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# Dietary fibre in fermented oat and barley β-glucan rich concentrates

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**Abstract**

The ability of different lactic acid bacteria to influence the physicochemical characteristics (content, viscosity and molecular weight) of dietary fibre in β-glucan-rich barley and oat concentrates was investigated. The cultures used were *Lactobacillus acidophilus* and the exopolysaccharide producing strain *Pediococcus damnosus* 2.6, together with the yoghurt culture, V2 (a mixture (1:1) of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*). Two methodologies, one including filtration and another centrifugation-dialysis, to quantify the dietary fibre, were compared. The centrifugation-dialysis method generally gave significantly ($P < 0.05$) higher values than the filtration method (for example, 79.8 g/100 g DW versus 59.6 g/100 g DW for the total fibre in the native barley fibre concentrate) with the exception of soluble barley fibres. The insoluble fibre content was found to decrease after fermentation (58.8 g/100 g DW to 39.0/37.0 g/100 g DW in barley and 26.0 g/100 g DW to 4.5/3.0 g/100 g DW in oats as analysed by the centrifugation-dialysis method). The soluble fibre in the barley fibre concentrate was apparently not affected by fermentation, while contents and maximum viscosities of the soluble fibre in oat fibre concentrates decreased after fermentation. However, the molecular weight was apparently not affected.


*Keywords:* Oats; Barley; Polysaccharides; Dietary fibre; β-glucans; Physicochemical characteristics; Viscosity; Molecular weight; Fermentation; Lactic acid bacteria; Cereals

## 1. Introduction

The beneficial effects of healthy diets on quality of life and on the cost-effectiveness of health care has prompted the food industry to face the challenge of developing new food products with special health-promoting characteristics. Meeting this challenge involves the identification of new sources of nutraceuticals, as well as other nutritional and natural materials with the desirable functional characteristics. Barley and oats are examples of such sources and could be good bases for functional food products.

Cereals are an important source of dietary fibre, contributing to about 50% of the fibre intake in western countries (Nyman, Björck, Siljeström, & Asp, 1989). The hemicellulosic polysaccharides of rye and wheat are composed mainly of pentosans (arabinoxylans), whereas those in oats and barley are composed mainly of β-glucans (Selvendran & Verena, 1990). Both barley and oats have been reported to be effective in lowering total serum- and LDL-cholesterol in humans and animals, the effect being attributed to the content of β-glucans (Behall, Scholfield, & Hallfrisch, 1997; Bell et al., 1999; Federal register, 1997; Maier, Turner, & Lupton, 2000; McIntosh, Whyte, McArthur, & Nestel, 1991). Furthermore, a study on hypercholesterolaemic men, for 5 weeks, has shown that a milk-like oat product that contributed with 3.8 g/d of oat β-glucans significantly reduced total serum- and LDL-cholesterol by 6% (Onning et al., 1999). Similarly, in a study by McIntosh et al. (1991), it was demonstrated that plasma LDL-cholesterol concentrations were lowered by 7% in mildly hypercholesteroaemic men, who consumed 8 g/d of barley β-glucans for 4 weeks.

Recent studies have shown that oat-based media could be suitable for the growth of lactic acid bacteria (LAB) and also for the formation of microbial or exopolysaccharides (EPS) (Mårtensson, Öste, & Holst,

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2002a; Mårtensson et al., 2002b). This is interesting because EPS are capable of improving the texture and viscosity of the final product (Ricciardi, Parente, & Clementi, 1994) and may also improve both sensory and nutritional properties. Some of the cultures used in the dairy industry have also been described as promoters of ropiness or mouth-feel, because of their texture-enhancing properties (Sutherland, 1998). Moreover, yoghurts with EPS-producing strains have demonstrated less shear-thinning behaviour in comparison with yoghurt made with non-EPS producing strains (Sutherland, 1977). This structural property would probably give rise to a new generation of *in situ* produced thickeners, which could decrease the need of stabilisers in yoghurt (Cerning, 1995). In addition, a study conducted by Nakajima, Suzuki, Kaizu, and Hirota (1992), in which rats were fed a ropy milk product containing a phospho-polysaccharide, revealed a reduction in serum lipids.

Lactic acid bacteria are also capable of producing EPS with a β-glucan structure, as described by Dueñas-Chasco et al. (1997), who isolated straight-chained β-(1 → 3) glucans with β-(1 → 2) glucose monomers linked to the interior chain from *Pediococcus damnosus* 2.6, that is β-glucans with somewhat different structure from those found in oats and barley that contain linear β-glucans with alternating (1 → 3) and (1 → 4) linkages. The physiological effects of this LAB produced EPS may depend on their ability to resist degradation by gastrointestinal enzymes, and thus behave like a type of dietary fibre. However, nothing is known about their physicochemical properties or physiological effects.

The aim of the present study was to investigate how different lactobacilli strains could affect the content of "dietary fibre components", especially the β-glucans, and their physicochemical characteristics (viscosity and molecular weight) in barley and oat fibre concentrates. Further, as there may be a degradation of dietary fibre polysaccharides to smaller fragments during fermentation, these may be soluble in 80% ethanol and as a consequence there will be loss of fibre (Johansson, 1987) when using one of the conventional fibre methods (Asp, Johansson, Hallmer, & Siljestöm, 1983; Englyst, Cummings, & Wood, 1987; Prosky, Asp, Schweizer, deVreis, & Furda, 1988; Theander, Åman, Westerlund, Andersson, & Pettersson, 1995). Therefore another methodology was also used, including centrifugation and dialysis instead of precipitation with ethanol and filtration.

## 2. Materials and methods

### 2.1. Bacterial strains

Lactobacilli strains with optimal growth temperatures of 28 and 43 °C were used. The EPS-producing strain, *Pediococcus damnosus* 2.6 (Pd 2.6), was obtained from the collection at the University of San Sebastian (Spain, UPV). The strain was stored at −80 °C in de man Rogosa Sharpe (MRS) broth (De Man, Rogosa, & Sharpe, 1960) containing 25% (v/v) glycerol. The commercial yoghurt culture V2 was a 1:1 mixture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (Visby Tønder nder, Denmark). *Lactobacillus acidophilus* (La5) was obtained from Christian Hansen A/S, Hørsholm, Denmark. V2 and La5 were stored at −80 °C according to the recommendation of the manufacturer. These two cultures were chosen because V2 is the starter culture commonly used in Sweden and La5 is known to improve aroma and acidity in the final product.

### 2.2. Sample preparation and fermentation procedure

Native oat and barley fibre concentrates, with dry matter contents between 8% and 10%, were obtained from Ceba Foods AB (Lund, Sweden). Oats had been treated enzymatically, ultrafiltrated and dialysed as previously described (Oste, 1999). Barley was treated in a similar manner. The substrates glucose or sucrose (1%, w/v) were added to the cereal fibre concentrates prior to inoculation with V2/Pd 2.6 and V2/La5, respectively. After heat-treatment at 90 °C for 5–10 min, the cereal fibre concentrates were cooled to fermentation temperature (28 or 37 °C for V2/Pd 2.6 and V2/La5, respectively) and then inoculated. The fermentations were performed over a period of about 20 h. A 0.02% portion of the V2 and La5 cultures was used as inoculum. Pd 2.6 inoculate (5% v/v) was taken from a fresh (20-h incubation) pre-inoculum. The pre-inoculum medium was cereal-based and inoculated with 5% of an exponentially growing Pd 2.6 in MRS broth (Merck, Darmstadt, Germany) and then incubated for approximately 20 h at 28 °C. The final pH of the fermented products was $4.0 \pm 0.3$. All samples were lyophilised using a Labconco lyphlock 12 freeze-dry system and milled to a particle size of less than 0.3 mm in a Cyclotec mill (Tecator AB, Höganäs, Sweden). Moisture content was determined by drying to constant weight at 105 °C.

### 2.3. Analytical methods

#### 2.3.1. Analytical steps

A simplified flowchart of the quantification and characterisation steps used is shown in Fig. 1.

#### 2.3.2. Protein

The nitrogen was assayed by the Kjeldahl procedure (Kjeltec System 1003, Tecator AB, Sweden) according to the manual. Protein was calculated as $N \times 6.25$.

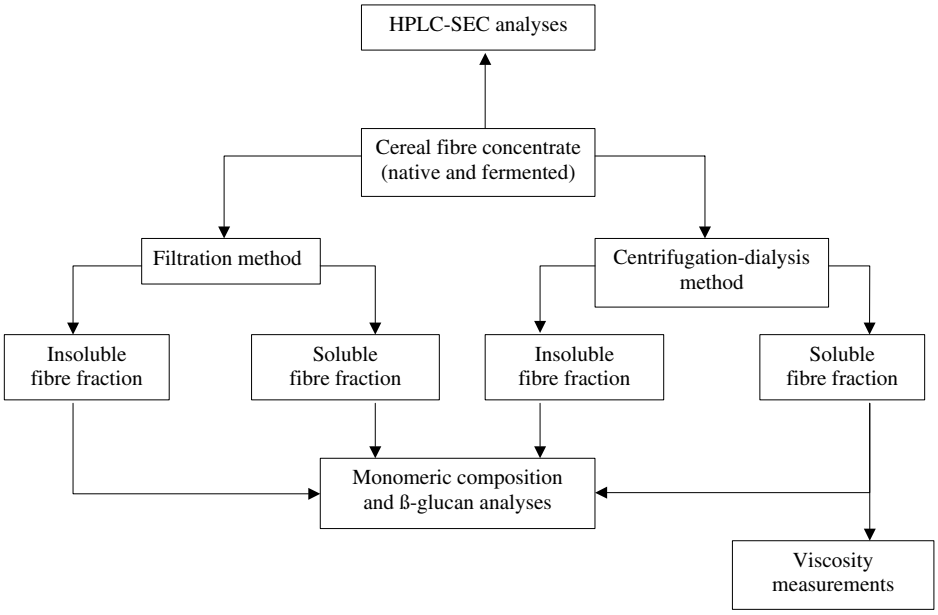


Fig. 1. Simplified flowchart of the steps used in the quantification and characterisation of native and fermented oat and barley fibre concentrates.

*2.3.3. Lipids*

Lipid content was determined gravimetrically by extraction in diethylether and petroleum ether (b.p. 40–60 °C; 1:1) after hydrolysis with 7.7 N HCl at 70–80 °C for 60 min (AOAC, 1980).

*2.3.4. Starch, ash, lactic acid and low-molecular weight carbohydrates*

Total starch was quantified as described by Björck and Siljeström (1992).

Ash content was determined by incineration at 550 °C for at least 5 h, cooling in a desiccator and then weighing.

The amount of lactic acid was analysed by a GLC method (Richarson, Calder, Stewart, & Smith, 1989).

The low molecular weight carbohydrates (glucose, sucrose and fructose) were analysed using an enzyme kit (Food Diagnostics ApS, Denmark).

*2.3.5. Dietary fibre – filtration method*

Dietary fibre was isolated using the principle of the method developed by Asp et al. (1983). However, as starch hydrolysis had already been carried out during the production of the cereal fibre concentrates, the termamyl step was excluded. Fibre fractions were also corrected for remaining proteins, lipids, starch and ash.

*2.3.6. Dietary fibre – centrifugation-dialysis method*

Samples (2 g) were weighed to 0.1 mg accuracy and transferred to an Erlenmeyer flask. Sodium phosphate buffer (pH 6.0) was added, with thorough mixing and the pH adjusted to 1.5 with HCI solution (0.2 N). The electrode was rinsed with a few millilitres of distilled water, pepsin (200 mg, 2000 FIP-U/g, Merck, Darmstadt, Germany) was added and the samples were incubated for 60 min in a water bath (40 °C) with agitation. The pH was then adjusted to 6.8 with NaOH and pancreatin (200 mg, activity equivalent to 8× USP specifications, Sigma, St Louis, USA) was added. The samples were then incubated at 40 °C for another 60 min, centrifuged for 30 min at 4000 rpm and the supernatants were collected. The pellets were washed by suspension and recentrifuged with $3 \times 30$ ml distilled water, 95% and 99% ethanol, air-dried overnight and then vacuum-dried (50 °C for 24 h). The pellets were cooled to room temperature in a desiccator and then weighed as the insoluble fibre fractions. The supernatants and distilled water washings were combined and dialysed (molecular weight cut-off = 1000 Da) against distilled water (5 l) for 36–48 h, changing the water twice daily. The dialysates were lyophilised and weighed as the soluble fibre fractions. The fibre fractions were corrected for remaining starch, protein, lipids and ash.

*2.3.7. Monomeric composition of the dietary fibre*

The composition of the isolated fibre fractions from the two methods was analysed by gas–liquid chromatography (GLC) on a DB-225 column (J&W Scientific, Folsom, CA, USA) for the neutral sugars as their alditol acetates, and spectrophotometrically for the uronic acids, as described by Theander et al. (1995).

*2.3.8. β-glucans*

The amounts of β-glucans in the soluble and insoluble fibre fractions were analysed using an enzyme kit (Megaenzyme International, Co. Wicklow, Ireland).

The assay procedure was based on the McCleary method for mixed-linkage β-glucans (McCleary & Codd, 1991), which has been approved by the AACC (method 32-23) and the AOAC (method 995.16).

*2.3.9. Molecular weight*

The native and fermented cereal fibre concentrates (0.5 g) were dissolved in 0.1 M NaOH containing 0.1% $NaBH_4$ with gentle stirring overnight and injected (70 μl) into the HPLC-SEC system without further manipulations.

The HPLC-SEC (size exclusion chromatography) instrument employed consisted of an M-590 pump, M-717 auto-sampler, μHydrogel 2000, 500 and 250 columns (7.8 × 300 mm) at 70 °C and M-474 fluorescence detector ($\lambda_{ex.} = 410$ nm/$\lambda_{em.} = 430$ nm). The eluent was 50 mM NaOH at a flow-rate of 0.5 ml/min. Calcofluor solution (120 mg/l calcofluor, Sigma fluorescent brightener 28) was used as post-column reagent and pumped by an M616 pump. The system was controlled and the data analysed with Millenium (version 3.2) chromatography manager software. Oat β-glucans of molecular weights ($M_w$) of 66,000 and 41,000 (Megaenzyme International Co., Wicklow, Ireland) were used for calibration.

*2.3.10. Viscosity measurement*

The isolated and freeze-dried fractions of the soluble fibres from the centrifugation-dialysis method were redissolved in 0.1 M Na-phosphate buffer (pH 6.0), giving a final concentration of 50 g/l (Svanberg, Gustafsson, Suortii, & Nyman, 1995). The solutions were allowed to equilibrate in a cold room (9 °C) for 24 h before measurements. Their viscosities were determined with a Haake viscometer (Viscotester VT501, Tillquist, Kista, Sweden) in the shear rate interval 10–1000 $s^{-1}$. The samples were measured with the low viscosity sensor system (NV). The analyses were performed in duplicate. The viscosity of the "random coil" polysaccharides was characterised by two parameters, the maximum "zero-shear" viscosity ($\eta_0$) and the shear rate ($\gamma_{1/2}$) at which the viscosity is reduced to $\eta_0/2$ (Morris, 1990).

*2.4. Calculations and statistical evaluation*

Minitab® software package version 13.0 (Minitab Inc, State College, PA, USA) was used to perform the statistical evaluation. The values were expressed as means and standard deviations. The means were analysed by one-way ANOVA, using the general linear model procedure. Tukey's procedure for multiple comparisons of means was used for the significance of difference ($P < 0.05$). Triplicate samples were run for the filtration method and duplicates for the centrifugation-dialysis method. The total dietary fibre was determined by summing the soluble and insoluble fibre values.

## 3. Results

*3.1. General composition*

The composition of the native and fermented oat and barley fibre concentrates is shown in Table 1. Minor differences were observed between the contents of proteins and minerals in each cereal group, while the content of total dietary fibre, lipids (oat fibre concentrate) and starch (barley fibre concentrate fermented with V2/Pd 2.6) was lower in fermented samples. The amounts of low-molecular weight carbohydrates were negligible in all samples. Considerable amounts of material in the fermented samples could not be quantified as protein, lipids, starch or dietary fibre. These amounts were

Table 1
Composition of the cereal fibre concentrates (g/100 g DW)

| Component | Barley fibre concentrate | | | Oat fibre concentrate | | |
|---|---|---|---|---|---|---|
| | Native | Fermented with V2/La5 | Fermented with V2/Pd 2.6 | Native | Fermented with V2/La5 | Fermented with V2/Pd 2.6 |
| Protein | 8 | 6 | 6 | 23 | 22 | 22 |
| Lipids | 10 | 8 | 4 | 21 | 12 | 9 |
| Glucose | tr[b] | tr[b] | 1 | 0 | 1 | tr[b] |
| Sucrose | tr[b] | 1 | tr[b] | tr[b] | tr[b] | 1 |
| Fructose | tr[b] | tr[b] | tr[b] | tr[b] | tr[b] | tr[b] |
| Starch | 11 | 9 | 5 | tr[b] | tr[b] | tr[b] |
| Dietary fibre[a] | 80 | 61 | 58 | 70 | 40 | 36 |
| Minerals | tr[b] | tr[b] | tr[b] | 3 | 4 | 4 |
| Lactic acid | na | 1 | 1 | na | 5 | 5 |
| Not determined | – | 14 | 25 | – | 16 | 23 |

na = not analysed.
[a] Sum of soluble and insoluble fibre from the centrifugation-dialysis method.
[b] tr = traces, less than 0.5 g/100 g.

higher with fibre concentrates fermented with V2/Pd 2.6 than with those fermented with V2/La5.

### 3.2. Content of dietary fibre using the two methods

#### 3.2.1. Insoluble fibre

The amounts of insoluble fibres quantified by the filtration method were significantly lower ($P < 0.05$) in the fermented samples (18.0/24.0 g/100 g DW in barley and 0.0 g/100 g DW in oats) than in the native samples (41.0 g/100 g in barley and 16.2 g/100 g DW in oats) (Table 2). The same trend was observed with the centrifugation-dialysis method (39.0/37.0 g/100 g DW in the fermented barley fibre concentrates versus 58.8 g/100 g DW in native barley fibre concentrate and 4.5/3.0 g/100 g DW in fermented oat fibre concentrates versus 26.0 g/100 g DW in native oat fibre concentrate). Furthermore, the centrifugation-dialysis method gave higher values than the filtration method. This was observed with both barley and oats.

#### 3.2.2. Soluble fibre

The amounts of soluble fibre quantified in the barley fibre concentrates were similar between the native (18.6 g/100 g DW) and the V2/Pd 2.6-fermented (16.0 g/100 g DW) fibre concentrates when quantified by the filtration method (Table 2). Similar trends could be seen with the centrifugation-dialysis method. Further, no significant differences in the soluble fibre content could be seen between the filtration and centrifugation-dialysis methods. An exception was the V2/La5-fermented barley fibre concentrate, where a higher content of soluble fibre was obtained by the filtration method.

The soluble fibres content in the fermented oat fibre concentrates (21.0/18.5 g/100 g DW), on the other hand, were generally significantly ($P < 0.05$) lower than in native fibre concentrate (28.4 g/100 g DW) when quantified by the filtration method (Table 2). Similar results were obtained with the centrifugation-dialysis method in which the content of soluble fibre was higher (44.0 g/100 g DW) in the native oat fibre concentrate than in the fermented oat fibre concentrates (35.0/33.2 g/100 g DW).

There was a linear correlation in the content of total fibres ($y = 0.9x - 14.8$; $r = 0.96$, where $x =$ amount quantified by the centrifugation-dialysis method and $y$, by the filtration method) and insoluble ($y = 0.7x - 3.2$; $r = 0.96$) fibres between the two methods used. The soluble dietary fibre content, however, correlated to a much lower extent ($y = 0.2x + 14.4$; $r = 0.18$).

### 3.3. Monomeric composition of the dietary fibre

The main component of the fibre in the insoluble and soluble fractions for both cereals was glucose (Table 3). The soluble fibre fractions of both cereals also contained significant amounts of uronic acids, arabinose and xylose. Further, in native barley fibre concentrate, considerable amounts of insoluble polymers containing arabinose and xylose were found by the centrifugation-dialysis method.

In the fractions isolated from the barley fibre concentrates, the soluble and insoluble fibre monomers decreased after fermentation, from 66.6 g/100 g DW to 50.4/42.5 g/100 g DW, as analysed by the centrifugation-dialysis method. The decrease was apparently due to a reduction of insoluble polymers containing glucose, arabinose and xylose. A small concurrent increase of glucose was revealed in the soluble fibre fraction after fermentation.

A decrease in the sum of fibre monomers could also be seen with oat fibre concentrate after fermentation, from e.g. 45.2 g/100 g DW to 24.4 g/100 g and 20.2 g/100 g DW, as analysed by the centrifugation-dialysis method. However, no concurrent increase in the soluble glucose content was obtained after fermentation of oats. Fermentation was of minor importance for other fibre components in oat fibre concentrate as these were present in very low amounts.

Table 2
Insoluble and soluble dietary fibre (g/100 g DW) in native and fermented barley and oat fibre concentrates[a]

| | | Barley fibre concentrate | | | Oat fibre concentrate | | |
|---|---|---|---|---|---|---|---|
| | Sample | Insoluble | Soluble | Total | Insoluble | Soluble | Total |
| Filtration method | Native | 41.0 ± 1.1a | 18.6 ± 1.0a | 59.6 ± 0.3a | 16.2 ± 1.1a | 28.4 ± 3.0ad | 44.6 ± 1.0a |
| | Fermented with V2/La5 | 18.0 ± 0.7b | 28.0 ± 0.7b | 46 ± 1.0b | 0.0b | 21.0 ± 3.5b | 21.0 ± 0.2b |
| | Fermented with V2/Pd 2.6 | 24.0 ± 1.2c | 16.0 ± 1.9a | 40 ± 1.0c | 0.0b | 18.0 ± 1.7b | 18.0 ± 2.0b |
| Centrifugation-dialysis method | Native | 58.8 ± 0.5d | 21.0 ± 0.3a | 79.8 ± 0.2d | 26.0 ± 0.1d | 44.0 ± 1.2c | 70 ± 1.0c |
| | Fermented with V2/La5 | 39.0 ± 2.0ae | 22.1 ± 0.3a | 61.1 ± 2.0a | 4.5 ± 0.3c | 35.0 ± 0.8cd | 39.5 ± 0.5ad |
| | Fermented with V2/Pd 2.6 | 37.0 ± 0.6e | 21.1 ± 0.5a | 58.1 ± 0.1a | 3.0 ± 0.05c | 33.2 ± 0.5d | 36.2 ± 0.3d |

[a] Values are expressed as means ± S.D. Mean values with different letters within the same cereal and fibre type are significantly different, $P < 0.05$ according to Tukey's procedure.

Table 3
Composition of insoluble and soluble dietary fibre isolated by the filtration and centrifugation-dialysis methods (in brackets) in fermented, native barley and oat fibre concentrates (g/100 g DW)

| | | Arabinose | | Xylose | | Glucose | | Uronic acids | | Cellulose[a] | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample | Insoluble | Soluble | Insoluble | Soluble | Insoluble | Soluble | Insoluble | Soluble | | |
| Barley fibre concentrate | Native | 0.0(6.0) | 1.7(1.3) | 0.0(7.0) | 2.3(2.0) | 35.0(39.0) | 11.0(7.0) | 3.0(3.0) | 1.0(1.3) | 5.0(6.0) | 54 (66.6) |
| | Fermented with V2/La5 | 0.0(1.0) | 1.0(1.2) | 0.0(0.5) | 1.3(1.2) | 21.0(26.1) | 13.4(13.0) | 1.1(4.0) | 4.5(3.4) | 7.0(5.0) | 42.3(50.4) |
| | Fermented with V2/Pd 2.6 | 0.0(1.0) | 1.0(1.2) | 0.0(1.0) | 1.0(1.3) | 21.0(24.0) | 9.0(10.0) | 3.0(2.0) | 1.5(2.0) | 2.0(2.0) | 36.5(42.5) |
| Oat fibre concentrate | Native | 0.0(0.4) | 0.0(1.3) | 0.0(0.4) | 0.0(1.0) | 14.1(13.0) | 24. 0(25.0) | 2.3(2.1) | 0.2(2.0) | 3.2(2.1) | 40.6(45.2) |
| | Fermented with V2/La5 | 0.0(0.2) | 0.5(1.0) | 0.0(0.2) | 0.0(0.0) | 0.0(1.0) | 14.0(18.0) | 0.0(1.0) | 2.0(3.0) | 0.0(1.0) | 16.5(24.4) |
| | Fermented with V2/Pd 2.6 | 0.0(0.0) | 0.0(0.0) | 0.0(0.0) | 0.0(0.0) | 0.0(0.0) | 11.2(18.0) | 0.0(0.0) | 1.0(2.2) | 0.0(0.0) | 12.2(20.2) |

[a] Cellulose calculated as total amount of glucose residues in the insoluble fraction of non-starch polysaccharides minus the amount of insoluble β-glucans.

The amount of cellulose calculated as the total amount of glucose residues in the insoluble fibre fraction of non-starch polysaccharides minus the amount of insoluble β-glucans, decreased considerably after fermentation of the barley fibre concentrate with V2/Pd 2.6. In oat fibre concentrate, only small amounts of cellulose were found.

### 3.4. β-glucan measurements

The total contents (sum of soluble and insoluble β-glucan contents) of β-glucans in the native barley and oat fibre concentrates were similar with both fibre assay methodologies, 38.0/39.3 g/100 g DW and 35.0/33.9 g/100 g DW, respectively (Table 4). After fermentation there was a decrease in the content of β-glucans, from e.g. 39.3 g/100 g DW to 31.5/30.7 g/100 g DW in the barley fibre concentrates. The decrease was similar with both fermented samples in both barley and oat fibre concentrates.

Most of the β-glucans in the native barley fibre concentrate were insoluble (~80–85%), while the opposite was observed for β-glucans in the native oat fibre concentrate, where most of the β-glucans (~68%) were found in the soluble fibre fractions.

There was a correlation between the total dietary fibres isolated by the filtration and centrifugation-dialysis methods ($r = 0.973$, $P < 0.01$) and the total amounts of β-glucans obtained from the filtration and centrifugation-dialysis methods, respectively ($r = 0.986$, $P < 0.01$).

### 3.5. Molecular weight analyses

The β-glucans from barley fibre concentrates had a molecular weight ($M_w$) of approximately 41,000 (Fig. 2). Soluble β-glucans in oat fibre concentrates had a $M_w$ of approximately 66,000. The HPLC-SEC analyses also revealed that the sizes of the soluble β-glucan fibre in the fermented and native oat fibre concentrates were similar to each other. The same conclusion was drawn for the soluble polysaccharides of the native and fermented barley fibre concentrates.

### 3.6. Viscosity measurements

The viscosities of the soluble fibre isolated from the fermented oat and barley fibre concentrates by the centrifugation-dialysis method are shown in Fig. 3. All materials showed a non-Newtonian shear-thinning be-

Table 4
β-glucan contents (g/100 g DW) in native and fermented barley and oat fibre concentrates

| | | Barley fibre concentrate | | | Oat fibre concentrate | | |
|---|---|---|---|---|---|---|---|
| | Sample | Insoluble | Soluble | Total[a] | Insoluble | Soluble | Total[a] |
| Filtration method | Native | 30.0 | 8.0 | 38 | 11.0 | 24.0 | 35 |
| | Fermented with V2/La5 | 14.0 | 14.0 | 28 | 0.0 | 12.0 | 12 |
| | Fermented with V2/Pd 2.6 | 19.4 | 8.0 | 27.4 | 0.0 | 11.0 | 11 |
| Centrifugation-dialysis method | Native | 33.3 | 6.0 | 39.3 | 10.9 | 23.0 | 33.9 |
| | Fermented with V2/La5 | 21.5 | 10.0 | 31.5 | 0.0 | 18.5 | 18.5 |
| | Fermented with V2/Pd 2.6 | 22.3 | 8.4 | 30.7 | 0.0 | 17.0 | 17 |

[a] Sum of insoluble and soluble β-glucan contents.

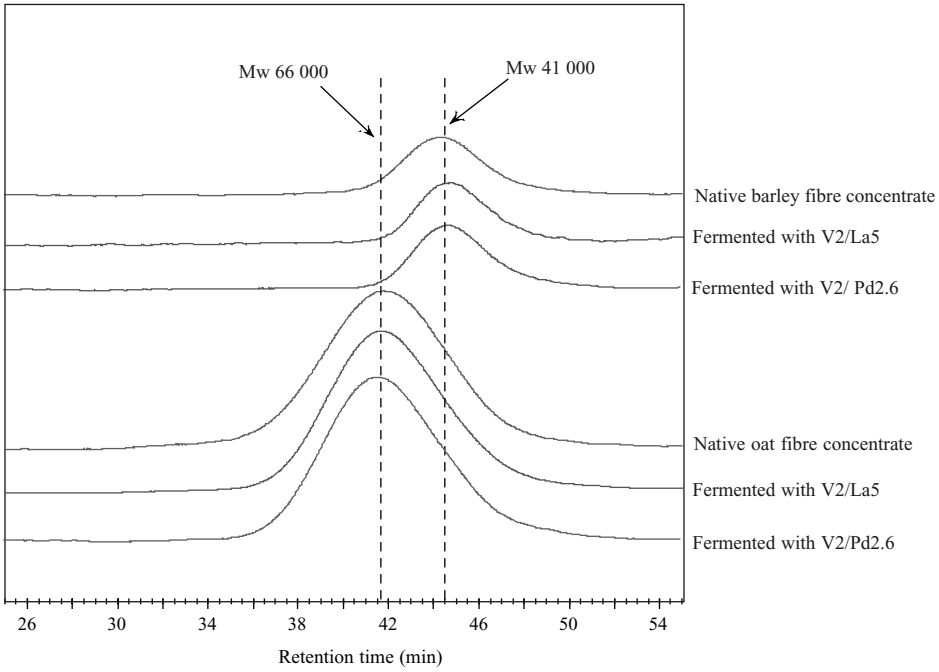


Fig. 2. Molecular weight distribution of the β-glucan fibre in the cereal fibre concentrates: native, fermented with V2/La5 and fermented with V2/Pd 2.6.

haviour; that is the viscosity decreased with increasing shear rate.

The maximum viscosities, $\eta_0$, and shear rates, $\gamma_{1/2}$, are listed in Table 5. For the native barley and oat samples, the maximum viscosities, $\eta_0$, were 59.1 and 282 mPas, respectively. Fermentation did not significantly affect the $\eta_0$ values of barley fibre concentrates, while that of oat fibre concentrates decreased by approximately one-third and one-fifth for the strains V2/La5 and V2/Pd 2.6, respectively.

## 4. Discussion

The possibility of using a cereal-based medium for the growth of LAB has been investigated in earlier studies (Mårtensson et al., 2002a, 2002b) and the ability of Pd 2.6 to produce EPS in such a medium has also been demonstrated (Mårtensson, Öste, & Holst, 2000). In another study (Önning, G., Mårtensson, O., Lambo, A., Biörklund, M., Dueñas-Chasco, M., Irastorza, A., Holst, O., Norin, E., Welling, G.,Åkesson, B. & Öste, O., unpublished results) on humans, an oat-based product fermented with the strains V2/Pd 2.6 reduced total cholesterol (5%) and increased faecal *Bifidobacterium* ssp. count.

The present study was undertaken to find out whether the growth of a combination of V2/La5 and V2/Pd 2.6 in oat and barley fibre concentrates had any effect on the insoluble and soluble dietary fibre contents, the viscosity of the soluble fibre and the average molecular weight of the β-glucans.

The proportion of soluble β-glucans was much higher in native oat fibre concentrate than in native barley fibre concentrate (Table 4). Purified mixed β-glucans from oats and barley have been shown to be linear, unbranched polysaccharides composed of alternating β-(1 → 4) linkages (∼70%) and β-(1 → 3) linkages (∼30%) (Aspinall & Carpenter, 1984; Clarke & Stone, 1966; Fleming & Manners, 1966; Luchsinger, Chen, & Richards, 1965). The β-(1 → 3) linkages occur singly (Dais & Perlin, 1982; Vårum & Smidsrød, 1988; Woodward, Fincher, & Stone, 1983) and these are responsible for the substantial chain flexibility and hence polysaccharide solubility (Buliga, Brant, & Fincher, 1986). Most of the (1 → 4)-linkages occur in groups of two or three (Aspinall & Carpenter, 1984; Dais & Perlin, 1982; Woodward et al., 1983), but longer chains of consecutive (1 → 4)-linked units have been found in barley β-glucans (Luchsinger et al., 1965; Woodward et al., 1983). Theoretical studies have suggested that differences in minor structural features, such as the cellulose-like sequences of (1 → 4)-linkages, might have a great influence on physical properties (Buliga et al., 1986). This could explain why 80–85% of the native barley β-glucans were insoluble as compared with the native oat fibre concentrate, which had just 31–32% of insoluble β-glucans.

The general reduction of total fibre content (especially the insoluble fibre) after the fermentation process, as seen with both methods in both barley and oats

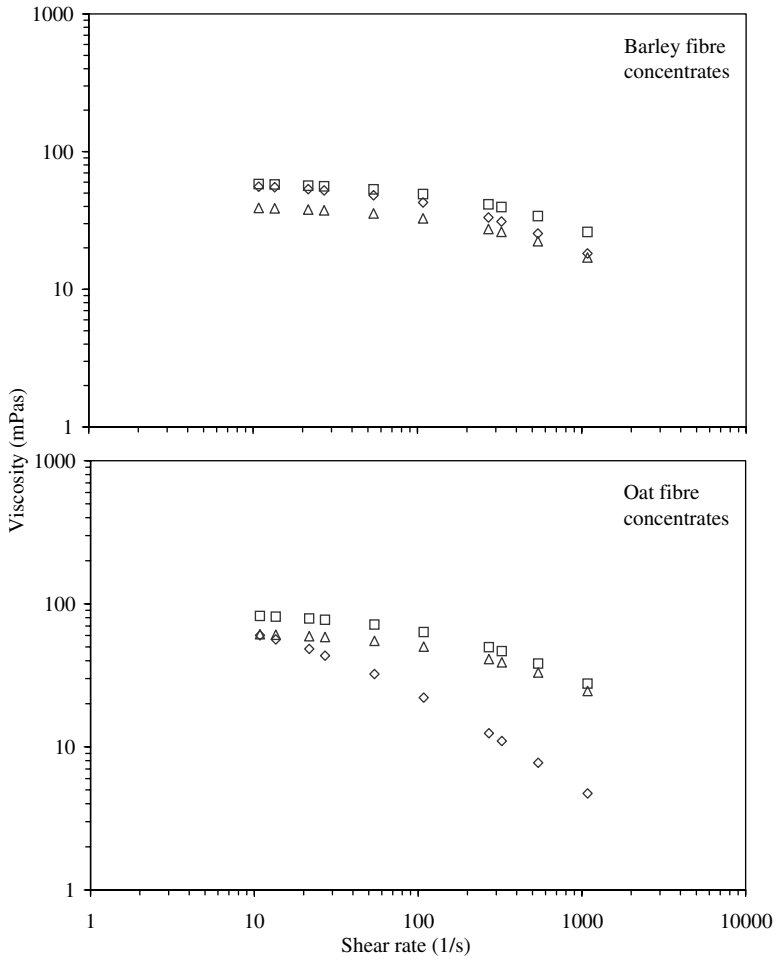


Fig. 3. Viscosity versus shear rate of the soluble fibres (50 g/l) from the centrifugation-dialysis method for native cereal fibre concentrates: (◇), fermented with V2/La5; (□), fermented with V2/Pd 2.6 (△).

Table 5
Maximum viscosity, $\eta_0$ and shear rate, $\gamma_{1/2}$, for the soluble fibre from the centrifugation-dialysis method for fermented and native barley and oat fibre concentrates[a]

| | Sample | Barley fibre concentrate | Oat fibre concentrate |
|---|---|---|---|
| Maximum viscosity, $\eta_0$ (mPas) | Native | 59.1 ± 0.3a | 282.0 ± 4a |
| | Fermented with V2/La5 | 59.2 ± 0.1a | 88.2 ± 0.7b |
| | Fermented with V2/Pd 2.6 | 54.1 ± 3.0a | 64.3 ± 0.4b |
| Shear rate, $\gamma_{1/2}$ ($s^{-1}$) | Native | 368.2 ± 1.4a | 18.1 ± 1.8a |
| | Fermented with V2/La5 | 755.1 ± 1.2b | 386.0 ± 3b |
| | Fermented with V2/Pd 2.6 | 433.2 ± 0.4ab | 575.2 ± 7c |

[a] Mean values in the same column, variable and cereal type, with different letters are significantly different from each other ($P < 0.05$).

(Table 2), could be a result of a breakdown of the cereal dietary fibre polysaccharides by the microorganisms in the fermentation medium. The mechanism behind this is not clear, but it is most likely an enzymatic breakdown. Thus, microorganisms are able to hydrolyse and metabolise insoluble polysaccharides by producing extracellular enzymes that are either cell-free or cell-associated (Schwarz, 2001). Anaerobic bacteria have been shown to possess unique extracellular, multi-enzyme, cellulose-degrading organelles called cellulosomes, which are protuberances formed on the cellulolytic bacterial cell wall when growing on cellulose-based materials (Schwarz, 2001). Crucial details about cellulose hydrolysis are still unknown, although the cellulosomes have been found to attach both to the cell envelope and to the substrate (Schwarz, 2001), forming a cellulose-enzyme-microbe ternary complex (Lynd, Weimer, van Zyle, & Pretorius, 2002). Enzymes which

are capable of hydrolysing the terminal, non-reducing end of polysaccharides, β-D-glucosidases (EC 3.2.1.21), have been isolated from some *Lactobacillus plantarum* (Coutinho & Henrissat, 1999). In this study, the insoluble fibres which probably consisted of longer chains of β-(1 → 4)-glucose linkages were preferentially degraded (Table 2). It is therefore tempting to speculate that a mechanism similar to that of cellulose degradation was responsible for the lower total fibre (Tables 2 and 3) and total β-glucan contents (Table 4) observed after fermentation. The high amounts of non-determined components in the fermented fibre concentrates (Table 1) could probably be glucose oligosaccharides with low $M_w$, resulting from the degraded polysaccharides that may have been lost in the precipitation as well as the dialysis steps (in the case of barley, it could also be arabinose and xylose). This needs further investigation.

The centrifugation-dialysis method gave higher insoluble (for both barley and oat fibre concentrates) and soluble (for oat fibre concentrate) fibre values than the filtration method (Table 2). The differences observed between the dietary fibre values could be due to the loss of material during the filtration and precipitation steps upon isolation and quantification of the fibres. In the filtration method, only polymers with a degree of polymerisation (DP) slightly greater than 20 are quantified at the ethanol concentration (80%) used (Johansson, 1987) while the centrifugation-dialysis method has a DP limit of about 5.

The amount of soluble β-glucans (Table 4) in the oat fibre concentrates decreased significantly after fermentation, indicating a considerable degradation of these polymers. This decrease in concentration, however, did not affect the molecular weight of the soluble β-glucans, as seen by the HPLC-SEC (Fig. 2). However, β-glucans with a molecular weight <10,000 cannot be detected by the calcofluor method and may explain these differences (Jørgensen & Aastrup, 1988). The lower maximum viscosity (Table 4) with the fermented oat fibre concentrates compared with the native samples gives further evidence for a degradation of β-glucans. Some of the β-glucans might have been more or less completely degraded, as they apparently could not be quantified, even by the centrifugation-dialysis method. This needs further investigation.

Cellulose and Klason lignin could constitute the portion of the dietary fibre content that was not β-glucans or starch. The proportions of cellulose in relation to total fibre in dehulled barley and oat dietary fibre have been found to be approximately 17 g/100 g (Oscarsson, Andersson, Salomonsson, & Åman, 1996) and 11 g/100 g DW, respectively (Åman, Theander, & Westerlund, 1989). The same figures for Klason lignin has been found to be approximately 4 g/100 g DW (Oscarsson et al., 1996) and 13 g/100 g DW in dehulled barley and oats, respectively (Åman et al., 1989). The proportions of cellulose in the barley and oat fibre fractions used in this study were calculated to be 9.0/9.0 g/100 g DW and 8.0/4.3 g/100 g DW, respectively (Table 3). Klason lignin was not quantified in the present study.

Fermentation with V2/Pd 2.6 strains (expected to produce EPS) did not result in any significant increase in the total amount of fibres, although an increase in the viscosity of the final products has been observed in previous studies (Mårtensson et al., 2002b). A possible explanation could be that, although the EPS were probably produced in small amounts, they formed weakly bonded associations with the more abundant proteins and cereal fibres, thus significantly affecting the overall product viscosity. Typical EPS concentrations of approximately 0.2–1 g/l have previously been observed in an oat-based medium (Mårtensson et al., 2002b).

The EPS produced during the V2/Pd 2.6 fermentations were not detectable using the HPLC-SEC calcofluor method, as judged by the very similar profiles in molecular weight between native and fermented samples (Fig. 2). This is most likely due to the molecular structure of the EPS, which consists of a backbone of β-(1 → 3)-linked glucose residues with branched β-(1 → 2)-linked glucose units. The calcofluor dye is not specific for straight-chained β-(1 → 3)-linked glucose linkages (Wood & Fulcher, 1978) and therefore the EPS could not be detected.

The viscosity of various soluble fibres is important in relation to carbohydrate and lipid metabolism. Viscosity is characterised by two parameters, the maximum "zero-shear" viscosity ($\eta_0$) and the shear rate ($\gamma_{1/2}$) at which the viscosity is reduced to $\eta_0/2$ according to Morris (1990). This facilitates the comparison of the viscosities of various polysaccharides. A significant relationship between changes in peak blood glucose and a combination of the logarithm of concentration and the logarithm of molecular weight of β-glucans was found by Wood, Beer, and Butler (2000). A positive correlation ($r = 0.99$) was also observed between the viscosity of barley breads and the glycemic index (Östman, E., Rossi, E., Larsson, H., Brighenti, F. & Björck, I., unpublished results).

Furthermore, viscosity changes with the shear rate and it is difficult to determine the actual shear rate of food in the stomach. This is probably fairly low, and comparisons at zero shear rates (maximum viscosity) may be relevant. In this study, the maximum viscosities for the barley fibre concentrates were not significantly different from each other but, for the oat fibre concentrates, the maximum viscosity decreased significantly during fermentation (Table 5). The HPLC-SEC analyses, on the other hand, did not reveal degradation of the soluble β-glucans to any great extent and this may be dependent on the limit of detection. Similar results have been seen in carrots. Thus, souring and microwave treatment had no effect on the molecular weight distribution of carrot polysaccharides compared

to blanching, while the maximum viscosity nevertheless, was reduced (Svanberg et al., 1995). Another study also indicated that functionally significant changes in the viscosity of β-glucan extracts from oat bran resulted from relatively small changes in molecular weight (Wood, Weisz, & Mahn, 1991). It may therefore be questioned whether molecular weight distribution is as appropriate as a viscosity analysis for ranking changes in physicochemical properties of relevance from a physiological point of view. The viscosity has been reported to increase about tenfold with a doubling of the $M_w$ (Eastwood & Morris, 1992).

## 5. Conclusion

Cereals provide suitable growth media for LAB. Although the total fibre concentrations for all samples and the maximum viscosity for the oat samples decreased after fermentation, the molecular weights were not significantly affected.

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