrogen supply (Micronis, Agen, France) in the 100 UPZ impact mill as described by Hemery et al. (2011).

2.2.2. Electrostatic separation of ground oat brans

A pilot-scale electrostatic separator (TEP System, Tribo Flow Separations, Lexington, USA) was used for the production of various fractions, using ultra-fine oat bran preparations as starting materials as described by Hemery et al. (2011). The feeding system of the separator was operated at 100 rpm, the bran particles were then conveyed by compressed air in a charging line where they were charged by tribo-electricity, by impacting against each other and impacting against the walls of the charging line. The charged bran particles were then introduced in a separation chamber containing two high voltage electrodes (15 kV), where the positively charged particles were attracted by the negative electrode and the negatively charged particles were attracted by the positive electrode. A particle recovery system equipped with two cyclones allowed to separately recover two fractions (one containing the positively charged particles and the other negatively charged particles). Starting from the raw material ‘F0’, fractions ‘F1A-’ and ‘F1B+’ were separated by the negative and positive electrodes, respectively. Fractions ‘F2AA-’ and ‘F2AB+’ were obtained by repeating the separation starting from fraction ‘F1A-’, whereas fractions ‘F2BA-’ and ‘F2BB+’ were obtained starting from fraction ‘F1B+’ (Fig. 1). A deposit was noticed on both

positive and negative electrodes: these particles were collected and named as 'left(-)' and 'right(+)' fractions, respectively.

2.4. Jet-milling after electrostatic separation

The oat bran fraction, which contained the highest concentration of β-glucan after the electrostatic separation, was further studied using a 100AFG Multi-Processing System (Hosokawa-Alpine AG), with a combination of jet-milling and air classification. The particles were forced to collide with each other under 6 bar pressure, and an air classifier wheel with 10 000 rpm rotor speed was used to allow only the finest particles to pass through the classifier wheel. The coarse fraction, which could not exit from the milling chamber, was collected as a separate fraction (Fig. 1).

2.5. Biochemical analyses

The amount of β-glucan was determined by an enzymatic method 32-23 (AACC, 2000) using the Megazyme β-Glucan mixed linkage assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The molecular weight (M_w) of β-glucan was analysed by the following procedure: 10–30 mg of each sample (depending on the β-glucan concentration) was dissolved into 10 ml 0.1 M NaOH in presence of 0.1% $NaBH_4$. Samples were kept under magnetic stirring at room temperature overnight. Before the measurements, samples were diluted and filtered through 0.45 µm syringe filter. After filtering, samples were analysed by high-performance size-exclusion chromatography (HP-SEC), which consisted of an Alliance 2690 separation module, using calcofluor staining (Calcofluor solution for post column staining was 30 mg Fluorescent Brightener 28 in one litre of 50 mM NaOH) and Scanning Fluorescence 474 detector (Waters Inc., Milford, MA, U.S.A.). The columns employed were (7.8 × 300 mm) µHydrogel 2000, µHydrogel 500 and µHydrogel 250 (Waters Inc.) in series at 60 °C. The eluent was aqueous 50 mM NaOH at a flow rate of 0.5 ml/min. Injections (100 µl) were made of sample and standard solutions (Suortti, 1993). The linear size-exclusion calibration curve ($r^2 > 0.95$) was constructed on the basis of β-glucan standards ranging from 33.6 to 667 kDa. The system was controlled and calculations were performed with Waters Empower software's GPC option. In principle the software sliced the sample peak into narrow slices. The peak molecular weight value and the area of each slice (i.e. content) were calculated by the software. Then the weight average molecular weight (M_w) was calculated over the whole β-glucan peak.

The amounts of neutral sugars were quantified as anhydro-sugars, after hydrolysis of the samples by sulphuric acid (1 M, 2 h, 100 °C) and conversion into alditol acetates (Blakeney, Harris, Henry & Stone, 1983). Anhydrosugar determination was done by gas-liquid chromatography (DB 225 capillary column) at 225 °C, using hydrogen as carrier gas and allose (5 mg) as internal standard. The content of arabinoxylan was assumed as the sum of anhydro-arabinose and anhydro-xylose. Nitrogen was analysed using a Kjeldahl autoanalyzer (Foss Tecator Ab, Höganäs, Sweden), and protein concentration was calculated as N x 6.25 according to method 46-11A (AACC, 2000). Starch was quantified using the Megazyme total starch assay kit according to method 76-13.01, and the total fat content using a Soxhlet extraction by heptane according to method 30-25.01 (AACC, 2000). The results were presented as an average of triplicate analyses.

2.6. Solubility of β-glucan

The solubility of β-glucan in oat bran fractions was evaluated by mixing 2 g of oat bran material (DM) in 1000 ml of distilled water at 90 °C or in 1000 ml of 0.1 M NaOH at room temperature (22 °C). Both of the solutions were stirred for 2 min with a hand held homogeniser (Heidolph Diax900 Ultra Turrax, Gemini BV, Apeldoorn, the Netherlands), using 12 000 rpm speed. The insoluble residues were separated by a centrifuge (Sorvall RC-12BP, DuPont, U.S.A.), using rotor speed 4000 rpm for 15 min. The soluble fractions were freeze-dried by a Christ Epsilon 2-25 freeze drier

(Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The percentage of soluble β-glucan was calculated based on the β-glucan concentration and the yield of the freeze-dried soluble fractions in comparison to the original bran fractions.

2.7. Particle size measurement

The particle size distribution was measured by a laser diffraction particle size analyser Mastersizer 2000 (Malvern Instruments Ltd., United Kingdom). Oat bran fractions were suspended in ethanol by the fully automated wet sample dispersion unit Hydro 2000S. The suspensions were analysed when the obscuration was between 10 and 20%, using a continuous sonication. The volume based particle size distribution was presented as cumulative undersize centiles (D_{10}, D_{50} and D_{90} values) with an average of triplicate analyses.

2.8. Colour measurement

The colour of the different oat fractions was measured directly from the dry powders with a CR-410 colorimeter (Minolta Ltd, Nieuwegein, Netherlands), using the L.a.b. colour system. The L-value gave information on the luminance, and the a- and b-values gave information about the colour of the sample (a: from green to red, and b: from dark blue to yellow).

2.9. Statistical analysis

The results were calculated as means of triplicate analysis results. The data was subjected to analysis of variance using the IBM SPSS Statistics 20 (IBM Corporation, Somers, NY, U.S.A.), and significant differences ($P < 0.05$) between individual means were identified by the Tukey's test.

2.10. Microscopic observation

Prior to light microscopy, the samples were embedded into 2% agar and then fixed in 3.0% (w/v) paraformaldehyde and 1.0% (v/v) glutaraldehyde in 0.1 M Na-K phosphate buffer (pH 7.0), dehydrated in graded ethanol series, and embedded in hydroxyethyl methylacrylate resin (Leica Historesin embedding kit, Heidelberg, Germany). The embedded samples were sectioned (2 μm) in a rotary microtome HM 355 (Microm Laborgeräte GmbH, Walldorf, Germany) using a steel knife. The sections were transferred onto glass slides, and stained either with light green (BDH Chemicals Ltd, Poole, Dorset, UK) / Lugol's iodine solution when observed in bright field or with Acid Fuchsin (BDH Chemicals Ltd., Poole, Dorset UK) / Calcofluor White (Fluorescent Brightener, Aldrich, Germany) for epifluorescence observation. Light green stains protein green/yellow, whereas Lugol's iodine solution stains starch in dark blue. Acid Fuchsin and Calcofluor White were used for staining protein red and β-glucan rich cell walls light blue, respectively, when the samples were imaged using exciting light at 400–410 nm and emission fluorescence recovered at >455 nm). The samples were then examined under a BX-50 microscope (Olympus Corp., Tokyo, Japan). Micrographs were obtained using a PCO SensiCam CCD colour camera (PCO AG, Kelheim, Germany) and the Cell^P imaging software (Olympus). At least five images were acquired from the sections cut from three blocks per sample. The most representative micrographs were selected for the comparison of the different fractions.

3. Results

3.1. Electrostatic separation of non-defatted oat bran particles

The non-defatted and heat-treated oat brans (Elovena Plus and OBC-20NEF) contained 8.5 and 10.1% total fat, respectively. In these samples, the β-glucan concentration increased only slightly when ultra-fine milled bran particles were separated in electric field in two steps (Figure 1, Table 1). The β-glucan concentration of Elovena Plus bran increased from the initial 5.8% up to 8.7% in

fraction ‘F2AB+’. The β-glucan concentration of OBC-20NEF increased from the initial 19.6% up to 24.5–25.0% in fractions ‘F2AB+’ and ‘F2BA-‘. The lowest β-glucan concentrations (5.4% in Elovena Plus and 16.3% in OBC-20NEF) were obtained from the fraction ‘F2BB+‘. By contrast, the starch concentrations of both of the non-defatted samples were highest (53.7 and 25.5%, respectively) in the fraction ‘F2BB+’. The lowest starch concentrations (33.7% in Elovena Plus and 8.4% in OBC-20NEF) were in the fraction ‘F2AA-’. In OBC-20NEF samples, arabinoxylan was enriched in the fractions containing the smallest amount of starch, i.e. fractions ‘F1A-’ (13.5% AX) and ‘F2AA-’ (17.6% AX). The arabinose/xylose (A/X) ratio was between 0.6–0.7 in all of the fractions.

The deposits, ‘right (+)’ and ‘left (-)’, collected from the electrodes contained less β-glucan than the non-defatted raw materials. The ‘right (+)’ deposits of Elovena Plus and OBC-20NEF contained only 1.7 or 9.5% β-glucan, whereas the ‘left (-)’ deposits contained 4.2 or 13.1% β-glucan, respectively. The differences in starch concentration of the ‘right (+)’ and ‘left (-)’ deposits were even bigger (62.9 vs. 29.7% and 41.5 vs. 6.8%, respectively). The protein concentration did not vary radically between the (+) and (-) deposits of non-defatted samples, but the protein concentration changed in a different way in Elovena plus (from 14.4 to 19.1%) than in OBC-20NEF (from 28.2 to 24.4%). The AX in the deposits of OBC-20NEF was concentrated in the ‘left (-)’ deposit (22.5%), whereas the ‘right (+)’ deposit contained only very little amount of AX (1.9%). Moreover, the A/X ratio was higher in the ‘left (-)’ (0.9) than in the ‘right (+)’ (0.6) deposit.

The microscopic pictures of OBC-20NEF (Fig. 2) showed that more starch granules were observed in the positive fractions compared to the negative fractions. Fraction F2AA- had lower amount of cell walls rich in β-glucan (blue) than the other fractions. However, the difference between the distribution of β-glucan in fractions ‘F2AB+’, ‘F2BA-‘ and ‘F2BB+’ was not clearly seen from the microscopic pictures, which allowed only qualitative sample description. However, the chemical characterisation showed that β-glucan was significantly concentrated in fractions ‘F2AB+’ and ‘F2BA-‘ (Table 1). On the other hand, the light green / Lugol’s iodine staining (showing starch as dark blue in Fig. 2, row B) as well as the results in Table 1 showed that fractions ‘F2BA-’ and ‘F2BB+’ contained more starch (17.8 and 25.5%) than fractions ‘F2AB+’ and ‘F2AA-’ (11.9 and 8.4%, respectively).

Protein fractionated differently in the two non-defatted oat bran preparations. In Elovena Plus bran, the fraction ‘F1A-‘contained the highest amount of protein (18.2%), whereas in OBC-20NEF, the highest protein concentration (27.0%) was detected in fraction ‘F1B+’. In general, the differences in protein concentrations were only minimal as compared, for example, to the starch concentrations in different fractions.

In Elovena Plus and OBC-20NEF, the particle size of negative fractions was smaller compared to positive fractions (D_{50} was 30–36 μm in ‘F2AA-’, and 58–64 μm in ‘F2BB+’, respectively) (Table 2). The colour analysis (Table 2) showed that in both of the non-defatted oat bran preparations, the positive fractions had higher L-values, indicating a lighter colour than in the negative fractions, with higher a- and b-values, i.e. the negative fractions had a darker and more brownish colour. Both of these results could be related to the higher amount of (white) starch granules in the positive fractions (Table 1).

3.2. Electrostatic separation of defatted oat bran particles

Compared to non-defatted heat-treated brans, the defatted non-heat-treated brans (SC-CO2-OBC-1 and SC-CO2-OBC-2) contained significantly less fat (2.9 and 4.4%, respectively), but some of the lipids were still remaining. Higher β-glucan concentrations were achieved in the electrostatic

separation of defatted oat brans (Table 1). The largest increases in the amount of β-glucan were in the fraction 'F2BB+' both with OBC-1 (from 21.3 to 31.2%) and with OBC-2 (from 35.0 to 48.4%), relating to an overall increase of about 40% compared to the initial OBC material. The lowest β-glucan concentration (13.5% with OBC-1 and 28.0% with OBC-2) was observed in the fraction 'F2AA-'. When the fraction 'F2BB+' of OBC-2 (with the highest β-glucan concentration) was subjected to a third separation step, the β-glucan concentration did not increase anymore, i.e. the β-glucan concentration was around 48.4% in both 'F3BBA-' and 'F3BBB+' fractions (data not shown in Table 1).

The starch concentration was highest (31.6–34.5% with OBC-1 and 9.5–9.9% with OBC-2) in fractions 'F1B+' and 'F2BB+' for both defatted samples. The lowest starch concentrations of OBC-1 and OBC-2 (24.8% and 3.9%, respectively) were in the fraction 'F2AA-'. Arabinoxylan was enriched in the fractions 'F1A-' and 'F2AA-' of OBC-2 (19.4 and 22.7%, respectively). The A/X ratio was between 0.6 and 0.7 in all fractions. The electrostatic separation had only minimal effect on the protein concentration in fractions of OBC-1 and OBC-2 (the protein concentrations were 16.1–18.6% and 16.8–18.9%, respectively).

The deposits, 'right (+)' and 'left (-)', collected from the electrodes contained less β-glucan than the defatted raw materials, except the 'right (+)' deposits of OBC-1 (30.4% β-glucan) (Table 1). The 'left (-)' deposit of OBC-1 contained only 7.5% β-glucan, and the 'left (-)' deposits of differently milled OBC-2 materials only 18.3–19.9% β-glucan. The 'right (+)' deposits of OBC-2 contained higher amount of β-glucan (24.6–25.1%), but the β-glucan concentration was much lower compared to the fractions 'F1B+' and 'F2BB+' due to the high starch concentration in the 'right (+)' deposits (36.9 and 19.9% for OBC-1 and OBC-2, respectively). The 'left (-)' deposits contained significantly less β-glucan (17.1 and 4.7%, respectively). The AX in OBC-2 was concentrated in the 'left (-)' deposit (25.0%), whereas the 'right (+)' deposit contained only a small amount of AX (3.9%). Moreover, the A/X ratio was 0.6 in the 'left (-)' and 0.9 in the 'right (+)' deposit. This was consistent with the results of non-defatted samples. The protein concentration did not vary radically between the (-) and (+) deposits of OBC-1 and OBC-2 samples (18.3–19.5%).

Microscopic observation showed that the fraction 'F2AA-'and deposit 'left (-)' was enriched in brown fibrous particles and less enriched in blue-coloured β-glucan (Fig.3, row A) and starch granules shown in black (Fig. 3, row B) as compared to other fractions. This indicated that the fraction 'F2AA-' and deposit 'left (-)' consisted mainly of particles from pericarp. The fraction 'F2BB+' and deposit 'right (+)' contained a high amount of intracellular material, as well as cell walls released from the degradation of aleurone and endosperm cells, shown as blue (Fig 3., row A). In addition, the starch granules (Fig 3., row B) were enriched in the positive fractions, indicating that part of the endosperm was still present in this fraction.

The particle size distributions of negatively and positively charged fractions were quite close to each other (D_{50}=29.5–39.9 µm for SC-CO2-OBC-1 and D_{50}=55.4–64.2 µm for OBC-2, respectively), and did not show any clear correlation between the particle size and chemical composition of the particles. The only clear differences were measured from the deposits collected from the electrodes: The 'right(+)' fraction of SC-CO2-OBC-1 contained larger particles (D_{50}=43.3 µm) as compared to 'left(-)' fraction (D_{50} = 28.1 µm). However, the 'right' and 'left' fractions of SC-CO2-OBC-2 had almost the same particle size (D_{50} = 39.1–39.8 µm). Results of the colour analysis (Table 2) followed a similar trend as with the non-defatted oat bran preparation. The positive fractions had higher L-values (lighter colour) than the negative fractions, which had higher a- and b-values (darker and more brownish colour).

3.3. Effect of grinding method on electrostatic separation

Reduction of particle size of the defatted oat bran concentrate (SC-CO2-OBC-2) had a clear effect on the separation capacity of the electrostatic separation. Initially, the particle size of OBC-2 could be described by D_{10} = 107 µm, D_{50} = 213 µm and D_{90} = 404 µm. Grinding at ambient temperature yielded a particle size of D_{10} = 14 µm, D_{50} = 60 µm and D_{90} = 141 µm, and grinding at cryogenic conditions yielded to D_{10} = 9 µm, D_{50} = 63 µm and D_{90} = 155 µm, respectively. Thus, the particle sizes after ambient and cryogenic ultra-fine grinding did not differ much from each other.

After electrostatic separation similar results were observed between the fractions of ambient and cryogenic milled materials. Almost equal concentrations of β-glucan (48.4 and 46.0%) were detected in the 'F2BB+' fractions when the electrostatic separation was performed after the ambient or cryogenic grinding of OBC-2 bran (Table 1). By contrast, the coarse initial OBC-2 material (D_{50} = 213 µm) showed less enrichment in β-glucan as after the ultra-fine grinding; from 35.0 to 37.3% of β-glucan at highest (data not shown in Table 1).

3.4. Further enrichment of β-glucan by jet-milling and air classification

The fraction 'F2BB+', which contained 48.4% β-glucan, was further fractionated by a combination of jet-milling (JM) and air classification. The JM-equipment allowed simultaneous collision of particles with each other, and air classification to separate the fine starch and protein particles from more fibrous cell wall particles. Using this kind of approach, a fraction 'F2BB+, JM, Coarse' with 56.2% β-glucan concentration (Table 1) was achieved by collecting the sample not passing through the classifier wheel (from the milling chamber of the JM-equipment). The starch and protein concentrations were significantly lower (3.3% starch and 12.9% protein) in the cell wall enriched fraction compared to the corresponding fine-fraction (19.0% starch and 18.4% protein, Table 1). The microscopic pictures (Fig. 4) clearly showed that the starch granules entrapped by the cell wall structures in fraction F2BB+ were released by jet-milling, and concentrated in the subsequent fine fraction 'F2BB+, JM, Fine', whereas the starch-poor cell walls were recovered from the fraction 'F2BB+, JM, Coarse'. Similar results were obtained when JM was performed as the first step, and electrostatic separation as the second step (dashed lines in Fig. 1). The maximal β-glucan concentration was 56.1% when JM was performed prior to electrostatic separation (data not shown in Table 1). Thus, the order of the processing steps (JM either before or after the electrostatic separation) did not influence the results or the β-glucan yields of the fractions.

The effect of air classification on the defatted ultra-fine oat bran concentrate was also investigated without electrostatic separation. The β-glucan-rich raw material (SC-CO_2-OBC-2) was ground with the ultra-fine milling equipment at ambient temperature and subsequently air classified. The highest β-glucan concentration (40.3%) was obtained when the classifier wheel speed was 10 000 rpm, the air flow 100 m^3/h, and the mass yield of the coarse (D_{50} = 70 µm) fraction was 84.6%. Correspondingly, the fine fraction (D_{50} = 13 µm, mass yield 15.4%) had only 11.1% β-glucan concentration (data not shown in Table 1). Thus, it was concluded that air classification itself was not efficient enough to reach higher than 40.3% β-glucan concentrations when using the same ultra-fine raw material as in the electrostatic separation and jet-milling trials.

3.5. Properties of β-glucan after electrostatic separation

The M_w of β-glucan (766–799 kDa) differed only slightly between the different fractions of SC-CO2-OBC-2, being higher in the positive than in the negative fractions. The positive fraction 'F2BB+' had a better solubility of β-glucan both in water at 90 °C (65.9%) and in 0.1 M NaOH at ambient temperature (96.6%) compared to the negative fraction 'F2AA-' (42.0 and 75.4%, respectively) (Table 1).

4. Discussion

In the current study, much higher concentrations of β-glucan (from 48.4 to 56.4%) were obtained than has been reported previously using dry fractionation methods. Air classification of defatted oats has previously yielded a bran fraction with 33.9% of β-glucan (Sibakov et al., 2011). In the current study, further enrichment of β-glucan in oat bran was obtained by lipid removal, intensive particle size reduction, electrostatic separation, and subsequent jet-milling and air classification.

4.1. Tribo-charging and electrostatic separation

The relationship between the surface properties of the cereal particles and their tribo-charging behaviour is still largely unknown. The ability of the material's surface to donate or accept electrons when it comes into contact with another material determines the positive or negative surface charge density that it will acquire during tribo-charging (Mazumder et al., 2006). Different chemical groups and the type of cell wall polysaccharides (branched and cross-linked vs. linear) may be responsible for the acquired charges of the different types of particles (Hemery et al., 2011). In the current study, β-glucan and starch were concentrated in the positive fractions (i.e. the negatively charged particles collected from the positive electrode) of the defatted oat brans (SC-CO2-OBC-1 and OBC-2), whereas AX was concentrated in the negative fractions (i.e. particles with a positive charge). Arabinoxylan and β-glucan were concentrated similarly to the current study after the electrostatic separation of wheat bran (Hemery et al., 2011).

The negative fractions of OBC-20NEF and SC-CO2-OBC-2 showed higher amounts of outer pericarp than the positive, as observed by microscopy (Fig. 2 and 3). This was confirmed by higher amounts of arabinose, xylose and galactose. These compounds are known to be characteristic of the complex heteroxylans of the pericarp (Fincher & Stone, 1986). By contrast, the positive fractions contained more intracellular contents and cell wall fragments from aleurone layer and starchy endosperm.

The difference in A/X ratio of oat brans between the deposits 'right(+)' and 'left(-)', 0.9 and 0.6, was opposite to the results with wheat bran (0.4 and 1.1, respectively) (Hemery et al., 2011). In their study, the wheat bran before electrostatic separation contained 12.7% arabinose and 19.8% xylose, whereas in the current study the defatted oat bran concentrate (OBC-2) contained initially 6.0% arabinose and 9.2% xylose. The original AX composition of wheat and oat brans does not give any explanation for the different behaviour of AX in the electrostatic separation. Thus, there might be other compounds, such as AX-linked phenolic acids, which can affect the charging behaviour of the bran particles (Antoine, Castellon, Toureille, Rouau & Dissado 2004). However, the difference in A/X ratios was rather low, and only significant between the deposit fractions (collected directly from the electrodes). Thus, the A/X ratio may only play a minor role compared to the composition of β-glucan and AX molecules in the cell wall complex. Antoine, Castellon, Toureille, Rouau and Dissado (2004) found that the outer pericarp layer of wheat bran had a large porosity and thus exhibited a poor permittivity. In contrast, the aleurone layer showed a substantial permittivity, about six times higher than the pericarp. The good capacity of aleurone layer was postulated to be due to the high amount of linear polymers in aleurone. The proportion of pericarp layer in oat bran is smaller than in wheat bran (Dornez et al., 2011; Miller & Fulcher, 2011), which could explain the observed differences between the fractionation of oat and wheat bran samples. Nevertheless, the difference in the charging behaviour of wheat and oat bran particles would still need further investigations.

4.2. Effect of grinding on obtained particle size distribution and separation behaviour

The particle size reduction by ultra-fine grinding at ambient temperature was rather independent of defatting, as the particle size of non-defatted materials (Elovena Plus, D_{50} = 35 µm, and OBC-20NEF, D_{50} = 39 µm) was smaller or similar to the defatted samples (SC-CO2-OBC-1, D_{50} = 33 µm, and SC-CO2-OBC-2, D_{50} = 60 µm). The smaller particle size in non-defatted bran preparations as well as in SC-CO2-OBC-1 compared to SC-CO2-OBC-2 was most likely due to higher concentration of starch and smaller concentration of cell wall particles. Typically, oat starch granules have a diameter between 3–10 µm (Zhou, Robards, Glennie-Holmes & Helliwell, 1998), which is less than the diameter of the particles containing more cell wall material.

The ultra-fine grinding of the defatted oat bran SC-CO2-OBC-2 at ambient and cryogenic conditions yielded almost identical particle size distributions (D_{50}-values 60 and 63 µm, respectively) (Table 2). The limitation in the particle size reduction could be due to the efficiency of the grinding equipment: At ambient conditions, OBC-2 was ground in a Turborotor G-55 mill which had a long grinding path leading to several collisions of particles between the rotor and the stationary elements in the grinding barrel. The particle size reduction in this mill could be improved by increasing the rotor speed (>1 800 rpm), but it was not investigated in this study.

On the other hand, the cryogenic grinding was performed in a 100UPZ mill, in which the material was forced though a 0.3 mm screen. The limiting factor in cryogenic grinding was not the rotor speed (already at maximum 18 000 rpm) but the screen size. However, the 0.3 mm screen size was already the smallest provided by the manufacturer. Thus, it was not possible to conclude if the particle size reduction was limited due to the grinding equipment, but it was anyway shown that the cryogenic conditions did not results in lower particle sizes compared to ambient grinding. Hemery et al. (2011) showed similar kind of restriction in the particle size reduction of wheat bran. They used ambient and cryogenic grinding of wheat bran with the same milling equipment (100UPZ) and similar grinding conditions (18 000 rpm rotor speed and 0.3 mm screen) as in the current study. They showed that the particle size of ultra-fine wheat bran was almost identical after ambient and cryogenic ultra-fine grinding (D_{50} = 51.4 and 54.9 µm, respectively). However, three successive grinding passes was needed in ambient conditions, and only one in cryogenic grinding.

Hemery et al. (2011) discovered that the ambient grinding resulted in more efficiently dissociated wheat bran tissues, whereas after the cryogenic grinding the bran particles were more composite and difficult to separate from each other. However, this kind of behaviour was not observed in the current study. The ultra-fine milled OBC-2 (ground at ambient or cryogenic conditions) was separated in the electrostatic field in a similar way, and the obtained maximal β-glucan concentrations were close to each other (48.4 and 46.0%, respectively) (Table 1).

Hemery, Mabille, Martelli and Rouau (2010) related the more efficient dissociation of wheat bran at ambient temperature to the glass transition temperatures of different bran layers. The pericarp layer of wheat bran did not display almost any resistance to fracture at -80 °C, whereas the aleurone layer showed an elastoplastic behaviour. The intermediate layers (testa and hyaline) seemed to have lost their plasticity and were brittle at this temperature. The observed loss of plasticity of the intermediate layer was probably due to lipidic compounds of the cuticles of testa and hyaline layer, which displayed a glass transition at -46 °C (Hemery, Mabille, Martelli and Rouau, 2010). Thus, the cryogenic grinding at -100 °C (Hemery et al., 2011) fractured the pericarp and intermediate layers into small composite particles, whereas the ambient grinding kept these layers more intact even though their overall particle size was reduced.

In the current study, defatting by supercritical CO_2 prior to grinding induced a loss of lipidic non-polar compounds which are known to be present in the intermediate layers (Hemery et al., 2009). Thus, it can be hypothesised that the defatting of oat bran concentrate might have removed the effect of these intermediate layers on the particle dissociation. The effect of cryogenic grinding on non-defatted oat bran concentrate could bring more insight of the phenomenon, but was not researched in the current study.

Miller and Fulcher (2011) explained that the portion of subaleurone starchy endosperm is larger in oat bran than in wheat bran, because the aleurone layer of oats does not separate as cleanly from the endosperm in oats as it does in wheat. This also explains the presence of larger amount of aleurone and subaleurone cell walls in oat bran concentrate (OBC-2), as shown in Fig. 3. The high amount of cell walls could have modified the relative ratio of different tissues, leading to different mechanical properties and possibly diminishing the effect of individual layers (e.g. testa and hyaline) in such a composite material. Similarly, Antoine, Lullien-Pellerin, Abecassis, and Rouau (2004) showed that wheat brans fractured more rapidly than isolated aleurone layers due to the presence of the friable pericarp and the possible mechanical constraints due to tissues surrounding the aleurone layer.

4.3. Enrichment of β-glucan and its use in food applications

The overall fractionation process was significantly dependent on the air classification steps prior to the electrostatic separation (Sibakov et al., 2011). Thus, it made sense to compare the results obtained by the electrostatic separation to the air classification of ultra-finely milled oat bran preparation SC-CO2-OBC-2. The air classification itself was not efficient enough to separate the aleurone and endosperm cell wall fragments from the pericarp particles, and thus the highest β-glucan concentration obtained was 40.4%. This was most probably due to the similarity in the size and density of the particles. When a combination of electrostatic separation with jet-milling and air classification was utilised, further enrichment of β-glucan (up to 56.1–56.2%) was obtained by removing starch from the β-glucan rich fraction. This proved that the ultra-fine grinding (ambient or cryogenic) was not enough to release the embedded starch from the cell wall complex. In addition, the simultaneous jet-milling and air classification may have contributed to the separation capacity, as the particles stayed more dissociated during the classification, avoiding formation of agglomerates. The enrichment was independent of the order of the processing steps (either electrostatic separation or jet-milling first). However, it would be more feasible to do the electrostatic separation first, because jet-milling is a time-consuming and energy-intensive method. Thus, a smaller material flow would go through the jet-milling process. As jet-milling processes are always expensive, the highly concentrated β-glucan should be used as a premium ingredient for nutraceuticals and functional food products. In addition, it is important to notice that the recovery of β-glucan, for example in the fraction ‘F2BB+, JM, Coarse‘ (13.0% of the original β-glucan) was much lower than in the electrostatically separated β-glucan rich fractions, such as ‘F2BB+’ with a 22.3% recovery of the original β-glucan (Table 1).

In food products, the high β-glucan concentration of ingredients would be especially advantageous when traditional oat bran preparations are difficult to use. For example, the regular oat bran can bring too much of other materials, mainly starch, in the matrix, making the formulation of the products challenging. The products which would benefit of the high β-glucan include yoghurts and meat products (Havrlentová et al., 2011), beverages (Sibakov et al., 2013), and biscuits (Pentikäinen et al., 2014).

The advantage of ingredients which are produced by completely dry enrichment methods is that the β-glucan molecules are retained in a more natural state compared to wet extraction. This study proved that the M_w of β-glucan remained at the original level (around 780 kDa), and β-glucan in the

most enriched fractions was well soluble in water at 90 °C (65.9%) and even more in 0.1 M NaOH (96.6%) at ambient temperature (Table 1). The reason for the different solubility between the high ('F2BB+') and low ('F2AA') β-glucan fractions could be linked to the amount of AX in these fractions. In addition, β-glucan could have been differently entrapped in the cell wall complex of pericarp rich particles ('F2AA') than in the aluerone and endosperm cell wall rich fraction ('F2BB+'). Another reason could be a higher cellotriose/cellotetraose (DP3/DP4) ratio in the beta-glucan molecules of the fraction 'F2AA'. Lazaridou, Biliaderis, Micha-Screttas and Steele (2004) explained the superior water solubility of oat beta-glucan by its lower DP3/DP4 ratio (2.1) compared to barley (2.8–3.0) and wheat beta-glucan (3.7). Similarly, the DP3/DP4 ratio was shown to be higher in oat pericarp layer (2.6) compared to the whole oat flour (2.2) (Wood, Weisz & Blackwell, 1994). However, the DP3/DP4 ratio was not studied in the current study.

5. Conclusions

Electrostatic separation showed good potential and selectivity in the enrichment of different grain compounds from oat bran. It improved the enrichment of oat bran β-glucan and was superior to traditional dry fractionation methods, such as air classification. However, size reduction of bran particles was needed to reach a good dissociation of bran components. Further studies should be performed to acquire more knowledge on the surface properties of oat bran particles, and enable better understanding of the tribo-charging and electrostatic separation mechanisms. The results clearly demonstrated that the lipid removal by supercritical carbon dioxide had a greater impact on the fractionation behaviour than the particle size. It was not possible enrich β-glucan higher than up to 25% when non-defatted oat bran concentrate (OBC) was used as raw material, even though it was ground into ultra-fine particles. In contrast, the defatted OBC showed the highest reported concentration of β-glucan (from 48.4 to 56.2%), when applying only dry fractionation methods. The high β-glucan content enables novel kind of formulations for nutraceuticals and functional foods with cholesterol lowering properties.

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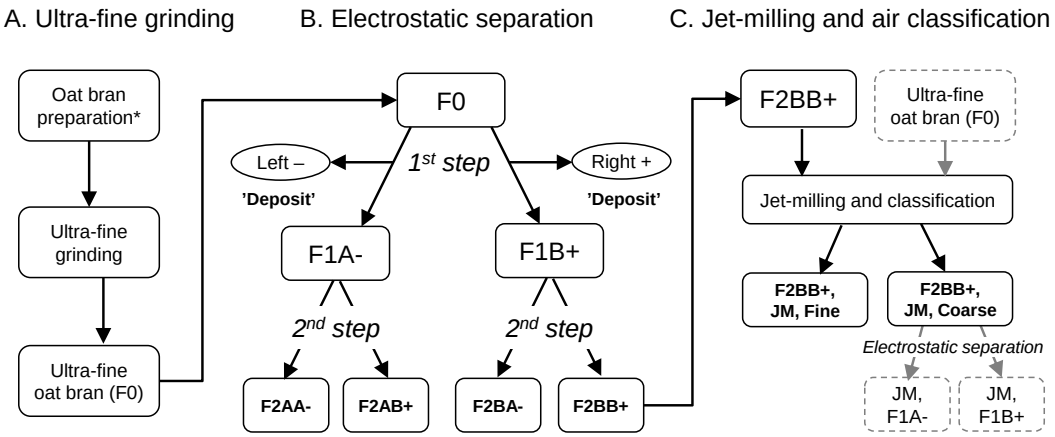

Fig. 1. The overall flow chart of the β-glucan enrichment process based on ultra-fine milling (A), electrostatic separation (B) and jet-milling with air classification (C). *Oat bran preparation means regular oat bran or oat bran concentrate (heat treated/non-defatted or non-heat treated/defatted).

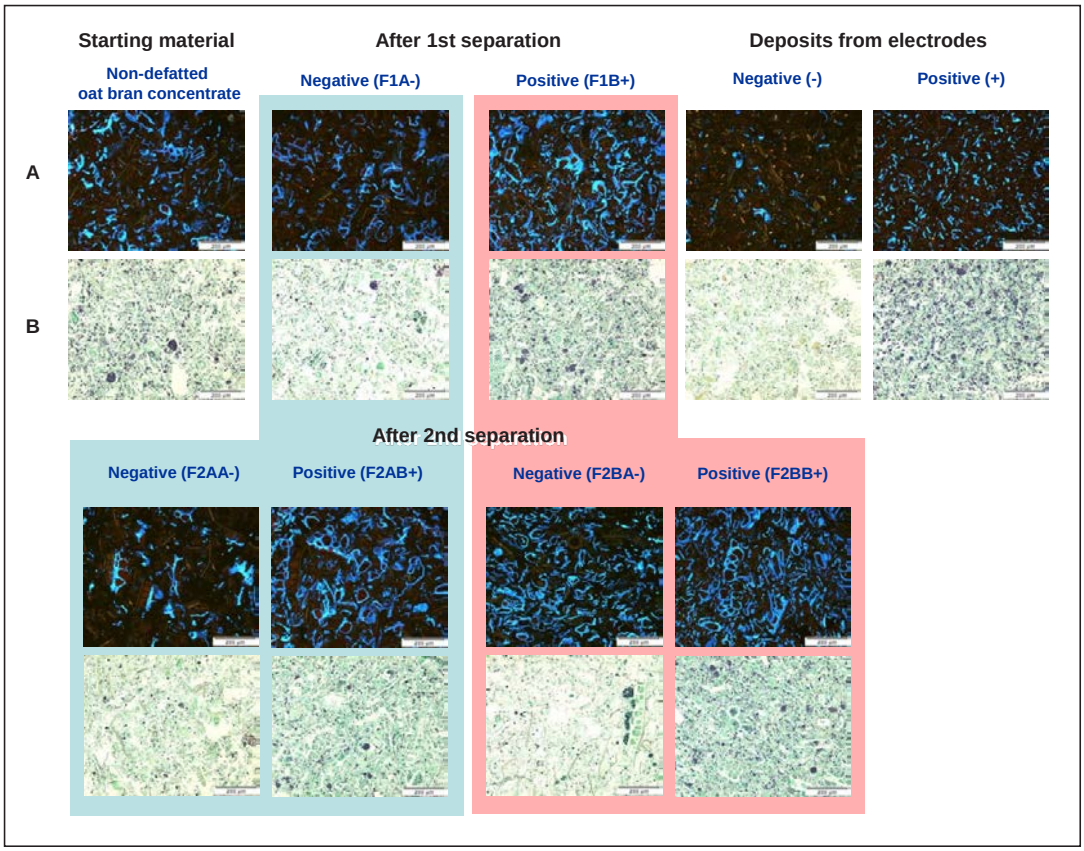

Fig. 2. Microscopic pictures of the non-defatted oat fractions (OBC-20NEF) oat fractions obtained from the electrostatic separation process (see Fig. 1B). Row A: Acid Fuchsin / Calcofluor White staining, showing protein as red and β-glucan rich cell walls as light blue. Row B: Light Green / Lugol's iodine staining, showing protein as green and starch as spherical objects in blue or brown.

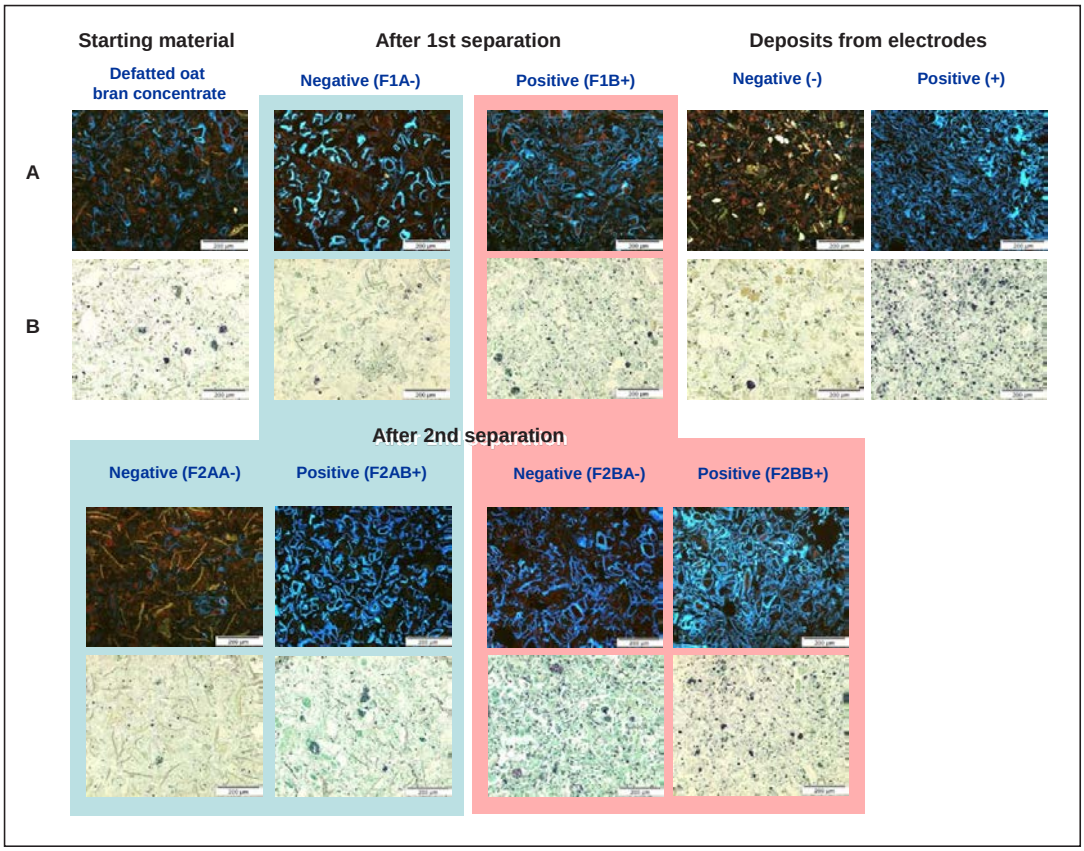

Fig. 3. Microscopic pictures of the defatted oat fractions (SC-CO2-OBC-2) obtained from the electrostatic separation process.

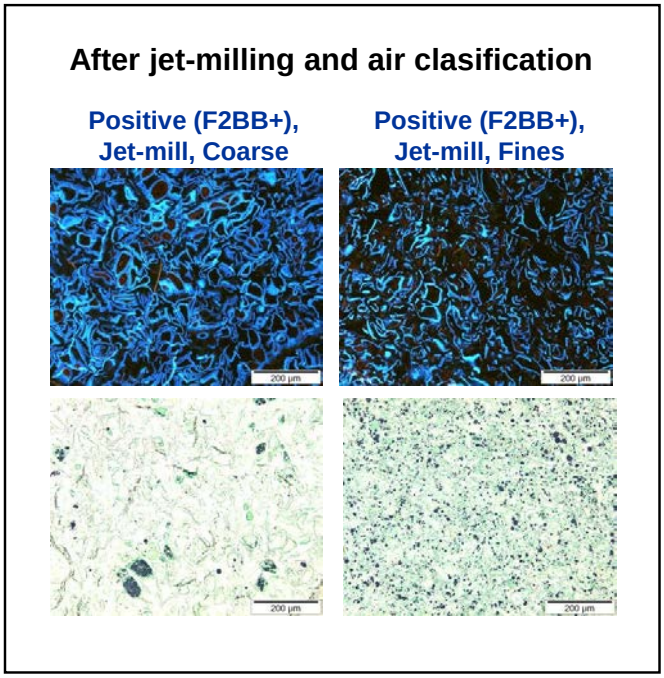

Fig. 4. Microscopic pictures of the defatted oat fractions (SC-CO2-OBC-2) obtained after electrostatic separation, jet-milling and combined air classification.

Table 1. The biochemical composition of the raw materials (F0) and electrostatically separated fractions. Values within the same row followed by a common letter are not significantly different ($P < 0.05$).

Non-defatted regular oat bran (Elovena Plus)	**F0**	**F1B+**	**F1A-**	**F2BB+**	**F2BA-**	**F2AB+**	**F2AA-**	**Right (+)**	**Left (-)**
Yield of the fraction (%[w])	50.0	34.0	16.0	23.5	10.5	11.0	5.0	-	-
Recovery of β-glucans (%[x])	74.4	42.7	31.6	32.5	18.3	24.5	10.1	-	-
β-Glucans (%DM)	5.8 d	4.9 c	7.7 f	5.4 cd	6.8 e	8.7 g	7.9 f	1.7 a	4.2 b
Total starch (%DM)	49.4 e	51.8 f	42.1 c	53.7 g	47.3 d	41.6 c	33.7 b	62.9 h	29.7 a
Protein (%DM)	16.9 c	16.6 bc	18.2 e	16.4 b	17.4 d	18.9 f	18.5 ef	14.4 a	19.1 g
Fat (%DM)	8.5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Non-defatted oat bran concentrate (OBC-20NEF)	**F0**	**F1B+**	**F1A-**	**F2BB+**	**F2BA-**	**F2AB+**	**F2AA-**	**Right (+)**	**Left (-)**
Yield of the fraction (%[w])	15.0	8.3	6.8	4.7	3.6	3.9	2.9	-	-
Recovery of β-glucans (%[x])	75.4	46.2	42.9	19.4	23.1	24.5	14.3	-	-
β-Glucans (%DM)	19.6 d	21.8 e	24.8 f	16.3 c	25.0 f	24.5 f	19.6 d	9.5 a	13.1 b
Total starch (%DM)	17.8 d	22.6 e	11.5 c	25.5 f	17.8 d	11.9 c	8.4 b	41.5 g	6.8 a
Arabinose + Xylose (%DM)	9.2 e	6.2 c	13.5 g	5.0 b	8.3 d	11.3 f	17.6 h	1.9 a	22.5 i
Arabinose/Xylose (wu)	0.7 ab	0.7 ab	0.6 a	0.7 ab	0.7 ab	0.6 a	0.6 a	0.9 bc	0.6 a
Protein (%DM)	26.3 d	27.0 e	25.2 b	27.7 f	25.8 c	25.4 bc	24.5 a	28.2 g	24.4 a
Fat (%DM)	10.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Defatted oat bran concentrate (SC-CO2-OBC-1)	**F0**	**F1B+**	**F1A-**	**F2BB+**	**F2BA-**	**F2AB+**	**F2AA-**	**Right (+)**	**Left (-)**
Yield of the fraction (%)	14.3	7.6	6.7	3.9	3.6	3.2	3.6	-	-
Recovery of β-glucans (%[x])	78.1	51.2	31.5	31.2	23.9	20.9	12.4	-	-
β-Glucans (%DM)	21.3 d	26.3 f	18.3 c	31.2 h	25.9 ef	25.4 e	13.5 b	30.4 g	7.8 a
Total starch (%DM)	28.0 c	31.6 e	25.5 b	34.5 f	30.4 d	28.7 c	24.8 b	36.9 g	17.1 a
Protein (%DM)	18.6 cd	18.0 bc	17.4 b	17.6 b	16.5 a	16.1 a	17.4 b	19.3 e	18.3 c
Fat (%DM)	2.9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Defatted oat bran concentrate (SC-CO2-OBC-2)	**F0**	**F1B+**	**F1A-**	**F2BB+**	**F2BA-**	**F2AB+**	**F2AA-**	**Right (+)**	**Left (-)**	**F2BB+, JM, Coarse**	**F2BB+, JM, Fine**
Yield of the fraction (%)	7.8	3.6	4.2	1.8	1.8	2.1	2.1	-	-	0.9	0.9
Recovery of β-glucans (%[x])	70.0	39.0	32.4	22.3	20.5	21.9	15.1	-	-	13.0	7.9
β-Glucans (%DM)											
after ambient grinding	35.0 g	42.2 i	30.1 d	48.4 k	44.4 j	40.7 h	28.0 c	24.6 b	19.9 a	56.2 l	34.2 f
after cryogenic grinding	33.1 d	39.3 f	27.7 c	46.0 g	34.4 e	33.5 d	18.6 a	25.1 b	18.3 a	-	-
β-Glucan Mw (kDA[y])	782 b	n.a.	n.a.	787 b	n.a.	n.a.	766 a	n.a.	n.a.	799 c	778 b
β-Glucan solubility (%[z]) in water	54.2 b	n.a.	n.a.	65.9 c	n.a.	n.a.	42.0 a	n.a.	n.a.	n.a.	n.a.
β-Glucan solubility (%[z]) in 0.1 M NaOH	86.1 b	n.a.	n.a.	96.6 c	n.a.	n.a.	75.4 a	n.a.	n.a.	n.a.	n.a.
Total starch (%DM)	7.8 f	9.9 g	5.3 d	9.5 g	6.5 e	4.7 c	3.9 b	19.9 i	4.7 c	3.3 a	19.0 h
Protein (%DM)	18.9 f	18.1 de	18.1 de	17.9 d	17.4 c	16.8 b	18.4 e	19.5 g	19.5 g	12.9 a	18.4 e
Arabinose + Xylose (%DM)	15.2 f	10.5 d	19.4 h	8.0 c	13.4 e	16.0 g	22.7 i	3.9 a	25.0 j	6.6 b	8.3 c
Arabinose/Xylose (wu)	0.7 ab	0.7 ab	0.6 a	0.8 bc	0.7 ab	0.7 ab	0.6 a	0.9 c	0.6 a	0.8 bc	0.7 ab
Fat (%DM)	4.4 a	n.a.	n.a.	4.9 b	n.a.	n.a.	4.2 a	n.a.	n.a.	n.a.	n.a.

[w]The initial yields of ElovenaPlus (50%) and OBC-20NEF (15%) (calculated from whole grain oats) are only estimated by the authors, as they are commercially produced ingredients.
[x]β-Glucan recovery was calculated based on the beta-glucan concentration of the whole grain oats, supposing that the initial beta-glucan concentration was 3.9%.
[y]β-Glucan Mw was analysed from the fractions made of SC-CO2-OBC2 after the ambient grinding.
[z]β-Glucan solubility (in water at 90 °C and in 0.1 M NaOH at room temperature) were analysed from the fractions made of SC-CO2-OBC2 after the ambient grinding
n.a. = not analysed

Table 2. Particle size distribution and colour values of the fractions obtained by electrostatic separation. Values within the same column followed by a common letter are not significantly different ($P < 0.05$).

Non-defatted regular oat bran	Particle size distribution (µm)			Colour values		
(Elovena Plus)	D_{10}	D_{50}	D_{90}	L	a	b
F0 (Raw material)	9.5 ± 0.2 d	35.3 ± 0.9 de	112.6 ± 1.4 hi	82.7 ± 0.4 ef	0.8 ± 0.0 g	11.9 ± 0.1 jk
F1B+	9.2 ± 0.3 cd	31.5 ± 0.7 cd	104.7 ± 1.2 gh	86.3 ± 0.5 h	0.5 ± 0.0 f	11.4 ± 0.1 ij
F1A-	10.6 ± 0.4 de	43.9 ± 2.1 f	123.9 ± 3.3 j	82.0 ± 0.1 e	1.3 ± 0.0 h	13.3 ± 0.1 l
F2BB+	10.9 ± 0.3 de	36.3 ± 0.8 de	110.8 ± 1.5 hi	87.4 ± 0.1 hi	0.2 ± 0.0 e	11.7 ± 0.1 j
F2BA-	10.6 ± 0.1 de	40.5 ± 0.5 ef	116.2 ± 0.8 i	85.6 ± 0.3 gh	0.6 ± 0.0 f	12.6 ± 0.1 k
F2AB+	12.2 ± 0.4 ef	51.6 ± 0.6 gh	127.5 ± 1.4 jk	84.7 ± 0.4 h	0.7 ± 0.0 fg	13.2 ± 0.1 l
F2AA-	12.0 ± 0.2 ef	58.0 ± 1.2 i	139.8 ± 3.4 l	78.7 ± 0.3 c	1.7 ± 0.0 i	15.8 ± 0.1 p
Right (+)	8.0 ± 0.4 cd	18.6 ± 0.2 b	34.2 ± 0.9 a	88.9 ± 0.3 ij	0.2 ± 0.0 de	9.9 ± 0.0 h
Left (-)	9.7 ± 0.2 d	34.0 ± 0.7 de	80.8 ± 1.2 de	66.4 ± 0.5 a	3.0 ± 0.1 l	17.6 ± 0.2 q

Non-defatted oat bran concentrate	Particle size distribution (µm)			Colour values		
(OBC-20NEF)	D_{10}	D_{50}	D_{90}	L	a	b
F0 (Raw material)	5.8 ± 0.5 bc	38.7 ± 0.3 e	104.9 ± 2.0 gh	86.6 ± 0.3 h	0.5 ± 0.0 f	12.3 ± 0.0 k
F1B+	5.2 ± 0.2 bc	31.6 ± 0.6 cd	100.2 ± 1.8 g	88.4 ± 0.1 i	0.3 ± 0.0 e	11.4 ± 0.0 ij
F1A-	8.8 ± 0.6 cd	53.6 ± 2.3 g	119.2 ± 2.9 ij	84.4 ± 0.2 fg	0.9 ± 0.0 g	14.4 ± 0.1 n
F2BB+	5.3 ± 0.4 bc	29.5 ± 0.3 cd	97.2 ± 1.2 fg	88.1 ± 0.2 i	0.2 ± 0.0 de	11.0 ± 0.1 ij
F2BA-	7.4 ± 0.1 c	44.4 ± 0.4 f	108.9 ± 1.8 h	87.0 ± 0.3 hi	0.4 ± 0.0 ef	12.5 ± 0.2 k
F2AB+	8.3 ± 0.3 cd	49.0 ± 0.5 g	112.7 ± 2.4 hi	85.8 ± 0.1 gh	0.6 ± 0.0 f	13.7 ± 0.1 m
F2AA-	14.0 ± 0.5 f	64.3 ± 0.9 j	127.0 ± 3.3 jk	82.0 ± 0.1 e	1.2 ± 0.0 h	16.1 ± 0.0 p
Right (+)	4.7 ± 0.1 b	16.8 ± 0.3 b	59.1 ± 0.6 b	90.7 ± 0.2 jk	-0.1 ± 0.0 cd	9.2 ± 0.0 g
Left (-)	6.6 ± 0.2 bc	29.4 ± 0.4 cd	77.6 ± 2.5 d	78.2 ± 0.5 c	2.0 ± 0.1 j	17.1 ± 0.1 q

Defatted oat bran concentrate	Particle size distribution (µm)			Colour values		
(SC-CO2-OBC-1)	D_{10}	D_{50}	D_{90}	L	a	b
F0 (Raw material)	5.0 ± 0.2 b	32.9 ± 0.4 d	96.2 ± 1.3 fg	87.7 ± 0.0 hi	0.5 ± 0.0 ef	10.0 ± 0.0 h
F1B+	5.3 ± 0.4 bc	38.0 ± 0.5 e	105.5 ± 0.7 gh	90.7 ± 0.0 jk	0.0 ± 0.0 d	7.9 ± 0.0 e
F1A-	5.4 ± 0.1 bc	32.5 ± 0.8 d	92.9 ± 2.6 f	85.5 ± 0.0 g	0.9 ± 0.0 g	11.8 ± 0.1 j
F2BB+	6.1 ± 0.2 bc	39.9 ± 0.5 ef	106.8 ± 0.8 h	92.2 ± 0.1 kl	-0.2 ± 0.0 c	6.4 ± 0.1 b
F2BA-	5.0 ± 0.3 b	33.2 ± 0.6 d	94.3 ± 1.1 fg	89.1 ± 0.0 ij	0.2 ± 0.0 de	9.5 ± 0.0 g
F2AB+	5.1 ± 0.2 b	29.5 ± 0.4 cd	85.1 ± 1.3 e	89.2 ± 0.0 ij	0.2 ± 0.0 de	9.5 ± 0.0 g
F2AA-	5.5 ± 0.6 bc	30.2 ± 1.2 cd	81.7 ± 3.8 de	83.7 ± 0.0 f	1.3 ± 0.0 h	13.3 ± 0.0 l
Right (+)	8.8 ± 0.4 cd	28.1 ± 0.7 c	69.4 ± 0.7 c	93.1 ± 0.0 l	-0.2 ± 0.0 c	5.5 ± 0.0 a
Left (-)	7.2 ± 0.1 c	43.3 ± 0.4 f	109.4 ± 1.5 hi	77.0 ± 0.0 b	2.3 ± 0.0 k	16.3 ± 0.1 p

Defatted oat bran concentrate	Particle size distribution (µm)			Colour values		
(SC-CO2-OBC-2)	D_{10}	D_{50}	D_{90}	L	a	b
F0 (Raw material)	14.0 ± 0.3 f	60.1 ± 0.3 i	141.4 ± 2.5 l	88.1 ± 0.1 i	0.2 ± 0.0 de	11.5 ± 0.1 ij
F1B+	11.3 ± 0.5 de	59.2 ± 0.5 i	144.0 ± 1.8 lm	89.9 ± 0.3 j	-0.2 ± 0.0 cd	9.4 ± 0.0 g
F1A-	12.8 ± 0.2 ef	55.4 ± 0.8 h	124.9 ± 2.9 j	84.4 ± 0.2 fg	0.8 ± 0.0 g	13.1 ± 0.0 l
F2BB+	12.0 ± 0.6 e	60.9 ± 0.1 ij	148.5 ± 1.5 m	91.6 ± 0.4 k	-0.8 ± 0.0 a	8.5 ± 0.0 ef
F2BA-	13.1 ± 0.3 ef	64.2 ± 0.5 j	148.6 ± 1.8 m	88.7 ± 0.2 i	-0.3 ± 0.0 bc	11.0 ± 0.1 ij
F2AB+	13.1 ± 0.5 ef	58.5 ± 0.3 i	132.9 ± 2.1 k	88.0 ± 0.3 i	0.2 ± 0.0 e	11.7 ± 0.0 j
F2AA-	16.4 ± 0.2 g	61.3 ± 0.9 ij	130.7 ± 2.7 jk	83.6 ± 0.2 fg	1.2 ± 0.0 h	14.9 ± 0.0 o
Right (+)	11.2 ± 0.4 de	39.1 ± 0.5 e	83.3 ± 0.9 de	92.4 ± 0.1 kl	-0.3 ± 0.0 bc	7.0 ± 0.0 c
Left (-)	8.0 ± 0.1 cd	39.8 ± 0.2 ef	92.5 ± 0.2 f	80.1 ± 0.5 d	1.6 ± 0.1 i	16.3 ± 0.3 p
F2BB+, JM, Coarse	32.8 ± 0.3 h	67.9 ± 0.8 k	137.1 ± 2.2 kl	92.6 ± 0.0 kl	-0.5 ± 0.0 b	7.5 ± 0.0 d
F2BB+, JM, Fine	2.6 ± 0.1 a	11.2 ± 0.3 a	31.2 ± 1.1 a	92.4 ± 0.0 kl	-0.8 ± 0.0 a	8.6 ± 0.0 f

PUBLICATION III

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Comparison of acid and enzymatic hydrolyses of oat bran β-glucan at low water content

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ABSTRACT

The effect of acid- or enzyme-catalysed hydrolysis on partial depolymerisation of β-glucan in oat bran was studied. Hydrolyses were performed at relatively low water content (50% dry matter) using high shear mixing in a twin-screw extruder. The hydrolysed oat brans were extracted with hot water and centrifuged to obtain a water-soluble phase and an insoluble residue. The time-dependent gelling of the water-soluble phase was monitored for 14 weeks at 5 °C. Acid-hydrolysis required a short reaction time (3 min) to depolymerise the β-glucan molecules from their original average M_w of 780,000 to 34,000 g/mol. After acid-hydrolysis, β-glucan had low polydispersity (4.0–6.7). Longer incubation time (3–4 h) was needed for enzymatic depolymerisation of the β-glucan molecules down to 71,000–49,000 g/mol. Enzymatic hydrolysis resulted in high polydispersity (19.0–24.2). The concentration and M_w of β-glucan significantly affected the gelling of hot water extracts. At 1.4–2.0% β-glucan concentration, solutions of β-glucan molecules with M_w > 50,000 g/mol agglomerated rapidly, whereas solutions of smaller molecules (34,000–49,000 g/mol) remained as stable dispersions for longer. Gelling was strongly concentration-dependent and at 1.4 to 1.6% beta-glucan concentration gelling occurred after 7 to 12 weeks of storage, whereas at 1.8 to 1.9% concentration gelling occurred already after 2 weeks of storage.

1. Introduction

Oats are a good source of β-glucan, because they have high initial β-glucan concentration, usually around 4.5–5.5% (Ajithkumar, Andersson, & Åman, 2005; Cho & White, 1993; Saastamoinen, Plaami, & Kumpulainen, 1992). Oat β-glucan is a linear polysaccharide consisting of β-D-glucopyranosyl units, which are joined by (1 → 3)- and (1 → 4)-β-D-linkages. The (1 → 3)-linkages increase the flexibility of the chain and make β-glucan water-soluble (Buliga, Brant, & Fincher, 1986). The main building blocks, cellotriose (DP3) and cellotetraose (DP4), comprise over 90% of the β-glucan molecule. A small proportion of oat β-glucan consists of longer cellulose-like sequences, mainly DP5–DP9, but even up to DP20 (Doublier & Wood, 1995; Lazaridou, Biliaderis, & Izydorczyk, 2003). The average molecular weight (M_w) of β-glucan in untreated oats is usually around 1,500,000–2,500,000 g/mol (Sikora, Tosh, Brummer, & Olsson, 2013). Oat β-glucan has a strong water-binding and viscosity-thickening capacity.

Several studies and meta-analyses have shown that oat β-glucan can reduce LDL cholesterol (low-density lipoprotein) of hyperlipidemic subjects (Brown, Rosner, Willett, & Sacks, 1999; Othman, Moghadasian, & Jones, 2011; Ripsin, et al., 1992). High or medium molecular weight (2,210,000 ≤ M_w ≤ 530,000 g/mol) oat β-glucan has been shown to induce stronger cholesterol lowering capacity compared to low M_w β-glucan (210,000 g/mol) in extruded breakfast cereals (Wolever et al., 2010). High M_w may also be linked to the cholesterol lowering capacity of breads and cookies (Kerckhoffs, Hornstra, & Mensink, 2003). However, Biörklund, van Rees, Mensink and Önning (2005) and Naumann et al. (2006) have shown that low M_w β-glucan (70,000 or 80,000 g/mol) can lower the LDL cholesterol when consumed in beverages. Thus, the food matrix seems to have an effect on the cholesterol lowering, and the role of M_w in predicting β-glucan's cholesterol lowering potency should be further studied.

In low moisture products, such as breads and snacks, much effort is made to retain the high molecular weight (HM_w) of β-glucan (Åman & Andersson, 2008; Duss & Nyberg, 2004). The M_w of β-glucan may be reduced due to endogenous enzyme activity, low pH or high temperature during processing (Åman, Rimsten, & Andersson, 2004; Flander, Salmenkallio-Marttila, Suortti, & Autio, 2007). In high moisture applications the use of HM_w β-glucans is challenging, because they tend to aggregate and form semi-solid or concentrated dispersions. Critical concentration (c^*) is used to describe the concentration at which β-glucan molecules begin to interact with each other. At c^*, the diluted solution turns into semi-diluted, and finally into concentrated solution

Abbreviations: DP, degree of polymerisation; M_w, weight average molecular weight; M_n, number average molecular weight; HM_w, high molecular weight; LM_w, low molecular weight; SDF, soluble dietary fibre.

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(c^{**}). For HM_w molecules (≈1,000,000–1,500,000 g/mol) the c^* and c^{**} values have been reported to be as low as 0.1–0.4% (Doublier & Wood, 1995; Ren, Ellis, Ross-Murphy, Wang, & Wood, 2003).

To obtain high concentration of β-glucan and yet avoid its aggregation in foods with very high water content, such as beverages, β-glucan needs to be in the diluted region (i.e. high c^* value and low viscosity). To obtain lower viscosity, the M_w of β-glucan must be reduced. This is possible, for example, by high pressure homogenization (Laakso & Lehtinen, 2005), by sonication (Vårum, Smidsrød, & Brant, 1992), by adding ascorbic acid (Kivelä, Gates, & Sontag-Strohm, 2009), by using thermo-mechanical degradation in extrusion (Tosh et al., 2010; Zhang, Bai, & Zhang, 2011), or by acid- or enzyme-catalysed depolymerisation at reduced water content (Kaukovirta-Norja et al., 2009; Lehtomäki & Myllymäki, 2009).

The aim of the current study was to compare the effects of acid- and enzyme-catalysed depolymerisations of β-glucan in an oat bran fraction with high β-glucan content. The stability of hot water-extracted bran hydrolysates at 1.4–2.0% β-glucan concentration was studied during 14 weeks of storage at 5 °C. The objective was to identify the hydrolysis conditions which would enable the production of stable β-glucan dispersions to be used in high-moisture foods, such as beverages.

2. Materials and methods

2.1. Raw materials

The raw material was dehulled, non-heat-treated commercial oat grains from Raisio Oyj (Kokemäki, Finland), the lipid content of which was reduced by supercritical carbon dioxide extraction at NATECO2 GmbH & Co. (Wolnzach, Germany). After the lipid extraction, a β-glucan-enriched fraction was obtained using pin disc milling and air classification at Hosokawa Alpine AG (Augsburg, Germany). First, the pin disc milled oats were separated by air classification into bran and endosperm fractions. Then, the bran fraction was milled and air classified again to obtain a highly enriched β-glucan fraction which contained 33.9% β-glucan, 23.0% protein, 9.2% starch and 4.6% total lipids (Sibakov et al., 2011).

2.2. Acid and enzymatic hydrolyses

The β-glucan-enriched oat bran fraction was first preconditioned by mixing it with 30% water (Fig. 1). The preconditioning ensured that water was evenly absorbed in the matrix. The acid-catalysed hydrolysis was performed with ortho-phosphoric acid (Merck KGaA, Darmstadt, Germany) and enzyme-catalysed hydrolysis with a commercial enzyme preparation (Depol 740L, Biocatalyst Ltd., Wales, UK). The enzyme preparation was produced by *Humicola* spp. and the following activities were detected: Xylanase 17,343 nkat/ml (birch glucurone xylan as substrate, pH 6, 50 °C; Bailey, Biely, & Poutanen, 1992), β-glucanase 6962 nkat/ml (1% barley β-glucan, pH 6, 50 °C; Zurbriggen, Bailey, Penttilä, Poutanen, & Linko, 1990), endoglucanase 614 nkat/ml (1% HEC, pH 6, 50 °C; IUPAC, 1987), β-glucosidase 472 nkat/ml (1 mM 4-nitrophenyl-β-D-glucopyranoside, pH 5, 50 °C; Bailey & Linko, 1990), ferulic acid esterase 52 nkat/ml (pH 5, 50 °C; Forssell et al., 2009), and α-arabinosidase 34 nkat/ml (p-nitrophenyl-α-L-arabinofuranoside, pH 5, 50 °C; Poutanen, Rättö, Puls, & Viikari, 1987).

The hydrolysis was carried out using an APV MPF 19/25 twin-screw extruder (Baker Perkins Group Ltd., Peterborough, U.K.). The feed rate of the preconditioned oat bran into the extruder was 24 g min^{-1}, speed of the twin-screws was 75 rpm, and residence time inside the barrel about 3 min. The temperature inside the extrusion barrel was set to 110–130 °C for acid-hydrolysis and to 50 °C for enzyme-hydrolysis. Water solution of phosphoric acid (8% w/v) or Depol 740L enzyme preparation (50 nkat β-glucanase activity/g oat bran) was fed into the extruder at a rate of 12 ml min^{-1}, resulting in a final water content of 50%.

After the extrusion, the dough-like mass was either ready for subsequent processing (acid-hydrolysis) or incubated in sealed containers at 50 °C for 1–4 h (enzyme-hydrolysis) as shown in Fig. 1. After incubation with the enzyme, the dough-like mass was manually fed into extruder again to inactivate the enzyme. The inactivation was

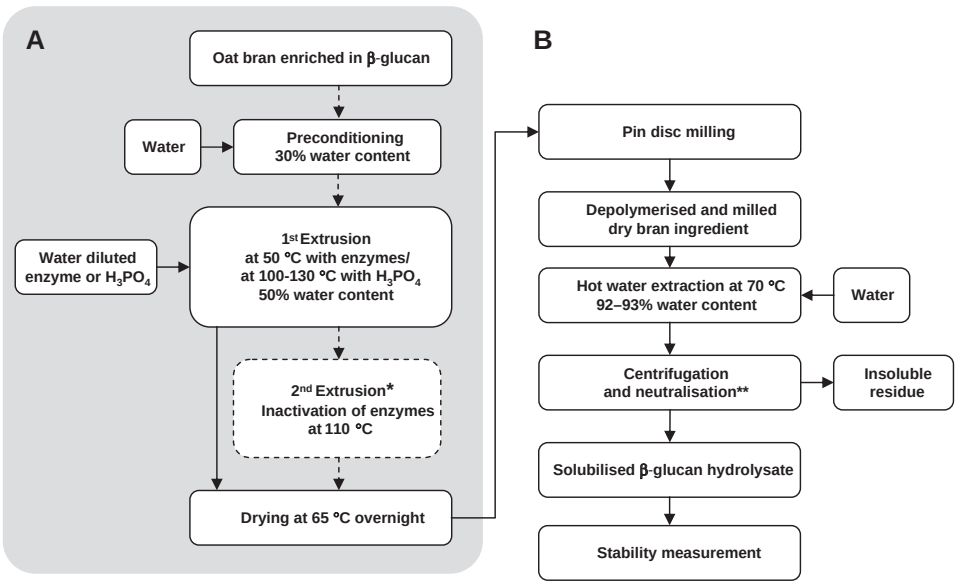


Fig. 1. Process flow chart of the depolymerisation of oat bran enriched in β-glucan. A. Preparation of depolymerised dry oat β-glucan ingredient. B. Extraction of dry ingredient into hot water and separation of solids to yield β-glucan-concentrated beverage base. *The second extrusion was performed after 1–4 h incubation to inactivate the enzyme. **Neutralisation of acid was needed when using phosphoric acid.

performed at 110 °C using 75 rpm speed for the twin-screws, resulting in a residence time of 3–4 min.

2.3. Preparation of the water extractable fractions

After extrusion, the moist, hydrolysed material was spread on stainless steel trays and dried overnight in an oven with recirculation air at 65 °C to avoid microbiological contamination and further depolymerisation of β-glucan. The dried material was first ground in a Wiley cutting mill (Arthur H. Thomas Company, Philadelphia, U.S.A.) and subsequently in a Hosokawa Alpine 100 UPZ-lb Fine impact mill with stainless steel pin discs (Hosokawa Alpine AG, Augsburg, Germany) so that the powder was easier to disperse in water. For the hot water extraction, 66.7 or 83.3 g (d.w.) of dried and milled oat bran material was mixed with 1 l of distilled water at 70 °C. The mixture was stirred for 2 min with a hand-held homogeniser (Heidolph Diax900 Ultra Turrax, Gemini BV, Apeldoorn, The Netherlands), using 12,000 rpm speed. The insoluble residue was separated with a centrifuge (Sorvall RC-12BP, DuPont, U.S.A.), at 4000 rpm (ca. 4000×*g*) for 15 min. The centrifugation was performed at room temperature without cooling to avoid gelling of the water-soluble fraction. The supernatant of acid-hydrolysed oat bran was neutralised from pH 2.1–2.2 to pH 5.0–5.2 with calcium hydroxide (Merck KGaA, Darmstadt, Germany), and the $Ca_3(PO_4)_2$ precipitate formed was separated by centrifugation as described above. Neutralisation was not needed for the enzyme-hydrolysed material, which had a pH of 5.6–5.8.

The supernatants were cooled to 5 °C and stored in a controlled cold room at 5 °C for 14 weeks. The viscosities of the solutions (Pa·s) were measured once a week in a Bohlin Visco 88 Viscometer (Malvern Instruments Ltd., UK) at 5 °C, using eight different shear rates (24–1300 s^{-1}). The solution flasks were mixed well before the analysis. Each time a new sample of 18 ml was taken to be analysed by the viscometer. After the analysis, the sample was thrown away, not returned back to the solution flask.

2.4. Analytical methods

2.4.1. Chemical characterisation

The water-soluble fractions as well as the insoluble residues were dried in a Christ Epsilon 2–25 freeze drier (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The freeze-dried samples were ground in an Ultra centrifugal mill ZM 200 (Retsch GmbH, Haan, Germany) using a 0.3 mm screen. The total β-glucan concentration was analysed by the spectroscopic method 32–23 (AACC, 2003) using Megazyme β-glucan mixed linkage assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The M_w of β-glucan in the hot water extracts was analysed by dissolving the samples at concentration levels of 12 and 8 g/l in 0.2% H_3PO_4 and 200 ppm Na-azide at room temperature overnight with magnetic stirring. The samples were analysed by liquid chromatography, which consisted of an Alliance 2690 separation module and M-2414 refractive index detector. The columns employed were (7.8 × 300 mm) three μHydrogel 2000, μHydrogel 250 and μHydrogel 120 (Waters Inc., Milford, MA, U.S.A.) and Aminex HPX-87H (Bio-Rad, Hercules, CA, U.S.A.) in series at 60 °C. The eluent was aqueous 0.2% H_3PO_4 at a flow-rate of 0.5 ml/min. Injections (50 μl) were made of sample and standard solutions (Suortti, 1993). The linear size-exclusion calibration curve ($r^2 > 0.96$) was constructed on the basis of pullulan standards ranging from 788,000 to 5900 g/mol and malto-oligomers ranging from maltoheptaose to maltose. The system was controlled and calculations were performed with Waters Empower software's GPC option. In principle the software sliced the sample peak into narrow slices. The peak molecular weight value and the area of each slice (i.e. concentration) were calculated by the software. Then the weight average molecular weight (M_w) and the number average molecular weight (M_n) were calculated over the whole β-glucan peak. Polydispersity, a measure of the distribution of individual molecular masses, was calculated as a ratio between M_w and M_n.

Monosaccharides were analysed by extracting 1 g of the cereal sample with 4 ml of cold water (+4 °C) and hydrolysing the water soluble fraction with 1.2 ml of 7.5 N H_2SO_4 at boiling water bath for 2 h. The sugars obtained from the hydrolysis steps and the monosaccharide standards (50 mg/ml; glucose, arabinose, xylose, galactose, mannose) were analysed as their alditol acetates as described by Blakeney, Harris, Henry, and Stone (1983). The dilutions for the standard curves were made from these monosaccharide solutions. Myo-inositol was used as an internal standard (0.5 mg/ml sample). The acetylated monosaccharides were analysed with gas chromatography using an Agilent 6890 GC (Palo Alto, CA, USA) equipped with a flame ionization detector (FID). The column was DB-225 (30 m × 0.32 mm; film thickness 0.15 μm; Agilent). Helium was used as a carrier gas 1.2 ml/min. Split injection (1:3) was performed at 250 °C and the FID-detector was operated at 250 °C. The analytes were separated at 220 °C for 15 min. The monosaccharides were identified according to their retention times and quantitated with standard curve. Free hexose-sugars were corrected by a factor of 0.9 to anhydro sugars, and pentose-sugars by a factor of 0.88. Nitrogen was analysed by a Kjeldahl autoanalyser (Foss Tecator Ab, Höganäs, Sweden), and protein concentration was calculated as N × 6.25 according to the method 46-11A (AACC, 2003). Starch was quantified using Megazyme total starch assay kit according to the method 76–13.01 (AACC, 2003). All chemical characterisations were made in triplicate.

2.4.2. Microscopic analyses

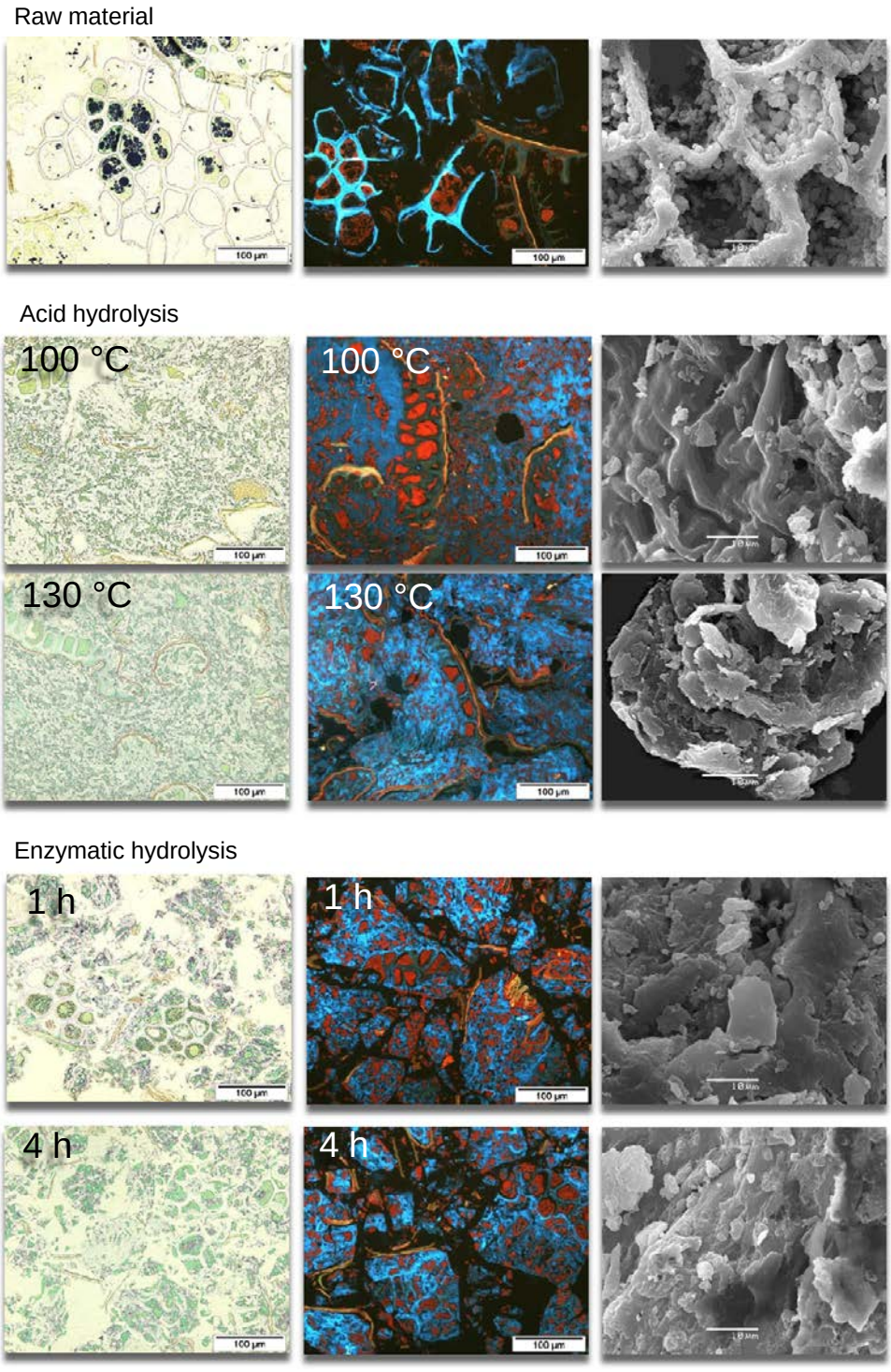
The samples were embedded into agar using a Historesin embedding kit (Leica instruments GmbH, Heidelberg, Germany), and the embedded samples were sectioned (2 μm) in a rotary microtome HM 355 (Microm Laborgeräte GmbH, Walldorf, Germany) using a steel knife. The sections were stained with Acid Fuchsin (BDH Chemicals Ltd., Poole, Dorset UK)/Calcofluor White (Fluorescent Brightener, Aldrich, Germany). Acid Fuchsin and Calcofluor White were used for staining protein red and β-glucan rich cell walls light blue, respectively, and the samples were imaged using exciting light (epifluorescence at 400–410 nm and fluorescence at >455 nm). The samples were then examined under a BX-50 microscope (Olympus Corp., Tokyo, Japan). Micrographs were obtained using a PCO SensiCam CCD colour camera (PCO AG, Kelheim, Germany) and the Cell^P imaging software (Olympus). The electron microscopy was performed using a Jeol 6360 Scanning Electron Microscope (Jeol Ltd., Tokyo, Japan). Prior to imaging, the samples were dried with absolute ethanol and coated with a thin layer of gold atoms using an Edwards Sputter Coater S150B (Massachusetts, U.S.A.).

2.5. Statistical analysis

The results were calculated as means of triplicate analysis results. The data was subjected to analysis of variance using the IBM SPSS Statistics 20 (IBM Corporation, Somers, NY, U.S.A.), and significant differences ($P < 0.05$) between individual means were identified by the Tukey's test.

3. Results and discussion

Low water content (1:1 water and bran) enabled a controllable depolymerisation of HM_w β-glucan into smaller molecules both in acid and enzymatic hydrolyses. Higher water content was also investigated in preliminary experiments. Hydrolysis at 90% water content, using the same concentration of phosphoric acid (8%) or the same enzyme dosage (50 nkat β-glucanase/g oat bran) and at the same temperatures (100–130 °C for acid-hydrolysis or 50 °C for enzymatic hydrolysis) as at 50% water content, resulted in rapid breakdown of β-glucan into short oligosaccharides (data not shown). The results of acid and enzymatic hydrolyses at 50% water content are described and discussed below.


Raw material
Acid hydrolysis
100 °C
100 °C
130 °C
130 °C
Enzymatic hydrolysis
1 h
1 h
4 h
4 h

3.1. Acid-catalysed depolymerisation of β-glucan

Acid-hydrolysis had a great effect on the cell wall structures of the oat bran concentrate. The cell walls were almost completely destroyed as compared to the unhydrolysed raw material. Irrespective of the different hydrolysis temperatures (100 or 130 °C), the microscopic pictures showed similar dispersed distribution of grain compounds (i.e. fragments of cell walls, proteins and starch) in the matrix (Fig. 2).

In the subsequent hot water extraction, 48.2–52.9% of the acid-hydrolysed oat bran material was solubilised and 61.6–68.9% of the total β-glucan in the oat bran fraction was extracted in the water solution (Table 1). The degree of oat bran solubilisation did not differ significantly between the hydrolysis temperatures (100, 120 and 130 °C) and the amounts of added bran (66.7 or 83.3 g/l). However, there seemed to be a trend that higher hydrolysis temperature slightly improved the solubilisation of oat bran. The highest solubilisation of β-glucan (68.9% of the total β-glucan) was obtained when the oat bran was hydrolysed at 120 °C. The solubilisation level was reduced to 63.4–64.3% when the bran was hydrolysed at 130 °C. This was most probably due to harsh hydrolysis conditions, where a small proportion of the β-glucan molecules might have been cleaved down to glucose or even smaller units. However, as the reduction in β-glucan's solubilisation was substantially low, it did not significantly reduce the β-glucan concentrations in the hot water extracts (Table 1).

The composition per dry matter of freeze-dried hot water extracts was: 41.6–45.7% β-glucan, 7.9–8.5% protein and 6.9–8.9% starch. The extracts contained 5.4–5.6% arabinose, 5.8–7.1% xylose, 8.3–8.6% mannose and 52.0–54.1% glucose (Table 2). Similar to the degree of β-glucan solubilisation (Table 1), the β-glucan concentration was highest (45.7%) after the hydrolysis at 120 °C and decreased slightly (to 41.6%) when the hydrolysis was performed at 130 °C. The protein content did not show any clear trend, being lowest (7.9%) after the hydrolysis at 120 °C, but remained at the same level (8.5%) after the hydrolysis at 100 and 130 °C. The starch and xylose concentrations increased when the hydrolysis temperature was raised. However, there were no significant changes in the concentrations of arabinose, mannose and glucose.

The insoluble residues, after the hot water extraction and centrifugation, contained 6.1–6.5% β-glucan, 30.2–35.0% protein and 7.6–11.1% starch, and their monosaccharide composition was: 2.4–3.6% arabinose, 4.4–6.8% xylose, 4.2–4.5% mannose and 18.9–22.6% glucose (Table 2). The differences in β-glucan concentrations were not statistically significant. The protein concentration increased with higher hydrolysis temperature. However, this was not in line with the results obtained from the freeze fried extracts. The reason might be linked to the solubility of different kinds of proteins, but this was not investigated more in detail in this study. Starch and xylose concentrations decreased with the increased hydrolysis temperature. This was in accordance with the increased starch and xylose concentrations in the freeze dried extracts. In addition, glucose concentration decreased slightly between 120 and 130 °C hydrolysis temperatures. As there were no significant differences between the glucose concentrations of the freeze dried extracts, it might be interpreted that some of the glucose molecules were degraded into smaller units at 130 °C.

The depolymerisation of β-glucan was highly dependent on the hydrolysis temperature. The average M_w of β-glucan in the hot water extracts was 110,000; 86,000 and 34,000 g/mol after hydrolysis at 100, 120 and 130 °C, respectively (Fig. 3). The corresponding polydispersity (M_w/M_n) values of the β-glucan molecules were 4.0, 4.3 and 6.7. The low polydispersity values were also detectable as relatively sharp peaks in the molecular weight distributions (Fig. 3).

3.2. Enzyme-catalysed depolymerisation of β-glucan

Enzymatic hydrolysis destroyed most of the cell wall structures, although considerably less severely than acid-hydrolysis. The microscopic analysis showed that some of the cell wall structures still remained intact after 1 h enzymatic hydrolysis, although they were mostly destroyed after 4 h hydrolysis (Fig. 2). In contrast to acid-catalysed hydrolysis, starch particles were still detectable after 1 h enzymatic hydrolysis.

In the subsequent hot water extraction, 29.0–47.1% of the enzyme-hydrolysed oat bran material was solubilised and 44.3–77.9% of the total β-glucan in the oat bran fraction was extracted in the water solution (Table 1). The degree of oat bran solubilisation did not differ significantly after the hydrolysis times of 2–4 h. However, after the 1 h hydrolysis time, a lower solubilisation of oat bran was obtained. The amount of added bran clearly affected the solubilisation of oat bran, being higher with the lower bran proportion (66.7 g/l) compared to the higher bran proportion (83.3 g/l). The reason was most probably related to the high water-absorption capacity of 'mildly' treated oat brans. For example, after being hydrolysed for 1 h with Depol 740L, only 29.0% of bran was solubilised when 88.3 g of oat bran was dissolved in 1 l of hot water. The solubilisation of β-glucan improved significantly when the hydrolysis time was increased from 1 to 3 h. A small reduction in β-glucan's solubilisation between 3 and 4 h hydrolysis time might be explained by a similar cleavage of β-glucan as with the acid. However, the reduction in the level of β-glucan solubilisation between 1 and 4 h hydrolysis time was substantially low, and did not significantly reduce the β-glucan concentrations in the hot water extracts.

The composition per dry matter of hot water extracts of freeze dried material was: 52.2–58.6% β-glucan, 5.0–8.9% protein and 3.8–6.7% starch. The water extracts contained 5.2–6.5% arabinose, 5.6–7.2% xylose, 11.2–11.8% mannose and 59.0–63.7% glucose (Table 2). The β-glucan concentration was reduced with longer hydrolysis time. This could be explained by the cleavage of some β-glucan molecules into glucose. However, the cleavage to glucose should have been low, because the β-glucosidase activity of Depol 740L (3.4 nkat/g oat bran) was almost 15-times lower compared to its β-glucanase activity (50 nkat/g oat bran). This was actually proved with the reference blanks of the β-glucan analysis, where no free D-glucose was detected. Smaller units than glucose were not produced by the enzyme, as might have been the case with acid, because there were no significant differences between the glucose concentrations of freeze dried extracts of enzyme hydrolysed oat brans. The protein concentrations of the freeze dried extracts increased along with the longer incubation time. This was not consistent with the values measured from the insoluble residues, because those did not show any significant differences in protein concentration. Similar to higher temperature in acid hydrolysis, longer hydrolysis time in enzyme hydrolysis resulted in slightly higher concentrations of starch and xylose in the freeze dried extracts.

The insoluble residues after the hot water extraction contained 11.6–16.4% β-glucan, 35.1–35.6% protein and 12.2–14.2% starch, and their monosaccharide composition was: 4.3–5.5% arabinose, 4.6–6.5% xylose, 5.1–5.4% mannose and 26.0–27.6% glucose (Table 2). The clearest difference in the chemical composition of the insoluble residues was in the concentrations of β-glucan. The β-glucan concentration was reduced from 16.4 to 11.6% when the hydrolysis time was prolonged from 1 to 4 h. The significantly higher β-glucan concentration in the insoluble residue of 1 h hydrolysed sample was likely due to the low solubilisation of oat bran after the hot water extraction, and due to the amount of higher M_w β-glucans that did not dissolve into hot water as easily as the lower

Fig. 2. Microscopic pictures of oat bran concentrates: intact oat bran, acid-hydrolysed oat bran (at 100 °C and 130 °C) and enzyme-hydrolysed oat bran (incubated at 50 °C for 1 h and 4 h). First row = column: Light Green/Lugol's iodine staining, showing protein as green and starch as spherical objects in blue or brown. Second row = column: Acid Fuchsin/Calcofluor White staining, showing protein as red and β-glucan rich cell walls as light blue colour. Third row = column: Scanning electron microscopic pictures. Bar = 100 μm (in the first and second rows = columns) and 10 μm (in the third row = column). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Solubilisation of oat bran and β-glucan into liquid phase after acid- and enzyme-hydrolyses and hot water extraction at 70 °C. The results are expressed as mean (n = 3) ± standard deviation. Values marked with different letters within the same row = column are significantly different (P < 0.05).

	Oat bran (g/l of water)	Solubilised oat bran (% of total)	Solubilised β-glucan (% of total)	β-Glucan in water extract (%)
Acid-hydrolysis				
H3PO4, 100 °C	66.7	50.5 ± 0.8 gh	63.8 ± 0.3 d	1.38 ± 0.07 a
H3PO4, 120 °C	66.7	51.3 ± 0.9 h	68.9 ± 0.2 ef	1.56 ± 0.05 ab
H3PO4, 130 °C	66.7	52.0 ± 0.7 h	63.6 ± 0.3 d	1.43 ± 0.06 ab
H3PO4, 100 °C	83.3	48.2 ± 1.1 fg	61.6 ± 0.7 c	1.83 ± 0.11 cd
H3PO4, 120 °C	83.3	51.6 ± 1.1 h	68.9 ± 0.7 e	2.06 ± 0.10 d
H3PO4, 130 °C	83.3	52.9 ± 0.4 h	64.3 ± 0.5 d	1.84 ± 0.04 cd
Enzyme-hydrolysis				
Depol 740L, 1 h	66.7	41.3 ± 0.9 c	71.1 ± 0.7 g	1.55 ± 0.09 ab
Depol 740L, 2 h	66.7	45.4 ± 0.8 de	76.5 ± 1.0 h	1.64 ± 0.07 bc
Depol 740L, 3 h	66.7	47.2 ± 0.6 ef	77.9 ± 0.4 h	1.66 ± 0.08 bc
Depol 740L, 4 h	66.7	45.7 ± 0.8 def	70.2 ± 0.3 ef	1.57 ± 0.06 ab
Depol 740L, 1 h	83.3	29.0 ± 1.7 a	44.3 ± 0.8 a	1.93 ± 0.10 d
Depol 740L, 2 h	83.3	37.6 ± 1.2 b	57.4 ± 0.6 b	1.94 ± 0.12 d
Depol 740L, 3 h	83.3	43.9 ± 0.9 cd	70.5 ± 0.3 fg	2.01 ± 0.06 d
Depol 740L, 4 h	83.3	44.1 ± 0.5 d	67.7 ± 0.4 e	1.94 ± 0.06 d

M_w molecules after longer hydrolysis times (2–4 h). Along with the longer hydrolysis time, there was a small decrease in the concentration of xylose in the insoluble residues. The trend in the concentration of xylose was similar to the results obtained with the acid hydrolysis at increased hydrolysis temperatures. The improved extraction of xylose could be explained by the high xylanase activity in Depol 740L.

The depolymerisation of β-glucan by the enzyme preparation was dependent on the incubation time at 50 °C. The average M_w of β-glucan in the hot water extracts was 218,000; 93,000; 71,000 and 49,000 g/mol after 10 min, 1 h, 3 h and 4 h incubation times, respectively. Compared to acid-hydrolysis, the enzyme-catalysed hydrolysis resulted in wider M_w distributions. The polydispersity value, being lowest with 10 min incubation time, varied between 19.0 and 24.2. The difference between 10 min and 1 h incubations was the most significant, as the average M_w fell below 100,000 g/mol and the proportion of small molecules increased (Fig. 3). The 'tails' at the ends of M_w profiles indicated that part of the β-glucan was depolymerised into shorter oligosaccharides, but their concentrations were not quantified in this work.

3.3. Comparison of acid and enzymatic hydrolyses

The degree and pattern of hydrolysis were different between acid and enzyme-catalysed hydrolyses. The yield of the water extract after acid-hydrolysis (48.2–52.6%) was higher than after the enzymatic hydrolysis (29.0–47.1%). This was probably due to the fact that the acid hydrolysed the grain compounds rather unspecifically (Johansson et al., 2006), whereas the enzyme preparation used, Depol 740L, degraded mainly polysaccharides. In addition, separation of the insoluble residue from the water dispersion of enzymatically hydrolysed oat bran was challenging at 1.9–2.0% β-glucan concentration, due to the high water absorption capacity of the β-glucan molecules with an intermediate M_w (71,000–93,000 g/mol).

Table 2
Monosaccharide compositions of hydrolysed oat brans after water extraction at 70 °C. The water-soluble (extract) and insoluble (residue) fractions were freeze-dried prior to analysis. The concentration of galactose was below the detection limit. The results are expressed as mean (n = 3) ± standard deviation. Values marked with different letters within the same row = column are significantly different (P < 0.05).

	Yield (wt.%)	β-Glucan (g/100 g)	Protein (g/100 g)	Starch (g/100 g)	Arabinose (g/100 g)	Xylose (g/100 g)	Mannose (g/100 g)	Glucose (g/100 g)
Raw material (OBC)	100.0	33.9 ± 0.2 e	23.0 ± 0.2 e	9.2 ± 0.1 f	5.4 ± 0.1 de	6.0 ± 0.1 d	8.6 ± 0.1 b	45.4 ± 0.7 d
Acid-hydrolysed, extract								
Hydrolysed at 100 °C	50.5	42.9 ± 0.6 g	8.5 ± 0.1 d	6.9 ± 0.1 c	5.6 ± 0.1 de	5.8 ± 0.2 d	8.3 ± 0.1 b	52.2 ± 1.3 e
Hydrolysed at 120 °C	51.3	45.7 ± 0.6 h	7.9 ± 0.1 c	7.8 ± 0.1 d	5.4 ± 0.1 de	6.0 ± 0.1 d	8.5 ± 0.3 b	54.1 ± 0.6 e
Hydrolysed at 130 °C	52.0	41.6 ± 0.3 f	8.5 ± 0.2 d	8.9 ± 0.3 e	5.5 ± 0.2 de	7.1 ± 0.3 f	8.6 ± 0.3 b	52.0 ± 1.6 e
Acid-hydrolysed, residue								
Hydrolysed at 100 °C	49.5	6.1 ± 0.1 a	30.2 ± 0.2 f	11.1 ± 0.1 h	3.6 ± 1.3 ab	6.8 ± 0.1 ef	4.2 ± 0.1 a	21.7 ± 0.1 b
Hydrolysed at 120 °C	48.7	6.5 ± 0.2 a	34.5 ± 0.3 g	10.8 ± 0.2 g	2.4 ± 0.3 a	4.4 ± 0.2 a	4.5 ± 0.1 a	22.6 ± 0.2 b
Hydrolysed at 130 °C	48.0	6.1 ± 0.1 a	35.0 ± 0.1 h	7.6 ± 0.1 d	2.4 ± 0.5 a	5.0 ± 0.2 bc	4.4 ± 0.2 a	18.9 ± 0.6 a
Enzyme-hydrolysed, extract								
Incubated for 1 h	41.3	58.6 ± 0.6 l	5.0 ± 0.1 a	4.1 ± 0.1 a	5.2 ± 0.3 de	5.6 ± 0.1 d	11.8 ± 0.8 c	63.7 ± 1.0 g
Incubated for 2 h	45.4	57.3 ± 0.1 k	6.0 ± 0.2 b	3.8 ± 0.2 a	6.0 ± 0.1 ef	6.7 ± 0.2 ef	11.5 ± 0.9 c	61.9 ± 1.8 g
Incubated for 3 h	47.1	56.2 ± 0.2 j	8.6 ± 0.1 d	4.4 ± 0.1 b	6.5 ± 0.5 ef	7.1 ± 0.1 f	11.4 ± 0.6 c	61.7 ± 0.7 fg
Incubated for 4 h	45.7	52.2 ± 1.1 i	8.9 ± 0.1 d	6.7 ± 0.2 c	6.2 ± 0.1 ef	7.2 ± 0.2 f	11.2 ± 0.4 c	59.0 ± 1.0 f
Enzyme-hydrolysed, residue								
Incubated for 1 h	58.7	16.4 ± 0.2 d	35.1 ± 0.1 i	12.2 ± 0.1 i	5.5 ± 0.2 de	6.5 ± 0.2 e	5.3 ± 0.4 a	26.8 ± 0.8 c
Incubated for 2 h	54.6	13.5 ± 0.1 c	35.2 ± 0.2 h	14.0 ± 0.1 k	5.3 ± 0.2 de	5.8 ± 0.1 d	5.4 ± 0.2 a	27.6 ± 0.1 c
Incubated for 3 h	52.9	11.8 ± 0.1 b	35.4 ± 0.1 h	14.2 ± 0.4 k	4.9 ± 0.5 cd	5.1 ± 0.2 c	5.1 ± 0.1 a	26.4 ± 0.4 c
Incubated for 4 h	54.3	11.6 ± 0.1 b	35.6 ± 0.2 h	13.6 ± 0.5 j	4.3 ± 0.1 bc	4.6 ± 0.1 ab	5.4 ± 0.1 a	26.0 ± 0.2 c

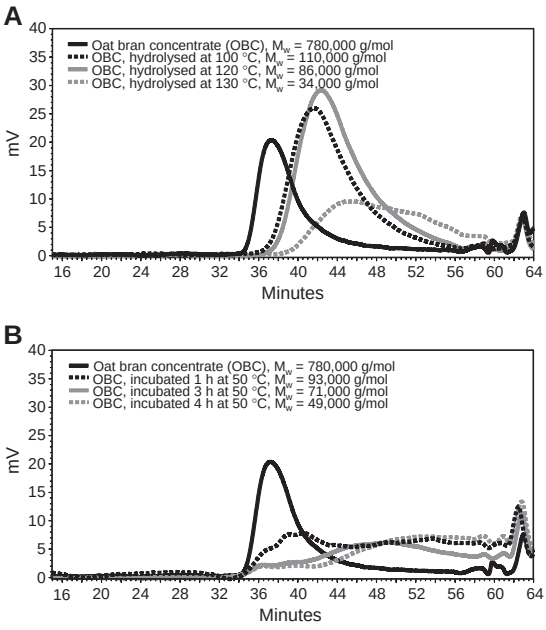


Fig. 3. M_w distributions of the hot water extracts of oat brans after A) acid- and B) enzyme-hydrolysis.

The β-glucan concentrations of freeze-dried extracts were 41.6–45.7 and 52.2–58.6% after acid and enzymatic hydrolyses, respectively. Thus, it was evident that both acid and enzymatic hydrolyses efficiently liberated β-glucan from the oat bran matrix. The β-glucanase in the enzyme preparation used, Depol 740L, was specific for (1 → 4)-β-D-linkages, whereas acid could cleave both (1 → 3)- and (1 → 4)-β-D-linkages (McCleary & Matheson, 1987; Tosh, Wood, Wang, & Weisz, 2004). Therefore the acid-hydrolysed β-glucan molecules may have contained relatively more (1 → 4)-β-D-linkages, meaning that the polymers were more linear. However, this was not fully proved by the current study.

The nature of the enzyme catalysed reaction resulted in wider M_w distributions (Fig. 3) and higher polydispersity values (19.0–24.2) as compared to acid-hydrolysis (4.0–6.7). The side activities of the enzyme preparation may also have affected the results, because the enzyme preparation had several polysaccharide-hydrolysing activities. In earlier studies, for example a very low endo-glucanase contamination in exo-acting cellobiohydrolase II preparation caused considerable changes in the hydrolysis products of β-glucan (Reinikainen, Henriksson, Siika-aho, Teleman, & Poutanen, 1995). However, as Depol 740L contained only low β-glucosidase side activity (around 3.4 nkat/g oat bran), it did not produce free D-glucose and all the β-glucans remained in polymeric or oligomeric form after enzymatic hydrolysis.

3.4. Low water and high substrate vs. high water and low substrate content

Low water content (50% dry matter) maintained the M_w of β-glucan higher than 34,000 g/mol, when using either 8% phosphoric acid at 100–130 °C for 3 min or an enzyme preparation, Depol 740L, with 50 nkat β-glucanase activity per gram of bran at 50 °C for 1–4 h. The same principle as in the current study was used in a patent of Kaukovirta-Norja et al. (2009). They used acid-hydrolysis of oat β-glucan at 45–50% water content. The M_w of β-glucan after acid-hydrolysis of different oat bran preparations at different temperatures and acid concentrations was in the range of 5000–360,000 g/mol. When 8% phosphoric acid was used at 110–130 °C, the M_w of β-glucan was between 37,000 and 135,000 g/mol (oat bran with 22% β-glucan) or between 28,000 and 105,000 g/mol (oat bran with 33% β-glucan). Thus, the results of Kaukovirta-Norja et al. (2009) were in agreement with the results obtained in the current study, in which the M_w of β-glucan was decreased from 110,000 to 34,000 g/mol at temperatures of 100–130 °C. Johansson et al. (2006) showed that at relatively low temperature (37 °C) no degradation of β-glucan was observed with 0.1 M HCl over a 12 h period. By contrast, at high temperature (120 °C) total hydrolysis to D-glucose occurred with 3 M HCl already after 1 h hydrolysis. Hydrolysis with 0.1 M HCl at 120 °C for 1 h produced a range of products, but as the water content was high, the majority of the resulting oligosaccharides had a low M_w (DP 1–7) compared to the molecules in the present study ($M_w \geq 34{,}000$ g/mol, DP ≥ 190). In addition, Tosh et al. (2004) hydrolysed oat β-glucan at high water content (1% dry matter) with 0.1 M HCl at 70 °C for 30–90 min, resulting in fragments with M_w-values from 1,200,000 to 30,000–170,000 g/mol.

The enzymatic hydrolysis at low water content was shown to be highly dependent on the plasticisation phenomenon at 45–50% water content. Similar findings were reported in a patent of Lehtomäki and Myllymäki (2009). They investigated the enzymatic hydrolysis of oat bran at 45–55% water content, using commercial cellulase and α-amylase preparations. They used a twin-screw extruder with a short reaction time (65 s) and an intermediate temperature (65 °C), and controlled the hydrolysis of β-glucan by the dosage of enzymes (17–17,000 nkat β-glucanase activity/g oat bran). The enzymes were inactivated immediately after the short hydrolysis by repeating the extrusion at 95 °C. Unfortunately, the patent did not report the M_w values of the enzyme-hydrolysed oat bran preparations.

Enzymatic hydrolysis at low water content is a complex reaction. In general, enzymes need water for their catalytic activity. Water also influences the structure of enzyme proteins via non-covalent bonding, disrupts the hydrogen bonds, facilitates the reagent diffusion, and influences the reaction equilibrium (Hari Krishna, 2002). Too low water content generally reduces the activity of enzymes, but the minimum hydration level for the enzymatic activity varies considerably between different enzyme preparations. Some enzymes can function even in solvent-free reaction conditions on solid substrates (Hobbs & Thomas, 2007). In addition to oat bran, Moore, Cheng, Su, and Yu (2006), as well as Santala, Lehtinen, Nordlund, Suortti, and Poutanen (2011) and Santala, Nordlund, and Poutanen (2012) have successfully treated wheat bran with hydrolytic enzymes at water contents of 30–90% to improve the bioaccessibility of antioxidants or the solubilisation of arabinoxylans. For example, Santala et al. (2011) showed that the enzymatic solubilisation of arabinoxylan at 40% water content was as efficient as at 90% water content. However, wheat bran is more resistant towards enzymatic hydrolysis than oat bran, because the major part of dietary fibre in oat bran consists of water-soluble β-glucan, whereas most of the fibre in wheat bran is water-insoluble arabinoxylan.

In addition to acid- and enzyme-assisted hydrolyses of β-glucan, Tosh et al. (2010) reported a study of extruded breakfast cereals in which the extrusion temperatures were 181–237 °C and water content was 7.0–18.7%. High specific mechanical energy resulted in the reduction of M_w of β-glucan from 1,930,000 to 251,000 g/mol. It was difficult to compare these results to the current study, because the recipe of the breakfast cereals contained corn flour in addition to the oat bran preparation. The low water content increased the torque during the extrusion, thus leading to much higher specific mechanical energy levels than in the current study. However, it was clearly seen that extrusion without acid or enzymes resulted in lower polydispersity and sharper M_w-distribution peaks compared to the present study (Fig. 3). Zhang et al. (2011) also reported that extrusion can increase the amount of soluble dietary fibre (SDF) in oat bran. Their results showed that the best yield of SDF was obtained with 10% water content. The yield of SDF increased (from 9.9 to 14.2%) when the extrusion temperature was increased from 100 to 140 °C, but somewhat decreased (12.4%

yield of SDF) when the temperature was 160 °C. Unfortunately, this study did not report the M_w-values of β-glucan.

3.5. Stability of depolymerised oat β-glucan dispersions

The intrinsic tendency to form gels and aggregates limits the use of β-glucans in food products with high water content. The size of aggregates has been shown to increase with increasing M_w and concentration (Wu et al., 2006). Similarly, in the current study, the viscosity of the water extracts of the hydrolysed oat brans depended on the average M_w and the concentration of β-glucan (Fig. 4). The hot water extracts prepared from the oat bran after 4 h enzyme-hydrolysis (M_w = 49,000 g/mol) retained their low viscosity only for 2 weeks at 1.9% β-glucan concentration but as long as for 12 weeks at 1.6% β-glucan concentration. Acid-hydrolysis at 130 °C (M_w = 34,000 g/mol), in turn, resulted in elevated viscosities after 3 or 7 weeks at 1.8 or 1.4% β-glucan concentration, respectively (Fig. 4). When the shear rate in the Bohlin 88 viscometer was gradually increased (from 24 to 1300 s^{-1}), lower viscosity values were measured, indicating shear thinning behaviour of the dispersions (data not shown). However, the solutions were considered as Newtonian fluids when their viscosity remained below the detection limit of the viscometer (<0.05 Pa·s).

Even though the average M_w of the acid-hydrolysed β-glucans was lower than after the enzymatic hydrolysis, they resulted in faster and more intensive gel-formation as compared to enzyme-hydrolysed β-glucan. The reason could be that acid-hydrolysed polymer populations had a sharper M_w distribution and more linear chains of β-glucan, which

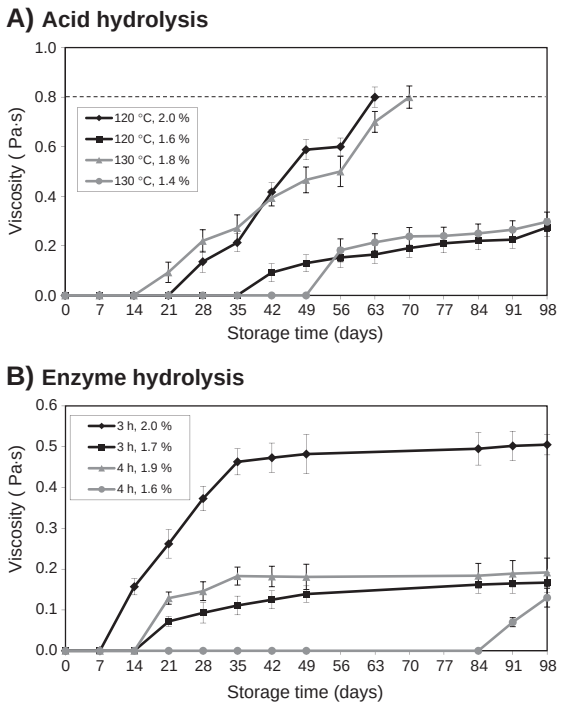


Fig. 4. The viscosity formation of A) acid- (120 and 130 °C) and B) enzyme-hydrolysed (3 and 4 h) oat bran extracts. The viscosity profiles were measured from acid-hydrolysed solutions with 1.4–2.0% β-glucan and from enzymatically hydrolysed solutions with 1.6–1.9% β-glucan. The viscosity values were measured at 24 s^{-1} shear rate in the viscometer. The dashed line (at 0.8 Pa s) shows the measurement limit of the viscometer. The results are expressed as means of duplicate analyses.

easily form aggregates with each other (Doublier & Wood, 1995). The agglomeration phenomenon has also been explained by the intermolecular interactions resulting from the increased mobility of hydrolysed macromolecules (Böhm & Kulicke, 1999; Doublier & Wood, 1995). As the hydrolysed macromolecules are more mobile, they have a greater probability to achieve the proximity of other compounds with regions required for aggregation (Doublier & Wood, 1995; Vaikousi, Biliaderis, & Izydorczyk, 2004).

The HM_w oat β-glucans (>250,000 g/mol) have been shown to form stronger gel networks, which consist of micro-aggregates with better organization, than their LM_w (35,000–140,000 g/mol) counterparts (Lazaridou et al., 2003). However, unhydrolysed HM_w oat β-glucan solutions (>1,200,000 g/mol) have not shown any tendency to form gel (Doublier & Wood, 1995). According to Agbenorhevi, Kontogiorgos, Kirby, Morris, and Tosh (2011), the solution viscosity was higher for HM_w samples when oat β-glucan molecules with different M_ws (142,000–2,800,000 g/mol) were compared at the same concentrations (0.01–8.0% w/v). Increased critical concentration (c^* = 0.25–1.10%) with decreased M_w of β-glucan (from 2,800,000 to 142,000 g/mol, respectively) showed that coil overlap occurred at lower concentrations in the case of HM_w samples (Agbenorhevi et al., 2011).

Similar critical concentrations (c^* = 0.5–2.0%) were also observed by Böhm and Kulicke (1999) for hydrolysed barley β-glucans (M_w range between 375,000 and 40,000 g/mol, respectively). Nevertheless, the solution stability of barley β-glucan differs from that of oat β-glucan. Barley β-glucan has a higher tri- to tetrasaccharide ratio (DP3/DP4) and more ordered cellotriose units compared to oats, and consequently a greater gelling and aggregate-forming tendency (Lazaridou & Biliaderis, 2004; Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004). Zheng, Hess, Khare, Hilbert, and Deguise (2004) patented an enzymatic method to reduce the M_w of barley β-glucan, based on the cellulase activity in the enzyme preparation. The M_w was preferably between 120,000 and 170,000 g/mol. In large scale, the dry matter concentration in hydrolysis could be up to 18%, which was however much lower than presented in the current study (50% dry matter). Zheng et al. (2004) reported that a 0.75–0.78% w/v solution of 120,000–170,000 g/mol β-glucan in water showed little or no precipitation when stored overnight (16 h) at 4 °C. However, they used a β-glucan ingredient which was highly purified (up to 75–78% β-glucan concentration) prior to the solution stability tests.

Tosh et al. (2004) investigated the ability of acid- (HCl) and enzyme-catalysed (lichenase and cellulase) hydrolyses to produce LM_w β-glucan molecules (31,000–237,000 g/mol). Independently of the hydrolysis method, all 6% oat β-glucan solutions with M_w < 150,000 g/mol formed gels at 5 °C in less than one week. The authors also showed that the time required for a gel to form became shorter in relation to the reduced M_w. These findings were opposite to the results obtained in the present study. It is difficult to compare the results, because the β-glucan concentration (6%) and purity of the β-glucan preparation (92%) were much higher than in the present study (max. 2 and 34%, respectively). Tosh et al. (2004) measured the apparent viscosity of the freshly dissolved 6% β-glucan at 25 °C, and the gel-samples in the rheological measurements were heated from 5 °C (at 5 °C/min) up to the melting temperature of the gel. In addition, they used lichenase enzyme which is known to cleave the (1 → 3)-β-D-linkages of β-glucan, leaving a higher amount of linear (1 → 4)-β-D-linked units in the dispersion, thus enhancing the gelling behaviour of the extracted β-glucan molecules.

In the present study, the oat bran material was extracted with hot water directly after the acid or enzymatic hydrolysis. In consequence, the resulting water extracts also contained other oat-based compounds, such as proteins, starch and arabinoxylan. These components may have prevented the interactions between the regions in the β-glucan molecules which are prone to agglomeration, thus limiting the increase in viscosity. In addition, the polydispersity values in the study of Tosh et al. (2004) were much lower (1.2–1.7) than those in

the present study (4.0–24.2). The oligosaccharides generated by the enzymatic hydrolysis in the present study may also have decreased the aggregation tendency of the β-glucan molecules.

4. Conclusions

This study showed that acid or enzymatic hydrolysis at low water content enabled controlled depolymerisation of β-glucan in oat bran. However, the reaction mechanisms of acid- and enzyme-catalysed hydrolyses were rather different. Acid hydrolysed both (1 → 3)- and (1 → 4)-β-D-linkages, whereas the enzyme preparation seemed to cleave mainly (1 → 4)-β-D-linkages. In acid-hydrolysis, the degradation was controlled by temperature. A clear change in the M_w of β-glucan (from 86,000 to 34,000 g/mol) as well as in the subsequent dispersion stability occurred between 120 and 130 °C. In the enzymatic hydrolysis, the degradation was mainly dependent on the incubation time. The M_w of β-glucan was reduced from 71,000 to 49,000 g/mol between 3 and 4 h incubations with the enzyme preparation. When stored at 5 °C, the hot water extracts of acid-hydrolysed oat bran (M_w = 34,000 g/mol) were stable for 2 weeks at 1.8% and for 7 weeks at 1.4% β-glucan concentration, respectively. Similarly, the water extracts of enzyme-hydrolysed oat bran (M_w = 49,000 g/mol) were stable for 2 weeks at 1.9% and for 12 weeks at 1.6% β-glucan concentration. Enzyme-catalysed hydrolysis was superior to acid-hydrolysis in requiring less harsh conditions, not producing inorganic side streams, and resulting in more stable extracts. The stability of the acid- and enzyme-hydrolysed oat bran extracts could also be affected by other bran components, such as proteins, starch and arabinoxylans. However, the associations between β-glucan and other bran components were not subject to this study, and should be studied in future work.

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PUBLICATION IV

Effect of oat bran fractions on extrudates made of defatted oats

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ORIGINAL PAPER

Effect of Oat Bran Fractions on Extrudates Made of Defatted Oats

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Abstract Oats are rich in dietary fibre (DF) especially in β-glucan which has several health-promoting effects. Oats are not commonly used in extruded snacks because they often result in a poor expansion and hard structure. In the present study, defatted wholegrain oat flour (WF) and defatted endosperm oat flour (EF) were used as starch sources for extrudates. Five differently treated oat bran fractions (untreated, ultra-fine ground, enzymatically hydrolysed and hot water-extracted solubles and residue) were added to EF (10 or 20 %), and their influence on the chemical, textural and structural properties of extrudates was investigated. Extrudates made of WF had a poor expansion (151 %) and hard texture (399 N), whereas EF formed a better expanded (199 %) and less hard product (149 N). Addition of oat bran concentrate (OBC) decreased the expansion (171–176 %) and resulted in a harder texture (200–265 N) compared to that of EF 100 % extrudates. The lower expansion of WF and OBC-enriched extrudates was due to the higher content of insoluble fibre. However, no statistical differences were detected between the untreated, ultra-fine ground and enzyme-hydrolysed OBC. The water-insoluble (WIS-OBC) and water-soluble (WS-OBC) fractions had opposite effects on the EF-based extrudates: 10 % addition of WIS-OBC fraction significantly decreased the expansion (163 %) and increased the hardness (313 N), whereas the addition of WS-OBC (10 or 20 %) enhanced the expansion (218–226 %) and resulted in less hard textures (131–146 N). The soluble fibres and low protein content in WS-OBC fraction were hypothesised to cause the improved expansion and decreased hardness. The results demonstrated that extrudates with acceptable expansion and hardness can be produced with defatted oat endosperm flour and oat bran fractions. However, the water-insoluble bran components had a negative effect on the textural properties of extrudates.

Keywords Defatted oat flour · Oat bran concentrate · Soluble dietary fibre · Insoluble dietary fibre · Beta-glucan · Extrusion processing

Abbreviations

DF	Dietary fibre
EF	Defatted oat endosperm flour
EH	Enzymatically hydrolysed
IDF	Insoluble dietary fibre
M_w	Molecular weight
OBC	Oat bran concentrate
SDF	Soluble dietary fibre
TDF	Total dietary fibre
UF	Ultra-fine ground
WF	Defatted wholegrain oat flour
WIS	Water-insoluble
WS	Water-soluble

Introduction

Extrusion is a high-temperature, short-time process which can be used for producing different types of snacks and breakfast cereals (Eastman et al. 2001). Starch is the most important

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ingredient in extrusion, ensuring good expansion characteristics and gas-holding properties (Guy 2001). The chemical composition of starch affects its behaviour in extrusion; starches rich in amylopectin usually undergo greater diametric expansion compared to amylose-rich starch (Launay & Lisch 1983). However, the expansion depends largely on the conditions during extrusion, such as temperature and water content (Babin et al. 2007). In fibre-enriched extrudates, it is recommended to use 60–70 % starch of the dry weight to improve expansion and textural properties (Yao et al. 2011).

Compared with other cereals, such as maize and wheat, oats give poorly expanded and non-crispy extrudates (Rzedzicki 1999; Zarzycki et al. 2010). The poor expansion of oats is mainly due to their high content of lipids (4–9 %) and (1→3)(1→4)-β-D-glucans (3–8 %), as well as to the relatively low content of starch (45–61 %) (Wood 1986; Peterson & Wood 1997; Peterson 2002). Addition of oat bran into starch matrix has been shown to increase the hardness and decrease the expansion of the extrudates (Lobato et al. 2011). Nevertheless, up to 18 % oat bran of solids can give expanded, porous and low-density extrudates when a high-starch ingredient, such as maize semolina, is used as the bulk carrier matrix (Rzedzicki 1999; Rzedzicki et al. 2000). However, Rzedzicki et al. (2000) recommended that 9–12 % oat bran of solids would be the practical application limit in highly expanded products.

Dietary fibres (DF) usually interfere with the expansion of the starch-based matrix. The effect of DF on the texture and structure of extrudates depends mainly on its interactions with starch and on the type and quantity of DF (Sozer & Poutanen 2013). Food matrices high in DF have poor gas-holding capacity during extrusion, which easily reduces the expansion. The reduced capacity of the bubbles to expand has been explained by the low adhesion between starch and bran and the reduced interactions between starch molecules. Thus, a high amount of bran may cause early bursting of air bubbles, resulting in shrinkage of the matrix (Robin et al. 2011a).

DF in bran also binds some of the water present in the matrix, and thus reduces its availability for expansion (Moraru & Kokini 2003). According to Lee (2000), the addition of cereal bran ingredients increases the nucleation degree of bubbles. However, if the nucleation starts too early at the die exit, it favours the longitudinal expansion and reduces the sectional expansion due to stretching of the bubbles in the direction of the melt flow (Robin et al. 2011b). In general, the addition of insoluble dietary fibre (IDF) leads to porous structures with smaller pore sizes and higher densities. For example, the density of wheat flour extrudates has been shown to increase exponentially in proportion to added wheat bran (Robin et al. 2011a).

Modification (e.g. particle size reduction or alkaline treatment) of a DF source prior to extrusion may lead to significant improvements in expansion compared to an unmodified DF source (Pai et al. 2009; Kale et al. 2011). Soluble dietary fibres (SDF), such as inulin, can also deliver a higher expansion and crispier texture compared to cereal brans, which mainly contain IDF (Blake 2006; Brennan et al. 2008). On the other hand, inulin and other relatively homogenous fibre polymers differ significantly from grain fibres, which are organised in a complex structure within the grain cell walls (Rosell et al. 2009). The effects of SDF on the expansion of extrudates are still rather poorly known, and the expansion is largely dependent on the type of starch matrix (Parada et al. 2011). In addition to the content of SDF, its molecular weight and structure can also have an effect on the expansion (Robin et al. 2012).

This study aimed at producing 100 % oat-based extrudates with high DF content and acceptable structural properties (high expansion and porosity) using differently modified oat bran fractions in a mixture with defatted oat endosperm flour. The aim was also to relate ingredient properties and interactions with other extrudate components to the texture and microstructure of the expanded product.

Materials and Methods

Raw Materials

The initial raw material, non-heat-treated and dehulled wholegrain oats, was obtained from Raisio plc (Kokemäki, Finland). All extrusion ingredients were prepared by removing the lipids of oats by supercritical CO_2 extraction and subsequently fractionating the different grain components by grinding and air classification according to Sibakov et al. (2011) (Fig. 1a). Defatted endosperm oat flour (EF) was used as the reference and main carrier matrix during extrusion processing. In addition, defatted wholegrain oat flour (WF) was used as another reference. Oat bran concentrate (OBC), containing 28.5 % β-glucan, was used as the source of DF in untreated and modified form. In order to study the effect of particle size, the OBC with an original particle size of D_{50}/D_{90}=213/404 μm was ground into an ultra-fine powder (32/81 μm) in a TurboRotor G-55 mill (Mahltechnik Görgens GmbH, Dormagen, Germany) using 60 Hz rotor speed and an average feed rate of 30 kg h^{-1}. This fraction was designated as ultra-fine ground OBC (UF-OBC) (Fig. 1a).

OBC was also enzymatically hydrolysed to reduce the molecular weight (M_w) of β-glucan. This was performed according to Sibakov et al. (2013), using Depol 740L enzyme preparation (Biocatalyst Ltd., Wales, UK) with a dosage of 50 nkat β-glucanase activity/gramme of oat bran. The hydrolysis was performed at 50 % water content in an APV MPF 19/25 twin-screw extruder (Baker Perkins Group Ltd, Peterborough, UK) at 50 °C with 2 min residence time (screw speed 75 rpm). The temperature was kept constant throughout the extruder barrel by water cooling jackets. After extrusion,

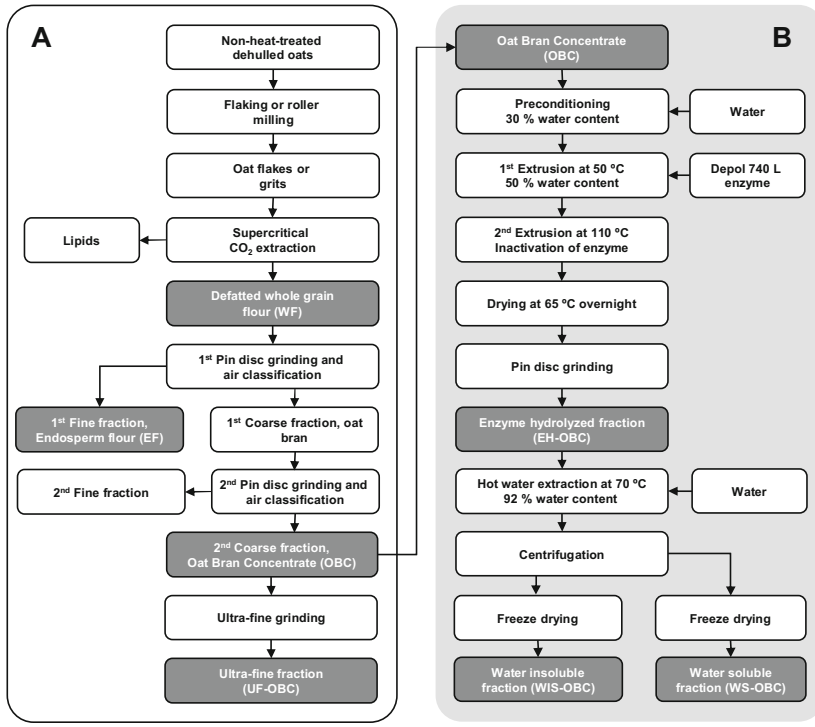

Fig. 1 Process flow chart of the fractionation of oats starting from non-heat-treated dehulled oats. **a** The defatted wholegrain oat flour (WF) was obtained after supercritical CO_2 extraction. Oat endosperm flour (EF) and oat bran concentrate (OBC) were prepared by subsequent pin disc grinding and air classification. OBC with high β-glucan content was obtained by repeating the grinding and air classification. **b** OBC with high β-glucan content was hydrolysed by Depol 740L enzyme at 50 % water content. The enzyme was allowed to act for 4 h and was then inactivated. The hydrolysed OBC was dried and ground into a fine powder (to obtain EH-OBC). This was further fractionated into water-soluble (WS-OBC) and water-insoluble (WIS-OBC) fractions by hot water extraction, centrifugation and freeze drying

the dough-like OBC-water mixture was incubated with the enzyme preparation at 50 °C in a sealed container for 4 h. Then, the enzyme was inactivated by feeding the mass manually through the same extruder at 110 °C (residence time 2–3 min, screw speed 75 rpm). After inactivation, the moist hydrolysed material was spread on stainless steel trays and dried overnight at 65 °C in an oven (Model 800, Memmert GmbH, Schwabach, Germany) with air recirculation. The dried material was first ground in a Wiley cutting mill (Arthur H. Thomas Company, Philadelphia, USA) and subsequently in a Hosokawa Alpine 100 UPZ-lb Fine impact mill with stainless steel pin discs (Hosokawa Alpine AG, Augsburg, Germany), using 17,800 rpm rotor speed. The dried and ground powder was designated as enzymatically hydrolysed OBC (EH-OBC) (Fig. 1b).

To separate the EH-OBC into water-insoluble and water-soluble fractions, 83 g dw of EH-OBC powder was dispersed in 1 l of distilled water at 70 °C. The mixture was stirred for 2 min with a handheld homogeniser (Heidolph Diax900 Ultra Turrax, Gemini BV, Apeldoorn, the Netherlands), using 12,000 rpm speed. The insoluble residue was separated with a Sorvall RC-12BP centrifuge (DuPont, USA), at 4000 rpm (ca. 4000×*g*) for 15 min at room temperature. The water-insoluble residue and the water-soluble extract were dried in a Christ Epsilon 2-25 freeze drier (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The freeze-dried samples were ground in a Hosokawa Alpine 100 UPZ-lb Fine impact mill with stainless steel pin discs (17,800 rpm) prior to using them as ingredients in the extrusion trials. The water-insoluble residue and the water-soluble extract of OBC were designated as water-insoluble OBC (WIS-OBC) and water-soluble OBC (WS-OBC), respectively (Fig. 1b).

Experimental Plan

The experiments were performed using raw materials from three different 'groups'. The first group (i.e. reference samples without OBC) consisted of starch-rich carrier matrices: defatted wholegrain oat flour (WF) and defatted endosperm oat flour (EF). The second group was differently treated oat bran concentrates: untreated (OBC), ultra-fine ground (UF-OBC) and enzymatically hydrolysed (EH-OBC). The third group was water-insoluble (WIS-OBC) and water-soluble (WS-OBC) fractions which were obtained when the EH-OBC material was extracted with hot water and subsequently freeze dried and ground into powder form.

Differently treated oat bran concentrates (untreated (OBC), ultra-fine ground (UF-OBC) and enzyme-hydrolysed (EH-OBC)) were added at 10 % solid content with EF 90 %. The water-insoluble (WIS-OBC) and water-soluble (WS-OBC) fractions were investigated by adding 10 % WIS- or WS-

OBC into EF. Moreover, the addition of 20 % WS-OBC was studied in the EF-based recipe. All recipes were prepared by adding distilled water so that the water content of the extruded mass was 16 %. All raw materials had a low initial water content (4.6–8.8 %).

Extrusion Experiments

Extrusion trials were conducted in a co-rotating twin-screw extruder (Poly Lab System, Thermo Prism PTW24, Thermo Haake, Germany) with a barrel length (L) of 672 mm, diameter (D) 24 mm and L/D ratio of 28:1. The extruder consisted of seven sections (each 96 mm long, six with individual temperature control) with a die exit of 5 mm in diameter (Fig. 2). The screw speed was 500 rpm and the total feed rate (including both solids and water) was 4.56 kg/h. The temperature profile in the barrel was 40, 70, 70, 100, 110, 130 and 130 °C (sections 1–6 and the die, respectively), and a cooling water circulation was utilised to keep the temperatures constant. The values of torque and die pressure were monitored and recorded during the extrusion. Specific mechanical energy (SME) was calculated with Eq. (1) according to Hu et al. (1993).

$$\mathrm{SME}\left(\mathrm{kW\,h\,kg^{-1}}\right) = \frac{\omega}{\omega_r} \times \frac{\tau}{100} \times \frac{Z_r}{Q} \quad (1)$$

where ω is the screw speed (in rotations per minute, rpm), ω_r is the maximum screw speed of the extruder used (1100 rpm), τ is the torque (in percent), Z_r is the maximum power capacity of the extruder (16 kW) and Q is the feed rate (kg/h).

Particle Size Measurement

The volume-based particle size distribution of the raw materials was analysed by a Beckman Coulter LS 230 (Beckman Coulter. Inc., CA, USA) using the liquid module and distilled water as a carrier (Sibakov et al. 2011). The particle size data was presented as cumulative undersize centiles (D_{50} and D_{90} values) with an average of triplicate analyses.

Chemical Analyses

Water contents of the raw materials were determined by drying the samples in an oven at 130 °C for 1 h (44-15.02, AACC, 2000). Water contents of final extrudates were measured by drying the samples in a vacuum oven (Salvis Vacucenter VC 50, Switzerland) at 50 °C for 72 h. All materials were maintained at room temperature in a desiccator for 1 h before weighing the dry weight. Total starch was quantified using the Megazyme total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) according to the method 76-13.01 (AACC, 2000). Nitrogen was analysed using a Kjeldahl autoanalyser (Foss Tecator Ab, Höganäs, Sweden), and protein content was calculated as $N \times 6.25$ according to method 46-11A (AACC, 2000).

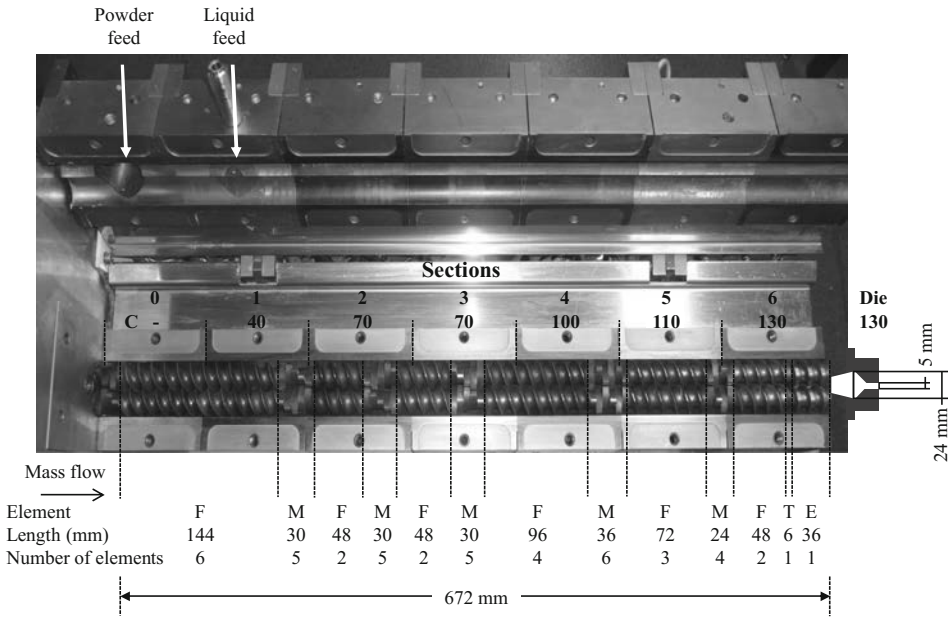

Fig. 2 Extruder screw design and barrel temperature profiles in sections 1–6 and the die. *F* feed screw elements with the depth of 5 and the pitch of 10 mm, *M* ellipse-shaped mixing element length of each D/4, *T* transition element, *E* extrusion screw elements (i.e. pressurising elements)

The content of β-glucan was analysed by an enzymatic method 32-23 (AACC, 2000) using the Megazyme β-glucan mixed linkage assay kit. The molecular weight (M_w) of β-glucan in the raw materials and extruded samples was analysed by the following procedure: 30 mg of each sample was dissolved into 10 ml 0.1 M NaOH in the presence of 0.1 % $NaBH_4$. Samples were kept under magnetic stirring at room temperature overnight. Before the measurements, samples were diluted and filtered through a 0.45-μm syringe filter. After filtering, samples were analysed by high-performance size-exclusion chromatography (HP-SEC), which consisted of an Alliance 2690 separation module, using calcofluor staining (calcofluor solution for post column staining was 30 mg Fluorescent Brightener 28 in 1 l of 50 mM NaOH) and Scanning Fluorescence 474 detector (Waters Inc., Milford, MA, USA). The columns employed were (7.8×300 mm) μHydrogel 2000, μHydrogel 500 and μHydrogel 250 (Waters Inc.) in series at 60 °C. The eluent was aqueous 50 mM NaOH at a flow rate of 0.5 ml/min. Injections (100 μl) were made of sample and standard solutions (Suortti, 1993). The linear size-exclusion calibration curve (r^2>0.95) was constructed on the basis of β-glucan standards ranging from 33.6 to 667 kDa. The system was controlled and calculations were performed with Waters Empower software's GPC option. In principle, the software sliced the sample peak into narrow slices. The peak molecular weight value and the area of each slice (i.e. content) were calculated by the software. Then, the weight average molecular weight (M_w) was calculated over the whole β-glucan peak, as well as the percentage proportions of the molecules in different M_w ranges (M_w>1000 kDa, 1000 kDa>M_w>100 kDa and M_w<100 kDa).

Total dietary fibre content (TDF) was measured by the method AOAC 2011.25 (AOAC 2011) using Megazyme's integrated procedure for the measurement of total, insoluble and soluble dietary fibre, including non-digestible oligosaccharides (DP ≥3). The contents of β-(1→3) and β-(1→4) D-glucose disaccharides (cellobiose and laminaribiose) were analysed to confirm the observed difference between the content of β-glucan (AACC method 32-23) and TDF (AOAC method 2011.25) in the extrudate samples containing 20 % WS-OBC. The content of disaccharides was determined by high-performance anion exchange chromatography (HPAEC) (Dionex ICS-3000) with pulse amperometric detection (PAD) (Dionex Corporation, Sunnyvale, CA, USA). The pre- and separation columns (Dionex CarboPac PA-1) were used at 30 °C with a flow rate of 1 ml/min using the following eluents: Milli-Q water, 100 mM NaOH, 300 mM Na acetate/100 mM NaOH and 300 mM NaOH. The gradients of different injected eluents were adopted from Tenkanen et al. (1997).

Textural Analyses

The diameters of the extrudates were measured by a vernier calliper using an average of 20 replicates. The samples (20 replicates) were cut into 10-mm-long pieces with a band saw (Power ST-WBS800, Taiwan Sheng Tsai Industrial Co. Ltd., Taiwan). The expansion was calculated by the Eq. (2) according to Kumagai et al. (1987).

$$\text{Expansion}\,(\%) = \frac{D_e}{D_d} \times 100\% \tag{2}$$

where D_e is the average diameter measured at three different points of the extrudate sample (in millimetre) and D_d is the diameter of the die (5 mm).

Hardness and crispiness of the extrudates were determined by a TA.XT2i Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) equipped with a 30-kg load cell (crosshead speed 1.0 mm/s) and a cylindrical 36-mm aluminium probe. The compression curve was linearized by a linear distance function of the Texture Exponent v. 5.1.2.0 software (Stable Micro Systems Ltd.). The length of the curve was used for the calculation of crispiness. Crispiness index (C_i) was calculated with Eq. (3) (Heidenreich et al. 2004). High crispiness is accompanied by a high C_i value, whereas low crispiness corresponds to a low C_i value.

$$C_i = \frac{L_N}{A \times F_{mean}} \tag{3}$$

in which

L_N	Normalised curve length (length of linearized curve/F_{max}) and F_{max}=maximum hardness of extrudates
A	Area under the force/deformation curve=toughness (N s)
F_{mean}	Sum of the actual force values divided by the number of peaks (N)

Structural Analyses

X-ray microtomography was used to investigate and quantify the porous structures of extrudates. Triplicate samples (10-mm-long pieces of each extrudates) were scanned with a SkyScan 1172 microtomograph (Bruker-microCT Ltd., Kontich, Belgium). The instrument was operated at 40 kV/250 μA. The pixel size was 11.65 μm, the exposure time 0.079 s and the total scanning time 18 min. After scanning, binarized projection images from each sample were reconstructed into a 3D object by NRecon reconstruction software (SkyScan, Belgium) and further analysed for porosity (%), cell wall thickness (mm) and pore diameter (mm) by Ctan

image analysis software (SkyScan, Belgium). In addition, the image analysis software provided the distributions of pore size and cell wall thickness.

The localization of grain cell wall components (mainly β-glucan), starch and proteins in the extrudates was investigated by bright field microscopy. Samples were embedded into agar using a HistoResin embedding kit (Leica instruments GmbH, Heidelberg, Germany), and then sectioned into 2-μm slices in a rotary microtome HM 355 (Microm Laborgeräte GmbH, Walldorf, Germany) using a steel knife. The sections were stained either with Light Green (BDH Chemicals Ltd., Poole, Dorset, UK)/Lugol's iodine solution or with Acid Fuchsin (BDH Chemicals Ltd., Poole, Dorset, UK)/Calcofluor White (Fluorescent Brightener, Aldrich, Germany). When imaged in bright field, Light Green stained protein green/yellow, whereas Lugol's iodine solution stained the amylose component of starch blue and amylopectin brown. Most starch appeared dark blue, because amylose masked amylopectin. Acid Fuchsin and Calcofluor White were used for staining protein red and β-glucan-rich cell walls light blue, respectively. The samples were imaged using exciting light (epifluorescence at 400–410 nm and fluorescence at >455 nm). At least five images were acquired from the sections cut from three blocks per sample. The most representative micrographs were selected for the comparison of the different fractions.

Statistical Analysis

The data was subjected to analysis of variance using the IBM SPSS Statistics 20 (IBM Corporation, Somers, NY, USA), and significant differences ($P<0.05$) between individual means were identified by the Tukey's test. The statistical analysis involved only within-batch variation of the samples, so conclusions about batch-to-batch variation could not be made. The batch-to-batch variation could have increased the total variation.

Results and Discussion

Chemical Composition of Raw Materials

Wholegrain oat flour (WF) had significantly higher β-glucan (3.2 vs. 1.3 % of dry matter) and TDF contents (6.0 vs. 2.5 %) than that of endosperm oat flour (EF) (Table 1). The proportion of IDF compared to SDF was significantly higher in WF (60:40 %) than in EF (52:48 %). In turn, the starch content was lower in WF (65.6 %) than in EF (70.6 %). The starch content of WF was lower and DF content higher than EF, because most of the bran particles were removed from WF to obtain EF by air classification (Sibakov et al. 2011). However, the protein contents of EF and WF (16.7–17.2 %) were similar.

Table 1 Chemical compositions and particle sizes of the raw materials

Raw material	Moisture (%)	Starch (g/100 g)	Protein (g/100 g)	Dietary fibre (total, soluble, insoluble)			Particle size D50/D90 (μm)	β-glucan (g/100 g)	β-glucan M_w average (kDa)	β-glucan M_w distribution (%)		
				TDF (g/100 g)	SDF (%)	IDF (%)				>1000 kDa	100–1000 kDa	<100 kDa
1. WF (whole grain oat flour)	8.7±0.1d	65.6±1.8e	17.2±0.1c	6.0±0.2b	40	60	37/269	3.2±0.1b	780±5d	36	19	46
2. EF (oat endosperm flour)	8.8±0.2d	70.6±1.0b	16.7±0.1b	2.5±0.4a	48	52	14/175	1.3±0.2a	8352±7e	38	18	44
3. OBC (oat bran concentrate)	4.6±0.1a	9.7±0.8b	23.2±0.1e	48.1±0.5d	51	49	213/404	28.5±0.3d	783±7d	37	19	44
4. UF-OBC (ultra-fine OBC)	6.3±0.1c	12.4±0.4cd	22.9±0.1d	50.4±0.4d	51	49	32/81	28.1±0.1d	782±6d	35	19	46
5. EH-OBC (enzyme-hydrolysed OBC)	6.4±0.1c	11.1±0.1bc	23.2±0.1e	33.2±0.9c	67	33	111/262	28.3±0.1d	635±6d	19	23	58
6. WIS-OBC (water insoluble OBC)	5.2±0.2b	13.7±0.2d	35.6±0.1f	31.8±2.7c	25	75	70/150	11.6±0.1c	727±3c	28	27	45
7. WS-OBC (Water-soluble OBC)	5.1±0.1b	6.7±0.1a	8.9±0.1a	53.5±0.2e	100	0	37/79	52.2±0.4e	455±11a	6	22	72

Values within the same column followed by a common letter are not significantly different ($P<0.05$). The data is presented as mean±standard deviation (triplicate analyses)

The particle size of WF (D_{50}/D_{90}=37/269 μm) was higher compared to EF (14/175 μm), due to the higher amount of coarse and fibrous bran particles.

The oat bran concentrates (untreated OBC, UF-OBC and EH-OBC) had identical β-glucan contents (28.1–28.5 %), but the content of TDF varied between 33.3 and 50.4 %, being the lowest in EH-OBC (Table 1). The amount of SDF was substantially higher in EH-OBC (33:67 %, IDF:SDF) than in untreated OBC and UF-OBC (49:51 % in both). The M_w of β-glucan in untreated OBC did not change due to the ultrafine grinding (782–783 kDa), but the enzymatic hydrolysis decreased the M_w down to 635 kDa. The particle sizes of OBC (D_{50}/D_{90}=213/404 μm), UF-OBC (32/81 μm) and EH-OBC (111/262 μm) differed significantly from each other. However, it was not possible to demonstrate whether the particle size of EH-OBC was reduced due to the enzymatic hydrolysis itself or due to the subsequent drying and grinding in a pin disc mill.

The water-soluble and water-insoluble OBC fractions (WS- and WIS-OBC) had significant differences in their chemical compositions (Table 1). WS-OBC had higher β-glucan (52.2 %) and TDF contents (53.5 %) compared to that of WIS-OBC (11.6 and 32.1 %, respectively). The DF in WS-OBC was 100 % soluble, whereas 75 % of the DF in WIS-OBC was in insoluble form. The M_w of β-glucan was higher in the WIS-OBC fraction (727 kDa) compared to the WS-OBC (455 kDa). This was mainly because the low-molecular-weight (LM_w) β-glucan molecules were extracted more easily into the water-soluble fraction than that of the high-molecular-weight (HM_w) molecules (Sibakov et al. 2013). In fact, some of the HM_w β-glucan molecules, which remained in the water-insoluble residue, could be observed as a thin slimy layer on the surface of the water-insoluble residue after separation of the water-soluble supernatant by centrifugation. This layer of partly soluble HM_w β-glucan molecules could at least partly explain the remarkable proportion of SDF (25 % of TDF) in the WIS-OBC fraction.

The protein and starch contents of WIS-OBC were significantly higher than that in WS-OBC (35.6 vs. 8.9 and 13.7 vs. 6.7 %, respectively). Thus, the enzymatic treatment did not improve the solubility of oat bran proteins, and starch also remained mainly entrapped in the insoluble bran matrix even after the hot water extraction. The particle size of freeze-dried WIS-OBC (D_{50}/D_{90}=70/150 μm) was significantly higher compared to that of WS-OBC (39/79 μm), because the WIS-OBC particles were harder and more difficult to grind.

Extrudates Made of Defatted Oat Flours

WF 100 % extrudates contained lower amounts of starch and higher amounts of TDF compared to that of EF 100 % extrudates and similar amounts of protein (Table 2). Similarly to the raw materials, the WF extrudates had a higher

Table 2 Chemical compositions of the oat extrudates

	Moisture (%)	Starch (g/100 g)	Protein (g/100 g)	Dietary fibre (total, soluble, insoluble)			β-glucan (g/100 g)	β-glucan M_w average (kDa)	β-glucan M_w distribution (%)		
				TDF (g/100 g)	SDF (%)	IDF (%)			>1000 kDa	100–1000 kDa	<100 kDa
1. WF 100 %	9.7±0.1b	63.3±0.8b	14.9±0.1f	8.8±0.6b	40	60	5.2±0.1d	800±5bc	45	23	31
2. EF 100 %	9.2±0.3a	73.2±0.2f	14.5±0.1e	3.9±0.6a	51	49	1.5±0.2a	911±5d	54	22	23
3. EF 90 % + OBC 10 %	10.3±0.1c	71.8±0.1e	14.2±0.1c	8.9±0.5b	52	48	4.6±0.3c	815±1bc	47	24	29
4. EF 90 % + UF-OBC 10 %	11.6±0.1d	73.4±0.3f	14.0±0.1b	9.4±0.9b	48	52	4.9±0.1cd	796±4bc	44	24	31
5. EF 90 % + EH-OBC 10 %	12.5±0.1e	71.4±0.4e	14.4±0.1d	8.1±1.4b	51	49	4.7±0.1c	792±6bc	43	22	36
6. EF 90 % + WIS-OBC 10 %	10.3±0.1c	68.5±0.1d	17.1±0.1g	4.1±0.5a	26	74	2.7±0.1b	833±6c	47	23	30
7. EF 90 % + WS-OBC 10 %	10.4±0.2c	66.6±0.6c	14.1±0.1b	6.5±0.7a	96	4	6.2±0.4e	770±47b	52	23	25
8. EF 80 % + WS-OBC 20 %	9.9±0.1b	59.6±0.3a	13.7±0.2a1	10.4±0.6b	99	1	11.4±0.1f	686±12a	33	21	47

Values within the same column followed by a common letter are not significantly different ($P<0.05$). The data is presented as mean±standard deviation (triplicate analyses)

WF wholegrain oat flour, *EF* oat endosperm flour, *OBC* oat bran concentrate, *UF* ultra-fine, *EH* enzyme hydrolysed, *WIS* water-insoluble, *WS* water-soluble

proportion of IDF (IDF:SDF 60:40 %) compared to that of EF extrudates (48:52 %). The M_w of β-glucan in EF and WF extrudates (800–911 kDa) was higher than that in the corresponding raw materials (780–835 kDa). Comparable results were reported by Yao et al. (2011), who showed that oat flour-based extruded breakfast cereals had elevated TDF contents and higher molecular weights than that of the raw materials. They explained this by a molecular fragmentation of β-glucan, followed by a possible inter-chain aggregation. The mechanism behind the inter-chain aggregation could be related to the depletion of β-1→3 linkages due to the extrusion, leading to a higher amount of shorter but more linear β-1→4 linked polymer segments forming intermolecular associations (Gaosong & Vasanthan 2000).

Surprisingly, the torque, pressure and SME values did not differ between EF- and WF-based extrudates (Table 3). However, the expansion, texture and microstructure of EF- and WF-based extrudates differed significantly from each other. According to the results presented in Table 4 and Fig. 3, EF-based extrudates had significantly higher expansion (199 %) compared to that of WF-based extrudates (151 %). The WF-based extrudates were harder (376 vs. 156 N) and less porous (48.2 vs. 75.3 %) than that of the EF-based extrudates (Tables 4 and 5). The pore size and cell wall thickness distributions revealed that WF-based extrudates had significantly higher proportions of small pores and thicker cell walls than that of EF-based extrudates (Fig. 4). In addition, the microscopic images (Fig. 5) showed that WF-based extrudates contained more intact bran layers. Based on the better texture and structure of the EF-based extrudates, EF flour was selected as the carrier matrix for the rest of the trials. In fact, the WF-based extrudates were so hard that they could not even be considered as a food product. Usually, the critical threshold hardness value for consumer acceptance is around 200 N (Jin et al. 1995).

Table 3 Torque, pressure and SME values measured each second during the extrusion

	Torque (Nm)	Pressure (bar)	SME (kWh/kg)
1. WF 100 %	44±1de	50±9abcd	0.70±0.01de
2. EF 100 %	43±1de	57±9bcd	0.68±0.01de
3. EF 90 % + OBC 10 %	44±2de	58±6d	0.70±0.03de
4. EF 90 % + UF-OBC 10 %	40±1bc	47±7abcd	0.63±0.02bc
5. EF 90 % + EH-OBC 10 %	38±1ab	39±7ab	0.60±0.02ab
6. EF 90 % + WIS-OBC 10 %	42±2cd	54±6bcd	0.67±0.03cd
7. EF 90 % + WS-OBC 10 %	48±2e	53±8abcd	0.77±0.04e
8. EF 80 % + WS-OBC 20 %	47±2e	42±7abc	0.75±0.03e

Values within the same column followed by a common letter are not significantly different ($P<0.05$). The data is presented as mean±standard deviation ($n=200$)

Extrusion with Untreated, Ultra-Fine and Enzymatically Hydrolysed Oat Bran Concentrates

The behaviour of oat bran concentrates: untreated (OBC), ultra-fine ground (UF-OBC) and enzymatically hydrolysed (EH-OBC), were studied in EF-based recipes. The starch, protein, TDF and β-glucan contents of OBC, UF-OBC and EH-OBC were not statistically different, and the ratio between of IDF and SDF was around 50:50 % in all of the 90 % EF + 10 % OBC extrudates (Table 2). Torque (38 %), pressure (39 bar) and SME values (0.60 kWh/kg) were lower when OBC was enzymatically hydrolysed compared to untreated OBC (44 %, 58 bar and 0.70 kWh/kg; Table 3). This could be due to the lowered viscosity resulting from depolymerised β-glucan molecules. However, the SME values of the three OBC-supplemented extrudates did not differ from each other (Table 3). The addition of any of these three OBC fractions increased the hardness of the EF-based extrudates (200–265 N) in comparison to EF 100 %, but the hardness values of OBC extrudates did not differ statistically from each other. The number of peaks in the compression curve was higher in EF-based extrudates (59±11) than in the extrudates with OBC, UF-OBC and EH-OBC (2±1). WF-based extrudates also showed a greater number of peaks (30±24), but due to large variation in the results, the difference was not significant compared to the other extrudates. The crispiness index of EF- and WF-based extrudates was not significantly different from the extrudates with OBC, UF-OBC or EH-OBC (Table 4).

The extrudates with UF-OBC and EH-OBC had higher values of porosity (68.5 and 71.3 %, respectively) compared to that of untreated OBC (64.7 %) (Table 5). The void area of pores in EF-based extrudates decreased, and cell wall thickness increased, when 10 % untreated OBC, UF-OBC or EH-OBC was added in the recipe (Table 5 and Fig. 3). This was an opposite result compared to that reported by Robin et al. (2011b), who showed that the cell wall thickness of refined wheat flour-based extrudates was reduced when incorporating wheat bran into the recipe. However, they observed a higher surface porosity in the extrudates containing wheat bran, due to earlier burst of the bubbles during their growth. Thus, it might be concluded that wheat bran has a different kind of effect on the extrudate matrix than that of oat bran, as in wheat bran more than 90 % of the DF is in insoluble form (Dreher 2001), whereas in oat bran, more than 50 % of the DF was in soluble form (Table 1).

The viscosity increase due to the addition of β-glucan might also have restricted the expansion of EF-based extrudates. For example, Pai et al. (2009) showed that untreated maize bran significantly increased shear and extensional viscosity of a bran-maize meal mixture, whereas the addition of alkali-treated maize bran increased the shear viscosity of the mixture only to a small extent but showed the highest extensional viscosity amongst the samples. High shear

Table 4 Expansion and textural properties of the oat extrudates

	Expansion (%)	Hardness (N)	Toughness (N s)	Number of peaks	Length of actual curve	Crispiness index (10-4)
1. WF 100 %	151±4a	399±62de	135±65bcd	30±24abcd	1016±322d	3.7±1.7a
2. EF 100 %	199±8d	148±37ab	199±72cd	59±11d	895±179d	13.3±7.8ab
3. EF 90 % + OBC 10 %	172±5bc	258±37c	118±27bcd	2±1a	518±73ab	1.6±0.7a
4. EF 90 % + UF-OBC 10 %	176±2c	265±49c	124±34bcd	2±1a	541±89abc	1.6±0.7a
5. EF 90 % + EH-OBC 10 %	171±3bc	200±33bc	76±20ab	2±1a	415±56a	3.7±2.1a
6. EF 90 % + WIS-OBC 10 %	163±4b	313±59cd	56±8a	4±2a	690±95cd	4.2±2.5a
7. EF 90 % + WS-OBC 10 %	218±7e	141±23a	75±38abc	27±14bc	527±236abcd	21.3±9.1b
8. EF 80 % + WS-OBC 20 %	226±10e	146±20a	58±19ab	12±7ab	417±134ab	12.8±3.5b

Values within the same column followed by a common letter are not significantly different ($P<0.05$). The data is presented as mean±standard deviation ($n=20$)

WF wholegrain oat flour, *EF* oat endosperm flour, *OBC* oat bran concentrate, *UF* ultra-fine, *EH* enzyme hydrolysed, *WIS* water-insoluble, *WS* water-soluble

viscosity usually leads to reduced expansion, whereas an adequate extensional viscosity enables better biaxial extension of the bubbles (Robin et al. 2012). In contrast, the addition of alkali-soluble maize bran resulted in mixtures having lower shear and extensional viscosities than 100 % maize meal. The difference in cell wall thicknesses of extrudates supplemented with wheat and oat bran could be explained by the findings of Robin et al. (2011c), who showed a significant effect of wheat bran on the shear viscosity of wheat flour extrudates but only when bran was added at a high content.

Ultra-fine grinding or enzymatic hydrolysis did not lead to clear differences in the X-ray tomography skeleton images (Fig. 3) or in the microscopic images (Fig. 5). Usually, the physicochemical compatibility between DF and starch can be improved by reducing the size of the particles rich in DF, thus increasing the contact surface between DF and starch. Statistically significant increases in the radial expansion were reported by Lue et al. (1991), Blake (2006) and Alam et al. (2013), when decreasing the average particle size of sugar beet fibre from 2000 to 74 μm (10 to 200 mesh), maize bran from 250 to 50 μm and rye bran from 750–1250 to 28 μm, respectively. The high proportion of SDF in oat bran (>50 % of TDF) might explain why the particle size reduction of OBC did not change the expansion in the current study.

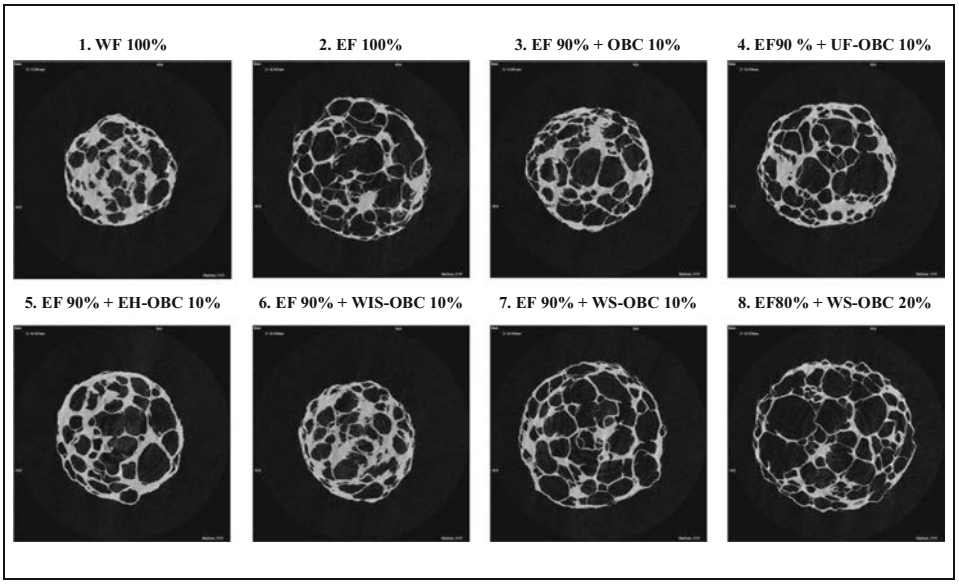

Fig. 3 X-ray tomography binarized projection images of extrudates made of different oat ingredients: *WF* wholegrain oat flour, *EF* endosperm oat flour, *OBC* oat bran concentrate, *UF* ultra-fine, *EH* enzyme hydrolysed, *WIS* water-insoluble, *WS* water-soluble

Table 5 Data from the X-ray tomographic measurements

Sample	Cell radius (mm)	Cell area (mm^2)	Cell wall thickness (mm)	Thickness/radius	Total porosity (%)
1. WF 100 %	0.29±0.01a	0.26±0.02a	0.48±0.02f	1.68±0.14e	48.2±0.6a
2. EF 100 %	0.55±0.06cde	0.98±0.27cde	0.32±0.01bc	0.59±0.08b	75.3±1.8e
3. EF 90 % + OBC 10 %	0.45±0.02bc	0.64±0.07bc	0.38±0.02d	0.84±0.08cd	64.7±2.8c
4. EF 90 % + UF-OBC 10 %	0.49±0.03bcd	0.75±0.13bcd	0.34±0.01c	0.69±0.06bc	71.3±2.2de
5. EF 90 % + EH-OBC 10 %	0.57±0.01de	1.01±0.01de	0.42±0.01e	0.74±0.04bc	68.5±0.4cd
6. EF 90 % + WIS-OBC 10 %	0.39±0.01ab	0.48±0.02ab	0.38±0.01d	0.97±0.01d	58.8±0.2b
7. EF 90 % + WS-OBC 10 %	0.49±0.07bcd	0.77±0.26bcd	0.29±0.01bc	0.60±0.12b	74.6±2.6e
8. EF 80 % + WS-OBC 20 %	0.60±0.05e	1.13±0.27e	0.22±0.01a	0.38±0.03a	81.3±0.1f

Values within the same column followed by a common letter are not significantly different ($P<0.05$). The data is presented as mean±standard deviation (triplicate analyses)

WF wholegrain oat flour, *EF* oat endosperm flour, *OBC* oat bran concentrate, *UF* ultra-fine, *EH* enzyme hydrolysed, *WIS* water-insoluble, *WS* water-soluble

Extrusion with Water-Insoluble and Water-Soluble Oat Bran Concentrates

The enzymatically hydrolysed and hot water-extracted water-insoluble (WIS-OBC) and water-soluble oat bran concentrates (WS-OBC) behaved differently in extrusion. When 10 % WIS-OBC was added into EF 90 % recipe, the starch content decreased from 73.1 to 68.5 %. The protein and β-glucan contents increased (from 14.5 to 17.1 % and from 1.5 to 2.7 %, respectively), but the TDF content did not change significantly (Table 2). The addition of 10 or 20 % WS-OBC resulted in even lower starch content (66.6 and 59.6 %), lower protein (14.1 and 13.7 %), higher β-glucan (6.2 and 11.4 %) and higher TDF (6.5 and 10.4 %) contents, respectively (Table 2).

It was notable that the TDF content (10.4 %) of extrudates with 20 % WS-OBC was lower than that of the β-glucan content (11.4 %). The method for analysing TDF content characterises only the fibre polymers with DP ≥3 (AOAC 2011), whereas the β-glucan method also includes disaccharides (McCleary & Glennie-Holmes 1985). Thus, the difference between the TDF and β-glucan contents could be explained by the presence of disaccharides produced by the extrusion of WS-OBC fibres. Indeed, the content of disaccharides (0.9 % of laminaribiose and 0.1 % of cellobiose) in extrudates with 20 % WS-OBC explained the observed difference between β-glucan and TDF contents.

Gajula et al. (2008) reported an increased SDF content when extruding wheat flour with 30 % addition of wheat bran, but the decrease in IDF fraction was even higher than the increase in the soluble fraction. They suggested that the fragmentation of DF by the shearing action of extrusion resulted in formation of lower-molecular-weight SDF residues, which could even further degrade to sugar derivatives, smaller units than glucose. However, the observed difference between TDF and β-glucan content in the current study could not be explained by degradation of β-glucan to smaller units than

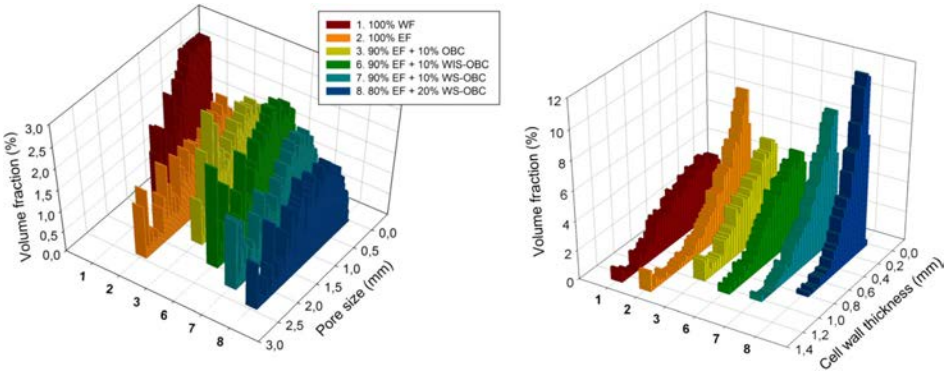

Fig. 4 Pore size and cell wall thickness distributions of the oat extrudates. Data of samples 4 and 5 (EF 90 % + UF-OBC 10 % and EF 90 % + EH-OBC 10 %) is not shown, because it was similar to that of sample 3. The narrow pore size distribution with small pore sizes and the broad cell wall distribution with thick cell walls (especially in 100 % WF-based extrudates) were unwanted properties of the extrudates. By contrast, extrudates with 80 % EF + 20 % WS-OBC showed wider pore size distribution with larger pores and narrower cell wall thickness distribution with thinner cell walls, thus exhibiting the desired properties of extrudates

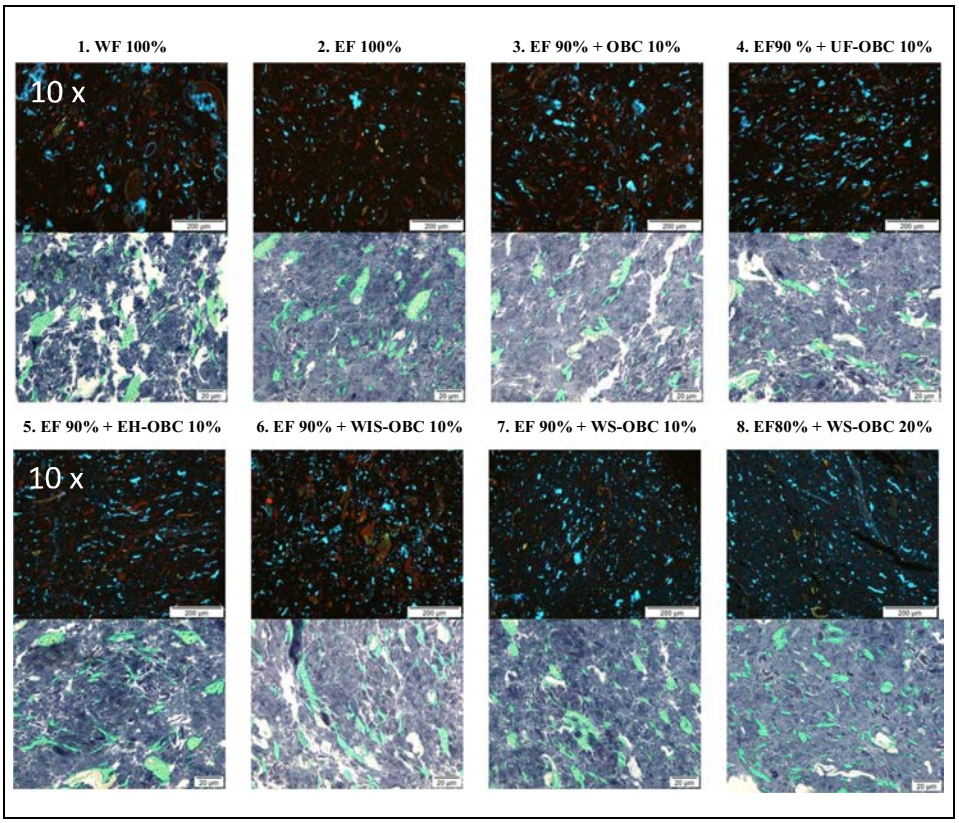

Fig. 5 Microscopic images of the oat extrudates. First row (×10 magnification, bar=200 μm): Acid Fuchsin/Calcofluor White staining, showing protein as *red* and β-glucan-rich grain cell walls as *light blue*. Second row (×100 magnification, bar=20 μm): Light Green/Lugol's iodine staining, showing protein as *green* and starch as *spherical objects in blue or brown*

glucose, because this would have been detected as a decreased β-glucan content.

During the processing of WS-OBC extrudates, the torque (47–48 %) and SME values (0.75–0.77 kWh/kg) were highest compared to other extrudates (Table 3). However, the values did not differ statistically from the EF and WF extrudates. Thus, no conclusions could be done, even though it might be speculated that the WS-OBC fraction increased the melt viscosity inside extruder, which might have led to higher expansion. The expansion of EF-based extrudates decreased with the 10 % addition of WIS-OBC (from 199 to 163 %). This was lower compared to the 10 % addition of untreated OBC (172 %) (Table 4 and Fig. 3). In contrast, the 10 or 20 % addition of WS-OBC significantly increased the expansion of EF-based extrudates (up to 218–226 %). The hardness of 100 % EF-based extrudates (originally 156 N) was increased up to 311 N when 10 % WIS-OBC was added and decreased down to 141–146 N when 10 or 20 % WS-OBC was added.

Although the differences in toughness of WS- and WIS-OBC-containing samples were not significant, the crispiness index of the WS-OBC-containing sample was highest at both addition levels (10–20 %) being close to the reference extrudate with EF 100 % (Table 4). This is likely because toughness is more dependent to the phase properties of the solid matrix than on the structural architecture. On the other hand, it is obvious that the use of WS-OBC fraction as a source of DF had a positive impact on the macro- and microstructural parameters, which in turn efficiently increased the crispiness index of the samples.

The 10 % addition of WIS-OBC reduced the porosity of the EF-based matrix from 75 to 59 %, whereas 10 % addition of WS-OBC resulted in the same porosity as in EF 100 % extrudates (75 %), and 20 % addition of WS-OBC resulted in an even higher porosity (81 %). The average cell wall thickness decreased when increasing the content of WS-OBC (Table 5). The proportion of very small pores was highest in 10 % WIS-OBC extrudates and significantly decreased in 10 % WS-OBC and even more in 20 % WS-OBC extrudates (Fig. 4). By contrast, the distribution of extrudate cell wall thickness shifted towards thinner cell walls when

comparing 10 % WIS-OBC to 10 and 20 % WS-OBC extrudates. The high protein content of WIS-OBC fraction probably fortified the negative effects on the expansion and porosity of the EF-based extrudates (Table 2).

Bright field microscopy showed that the amount of proteins (red) was higher and the amount of β-glucan-rich cell walls (light blue) was lower in 10 % WIS-OBC than in 10 or 20 % WS-OBC extrudates (Fig. 5). According to Camire (1991), protein molecules unfold and align themselves in the direction of flow during extrusion and form new intermolecular bonds. This kind of alignment was also visible in the samples containing WIS-OBC. Particularly, the Light Green/Lugol's iodine staining showed that proteins (green) formed a network type of structure, and they were at least partly aligned in the direction of the extrusion flow. The intensity of the blue colour of Acid Fuchsin/Calcofluor White in 20 % WS-OBC extrudates was higher compared to other samples. However, it appeared that the blue colour was not bound to the grain cell wall structure, but rather distributed throughout the matrix. This was interpreted to be due to the presence of water-soluble LM_w β-glucan molecules, which were extracted out of the grain cell wall complexes.

Insoluble and Soluble Dietary Fibre in Extruded Products

The extrudates which contained the highest proportions of IDF (i.e. WF 100 % and EF 90 % + WIS-OBC 10 %) exhibited the lowest expansions (151 and 163 %), highest hardness (376 and 311 N) and lowest porosity values (48.2 and 58.8 %) (Tables 4 and 5, Fig. 3). The poor expansion and hard texture of WF 100 % extrudates showed that the combination of EF 90 % + OBC 10 % gave better results, even though the TDF and β-glucan contents were similar (Table 2). Thus, the higher proportion of IDF (in WF-based extrudates), as compared to SDF, appeared to be the most probable explanation for the decrease in expansion and porosity and for the increase in hardness. Moraru & Kokini (2003) also concluded that IDF reduces the extensibility of grain cell walls during expansion and thus leads to lower expansion than SDF. Similarly, Santala et al. (2014) demonstrated that enzymatic modification of wheat bran improved the expansion and crispiness of wheat bran-supplemented extrudates and that the improvements correlated with the increased level of water-extractable arabinoxylan in the bran.

In the present study, the size of the pores was largest in samples containing 20 % WS-OBC (Fig. 3), which could be due to the earlier attainment of the glassy state compared to 100 % EF. Pai et al. (2009) concluded that in order to obtain good extrudate expansion, the shear viscosity of the melt should be low enough to promote bubble growth but high enough to prevent bubble collapse after coming out of the die.

Extrusion of oat bran DF has also been shown to increase the content of total and soluble β-glucan as well as the content of TDF and SDF (Gualberto et al. 1997; Zhang et al. 2011). Gualberto et al. (1997) showed that the insoluble part of oat bran DF was converted into a more soluble form during extrusion when the water content of the feed was around 22 % and the extrusion temperature was 180 °C. The SDF content was lower and the IDF content higher when the water content of the feed was around 41 % and the temperature 194 °C. Interestingly, they did not observe changes in the DF composition of wheat and rice bran under the same extrusion conditions. Zhang et al. (2011) also showed that the content of SDF in extruded oat bran was dependent on the water content of the feed. The highest SDS content was obtained at 140 °C with 10 % water content of the feed. The SDF content was reduced when the water content was 30 %. Unfortunately, the study of Zhang et al. (2011) did not report the effect of extrusion on the TDF contents.

Conclusions

The defatted oat fractions were suitable ingredients for expanded snack products, but their performance depended on the content and solubility of DF. Addition of differently treated OBC fractions into EF-based extrudates resulted in similar DF and β-glucan levels as in the WF-based extrudates, but the EF + OBC extrudates were better expanded and less hard. This was mainly due to lower proportion of IDF in the OBC-supplemented extrudates. The modification of OBC by ultrafine grinding or by enzymatic hydrolysis as such did not further improve the expansion and hardness of the EF + OBC extrudates. However, the expansion was significantly improved and the hardness reduced when supplementing the EF-based extrudates with WS-OBC. The improved expansion and textural properties were hypothesised to be due to the increased level of SDF, as has been previously demonstrated with wheat bran. The precise mechanism behind the observed positive effects remains to be further elucidated.

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VTT Science 67

Title	**Processing of oat dietary fibre for improved functionality as a food ingredient**
Author(s)	Juhani Sibakov
Abstract	A dry fractionation process was developed based on defatted oats. Lipid removal by supercritical carbon dioxide extraction enabled concentration of the main components of oats: starch, protein, lipids and cell walls into specific fractions. A defatted oat bran concentrate (OBC) with 34% beta-glucan was obtained after two grinding and air classification steps. Ultra-fine grinding was needed to further dissociate the macronutrients of oat bran particles. Electrostatic separation was used to separate particles rich in beta-glucan and starch from those rich in arabinoxylan. The beta-glucan from defatted OBC was enriched from 34 to 48% after two steps of electrostatic separation. The 48% beta-glucan fraction was further enriched by a combination of jet-milling and air classification, yielding a fraction with up to 56% beta-glucan. OBC was further processed by partial depolymerisation of beta-glucan with acid- or enzyme-catalysed hydrolysis at relatively low water content using a twin-screw extruder as a bioreactor. The hydrolysed oat brans were extracted with hot water and centrifuged to obtain a water-soluble phase and an insoluble residue. The time-dependent gelling of the water-soluble phase was monitored for 14 weeks at 5 °C. Acid hydrolysis depolymerised the beta-glucan molecules from their original average molecular weight (Mw) of 780 to 34kDa (polydispersity 4.0–6.7), and enzymatic hydrolysis down to 49 kDa (polydispersity 19.0–24.2). At 1.4–2.0% beta-glucan concentration, solutions of beta-glucan molecules with Mw>50 kDa agglomerated rapidly, whereas solutions of smaller molecules (34–49 kDa) remained as stable dispersions for longer. Gelling was strongly concentration-dependent; at 1.4 to 1.6% beta-glucan concentration gelling occurred after 7 to 12 weeks of storage, whereas at 1.8 to 1.9% concentration gelling occurred already after 2 weeks. OBC was used in extruded products in five different forms (untreated, ultra-fine ground, enzymatically hydrolysed and hot-water extracted solubles and insoluble residue). Addition of untreated OBC decreased the expansion (172%) and resulted in harder texture (258 N) compared to extrudates based on 100% endosperm flour (EF) (199% and 148 N, respectively). When OBC was separated into water-insoluble (WIS-OBC) and water-soluble (WS-OBC) fractions, significant differences were observed in the resulting extrudates. Ten percent addition of WIS-OBC fraction significantly decreased the expansion (163%) and increased the hardness (313 N) of EF-based extrudates, whereas 10 or 20% addition of WS-OBC enhanced the expansion (218–226%) and resulted in less hard textures (131–146 N). The improved texture was most probably due to the high amount of soluble fibres and low protein content.
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VTT Science 67

Nimeke	**Kauran ravintokuidun prosessointi elintarvikekäytön parantamiseksi**
Tekijä(t)	Juhani Sibakov
Tiivistelmä	Kauran erottamista eri komponentteihin tutkittiin kuivafraktioinnin avulla. Rasvan uutto ylikriittisen hiilidioksidin avulla mahdollisti pääkomponenttien eli tärkkelyksen, proteiinin, rasvan ja soluseinämien konsentroinnin erillisiin fraktioihin. Rasvan uuton sekä kaksinkertaisen jauhatuksen ja ilmaluokittelun jälkeen saatiin kauralesekonsentraatti (OBC), joka sisälsi 34 % beeta-glukaania. Jotta kauralesepartikkelien ravintoainekomponentteja pystyttiin rikastamaan edelleen, tarvittiin avuksi erittäin tehokasta jauhatusta. Sähköstaattista erotusta käytettiin jauhatuksen jälkeen erottamaan toisistaan beeta-glukaanin ja tärkkelyksen tai arabinoksylaanin suhteen rikastuneet partikkelit. Rasvattoman OBC:n beeta-glukaanipitoisuus kasvoi sähköstaattisessa erottelussa 34 %:sta 48 %:iin. Tämä fraktio rikastettiin edelleen ilmasuihku-jauhatuksen ja ilmaluokituksen yhdistelmällä siten, että beeta-glukaanipitoisuus oli 56 %. OBC:n sisältämä beeta-glukaani pilkottiin hallitusti happo- tai entsyymikatalysoidulla hydrolyysillä suhteellisen matalassa vesipitoisuudessa hyödyntäen kaksoisruuviekstruuderia bioreaktorina. Hydrolysoidut kauraleseet uutettiin kuumalla vedellä ja tämän jälkeen vesiliukoinen faasi ja veteen liukenematon jäännös sentrifugoitiin toisistaan erilleen. Happohydrolyysi pilkkoi beeta-glukaanimolekyylejä niiden alkuperäisestä molekyylipainostaan (Mw=780 kDa) aina 34 kDa:iin asti, jolloin pilkottujen molekyylien polydispersiteetti oli 4,0–6,7. Entsyymihydrolyysi pilkkoi beeta-glukaanimolekyylejä 49 kDa:iin asti, ja niiden polydispersiteetti oli 19,0–24,2. Vesiliukoisen faasin geeliytymistä seurattiin 5 °C:ssa 14 viikon ajan. Kun vesiliuoksen beeta-glukaanipitoisuus oli 1,4–2,0 %, beeta-glukaanimolekyylit, joiden Mw oli yli 50 kDa, agglomeroituivat hyvin nopeasti. Pienemmän Mw:n molekyylit (34–49 kDa) puolestaan pysyivät stabiileina dispersioina pidempään. Geeliytyminen oli vahvasti sidoksissa beeta-glukaanipitoisuuteen. Kun beeta-glukaanipitoisuus oli 1,4 % (happohydrolysoitu) tai 1,6 % (entsyymihydrolysoitu), liuos geeliytyi 7–12 viikon säilytyksen jälkeen. Suuremmassa beeta-glukaanipitoisuudessa (1,8–1,9 %) geeliytyminen tapahtui jo kahden viikon jälkeen sekä happo- että entsyymihydrolysoidulla näytteellä. Ekstrudoiduissa naksuissa OBC:tä lisättiin viidessä eri muodossa (käsittelemättömänä, hyvin hienoksi jauhettuna, entsymaattisesti hydrolysoituna sekä vesiliukoisena kylmäkuivattuna uutteena että veteen liukemattomana kylmäkuivattuna jäännöksenä). Kun 10-prosenttista käsittelemätöntä OBC:ta lisättiin rasvattomaan kauran ydinjauhoon, ekstrudoitujen naksujen ekspansio pieneni (172 %) ja naksuista tuli kovempia (258 N) verrattuna 100-prosenttisesta ydinjauhosta tehtyihin naksuihin (199 % ekspansio ja 148 N kovuus). Kun OBC:sta erotettua veteen liukenematonta (WIS-OBC) tai vesiliukoista (WS-OBC) fraktiota lisättiin ydinjauhoon, havaittiin näin saaduissa naksuissa merkittäviä muutoksia verrattuna muihin OBC-fraktioihin. Ekspansio pienentyi erityisen paljon (163 %) ja naksuista tuli kivikovia (313 N), kun 10-prosenttista WIS-OBC:ta lisättiin ydinjauhoa sisältävään reseptiin. Vesiliukoisen fraktion (WS-OBC) lisäys (10 tai 20 % ydinjauhosta) puolestaan paransi ekspansiota (218–226 %) ja johti vähemmän koviin naksuihin (131–146 N).
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Processing of oat dietary fibre for improved functionality as a food ingredient

A dry fractionation process was developed based on defatted oats. Lipid removal by supercritical CO_2 extraction enabled concentration of the main components of oats: starch, protein, lipids and cell walls. A defatted oat bran concentrate (OBC) with 34% beta-glucan was obtained after dry fractionation steps. Ultra-fine grinding was needed to further dissociate the macronutrients. Electrostatic separation was used to separate particles rich in beta-glucan from those rich in arabinoxylan. The beta-glucan from defatted OBC was enriched up to 48% by electrostatic separation, and further enriched by a combination of jet-milling and air classification, yielding a fraction with up to 56% beta-glucan. OBC was partially hydrolysed with acid or enzyme at relatively low water content using a twin-screw extruder as a bioreactor. The hydrolysed oat brans were extracted with hot water to obtain a water-soluble phase and an insoluble residue. The gelling of the water-soluble phase was monitored for 14 weeks. Acid hydrolysis depolymerised the beta-glucan molecules down to 34kDa and enzymatic hydrolysis down to 49 kDa. At 1.4–2.0% beta-glucan concentration, solutions of beta-glucan molecules with Mw>50 kDa agglomerated rapidly, whereas solutions of smaller molecules remained as stable dispersions for longer. Gelling was strongly concentration-dependent. OBC was used in extruded products in five different forms. Addition of untreated OBC decreased the expansion and resulted in harder texture compared to extrudates based on 100% endosperm flour (EF). Ten percent addition of water-insoluble OBC fraction significantly decreased the expansion and increased the hardness of EF-based extrudates, whereas 10 or 20% addition of water-soluble OBC fraction enhanced the expansion and resulted in less hard textures.

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