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ScienceDirect
Food Chemistry 105 (2007) 1439–1445
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of processing on the extractability of oat β-glucan

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Received 17 November 2006; received in revised form 19 February 2007; accepted 8 May 2007

Abstract

The effects of cooking, baking, fermentation and drying on the extractability of β-glucan were studied. The β-glucan was isolated from porridge, bread and fermentate both fresh and dry. Analyses were performed using three hydrolysis methods: acid hydrolysis (AH) with H_2SO_4, lichenase hydrolysis (LH) and the modified AOAC 995.15 method (MH). As shown by the DP3:DP4 ratio, processing did not affect the structure of soluble β-glucan. Cooking increased the amount of soluble β-glucan but baking decreased it. Thus, cooking appears to be the most favourable process when health effects are concerned.

The results of the three methods were compared statistically. The AH and MH methods, which are based on measurement of glucose, gave similar results. LH, which is based on analysis of oligosaccharides, gave higher results than AH and MH.

Keywords: β-glucan; Processing; Extractability; Oats; Similarity

1. Introduction

Many studies have shown the beneficial health effects of oats (Cavallero, Empilli, Brighenti, & Stanca, 2002; Mälkki et al., 1992; Wood, 1994; Wood, Beer, & Butler, 2000). The Food and Drug Administration (FDA) of the USA has allowed a health claim that oat β-glucan may lower the risk for coronary heart disease (FDA, 1997). The lowering of blood cholesterol and glucose levels is related to the major component of soluble dietary fibre of oats, i.e., mixed-linked (1 → 3), (1 → 4)-β-D-glucan (Ripsin et al., 1992). These effects are believed to be caused by the viscosity of β-glucan (Wood, 2004). Viscosity is affected by concentration and molar mass (M_w); thus the extractability of β-glucan is essential. To acquire the desired physiological effects, both the amount and M_w of β-glucan must be at levels high-enough to produce the viscosity needed for these effects (Wood, 2004).

The effects that processing may have on extractability and M_w of β-glucan have been studied. Andersson et al. (2004) showed that neither dough making nor baking affected the cellotriosyl/cellotetraosyl ratio of barley β-glucan. They also concluded that β-glucan may be degraded by endogenous enzymes in the barley and/or wheat flour used in baking. Beer, Wood, Weisz, and Fillion (1997) showed that baking of muffins increased the extractability but decreased the M_w of β-glucan. In their study, frozen storage decreased the extractability but did not change the M_w of β-glucan. They also found that cooking of porridge did not affect the extractability or the M_w of β-glucan. Kerkhoffs, Hornstra, and Mensink (2003) found that baking of bread and cookies decreased the lowering of cholesterol. They also found that the M_w of β-glucan was decreased by baking but not by freezing. Robertson, Majsak-Newman, Ring, and Selvendran (1997) reported that cooking and digestion in the upper gut increase the extractability of barley β-glucan. Lambo, Öste, and Nyman (2005) reported a decrease in the amount of soluble β-glucan and loss of insoluble β-glucan in oats, caused by fermentation.

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0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.05.021

In structural studies, acid hydrolysis is used to determine the monosaccharide content of polysaccharides (Blakeney, Harris, Henry, & Stone, 1983; Johansson et al., 2000; Pettersen & Schwandt, 1991). In enzymatic hydrolysis, lichenase is frequently used for β-glucan (Izydorczyk, Macri, & MacGregor, 1998; Johansson, Tuomainen, Ylinen, Ekholm, & Virkki, 2004; Wood, Weisz, & Blackwell, 1994). This specific enzyme breaks the 1,4-bonds adjacent to a 1,3-bond. The products are 1,4-linked glucose-oligosaccharides with one 1,3-link at the reducing end. Both glucose and the oligosaccharides can be analysed using high-performance anion-exchange chromatography equipped with pulse amperometric detection (HPAEC–PAD) (Izydorczyk et al., 1998; Johansson et al., 2004; Wood et al., 1994). The amount of β-glucan can be measured using the AOAC (the Association of Official Analytical Chemists) 995.15 method (McCleary & Codd, 1991).

The present work is part of a large project studying the health effects of oat β-glucan in healthy adults. In clinical tests, the subjects consumed β-glucan-rich porridge, bread and a fermented oat product to gain a daily dose of $\geqslant 3$ g of β-glucan. The results will be published elsewhere.

The main aim here was to determine the effects of baking, cooking, fermentation and drying on the extractability of oat β-glucan. For this, soluble and insoluble β-glucans were isolated from the samples used in the clinical tests, i.e., porridge, bread and fermentate, and also from their starting materials, i.e., flakes, bran concentrate and bran. A second aim was to compare three different methods to analyse the amount of β-glucan in processed samples with high-β-glucan content. Corresponding comparisons have been made previously for commercial β-glucan and β-glucan isolated from oat bran and whole grain oats by our group (Johansson et al., 2006) and it seemed reasonable to test them with processed foods as well. An enzymatic method involving lichenase, which produces oligosaccharides, was compared to two glucose producing methods, e.g., acid hydrolysis and another enzymatic method involving both lichenase and β-glucosidase. The similarity of the results given by these methods was analysed statistically using a method described by Rita and Ekholm (2007).

2. Materials and methods

2.1. Materials

Commercial samples of oat bran B1 (8.0% β-glucan) and flakes F (5.7% β-glucan,) were from Raisio Ltd, Nokia, Finland. Additional oat bran high in β-glucan B2 (12.3% β-glucan) was obtained from Finn Cereal Ltd (Vantaa, Finland). Fermented oat bran (FB1) was taken from commercial processing before flavouring (Bioferme Oy, Piispanristi, Finland, Avenly Oy Ltd, Helsinki, Finland). The abbreviations of materials are listed in Table 1.

Water was purified with a Milli-Q-Plus system (Millipore Corporation, Bedford, MA, USA). The malto-oligosaccharides with degrees of polymerization (DP) 3–6 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Glucose was obtained from J. T. Baker (Deventer, The Netherlands). Lichenase (E.C. 3.2.1.73) was obtained from Megazyme International (Wicklow, Ireland). The β-glucan content in the samples was determined using a β-glucan assay kit obtained from Megazyme International. All other chemicals used were the purest obtainable.

Table 1
Abbreviations for names of samples

Starting materials	F	Flake
	B1	Bran
	B2	Bran concentrate
Porridge	P	Porridge
	Pdry	Porridge dry
Bread	BB2	Bread
	BB2dry	Bread dry
Fermentate	FB1	Fermentate
	FB1dry	Fermentate dry

2.2. Methods

2.2.1. Preparation of samples

The sample materials were prepared at the test bakery in Cereal Technology Department at the University of Helsinki. Oat porridge (P) was prepared by adding the oat flakes (11% of oat flakes by total volume) into boiling water and cooking the porridge for 10 min. Oat bread (BB2) was baked using commercial wheat flour, oat bran concentrate (B2), salt, yeast and water. The bread was baked with straight-dough process and oat bran concentrate was added to reach β-glucan content of 1.7% db. Commercial yoghurt type oat bran fermentate (FB1) made of bran B1, was used as such. The soluble and insoluble fibre fractions were extracted from porridge, bread and fermented oat, both fresh and after drying overnight at 60 °C (The American Association of Cereal Chemists (AACC) 44-15A) and also from the starting materials. The dried sample materials were ground with a Tecator Cyclotec® mill (Foss North America, Eden Prairie, MN, USA) to pass a 0.5 mm screen before extraction of the fibre fractions.

Soluble fibre was extracted from the sample materials, both fresh and dry, by enzymatic digestion with the modified method of Asp, Johansson, Hallmer, and Siljeström (1983) (Anttila, 2006, unpublished). The starch was hydrolysed with heat-stable amylase in a boiling-water bath and the remaining protein with pepsin and pancreatin enzymes. The solutions were centrifugated and the insoluble pellet was washed four times with 100 ml of Milli-Q-water, then centrifuged to remove all the remaining soluble materials and dried over night at 70 °C. The extraction was performed 16 times for all the sample materials and pooled for structural analysis. The samples obtained are listed in Table 1.

The soluble β-glucans were precipitated from the soluble fibre fractions with aqueous 60% ethanol. Ethanol was slowly added with stirring and the solution kept overnight at 4 °C and centrifuged. The precipitate was washed twice with water, centrifuged and freeze-dried.

The insoluble β-glucans were extracted from the insoluble fibre fractions as previously described by Johansson et al. (2004) and Virkki, Johansson, Ylinen, Maunu, and Ekholm (2005). The fractions were first extracted with $Ba(OH)_2$ to remove the arabinoxylans and the precipitates further extracted with water to dissolve the β-glucans. The solutions were dialysed extensively against water and freeze dried.

2.2.2. Hydrolyses

Acid hydrolysis (AH) is used for analysing the monosaccharide content of samples. It was performed here with 1.5 M H_2SO_4 as described by Johansson et al. (2006). The hydrolyses were performed for 1 h at 120 °C in an autoclave. The glucose thus produced was analysed with HPAEC–PAD using hydrolysed glucose as standard. The free glucose residues were corrected by a factor of 0.9 to anhydro sugar, as present in polysaccharides (McCleary & Codd, 1991).

Enzymatic hydrolysis with lichenase (LH) allows the determination of the amounts of oligosaccharides and can be used to analyse the structure and content of β-glucan. The hydrolysis was performed as described by Johansson et al. (2000). The β-glucans were dissolved in phosphate buffer (4.0 ml, 20 mM, pH 6.5) and incubated with lichenase (100 U) at 60 °C for 2 h. The resulting solution was again incubated in a boiling-water bath for 10 min to inactivate the lichenase. The oligosaccharides thus produced were analysed with HPAEC–PAD.

The β-glucan contents of the samples were determined using the modified AOAC 995.15 method (McCleary & Codd, 1991) (MH) as described by Johansson et al. (2006). The modified method allows samples with high-β-glucan content to be analysed reliably. The samples were treated with isopropanol (IPA) prior to analyses at room temperature overnight, then dried first in an oven and then in a vacuum oven at 80 °C for 4 h. In the MH method the amount of lichenase was 0.2 ml (10 U), the amount of buffer in lichenase hydrolysis was 8 ml and the incubation time was 3 h. When appropriate, the solutions were diluted 2–4-fold for treatment with β-glucosidase. The glucose produced was determined spectrophotometrically at 510 nm as in the original method.

2.2.3. Analytical methods

HPAEC–PAD analyses of glucose from the AH and oligosaccharides from the LH were performed as previously described by Johansson et al. (2004, 2006). The analytical column was a CarboPac PA1 (250 × 4 mm, i.d.) and the guard column a PA1 (25 × 3 mm, i.d.) (Dionex, Sunnyvale, CA, USA). All samples were filtered before analysis (0.2 μm Acrodisc 13 GHP filter; Pall Corporation, Ann Arbor, MI, USA).

The monosaccharide analyses after AH were performed using isocratic elution with 8 mM NaOH. Quantitation was performed with glucose hydrolysed by the same process as the samples. Three parallel samples were analysed.

The oligosaccharide analyses were performed with a linear gradient using 50 mM NaOH and 500 mM sodium acetate in 150 mM NaOH. Quantitation was performed by establishing calibration curves for malto-oligosaccharides with DP 3–6 since true standards 3-*O*-β-cellobiosyl-D-glucose, 3-*O*-β-cellotriosyl-D-glucose, etc., are not available. All analyses were performed in triplicate.

2.2.4. Statistical analyses

The AH, MH and LH methods used for determination of the amount of β-glucan were applied for samples of soluble β-glucans from fresh and dry porridge, bread and fermentate as well as their starting materials, i.e., flakes, bran concentrate and bran. For the MH method, no observations were available for dried fermentate, due to the limited amount of the sample. Hence, nine samples with three replicates were analysed with AH and LH whereas for MH, there were only eight samples. In total, 78 determinations were made. All statistical analyses were performed using Statistix 8.0 software (Analytical Software, Tallahassee, USA).

In comparing the methods we hypothesized that (1) the results of the AH and MH methods would be similar, because they are both based on total hydrolysis to glucose, and that (2) the similarity between the AH and LH methods would not be as close as between AH and MH because in LH the hydrolysis products are oligosaccharides instead of glucose.

The statistical analysis of such similarity studies can be found in Ekholm, Jouttijärvi, Priha, Rita, and Nurmesniemi (2007), which is based on Schuirmann (1987). The main point in them is that lack of statistically significant difference between the methods is not enough to show their similarity. Since we had no basis for assigning a numerical value for testing the similarity parameter θ (Rita & Ekholm, 2007), an explorative approach was taken: it answers the question 'how close to each other can the results of the two methods be expected to be in the light of the present data'. The answer is given as the potential data supported similarity limit (PDS).

The effects of processing (i.e., cooking, baking, fermentation) on the amount of β-glucan were studied for the starting materials versus products as well as for fresh versus dried products. The change in amount of β-glucan was measured as the difference in percentages based on AH.

Cooking and fermentation were expected to increase the amount of water-soluble β-glucan (Degutyte-Fomins, Sontag-Strohm, & Salovaara, 2002; Robertson et al., 1997), whereas baking was expected to decrease it (Kerkhoffs et al., 2003). These one-sided hypotheses were tested separately for each pair of starting material and product. On

the other hand, drying was expected to decrease the amount of soluble β-glucan, but not considerably (Beer et al., 1997). These hypotheses postulated a (left) similarity for the fresh and dried samples. Again, only an explorative approach was possible, resulting in PDS left similarity limit for the fresh and dried samples. The approach is an obvious modification of the two-sided approach of Rita and Ekholm (2007).

3. Results

3.1. Structural analyses

The β-glucans extracted from the soluble and insoluble fibre fractions were analysed using three methods. First they were acid hydrolysed (AH) to glucose, secondly analysed for their β-glucan contents as glucose by the modified AOAC method (MH), and thirdly digested with lichenase to oligosaccharides (LH). The results of these analyses are shown in Table 2. The yields of soluble β-glucan from the precipitate received after ethanol precipitation from the soluble fibre fraction ranged from 37% to 77% of the precipitate. For the insoluble fraction the yields of β-glucan were from 20% to 49%. Other monosaccharides than glucose, or oligosaccharides other than those expected, were not found in the chromatograms. The amount of insoluble β-glucan in dry porridge (Pdry) obtained with LH was considerably lower than in the other samples.

Digestion with lichenase produces the structural components of β-glucan, i.e., oligosaccharides with DP $\geqslant$ 3. The percentages of oligosaccharides as well as the ratios DP3:DP4, DP3:DP5 and DP3:DP6 are shown in Table 3. The DP3:DP4 ratio is higher for the insoluble than for the soluble samples. For the ratio DP3:DP6, the values are between 21 and 47 except for the dry fermented sample whose value is 6.7, due to the high-amount of DP6; the ratios DP3:DP5 do not differ significantly.

Varying amounts of arabinose and xylose were found in some samples and were calculated by comparing the heights of their signals in the chromatograms to those of arabinose and xylose signals in a standard solution. The sum of arabinose and xylose was about 7% in soluble β-glucan samples from fresh bread and dry bread but in the remaining samples the amounts were negligible. All insoluble β-glucan samples contained arabinose and xylose. The sum was 3–4% for oat bran, oat bran concentrate, and fresh porridge whereas for fresh and dry breads they were 16% and 13%, respectively. For dry porridge and dry fermentate the sums were 10–11%.

Table 2
Percentages of soluble β-glucan from the soluble fibre fraction and of insoluble β-glucan from the insoluble fibre fraction analysed with three methods: AH = acid hydrolysis; MH = modified AOAC 995.15; LH = lichenase hydrolysis

Sample	AH% ± s.d.	MH% ± s.d.	LH% ± s.d.
Soluble			
F	45.6 ± 3.4	38.4 ± 0.2	43.6 ± 5.3
P	55.5 ± 3.8	50.8 ± 2.0	66.4 ± 2.5
Pdry	69.1 ± 4.6	62.7 ± 1.5	77.7 ± 0.8
B2	53.7 ± 1.1	55.6 ± 2.3	62.6 ± 9.1
BB2	49.3 ± 4.5	46.7 ± 4.0	46.6 ± 4.1
BB2dry	43.1 ± 3.7	37.8 ± 2.8	37.6 ± 0.6
B1	44.9 ± 1.2	44.8 ± 0.5	46.6 ± 6.6
FB1	69.8 ± 3.0	65.1 ± 1.8	71.3 ± 7.4
FB1dry	55.8 ± 4.8	n.d.	56.0 ± 6.6
Insoluble			
F	n.d.	n.d.	39.2 ± 9.8
P	35.2 ± 10.7	40.2 ± 1.0	41.3 ± 10.3
Pdry	30.2 ± 3.8	27.6 ± 1.1	4.8 ± 3.3
B2	45.1 ± 10.1	48.9 ± 0.7	48.5 ± 7.9
BB2	23.9 ± 3.8	19.8 ± 0.7	24.7 ± 7.3
BB2dry	27.6 ± 2.9	26.9 ± 0.8	20.0 ± 3.7
B1	51.0 ± 1.5	n.d.	39.9 ± 9.2
FB1	n.d.	n.d.	22.4 ± 9.5
FB1dry	36.7 ± 1.9	38.8 ± 3.0	39.3 ± 7.9

The results of LH are the sums of oligosaccharides with DP 3–6. n.d. = not determined.

3.2. Method comparison

Table 4 summarizes the results of the statistical analyses for comparison of methods. Across all eight samples the difference between the AH and MH methods was only 2.47%. This corresponds to PDS similarity limit of 4.89%, which is far better than the corresponding limit of 11.13% for AH–LH comparison. This is consistent with our hypothesis on the similarity behaviour of the three methods: the glucose producing methods AH and MH are more similar than AH and LH, which is the method producing oligosaccharides. Among the subgroups, AH and MH showed the tightest similarity for starting materials and fresh products. The similarity was poorest for dried products, which could be explained only partly by the smaller number of observations ($n = 6$), since the standard deviation (s.d.) among dried product measurements was only 5.35, similar to that for starting materials.

Comparison of AH and LH showed somewhat differing results, especially for dried products. Among them, despite the smaller number of observations, the PDS limit was almost as good as for starting materials, whereas for fresh products, the PDS limit was worst of all. This was partly due to the large standard deviation, but the observed difference (−10.94) was largest as well. Methods AH and MH thus show closer similarity than AH and LH, except for dried products.

3.3. Processing

Table 5 shows the results of statistical analyses for the effects of processing. For AH, the data were in accordance with all the hypotheses regarding the direction of effects during processing. One of these, bran to fermentate, was not statistically significant, due to a considerably larger s.d. than for cooking and baking. For the other two methods, MH and LH, the results were qualitatively similar to

Table 3
Yields of oligosaccharides DP3–DP6 produced with lichenase hydrolysis as analysed with HPAEC–PAD

	DP3 (m%)	DP4 (m%)	DP5 (m%)	DP6 (m%)	Total (m%)	DP3/DP4 (m%)	DP3/DP5 (m%)	DP3/DP6 (m%)
Soluble								
F	25.4	16.2	1.3	0.8	43.6	1.6 (2.1)[a]	19.8	33.2
P	37.6	25.8	1.9	1.1	66.4	1.5 (1.9)[a]	19.6	33.2
Pdry	43.9	30.2	2.2	1.5	77.7	1.5 (1.9)[a]	20.0	29.7
B2	35.7	23.9	1.8	1.2	62.6	1.5 (2.0)[a]	20.3	30.5
BB2	27.0	17.4	1.4	0.7	46.6	1.6 (2.1)[a]	19.1	37.0
BB2dry	21.7	14.2	1.1	0.5	37.6	1.5 (2.0)[a]	19.2	44.4
B1	26.7	17.3	1.3	1.3	46.6	1.5 (2.0)[a]	19.8	20.8
FB1	40.6	27.2	2.1	1.3	71.3	1.5 (2.0)[a]	19.1	30.4
FB1dry	31.8	17.8	1.7	4.8	56.0	1.8 (2.4)[a]	18.6	6.7
Insoluble								
F	24.1	13.1	1.4	0.7	39.2	1.8 (2.4)[a]	17.7	34.8
P	23.6	15.4	1.4	0.9	41.3	1.5 (2.0)[a]	16.8	25.8
Pdry	3.1	1.5	0.2	0.1	4.9	2.0 (2.6)[a]	14.7	42.3
B2	27.8	17.7	1.8	1.1	48.5	1.6 (2.1)[a]	15.3	24.8
BB2	15.7	7.7	0.9	0.5	24.7	2.0 (2.7)[a]	17.0	34.6
BB2dry	13.0	5.9	0.7	0.3	20.0	2.2 (2.9)[a]	17.8	37.2
B1	25.7	12.3	1.3	0.5	39.9	2.1 (2.8)[a]	19.8	47.4
FB1	14.7	6.8	0.7	0.3	22.4	2.2 (2.9)[a]	20.9	46.4
FB1dry	22.3	14.6	1.5	0.9	39.3	1.5 (2.0)[a]	14.6	24.7

Values for soluble samples are calculated from the precipitate received after ethanol precipitation. For insoluble samples the values are calculated from the freeze dried materials.

[a] Values in parentheses are mol%.

Table 4
Comparison of the AH, MH and LH methods (AH = acid hydrolysis; MH = modified AOAC 995.15; LH = lichenase hydrolysis) based on difference in percentages of β-glucan ($n = 3$)

Samples	Number of observations per method	Difference of AH and MH ± s.d.	PDS similarity limit θ[a]	Difference of AH and LH ± s.d.	PDS similarity limit θ[a]
All samples	24	2.47 ± 6.91	4.89	-5.55 ± 15.94	11.13
Starting material	9	1.83 ± 4.78	4.79	-2.83 ± 8.71	8.23
Fresh product	9	0.86 ± 9.21	6.56	-10.94 ± 23.61	25.58
Dried product	6[b]	5.87 ± 5.35	10.27	-1.55 ± 8.38	8.44

[a] Potential data-supported similarity limit, i.e., the smallest (positive) percentage θ such that the difference in β-glucan percentages based on AH and MH (or AH and LH) could statistically be shown to be within the interval $(-\theta, \theta)$. Small values of θ indicate close similarity between the method results. For details see Rita and Ekholm (2007).

[b] Porridge and bread only.

Table 5
Effect of processing on the amount of soluble β-glucan (average product percentage minus starting material percentage) ($n = 3$)

Method of processing	Method AH		Method MH	Method LH
	Difference ± s.d. (hypothesized sign)[a]	PDS change to the hypothesized direction[b]	Difference ± s.d.	Difference ± s.d.
Cooking	$9.83 \pm 0.81^{***}$ (>0)	8.46	12.40 ± 0.92	22.87 ± 3.07
Baking	$-4.63 \pm 2.66^{*}$ (<0)	−0.14	-8.93 ± 4.86	-16.03 ± 5.27
Fermentation	15.63 ± 15.12^{ns} (>0)	−9.85	20.30 ± 1.39	38.33 ± 17.53

ns: not significant.

[a] Statistical significances are given for one-sided tests.

[b] Potential data-supported change, i.e., the largest percentage by which the difference in β-glucan percentages could statistically be shown to differ from zero. The value is calculated as the endpoint of the one-sided (corresponding to the direction of the hypothesis) 95% confidence interval.

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

AH and again the hypothesized directions for the effects of processing were met. For fermentation, the results remain uncertain.

The statistical analyses of dried products showed that the amount of soluble β-glucan was decreased in drying for bread and fermentate. Due to large s.d., the PDS left similarity limits

were large (10.89 for porridge, −14.06 for bread, −39.35 for fermentate). This is not surprising, because with such a small data group, the precision remains low. For porridge, our hypothesis of decrease was not met, since the amount of soluble β-glucan increased. Again, fermentate showed the widest variation.

4. Discussion

Processing clearly affected the extractability of β-glucan. The amount of soluble β-glucan in flakes was lower than in porridges. Thus the hypothesis that cooking increases extractability, was met. β-Glucan is integral with cellulose and other noncellulosic polysaccharides in the cell wall and cooking releases it from the matrix (Buckeridge, Rayon, Urbanowicz, Tiné, & Carpita, 2004; Fincher & Stone, 1986; Robertson et al., 1997). Baking with wheat flour decreased the amount of soluble β-glucan as hypothesized. The enzymes of wheat flour used in baking of bread can cause loss of β-glucan (Andersson et al., 2004). Beer et al. (1997) showed that baking of bread decreases the M_w of β-glucan and increases its extractability by in vitro digestion when no wheat flour is used. When flour was added, the amount of soluble β-glucan did not change. For fermentation, the hypothesis was also met since our results showed increased extractability; however, the large s.d. made the results unreliable. Degutyte-Fomins et al. (2002) showed that fermentation of oat bran by rye sourdough starter increased the solubility. These findings are, however, not in agreement with those of Mårtensson, Öste, and Holst (2002) and Lambo et al. (2005), who showed that fermentation decreases the amount of β-glucan. Drying decreased the amount of soluble β-glucan in bread and fermentate, which was in accordance with our hypothesis. This observation is in agreement with Beer et al. (1997), who showed that freezing, in which water is repelled from the polysaccharide structure, decreases the amount of soluble β-glucan in muffins. However, for porridge the amount of soluble β-glucan increased in drying.

The higher DP3:DP4 ratio (Table 3) noted for insoluble than for soluble samples is in agreement with our previous results for whole-grain β-glucans of oats and barley (Johansson et al., 2004). A higher ratio indicates additional cellotriose units which may increase the possibility for helix-formation and stronger aggregation (Böhm & Kulicke, 1999; Cui, Wood, Blackwell, & Nikiforuk, 2000; Tvaroska, Ogawa, Deslandes, & Marchessault, 1983). The only exception was the dry fermented sample in which the ratio was higher for the soluble (1.8) than for the insoluble sample (1.5) and also highest for all soluble samples. This indicates that drying of fermentate caused β-glucans rich in cellotriose units to be concentrated in the soluble rather than the insoluble fraction. In other samples there was no difference in the DP3:DP4 ratios between starting materials and processed samples indicating that the structure of soluble β-glucan is not affected by processing. Andersson et al. (2004) studied breads made of barley and wheat. They found no change in the DP3:DP4 ratio of β-glucan (total β-glucan without prior isolation) of dough after mixing and fermentation as compared to flour. The DP3:DP6 ratio was similar for all samples except for the dry fermented sample where the value was 6.7.

There are differences in the DP3:DP4 ratios for insoluble β-glucans, but they do not allow conclusions to be drawn on the effects of processing. The ratios are around 2 for all samples except for porridge (1.5), bran concentrate (1.6) and dry fermentate (1.5).

The yields of β-glucan were low for both the soluble and insoluble samples.For the soluble samples, this may have been due to co-precipitation. The amount of proteins was negligible in the β-glucan fractions. The co-precipitated substances remained in the precipitate since the samples were not purified. The methods used for analyses have been successfully applied by Johansson et al. (2004, 2006) and Virkki et al. (2005) and have proven their reliability.

The insoluble samples contained small amounts of arabinoxylan. This finding is in agreement with the work of Johansson et al. (2004) for whole-grain oats and barley, where it was shown that part of the arabinoxylan is co-extracted with β-glucan. The soluble samples extracted from breads contained considerable amounts of arabinoxylan which derives from the wheat flour used for baking the breads. Arabinoxylan was not found in the other soluble samples.

5. Conclusions

All processing methods affected the extractability of β-glucan. Cooking released more soluble β-glucan while baking decreased the amount of soluble β-glucan, probably due to enzyme activity in the wheat flour towards β-glucan. Fermentation appeared to increase the amount of soluble β-glucan but the results are unreliable. In the present study, drying decreased the amount of soluble β-glucan in bread and fermentate but not in porridge. Thus, cooking is probably the most favourable process when it comes to health effects in which solubility plays a major role.

Structural differences between soluble and insoluble β-glucans were seen in the DP3:DP4 ratio. Processing did not result in differences in the DP3:DP4 ratio of soluble β-glucans except for dry fermentate.

The hypothesis concerning the similarity of the results of the glucose producing methods AH and MH gained support in this study. As hypothesized, there was less similarity between the glucose-based method AH and the oligosaccharide-based method LH than between AH and MH.

Acknowledgement

This work was financially supported by the National Technology Agency of Finland.

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