Title	Lipid removal enhances separation of oat grain cell wall material from starch and protein
Author(s)	Sibakov, Juhani; Myllymäki, Olavi; Holopainen, Ulla; Kaukovirta-Norja, Anu; Hietaniemi, V.; Pihlava, J.M.; Poutanen, Kaisa; Lehtinen Pekka
Citation	Journal of Cereal Science vol. 54(2011):1, pp. 104-109
Date	2011
URL	http://dx.doi.org/10.1016/j.jcs.2011.04.003
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Elsevier Editorial System(tm) for Journal of Cereal Science
Manuscript Draft

Manuscript Number: JCS10-390R1

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

Article Type: Standard Research Paper

Keywords: oats; beta-glucan; supercritical carbon dioxide extraction; air classification

Corresponding Author: Mr. Juhani Kristian Sibakov, M.Sc. (Tech.)

Corresponding Author's Institution: VTT Technical Research Centre of Finland

First Author: Juhani Kristian Sibakov, M.Sc. (Tech.)

Order of Authors: Juhani Kristian Sibakov, M.Sc. (Tech.); Olavi Myllymäki, M.Sc.(Tech); Ulla Holopainen, M.Sc.; Anu Kaukovirta-Norja, D.Sc.(Tech); Veli Hietaniemi, M.Sc.; Juha-Matti Pihlava, D.Sc.; Kaisa Poutanen, D.Sc.(Tech); Pekka Lehtinen, D.Sc.(Tech)

Abstract: Effects of lipid removal on the fine milling and air classification processing of oats were studied. Lipid removal by super¬critical carbon dioxide (SC-CO2) 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.

Dear Editor,

Please find enclosed our manuscript entitled "Lipid removal enhances separation of oat grain cell wall material from starch and protein" for submission in Journal of Cereal Science. We hope that you will find it suitable for publication.

Yours sincerely

Juhani Sibakov

Dear Editor-in-Chief,

Thank you for your decision to review our paper entitled "Lipid removal enhances separation of oat grain cell wall material from starch and protein".

To address the reviewers' concerns, we have made the following revisions:

COMMENTS FROM THE REVIEWER #1:

"It would better qualify for publication in the Journal of Cereal Science if there was a more detailed account of what is already known about the milling properties of grain after defatting, and if there was a thorough attempt to explain the results i.e. why does defatting result in a shift in milling performance?"

ANSWER:

The lines 310-319 and 368-369 in the *discussion* are dedicated to explain more thoroughly why the defatting resulted in a shift in milling and classification performance. The biggest reason seems to be the reduced cohesion between different grain compounds, especially starch granules, after the lipids have been extracted away. In addition, the defatted material acquired significantly smaller particle size during the pin disc milling compared to the non-defatted material at the same conditions, thus enabling a more efficient fractionation.

DETAILS OF ALL THE CHANGES MADE DURING REVISION:

All the changes are marked as blue in the manuscript file.

Lines 15-16:

Abbreviations were changed into alphabetical order

Lines 32-34:

Added text: "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."

Lines 57-60:

Removed text: "In contrast to other cereals, in which the highest proportion of lipids is localized in the vicinity of the embryo, the lipids of oats are also distributed throughout the endosperm (Peterson, 2002)."

Added text: "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).”

Line 76:
“SC-CO2” → “SC-CO_2“

Line 78-79:

“SC-CO2” → “SC-CO_2“

Line 80:

“SC-CO2” → “SC-CO_2“

Lines 84-88:

Added text (moved here to *introduction* from the original *discussion*):
“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., 2001; Redmond 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.”

Line 110 (Heading 2.2.):

“Overall description of the fractionation process” → “Overall description of the extraction and fractionation processes”

Line 111:

Added text: (2 kg batch size)

Line 112:

Added text: (2310 kg batch size)

Line 113:

“supercritical (sc) CO_2” → “SC-CO_2“

Line 120 (Heading 2.3.):

“supercritical CO_2” → “SC-CO_2“

Line 121:

“supercritical CO_2-extraction” → “SC-CO_2-extraction“

Line 142:

Added text: “stainless steel”

Line 180:

“Cell^P” refers to the software of Olympus. So, there’s nothing wrong with this.

Lines 209-210:

“intensive lump formation” → “the formation of several lumps”

Lines 233-234:

Deleted text: “and also leaves residues of ethanol in the oat flakes, which could lead to off-flavours in the milling fractions.”

Line 238:

“sc-CO_2” → “SC-CO_2“

Line 302:

Added text: “previously”

Lines 307-317:

Added text: “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 present study. Defatting affects also the starch granules, so that they become less aggregated which consequently enhance 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).

Deleted text: “In non-defatted flour, cellular structures with embedded starch granules were evident even after extensive milling.”

Lines 340-345:

Added text: “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).”

Lines 345:

“Due to” → “However, due to”

Lines 363-365:

Added text: “This was most probably due to the improved milling behaviour, smaller flour particle size and altered starch granule aggregation properties, enabling better classification.”

Lines 366-367:

“The β-glucan rich fraction enables the production of “ → “The high β-glucan content enables formulations for”

Lines 378-379:

Added text: “Heikki Aro is acknowledged for his important contribution in the pilot-scale SC-CO_2 extraction.”

Lines 396-397:

Added reference: ”Doehlert, D.C., Moore, W.R., 1997. Composition of oat bran and flour prepared by three different mechanisms of dry milling. Cereal Chemistry 74, 403-406.”

Lines 445-447:

Added reference: ”Price, P.B., Parsons, J., 1979. Distribution of lipids in embryonic axis, bran-endosperm, and hull fractions of hulless barley and hulless oat grain. Journal of Agricultural and Food Chemistry 27, 813-815.”

Lines 461-463:

Added reference: ” Stevenson, D.G., Eller, F.J., Radosavljević , M., Jane, J. & Inglett, G.E., 2007. Characterization of oat products with and without supercritical carbon dioxide extraction. International Journal of Food Science and Technology, International Journal of Food Science and Technology 42, 1489-1496.”

Table 1:

“sc-CO_2” → “SC-CO_2“ (inside the table)

Table 2:

“supercritical CO_2” → “SC-CO_2“ (in the caption)

"sc-CO_2" → "SC-CO_2" (inside the table)

Table 3:

"2) Sc-CO_2" → "2) SC-CO_2" (inside the table)

Table 4:

"n.a." = "not analysed"

We wish that these revisions are sufficient for accepting this manuscript for publication.

Yours sincerely,

Juhani Sibakov

Highlights:

>The lipids of oats were removed by supercritical carbon dioxide extraction.
>Defatting enabled concentration of starch, protein, lipids and cell walls into specific fractions.
>Highest β-glucan concentration of defatted, cell wall-enriched fraction was 33.9 %.
>Highest protein concentration obtained from the endosperm fraction was 73.0 %.
>The process was industrially applicable.

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

Sibakov, J.[a], Myllymäki, O.[a], Holopainen, U.[a], Kaukovirta-Norja, A.[a], Hietaniemi, V.[b], Pihlava, J.-M.[b], Poutanen, K.[a], Lehtinen, P.[a]

[a]VTT Technical Research Centre of Finland, P.O. Box 1000 (Tietotie 2), FI-02044 VTT, Finland

[b]MTT Agrifood Research Finland, FI-31600 Jokioinen, Finland

Corresponding author: Juhani.Sibakov@vtt.fi, P.O. Box 1000 (Tietotie 2), FI-02044 VTT, Finland, Tel. +358 20 722 4080, Fax. +358 20 722 7071

Keywords: oats, beta-glucan, supercritical carbon dioxide extraction, air classification

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

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 super critical 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 specialty 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., 2001; Redmond 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 in 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 (Kokemäki, 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 2,310 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

In 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). In 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

In 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 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.

In 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 11200 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 protein-enriched fraction from the first fine fraction was made only in 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 x 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 beta-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. In 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 to 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 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. ~~and also leaves residues of ethanol in the oat flakes, which could lead to off-flavours in the milling fractions~~.

3.2. Demonstration in 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 in 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 in pilot scale; 2200 rpm in both of the separations. Particle size distributions were similar to those in 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

252 Contraplex 250 CW pin disc mill (e.g. for the second coarse fraction D_{50} / D_{90} = 273 / 441 μm in
253 pilot scale vs. 197 / 323 μm in industrial scale).

254

255 The protein-enriched fraction was separated by re-classifying the endosperm flour (first fine
256 fraction) obtained after the first air classification. Protein enrichment was possible only for the
257 defatted sample. The rotor speed of the classifier played the most significant role in the separation
258 of protein particles from starch granules. By using a classifier speed of 6 600 rpm, a 5.0 % mass
259 yield of very fine fraction with 73.0 % of protein was obtained. Higher mass yields with lower
260 protein concentration were obtained by lowering the rotor speed. For example by using a rotor
261 speed of 5600 rpm a protein concentrate with 49.3 % of protein was produced with a mass yield of
262 14.4 %.

263

264 **4.3. Characterization of fractions produced in industrial scale**

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

271

272 The most pure starch and protein fractions were obtained by re-classifying the first fine fraction.
273 The protein was recovered in the fine fraction, whereas starch was concentrated into the coarse
274 fraction. The separation was very efficient and resulted in starch- and protein-enriched fractions
275 with 77.2 % of starch (coarse) and 73.0 % of protein (fine). The protein concentrate contained a few
276 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.

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 (Mälkki et al., 2004; Wu and Doehlert, 2002; Wu and Stringfellow, 1995; Lehtomäki et al., 1990). 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 present study. Defatting affects also the starch granules, so that they become less aggregated which consequently enhance 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 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.

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 (Mohamed et al., 2009; Lapveteläinen and Aro, 1994). 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.

Acknowledgments

Funding from Academy of Finland is gratefully acknowledged. Hannele Virtanen, Arja Viljamaa, Eeva Manninen, Liisa Änäkäinen and Eero Mattila at VTT are acknowledged for their excellent assistance in chemical and microscopic analyses and Michael Bailey for critical reading of the manuscript. Heikki Aro is acknowledged for his important contribution in the pilot-scale SC-CO_2 extraction. In addition, the technological knowledge of Alfred Schorer and Michael Kuhnen at Hosokawa Alpine AG, Germany, significantly promoted the industrial scale trials.

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Figure captions

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.

Fig. 2. Microscopic pictures of the oat fractions obtained from industrial scale dry fractionation process (see Fig. 1). Row A: Acid Fuchsin / Calcofluor White staining, showing protein as red and beta-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.

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

Table 2. The concentrations of different lipids 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	SC-CO_2 -extracted flour	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

578 **Table 3.** The effect of supercritical CO_2 extraction on the concentration of β-glucan in coarse oat
579 fractions after first and second air classification steps: 1) oat flour without lipid extraction, 2) SC-
580 CO_2 extracted oat flour. Columns A, B, C and D correspond to different mass yields, which were
581 obtained by varying the classifier rotor speed.
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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
	beta-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
	beta-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
	beta-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
	beta-glucan (%)	30.0 ± 0.3	**31.2 ± 0.6**	30.1 ± 0.3	27.7 ± 0.8

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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

Table 1.

	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

Table 2.

	Unextracted flour	SC-CO_2 -extracted flour	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.

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
	beta-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
	beta-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
	beta-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
	beta-glucan (%)	30.0 ± 0.3	**31.2 ± 0.6**	30.1 ± 0.3	27.7 ± 0.8

Table 4.

	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

Figure 1.

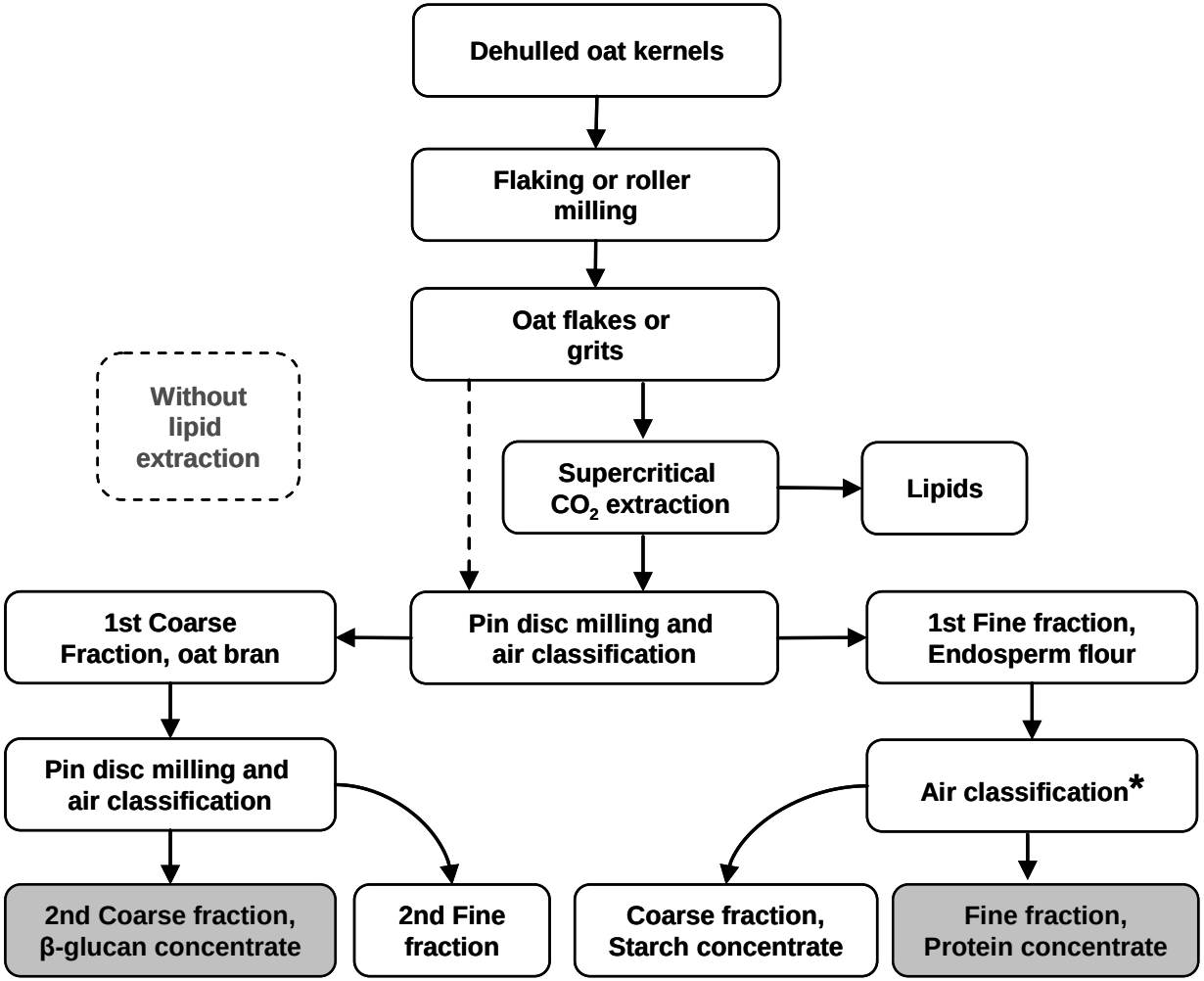
Dehulled oat kernels
Flaking or roller milling
Oat flakes or grits
Without lipid extraction
Supercritical CO_2 extraction
Lipids
1st Coarse Fraction, oat bran
Pin disc milling and air classification
1st Fine fraction, Endosperm flour
Pin disc milling and air classification
Air classification*
2nd Coarse fraction, β-glucan concentrate
2nd Fine fraction
Coarse fraction, Starch concentrate
Fine fraction, Protein concentrate

Figure 2.

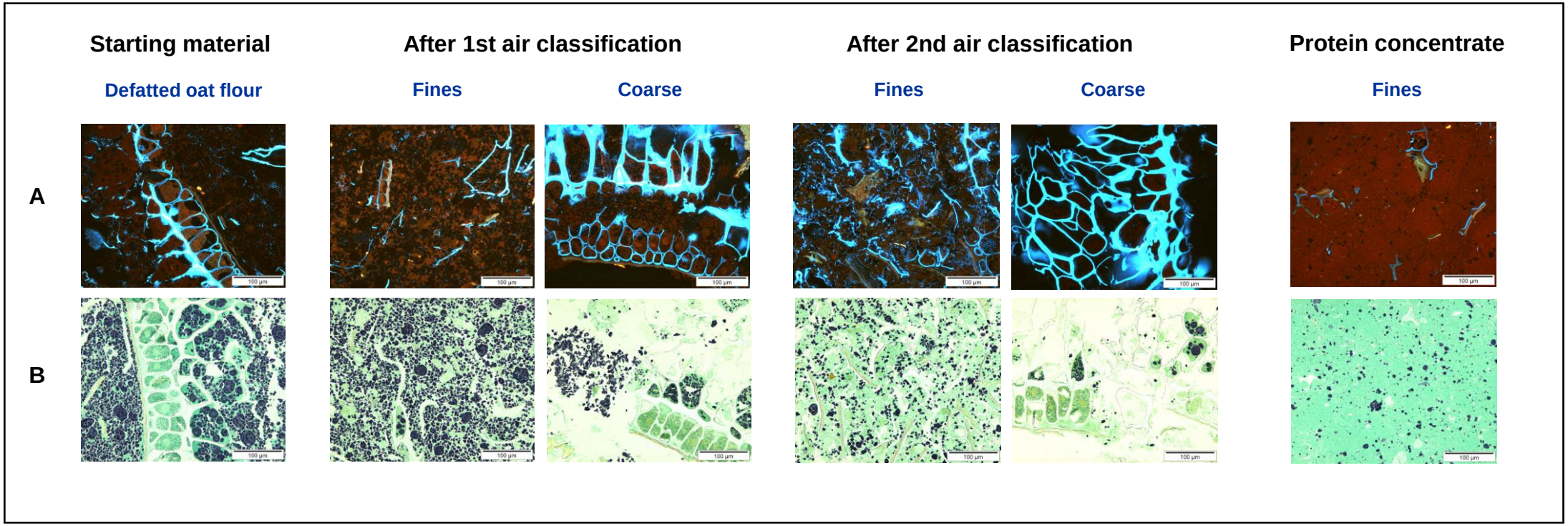
Starting material
Defatted oat flour
After 1st air classification
Fines
Coarse
After 2nd air classification
Fines
Coarse
Protein concentrate
Fines
A
B

Figure 2. in greyscale