

# Effects of particle size on determination of the contents of grain and legume dietary fibre and resistant starch

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## Effects of particle size on determination of the contents of grain and legume dietary fibre and resistant starch

**Abstract:** Dietary fibre comprises non-digestible carbohydrates, including resistant starch, and lignin, and it is an important constituent of a healthy diet. The aim was to define the influence of particle size on contents determined for dietary fibre and resistant starch in unprocessed grain and canned legumes. Five samples of unprocessed and processed grains were analysed, as oatmeal, buckwheat, dehulled barley, wheat and spelt, and three canned legumes, as beans, chickpeas and peas, with and without their brine. Samples were initially milled un-screened, and then again through 500 µm or 350 µm screens. For unprocessed grain samples, there was generally no influence of particle size, except for the 350-µm milling of dehulled barley, with significantly decreased contents determined for insoluble dietary fibre and resistant starch presumably due to damaging of starch granules and disrupting crystalline formation of starch. For canned legumes with and without their brine, particle size had little effect on contents determined for dietary fibre and resistant starch.

**Key words:** dietary fibre, resistant starch, particle size, canned legumes, milling, grains

## Vpliv velikosti delcev na določitev vsebnosti prehranske vlaknine in rezistentnega škroba v žitih in stročnicah

**Izvleček:** Prehranska vlaknina je sestavljena iz neprebavljivih ogljikovih hidratov, vključno z rezistentnim škrobom, in lignina ter je pomemben del uravnotežene prehrane. Cilj naše raziskave je bil preveriti vpliv velikosti delcev vzorca na določanje vsebnosti prehranske vlaknine in rezistentnega škroba v vzorcih žit in procesiranih žit ter konzerviranih stročnic. Analizirali smo pet vzorcev žit, in sicer ovsene kosmiče, ajdo, ješprenj, pšenico in piro ter tri vzorce konzerviranih stročnic, fižol, čičeriko in grah. Vzorci stročnic so bili analizirani z nalivom in brez naliva. Vzorci so bili mleti na nedefinirano velikost ter na 500 µm ali 350 µm. V skupini žit ni bilo zaznani vpliva velikosti delcev, razen pri vzorcu ješprenja, mletega na velikost 350 µm, kjer sta bili določeni značilno manjši vsebnosti netopne prehranske vlaknine in rezistentnega škroba. Predvidevamo, da je to posledica poškodb škrobnih zrn in porušenja kristalinične formacije škroba. V vzorcih konzerviranih stročnic z ali brez naliva je imela velikost delcev zanemarljiv učinek na določeno vsebnost prehranske vlaknine in rezistentnega škroba.

**Ključne besede:** prehranska vlaknina, rezistentni škrob, velikost delcev, konzervirane stročnice, mletje, žita

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## 1 INTRODUCTION

Dietary fibre is recognized as an important component of the human diet, due to its beneficial effects on the gastrointestinal tract and its positive effects on reducing the risk of developing non-communicable diseases. Dietary fibre consists of carbohydrates that are resistant to hydrolysis by the human endogenous enzymes and that are not readily absorbed in the small intestine, as also for resistant starch. However, the fermentation processes by microbiota in the colon can partially use the dietary fibre for energy production, while metabolising it to bioactive compounds, such as short-chain fatty acids (Fuller et al., 2016; Kendall et al., 2010). The definition of dietary fibre describes it as carbohydrate polymers with ten or more monomeric units that are not hydrolysed by the endogenous enzymes in the small intestine of humans, and that belong to following four categories:

- (i) Edible carbohydrate polymers that occur naturally in the food consumed;
- (ii) Carbohydrate polymers that have been obtained from raw food material by physical, enzymatic or chemical means, and that have been shown to have beneficial physiological effects on health, as demonstrated by the generally accepted scientific evidence available to competent authorities;
- (iii) Synthetic carbohydrate polymers that have been shown to have beneficial physiological effects on health, as demonstrated by generally accepted scientific evidence available to competent authorities.
- (iv) Carbohydrate polymers with three to nine monomeric units that belong to one of the previous categories, if the national authorities decide to include them in these definitions (De Menezes et al., 2013).

Dietary fibre can be classified based on its solubility in water. Insoluble dietary fibre (IDF) consists of mainly cellulose, lignin and some hemicelluloses, while (water)-soluble dietary fibre (SDF) consists of mainly pectin, gum and mucilage. The solubility of dietary fibre governs its physicochemical properties, as IDF has strong hygroscopic properties and can thus swell and absorb water. SDF, on the other hand, can form a gel network or a dense network under some physicochemical conditions, and can thus bind water in this way (Thebaudin et al., 1997).

Increased intake of dietary fibre promotes more frequent defecation and lowers the risk of diabetes mellitus, obesity, coronary heart disease and various cancers; this increased intake also lowers cholesterol levels in the blood (Dahl & Stewart, 2015; Fernstrand et al., 2017; Perry & Wang, 2012; Tarcea et al., 2017). In terms of the physiological effects on blood sugar levels and lipid levels, SDF shows potential for great benefits through its regulatory effects (Kapoor et al., 2016). The particle size of the di-

etary fibre itself can have an influence on the physiological properties of the colon. Smaller particles of bran have been shown to increase the microbiological production of short-chain fatty acids, which appears to be due to the increased surface area of the smaller particles. Particle size of corn bran can also influence its swelling, with a consequent influence on faecal wet mass and faecal bulking, with increases in liver cholesterol and butyrate levels (Ebihara & Nakamoto, 2001; Stewart & Slavin, 2009).

The dietary fibre content of food is generally determined by enzymatic–gravimetric methods, where the macro nutrients are digested *in vitro* or removed, and the remaining portion of the sample represents the dietary fibre. The most common of these methods are AOAC 985.29 and AOAC 991.43 (Westenbrink et al., 2013). Recently, new integrated methods that comply with the new definition of dietary fibre that also includes low molecular weight SDF are gaining value in food composition analyses (Zielinski & Rozema, 2013; Macagnan et al., 2016). The enzymatic breakdown of starch and protein is very important for accurate determination of the dietary fibre content of a food. In the protocol for methods AOAC 985.29 and AOAC 991.43, sample milling is defined, for particles to pass through a 500 µm screen (AOAC 991.43).

Effects of sample particle size on the determination of dietary fibre content have been reported for animal feed samples, where it was shown that the particle size of the feed has effects on hemicellulose, cellulose and lignin determination. With decreasing particle size, the fibre contents determined also decreased. This was consistent regardless of forage cultivar, season of the animal feed preparation and annual variations in the animal feed (Ehle, 1984). Differences in dietary fibre content have also been reported for wheat bran that was milled to particle sizes of 50, 160, 400 and 750 µm, although here these differences were seen for SDF, while total dietary fibre (TDF) remained unchanged (Coda et al., 2014). The main concern about the accuracy of methods AOAC 985.29 and 991.43 is that these only quantify part of the resistant starch, while the proportion that is included in the dietary fibre determination is not known (Champ et al., 2003).

The particle size of a sample can have effects on the enzyme activity of  $\alpha$ -amylase. For particles > 500 µm in size, starch granules can potentially remain entrapped and will not be reached by the enzyme. Then, for particles < 350 µm in size, the  $\alpha$ -amylase activity approaches a constant value, which shows influence of particle size on releasing starch granules from plant cells, making them more susceptible to hydrolysis with  $\alpha$ -amylase (Al-Rabadi et al., 2009). Starch digestibility is related to particle size, due to the increase in surface area with the

decrease in particle size. Here, this larger surface area of the finer ground particle allows for more rapid digestion of the starch and greater penetration of the amylase into the starch granules, therefore improving the starch digestibility (Mahasukhonthachat et al., 2010).

As well as particle size, the food matrix and technological processing are also important factors in enzymatic breakdown of starch (Singh et al., 2010). Lipids and proteins can also have an influence on starch digestibility. In combination with lipids, starch can form amorphous structures that are similar to slowly digestible starch. A similar effect has been reported for starch interactions with protein, where the protein can form a structural 'cage' around starch granules, and thus prevent enzymatic digestion (Zhang et al., 2009). Changes in particle size, for dietary fibre analysis, can lead to disruption of cell wall, therefore freeing starch granules from the cell, also milling of the samples on smaller particle size can disrupt starch – protein complex or damage starch granules and not least milling can destroy crystalline formation of starch (Li et al., 2014).

However, to the best of our knowledge, there are no studies on the influence of particle size of whole food samples on the dietary fibre content determined using enzymatic–gravimetric methods. The main aims of our study were thus to determine whether the particle size of five grains (i.e., oatmeal, buckwheat, dehulled barley, wheat, spelt) and three canned legumes (i.e., canned beans, chickpea, pea; with and without their brine) have an influence on the contents determined for dietary fibre, and resistant starch. Grain is frequently used as a model food for dietary fibre determination, while canned legumes are precooked food and therefore represent a different analytical matrix compared to grain. Furthermore, the effects of the brine present in the cans on the legume content of dietary fibre, and resistant starch were determined.

## 2 MATERIALS AND METHODS

### 2.1 SAMPLES AND SAMPLE PREPARATION

For evaluation of the effects of particle size on the contents determined for dietary fibre and resistant and digestible starch, the grain (oatmeal, buckwheat, dehulled barley, wheat, spelt) and canned legumes (beans, chickpea, pea; both with and without their brine) were air dried at 40 °C for 48 h. Their moisture contents were then determined gravimetrically from the difference in mass before and after drying at 105 °C for 5 h, along with the dry matter.

Each sample was initially milled in a cyclone mill

(AR100G31; Moulinex, Ecully, France) for 30 s, with no screening for particle size separation (i.e., unscreened). For 500 µm or 350 µm particle size the samples were milled as described, then passing of the samples through the mesh of desired size was ensured. If part of the sample was unable to pass the mesh, the milling was continued until all of the sample passed selected mesh. These milled samples were stored in plastic containers at –20 °C until analysis.

### 2.2 DETERMINATION OF DIETARY FIBRE

The dietary fibre was determined according to enzymatic–gravimetric method AOAC 991.43 (AOAC 991.43), in quadruplicate. The method was modified slightly: for the enzyme digestion, 50 ml centrifuge tubes were used instead of Erlenmeyer flasks, according to our previous study (Ferjančič et al., 2018). This method is based on three enzymes used under different conditions: heat stable  $\alpha$ -amylase, a protease, and an amyloglucosidase (all enzymes Cat N° 112979; Merck KGaA, Darmstadt, Germany).

The dietary fibre fractions were obtained as indigestible residues after this enzymatic digestion of the non-dietary fibre components. The IDF was obtained by filtration, and the SDF was precipitated from the filtrate with 96 % ethanol. Determination of the residual ash content (ashed at 525 °C in a muffle furnace for 5 h, and weighed) and residual protein content from the nitrogen content after Kjeldahl, was carried out on the residues, for the corresponding data correction. The TDF was defined as the sum of IDF and SDF. All of these data are presented as fresh mass (FM).

### 2.3 DETERMINATION OF RESISTANT STARCH

The resistant starch was determined according to AOAC 2002.02 method (McCleary et al., 2002), in quadruplicate, and with modifications for the sample milling. The proposed particle size in the original method is given as <1 mm for dry samples, and < 4.5 mm for fresh samples. The samples in the present study were milled as described above.

Resistant starch assay kit was used for resistant starch determination (K-RSTAR; Megazyme, Bray, Ireland). Samples were incubated with pancreatic  $\alpha$ -amylase and amyloglucosidase for 16 h at 37 °C, for hydrolysis of digestible starch to glucose. Resistant starch is recovered in form of a pellet, obtained by centrifugation of the sample. First centrifugation is followed by two suspensions of the pellet in 50 % ethanol (v/v) and

centrifugation. Resistant starch in the remaining pellet is dissolved in 2 M KOH and hydrolysed to glucose with amyloglucosidase. D-glucose is measured with glucose oxidase/oxidase reagent (GOPOD), which develops a pink colour in presence of glucose that can be measured by spectrophotometer. By obtaining concentration of glucose, content of resistant starch can be calculated.

For the contents determined for resistant starch, the light absorbance was measured using a UV-Vis spectrophotometer (Carry 8458; Agilent, Victoria, Australia) at 505 nm (instead of 510 nm), following identification of the absorbance peak maximum at this wavelength. All of these data are presented as FM.

## 2.4 STATISTICAL ANALYSIS

Statistical analyses were performed using the R commander software (version 3.3.3). To ensure the appropriateness of ANOVA, variances between the treatments were determined using Levene's tests ( $\alpha > 0.05$ ). Further ANOVA was performed with *post-hoc* Tukey's tests. The threshold for statistical significance was  $p \leq 0.05$ . For comparisons of the influence of the brine, F-tests were performed to ensure the homogeneity of variance, followed by Student's t-tests. The threshold for statistical significance was  $p \leq 0.05$ .

## 3 RESULTS AND DISCUSSION

### 3.1 EFFECTS OF MILLING ON THE CONTENTS DETERMINED FOR DIETARY FIBRE AND RESISTANT STARCH OF THE GRAIN

The data for the dietary fibre and resistant starch contents determined in grains are presented in Table 1. Across these five grain samples (i.e., oatmeal, buckwheat, dehulled barley, wheat, spelt) that were milled to the three different particle sizes (unscreened, or 500  $\mu\text{m}$ , 350  $\mu\text{m}$  screening), the highest TDF was determined for dehulled barley (10.15–13.40 g 100 g<sup>-1</sup> FM), followed by oatmeal (9.13–10.01 g 100 g<sup>-1</sup> FM) and wheat (8.96–12.36 g 100 g<sup>-1</sup> FM). These determined contents for TDF in the oatmeal grain are similar to those reported for nordic countries (10.8–12.3 g 100 g<sup>-1</sup> FM) (Rainakari et al., 2016). Similarly, Škrabanja et al. (2004) reported TDF of buckwheat as 2.7 to 21.3 g 100 g<sup>-1</sup> FM across different milling fractions, which corresponds to TDF determined for buckwheat in

the present study (3.92–4.59 g 100 g<sup>-1</sup> FM). TDF determined for the dehulled barley was also similar to a previous determination (10.8–12.3 g 100 g<sup>-1</sup> FM) (Yalcin et al., 2006), as also for wheat (9.2–20.0 g 100 g<sup>-1</sup> FM) (Ciudad-Mulero et al., 2019) and spelt (8.8–14.9 g 100 g<sup>-1</sup> dry mass [DM]) (Shewry & Hay, 2015). For dehulled barley, there was a significant decrease across the two specific particle sizes from 500  $\mu\text{m}$  to 350  $\mu\text{m}$  screening, respectively, for IDF and TDF determination.

Similarly, for resistant starch determination, significant decreases across the unscreened to fine milling (i.e., 350  $\mu\text{m}$  screening) of the samples were seen for dehulled barley, and also for wheat (Table 1). Such influences of the particle size on the contents determined for dietary fibre and resistant starch should be related to the enzyme kinetics of the  $\alpha$ -amylase. Larger particles would be expected to be less digested due to the slower penetration for larger particles, and therefore here it is possible that some starch in the samples with larger particle sizes remained undigested (Al-Rabadi et al., 2009). As well as consideration of the enzyme penetration for digestion of the starch in these samples, milling can also cause mechanical damage to starch granules, which can result in conversion of resistant starch to digestible starch (De La Hera et al., 2013). Differences in DF and resistant starch determination can be explained by influence of milling on starch digestion kinetics. Dhital et al. (2011) reported influence of cryo-milling of starch granules on molecular structure of starch itself. Their results suggest influence of milling on disruption of helical and crystalline structures of the starch, without breaking the covalent bond of starch molecules due to mechanical force. Furthermore, resistance of starch to hydrolysis is not purely molecular level effect but foremost inability of enzyme to digest starch due to mechanical obstacles, mainly absence of pores on starch granules, which are commonly present in rapid digested starch. Also, internal starch granules in the plant cell are commonly resistant to hydrolysis due to inaccessibility for enzymes. Milling however, can cause starch granule damage therefore facilitating starch hydrolysis (Dhital et al., 2010a). Also, an important note to starch digestion is resistance of starch to hydrolysis. Mechanisms behind the resistant starch are physical in nature (inaccessibility of starch to enzymes, recrystallization, physical entrapment and complexes with other macronutrients), therefore it should be possible for mechanical manipulation of sample particle size to have an effect on starch digestion kinetics (Dhital et al., 2017). The absence of significant differences in other grain samples can be explained by differences in structural features (Dhital et al., 2010b).

Table 1: Unprocessed grain dry matter, dietary fibre content, and resistant starch content according to milled screen setting for particle size

Sample	Milling screen setting ( $\mu\text{m}$ )	Dry matter ( $\text{g } 100 \text{ g}^{-1}$ )	Dietary fibre ( $\text{g } 100 \text{ g}^{-1}$ FM)			Resistant starch ( $\text{g } 100 \text{ g}^{-1}$ FM)
			Insoluble	Soluble	Total	
Oatmeal	Unscreened	96.4	4.35 $\pm$ 0.49 <sup>a</sup>	4.80 $\pm$ 0.31 <sup>a</sup>	9.15 $\pm$ 0.33 <sup>a</sup>	0.24 $\pm$ 0.01 <sup>b</sup>
	500	96.4	4.92 $\pm$ 0.81 <sup>a</sup>	5.09 $\pm$ 0.46 <sup>a</sup>	10.01 $\pm$ 1.23 <sup>a</sup>	0.15 $\pm$ 0.03 <sup>a</sup>
	350	96.4	4.42 $\pm$ 0.35 <sup>a</sup>	4.70 $\pm$ 0.32 <sup>a</sup>	9.13 $\pm$ 0.33 <sup>a</sup>	0.23 $\pm$ 0.03 <sup>b</sup>
Buckwheat	Unscreened	96.8	2.75 $\pm$ 0.20 <sup>a</sup>	1.17 $\pm$ 0.14 <sup>a</sup>	3.92 $\pm$ 0.25 <sup>a</sup>	0.72 $\pm$ 0.20 <sup>a</sup>
	500	96.8	3.32 $\pm$ 0.35 <sup>b</sup>	1.28 $\pm$ 0.29 <sup>a</sup>	4.59 $\pm$ 0.32 <sup>b</sup>	0.84 $\pm$ 0.02 <sup>a</sup>
	350	96.8	3.06 $\pm$ 0.18 <sup>ab</sup>	1.22 $\pm$ 0.25 <sup>a</sup>	4.28 $\pm$ 0.35 <sup>ab</sup>	0.62 $\pm$ 0.16 <sup>a</sup>
Dehulled barley	Unscreened	93.9	8.23 $\pm$ 0.44 <sup>c</sup>	5.17 $\pm$ 0.15 <sup>a</sup>	13.40 $\pm$ 0.33 <sup>c</sup>	2.41 $\pm$ 0.49 <sup>b</sup>
	500	93.9	5.88 $\pm$ 0.13 <sup>b</sup>	4.95 $\pm$ 0.44 <sup>a</sup>	10.83 $\pm$ 0.38 <sup>b</sup>	1.52 $\pm$ 1.64 <sup>ab</sup>
	350	93.9	4.94 $\pm$ 0.32 <sup>a</sup>	5.21 $\pm$ 0.13 <sup>a</sup>	10.15 $\pm$ 0.22 <sup>a</sup>	0.19 $\pm$ 0.12 <sup>a</sup>
Wheat	Unscreened	88.6	7.98 $\pm$ 0.47 <sup>a</sup>	1.25 $\pm$ 0.27 <sup>a</sup>	9.23 $\pm$ 0.56 <sup>a</sup>	11.94 $\pm$ 0.84 <sup>b</sup>
	500	88.6	7.64 $\pm$ 0.42 <sup>a</sup>	1.46 $\pm$ 0.30 <sup>a</sup>	8.96 $\pm$ 0.35 <sup>a</sup>	10.95 $\pm$ 0.73 <sup>b</sup>
	350	88.6	10.64 $\pm$ 0.10 <sup>b</sup>	3.53 $\pm$ 0.42 <sup>b</sup>	12.36 $\pm$ 0.28 <sup>b</sup>	6.71 $\pm$ 0.68 <sup>a</sup>
Spelt	Unscreened	89.2	8.02 $\pm$ 0.78 <sup>b</sup>	1.60 $\pm$ 0.31 <sup>a</sup>	9.62 $\pm$ 0.99 <sup>b</sup>	12.06 $\pm$ 0.47 <sup>a</sup>
	500	89.2	7.80 $\pm$ 0.13 <sup>b</sup>	1.70 $\pm$ 0.14 <sup>a</sup>	9.52 $\pm$ 0.06 <sup>b</sup>	11.60 $\pm$ 0.37 <sup>a</sup>
	350	89.2	4.16 $\pm$ 0.36 <sup>a</sup>	1.39 $\pm$ 0.11 <sup>a</sup>	5.55 $\pm$ 0.20 <sup>a</sup>	12.27 $\pm$ 1.25 <sup>a</sup>

FM, fresh mass

Data are means  $\pm$  SD (n = 4). Means with different letters within each sample are significantly different across the particle sizes ( $p \leq 0.05$ ; ANOVA with *post-hoc* Tukey's tests)

### 3.2 EFFECTS OF MILLING ON THE CONTENTS DETERMINED FOR DIETARY FIBRE AND RESISTANT STARCH OF THE LEGUMES

The data for dietary fibre and resistant starch for the legume samples (i.e., canned beans, chickpeas, peas; with and without their brine) are presented in Table 2. Across these three legume samples that were milled to the three different particle sizes (unscreened, or 500  $\mu\text{m}$ , 350  $\mu\text{m}$  screening), the highest TDF was determined for chickpeas (5.90-6.74 g 100 g<sup>-1</sup> FM) and beans (6.09-6.65 g 100 g<sup>-1</sup> FM), both without the brine. These relative levels for TDF were generally paralleled for IDF and SDF, with the exception of the legume samples without the brine.

The dietary fibre data for these legumes are in agreement with other studies, where IDF and SDF were determined for raw beans (11.4-19.9 g 100 g<sup>-1</sup> DM; 2.42-3.40 g 100 g<sup>-1</sup> DM; respectively) and raw peas (20.3 g 100 g<sup>-1</sup> DM; 1.73 g 100 g<sup>-1</sup> DM), and IDF for raw chickpeas (13.9 g 100 g<sup>-1</sup> DM) (Li et al., 2002; De Almeida et al., 2006; Kleintop et al., 2013).

Significant differences across the two specific particle sizes from 500  $\mu\text{m}$  to 350  $\mu\text{m}$  screening were seen for TDF and IDF determined, as increases for beans without brine, and decreases for chickpeas with brine. Significant increases were also seen according to decreased particle size for SDF determined for beans with brine and chickpeas without brine.

For resistant starch determination, again from 500  $\mu\text{m}$  to 350  $\mu\text{m}$  screening, significant difference was only seen as a decrease for peas without brine.

Considering these data, generally the effects of the different milling processes of these foods for the determination of the dietary fibre and resistant starch contents were not uniform. For example, for beans, this was seen for SDF determined with brine and for IDF, SDF and TDF determined without brine (lowest as 500  $\mu\text{m}$  milling), and for chickpeas, numerically (but not significantly) for IDF and SDF determined without brine (lowest as 500  $\mu\text{m}$  milling). On the other hand, across the unscreened to fine milling (i.e., 350  $\mu\text{m}$  screening) of the samples, for chickpeas with brine this showed a significant increase in SDF and decrease in IDF, thus corresponding to decreased particle size. For the beans, chickpeas and peas, the uniform resistant starch determined would be a consequence of the food preparation, which transformed the resistant raw starch granules into digestible starch (Brouns et al., 2002). For the samples of grains and processed grains (previous chapter), in terms of the relatively uniform contents determined for resistant starch despite the different particle sizes might relate to starch gelatinisation. Starch gelatinisation occurs when starch granules receive enough energy to break their in-

termolecular bonds, thus undergoing irreversible loss of the native structure. As a result of this gelatinisation, the starch granules become more readily digestible (Rooney & Pflugfelder, 1986). All legume samples were cooked beforehand, due to the fact that they were canned, therefore starch in said samples should be gelatinised. Similar effect could be induced in oatmeal, however the amount of water present in thermal treatment is different, thus not allowing for full gelatinisation of starch.

The changes for the legume samples without the brine, as the canned beans, chickpeas and peas, were also examined for the dietary fibre and resistant starch contents determined (Table 2). Significant differences were observed without the brine compared to the samples with brine for TDF, IDF, SDF and resistant starch determined. The TDF and IDF determined were significantly higher in all of these samples without the brine, while the SDF determined was significantly lower only for the beans and chickpeas without the brine. These data for TDF, IDF and resistant starch can be explained in terms of the differences in the dry matter contents. The samples without the brine had higher dry matter contents in comparison to the corresponding samples with the brine (Table 2), and therefore there was an effect of dilution. At the same time, the differences in the SDF determined for these legume samples can be explained in terms of the solubility of this dietary fibre in the brine. A large proportion of SDF in the legumes in brine was dissolved in the brine, and this was thus lost when the brine was discarded prior to the analyses (i.e., some 50 %-80 % of SDF lost in the brine). Also, the canned legumes used in the present study had been cooked and sterilised, with cooking previously shown to lower SDF (Martin-Cabrejas et al., 2006; Wang et al., 2008). At the same time, Shin et al. (2003) reported no changes in resistant starch contents in their samples with regard to the presence of brine, due to the low solubility of resistant starch in water.

## 4 CONCLUSIONS

The particle sizes of the grain samples generally had non-uniform effects on the determined dietary fibre and resistant and digestible starch. Some particle size effects were seen for dehulled barley, where decreasing the particle size from unscreened to 500  $\mu\text{m}$  to 350  $\mu\text{m}$  screening, the IDF determined significantly decreased, as also for the TDF determined in these samples. With the exception of oatmeal, all of the grain showed some susceptibility to these changes in particle size according to the IDF and TDF determined; however, overall, only the smallest particle size had any effect on dietary fibre determina-

**Table 2:** Legume dry matter, dietary fibre content, and resistant starch content according to milled screen setting for particle size

Sample	Brine	Milling screen setting ( $\mu\text{m}$ )	Dry matter ( $\text{g } 100 \text{ g}^{-1}$ )	Dietary fibre ( $\text{g } 100 \text{ g}^{-1} \text{ FM}$ )			Resistant starch ( $\text{g } 100 \text{ g}^{-1} \text{ FM}$ )
				Insoluble	Soluble	Total	
Beans	With	Unscreened	17.4	$3.02 \pm 0.18^a$	$1.28 \pm 0.03^b$	$4.30 \pm 0.21^a$	$0.51 \pm 0.02^a$
		500	17.1	$2.95 \pm 0.19^a$	$1.15 \pm 0.04^a$	$4.10 \pm 0.15^a$	$0.47 \pm 0.07^a$
		350	17.6	$3.06 \pm 0.12^a$	$1.28 \pm 0.07^b$	$4.34 \pm 0.15^a$	$0.52 \pm 0.01^a$
	Without	Unscreened	27.8	$5.98 \pm 0.14^{b,***}$	$0.67 \pm 0.05^{a,***}$	$6.65 \pm 0.10^{b,***}$	$1.34 \pm 0.03^{a,***}$
		500	27.7	$5.39 \pm 0.30^{a,***}$	$0.70 \pm 0.04^{a,***}$	$6.09 \pm 0.27^{a,*}$	$1.32 \pm 0.05^{a,***}$
		350	28.5	$5.83 \pm 0.09^{b,***}$	$0.68 \pm 0.09^{a,***}$	$6.51 \pm 0.06^{b,***}$	$1.32 \pm 0.04^{a,***}$
Chickpeas	With	Unscreened	8.8	$1.92 \pm 0.05^b$	$0.38 \pm 0.02^a$	$2.30 \pm 0.04^b$	$0.40 \pm 0.03^a$
		500	9.2	$1.86 \pm 0.06^b$	$0.41 \pm 0.02^{ab}$	$2.26 \pm 0.04^b$	$0.40 \pm 0.06^a$
		350	9.6	$1.50 \pm 0.18^a$	$0.45 \pm 0.03^b$	$1.95 \pm 0.15^a$	$0.36 \pm 0.01^a$
	Without	Unscreened	31.4	$6.49 \pm 0.22^{a,**}$	$0.32 \pm 0.14^{a,*}$	$5.90 \pm 0.69^{a,*}$	$1.69 \pm 0.06^{a,***}$
		500	30.9	$5.70 \pm 0.72^{a,***}$	$0.20 \pm 0.06^{a,*}$	$6.74 \pm 0.19^{a,***}$	$1.68 \pm 0.06^{a,***}$
		350	31.5	$6.26 \pm 0.16^{a,***}$	$0.40 \pm 0.05^b$	$6.65 \pm 0.16^{a,***}$	$1.62 \pm 0.11^{a,***}$
Peas	With	Unscreened	13.2	$3.44 \pm 0.14^a$	$0.49 \pm 0.02^a$	$3.92 \pm 0.13^a$	$1.06 \pm 0.04^b$
		500	13.0	$3.42 \pm 0.14^a$	$0.48 \pm 0.04^a$	$3.89 \pm 0.12^a$	$1.00 \pm 0.02^a$
		350	13.5	$3.44 \pm 0.09^a$	$0.48 \pm 0.02^a$	$3.93 \pm 0.08^a$	$1.01 \pm 0.04^{ab}$
	Without	Unscreened	20.2	$5.15 \pm 0.14^{a,*}$	$0.39 \pm 0.03^a$	$5.53 \pm 0.12^{a,***}$	$1.21 \pm 0.03^{b,***}$
		500	20.5	$4.83 \pm 0.12^{a,***}$	$0.46 \pm 0.11^a$	$5.29 \pm 0.07^{a,***}$	$1.22 \pm 0.01^{b,***}$
		350	20.7	$4.87 \pm 0.30^{a,***}$	$0.55 \pm 0.12^a$	$5.42 \pm 0.20^{a,***}$	$1.16 \pm 0.02^{a,***}$

FM, fresh mass

Data are means  $\pm$  SD ( $n = 4$ ). Means with different letters within each sample are significantly different across the particle sizes ( $p \leq 0.05$ ; ANOVA with *post-hoc* Tukey's tests) Student's t-test \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , significant differences between sample without versus with brine sample, within each particle size

tion. The particle size in the present study had little or no systematic effect on resistant starch determined.

This possibility of manipulation of dietary fibre determined was generally shown when these grain samples were milled according to the 350 µm screening. Therefore, for dietary fibre determined for grain samples, it is advisable to maintain the particle size at the level of the 500 µm screening, rather than for the 350 µm screening. In these canned (pre-cooked) legumes, particle size had little effect on the contents determined for dietary fibre, and also for resistant starch. However, overall, particle size can have some influence on the contents determined for dietary fibre and resistant determination, in terms of possible sources of error in such analyses, especially for grains.

The additional part of the present study showed that consumption of these canned legumes with the brine increased the SDF intake, although due to the dilution effects seen in the DW analysis, less TDF would be consumed for the same quantity of food.

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## 6 CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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