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Ball Milling Improves Extractability and Affects Molecular Properties of Psyllium (*Plantago ovata* Forsk) Seed Husk Arabinoxylan

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Psyllium (*Plantago ovata* Forsk) seed husk (PSH) is very rich in arabinoxylan (AX). However, its high gelling capacity and the complex nature of the AX make it difficult to process. In this study, ball milling was investigated as a tool for enhancing PSH AX water extractability and molecular mass (MM). A 48 h laboratory-scale ball mill treatment under standardized optimal conditions reduced the PSH average particle size from 161 μm for the untreated sample to 6 μm. Concurrently, it increased the water-extractable AX (WE-AX) level from 13 (untreated PSH) to 90% of the total PSH AX. While the WE-AX of the untreated PSH had a peak MM of 216 kDa and an arabinose to xylose (A/X) ratio of 0.20, WE-AX fragments from ball mill-pretreated PSH had a peak MM of 22 kDa and an A/X ratio of 0.31. Ball milling further drastically reduced the intrinsic viscosity of PSH extracts and their water-holding capacity. Prolonged treatment brought almost all AX (98%) in solution and yielded WE-AX fragments with an even higher A/X ratio (0.42) and a lower peak MM (11 kDa). While impact and jet milling of PSH equally led to significant reductions in particle size, these technologies only marginally affected the water extractability of PSH AX. This implies that ball milling affects PSH particles and their constituent molecules differently than impact and jet milling.

KEYWORDS: Psyllium seed husk; *Plantago ovata* Forsk; arabinoxylan; ball milling; impact milling; jet milling; particle size distributions; extractability; viscosity

INTRODUCTION

Psyllium (*Plantago ovata* Forsk) seed husk (PSH) is a rich source of dietary fiber. It contains a high level of a complex heteroxylan with arabinose and xylose as the main monosaccharides, which is further referred to as arabinoxylan (AX) (*1*−*4*). PSH AX is a highly branched polysaccharide with a main chain of densely substituted β-(1,4)-linked xylopyranose residues. Substituents include single arabinofuranose and xylopyranose residues or short side chains consisting of these monosaccharides and rhamnose and galacturonic acid residues. These substituents are attached at positions C(*O*)-2 and/or C(*O*)-3 of the main chain xylopyranose residues (*1, 4, 5*). The high AX content of PSH (62−63%) (*2, 4*) makes PSH a potentially interesting material for the production and study of arabinoxylo-oligosaccharides, AX degradation products. Such nondigestible oligosaccharides are considered as prebiotics if they exert a beneficial health effect on their host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (*6, 7*). Wheat bran-derived arabinoxylo-oligosaccharides have been shown to selectively stimulate *Bifidobacterium* species in in vitro pure cultures (*8*−*10*), in an in vitro continuous fermentation system mimicking the human gastrointestinal tract (*11*), and in in vivo trials with rats (*12*), mice (*8*), and chickens (*13, 14*) and to decrease carcinogen formation in the human colon by increased ammonium secretion via the feces (*15*).

Previous work by the present authors (*16*) showed that enzyme cocktails or strong acidic conditions increase the extractability of PSH AX by reducing their molecular mass (MM). However, the strong gel-forming capacity of PSH (*17, 18*) reduces the efficiency of such treatments. Reducing the gel-forming capacity of PSH AX would be a key step in enhancing the extractability of PSH.

Ball milling (*19*−*30*), high-pressure micronization (*27*−*29*), and jet milling (*27*−*29*) affect polysaccharide-containing particles and in some cases also the polysaccharide molecules themselves. Ball milling reduces the particle size of maize and lucerne stems (*30*), wheat bran particles (*25*), and fruit and carrot insoluble fiber (*27, 28*). It breaks covalent bonds in maize (*19*) and wheat starches (*23*) and decreases molecular sizes as also noted for ball-milled pectin molecules (*26*). As a result, the treatment also has a significant impact on the functional properties of polysaccharides (*21*). Ball milling of the starting materials reduces the viscosities of pectin solutions (*26*) and maize (*31*) and cassava starch pastes (*22*). For the latter, the viscosity decreases were larger with increasing treatment time (*22*). Ball milling cassava starch (*21, 22*), wheat starch (*23, 24*),

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and fruit and carrot insoluble fibers (*27, 28*) increases their water solubility. Furthermore, ball mill treatment increases maize starch amylase sensitivity (*19, 20*), as well as the in vitro ruminal degradability of cell wall polysaccharides (*30*).

The objective of the present study was to investigate the potential of ball milling for production of AX degradation products with possible potential for physiological functionality and to compare its effect on the structural and physicochemical characteristics of PSH and PSH AX with that of impact and jet milling.

EXPERIMENTAL PROCEDURES

Materials. Milled PSH (85% husk material, overall particle size <250 μm) was obtained from Biofiber-Damino (Gesten, Denmark). Chemicals and reagents were purchased from Sigma-Aldrich (Bornem, Belgium) and were of at least analytical grade.

Standard Analyses. Moisture and ash contents of PSH were analyzed according to AACCI methods 44-19 and 08-01, respectively (*32*). The protein content was determined using the Dumas combustion method, an adaptation of the AOAC Official Method (*33*) to an automated Dumas protein analysis system (EAS VarioMax N/CN, Elt, Gouda, The Netherlands), using 6.25 as a factor for conversion of nitrogen to protein content. The total noncellulosic carbohydrate content and uronic acid content of PSH were determined as described earlier (*16*).

Milling of PSH. *Ball Milling.* PSH was ground in a laboratory Retsch PM 100 planetary ball mill (Retsch, Haan, Germany), equipped with a 250 mL zirconium container with six zirconium balls (20 mm diameter). In a first experiment, PSH (50 g) was placed in the milling jar and milled at different rotational speeds (350, 400, 450, 500, and 550 rpm) for 24 h. After each 60 min, a 10 min rest was introduced to allow the ball mill to cool. All treatment times given are effective milling times and do not include rest times. The influence of the degree of filling of the milling jar was investigated by milling different amounts of PSH [40, 50, 60, and 70 g, which correspond to a total filling degree (including the ball volumes) of 30, 35, 40, and 45%, respectively, of the jar volume capacity] at 500 rpm for 24 h. To investigate the influence of milling time, PSH (40 g) was ground (500 rpm) for up to 168 h. After 2, 4, 6, 8, 12, and 24 h and from then on, every 24 h, a sample (1.0 g) was withdrawn for further analysis.

Impact Milling. PSH was impact milled on a Hosokawa Alpine type 100 UPZ impact mill (Hosokawa, Augsburg, Germany), operated at 18000 rpm and equipped with 0.5 mm sieve. The grinding of materials in an impact mill occurred through high-speed mechanical impact on the particles. The material was milled until it passed the sieve.

Jet Milling. The PSH sample first impact milled (two passages) was jet milled on a Hosokawa Alpine laboratory mill (Hosokawa) operated with an air jet milling 100 AFG module. Jet milling is a fluid energy impact-milling technique. Particles are accelerated in a high-velocity air stream, and size reduction is the result of collisions between particles of the process material itself (*34*). The feeding pressure was 6 bar, and the classifier was run at 3000 rpm. The material was recirculated until all particles passed through the classifier.

Particle Size Distribution Analyses of PSH. Particle size distributions of untreated PSH, ball-milled PSH (2, 4, 8, 12, and 48 h; 500 rpm; 30% of jar volume capacity), impact-milled PSH, and jet-milled PSH were determined by laser diffraction in a Malvern 2000 Mastersizer (Goffin/Meyvis, Hoeilaart, Belgium). Volume percentages of particles with diameter sizes between 0.49 and 840.90 μm were obtained. A particle size dispersion index was determined as $(d_{90} - d_{10})/d_{50}$, with d_{90}, d_{50}, and d_{10} representing the particle size limit that included 90, 50, and 10% of the particles in the distribution, respectively (*25*).

Determination of Water Extractability of PSH AX and A/X Ratio of Solubilized AX Fragments. Treated and untreated PSH samples were suspended in water (0.4% w/v) and shaken for 60 min at 6 °C to extract water-extractable AX (WE-AX). After centrifugation (24000*g*; 10 min; 6 °C), total and monomeric (free) carbohydrate contents of the extracts were determined as described earlier (*16*). A combination of data for total and monomeric monosaccharides allowed calculation of the arabinose to xylose ratio (A/X ratio):

$$\text{A/X ratio} = \frac{\text{total arabinose level} - \text{monomeric arabinose level}}{\text{total xylose level} - \text{monomeric xylose level}}$$

Measurements were performed in duplicate. Standard deviations of WE-AX levels (expressed as % of total PSH AX content) were smaller than 1.0%. Standard deviations of A/X ratios were below 0.01.

Determination of MM by High-Performance Size-Exclusion Chromatography (HPSEC). Apparent MM distributions of WE-AX in extracts from untreated and treated PSH samples were studied by HPSEC on a Shodex SB-806 HQ column (300 mm × 8.0 mm i.d.) with a Shodex SB-G guard column (50 mm × 6 mm i.d.) from Showa Denko K.K. (Kawasaki, Japan). Samples were suspended in water (0.4% w/v; 60 min; 6 °C), centrifuged (24000*g*; 10 min; 6 °C), and filtered through a 0.45 μm membrane (Regenerated cellulose, Grace Davison Discovery Sciences, Deerfield, MA). Elution of the samples (20 μL) was with 25 mM ammonium acetate (pH 5.0, 0.5 mL/min, 30 °C) on a Kontron 325 pump system (Kontron, Milan, Italy) equipped with autoinjection. The separation was monitored with an evaporative light scattering detector (Alltech ELSD 2000ES, Grace Davison Discovery Sciences). MM markers (1.5 mg/mL) were Shodex (Showa Denko K.K.) standard P-82 pullulans with MMs of 788, 404, 212, 112, 47.3, 22.8, 11.8, and 5.9 kDa and glucose (0.18 kDa).

Determination of Apparent Intrinsic Viscosity (η_{int}). The viscosities of PSH WE-AX-containing aqueous extracts (0.4% w/v, prepared as described above) were measured using an Ostwald type capillary viscometer (AVS 400, Schott Geräte, Hofheim/Ts, Germany). Flow times of supernatants (5.0 mL) were measured at 30 °C. The relative viscosity (η_{rel}), that is, the flow time of supernatant divided by the flow time of deionized water under the experimental conditions, was used to calculate the specific viscosity (η_{sp}), that is, $\eta_{sp} = \eta_{rel} - 1$. Apparent intrinsic viscosities (η_{int}, dL/g) were calculated using the Morris equation (*35*): $\eta_{int} = 1/c \times [2 \times (\eta_{sp} - \ln \eta_{rel})]^{0.5} \times 10$, where c represents the AX concentration (mg/mL), assuming that only AX contributes to the supernatant viscosities. Measurements were done in triplicate. Standard deviations were smaller than 0.4 dL/g.

Determination of Water-Holding Capacity. The water-holding capacity was determined after extraction (0.4% w/v; 60 min; 6 °C) and centrifugation (24000*g*; 10 min; 6 °C) of untreated and treated PSH and calculated as the ratio of the amount of water (mL) retained by the amount of PSH (g dry matter) suspended before extraction. Measurements were performed in duplicate. Standard deviations were smaller than 0.8 mL/g dm.

RESULTS

Preliminary Optimization of Laboratory-Scale Ball-Milling Parameters in Terms of AX Extractabilities. PSH is characterized by a high level of AX (57.5%) with an average A/X ratio of 0.43 and minor levels of proteins (8.7%) and uronic acids (4.9%) (**Table 1**). The WE-AX level of untreated PSH was 13.2% (expressed as a ratio on total PSH AX) (**Figure 1**).

The influence of the ball mill rotational speed on the extractability of PSH AX was investigated by milling PSH for 24 h at different speed values, ranging from 350 to 550 rpm (**Figure 1a**). With increasing speed, the WE-AX level clearly increased from 26% (expressed as a ratio on total PSH AX) at 350 rpm up to 50% (of all PSH AX) at 500 rpm as compared to 13% for the untreated sample. We used 500 rpm in all further experiments.

Milling different amounts of PSH (24 h; 500 rpm) allowed investigation of the influence of the degree of filling of the milling jar (**Figure 1b**) on PSH AX extractability. Upon ball mill treatment at 30 and 35% of the jar volume capacity, respectively, 73 and 50% of all PSH AX were extractable. Milling at 40% of the jar volume capacity or more did not affect the water extractability of the AX. Triplicate millings showed

Table 1. Chemical Composition of PSH[a]

	PSH
ash (% dm)	3.6 ± 0.1
protein (% dm)	8.7 ± 0.2
total noncellulosic carbohydrate (% dm)[b]	65.6 ± 0.5
arabinose (% dm)	19.8 ± 0.1
xylose (% dm)	45.6 ± 0.4
AX (% dm)[c]	57.5 ± 0.4
A/X ratio	0.43 ± 0.01
mannose (% dm)	1.2 ± 0.1
galactose (% dm)	4.7 ± 0.1
glucose (% dm)	2.0 ± 0.1
rhamnose (% dm)	1.0 ± 0.1
uronic acid (% dm)	4.9 ± 0.5

[a] Values are expressed as means ± standard deviations. [b] Total noncellulosic carbohydrate = 0.88 × (% arabinose + % xylose) + 0.89 × (% rhamnose) + 0.9 × (% mannose + % galactose + % glucose) with the factors 0.88, 0.89, and 0.9 to correct for hydration water. [c] AX = 0.88 × (% arabinose + % xylose) with the factor 0.88 to correct for hydration water.

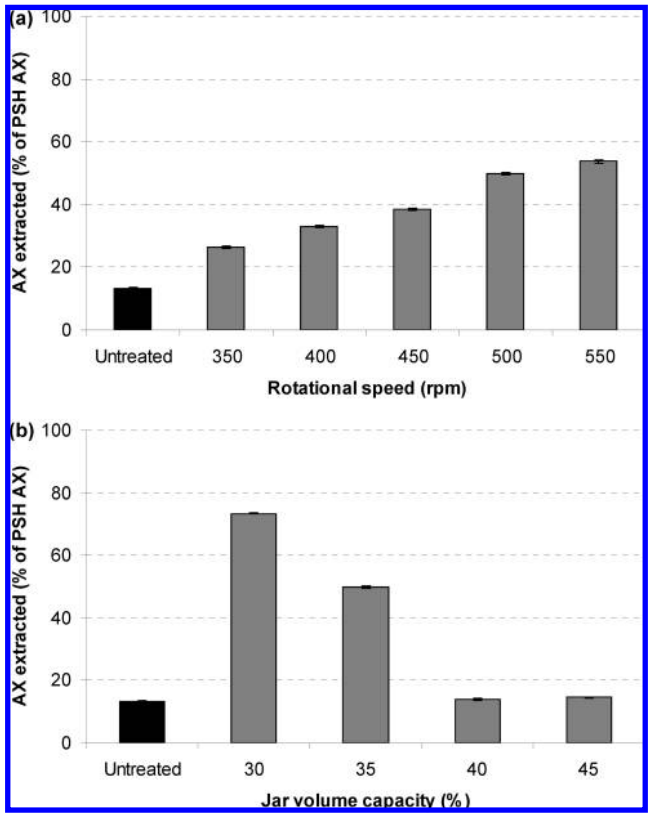

Figure 1. Influence of ball-milling parameters on the extractability of PSH AX (expressed as % of total PSH AX content). Untreated PSH is added as a reference. (**a**) Extractability of PSH AX after milling PSH (24 h; 30% jar volume capacity) with different velocities (350−550 rpm). (**b**) Extractability of PSH AX after milling PSH (24 h; 500 rpm) with different filling degrees of the milling jar (30−45%).

that milling with 30% filling was well reproducible in terms of the levels of WE-AX that could be measured (coefficient of variance <3.0%).

Changes in PSH AX Extractability and Molecular Properties upon Milling. PSH was ball milled for up to 168 h (500 rpm; 30% jar volume capacity), and samples were withdrawn after different times. The AX content (**Figure 2**) and the average A/X ratio (**Table 2**) of aqueous extracts increased with treatment time, implying that AX gradually became extractable in water and that the increasingly solubilized fragments contained increasing levels of arabinose. Under the dilute conditions used, only 13% of all PSH AX could be extracted from the untreated sample (average A/X = 0.20). This level increased to 20, 35, 73, and even 90% for fractions ball milled for 4, 8, 24 (average A/X = 0.29), and 48 h of treatment, respectively. Prolonged treatment (168 h) brought almost all AX (98%, A/X = 0.42) in solution. In contrast, impact and jet milling of PSH had hardly any effect on the extractability of PSH AX and, accordingly, on their A/X ratio (**Table 2**).

Because the degree of filling of the milling jar was shown above to influence the milling process, withdrawal of material for sampling can be assumed to have affected PSH AX extractability and properties. However, the difference in sample amount due to withdrawal of sample aliquots during the consecutive stages of the ball-milling process was limited, and only a slight overestimation can be expected.

Apparent MM distribution curves of untreated and ball-milled PSH AX extracts showed a shift to decreased MM and polydispersity with ball mill pretreatment time (**Figure 3**). AX fragments extracted from the untreated sample had an apparent peak MM of 216 kDa (**Table 2**). AX from material subjected to ball milling for 2 h had an apparent peak MM of 289 kDa. AX extracted from materials ball milled for more extended periods of time had apparent peak MMs, which decreased with the time that the materials had been subjected to ball mill treatment. After 168 h, solubilized AX fragments had an apparent peak MM of 10.8 kDa. In contrast, neither impact nor jet milling decreased the apparent peak MM.

Ball mill treatment of PSH affected the viscosity of its aqueous extracts. The effect was most clearly observed when considering the η_{int} (**Table 2**), which takes the AX content of the extracts into account. As a result of 24 h of ball mill treatment, η_{int} of the aqueous extracts was 83% lower (3.8 dL/g) than that of the untreated sample (22.4 dL/g). This decrease continued with increasing treatment time, resulting in viscosities as low as 0.2 dL/g for extracts isolated from PSH ball milled for 168 h. In contrast, the effect of impact milling and jet milling on the viscosity of AX containing extracts was limited (**Table 2**).

Untreated PSH retained 31.1 mL water per gram dry matter (**Table 2**). This value slowly decreased for PSH ball milled for a few hours. PSH ball milled for 24 h retained 11.7 mL/g dm. Prolonged treatment (168 h) of PSH further reduced this value to 14% (4.4 mL/g dm) of the untreated sample. Impact and jet milling only had a small effect on the water-holding capacity of PSH (**Table 2**).

Changes in PSH Particle Size upon Milling. An explanation for the different behavior of ball-milled PSH AX as compared

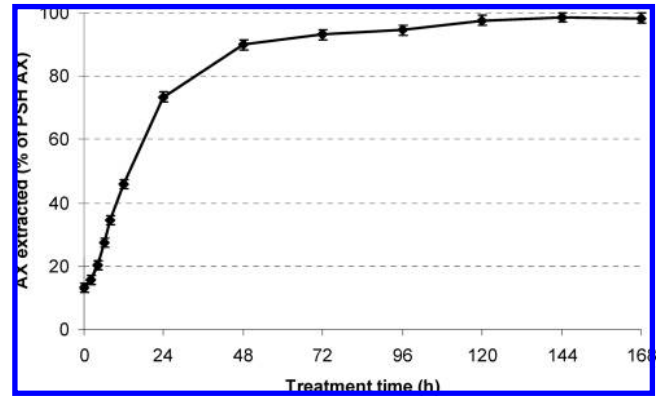

Figure 2. Influence of ball milling (500 rpm; 30% jar volume capacity) time (2 up to 168 h) on the extractability of PSH AX (expressed as % of total PSH AX content).

Table 2. AX Extractability and Structural, Physicochemical, and Particle Size Parameters of Untreated, Ball-Milled (500 rpm; 30% Jar Volume Capacity), Impact-Milled, and Jet-Milled PSH

		ball milling									
	control	2 h	4 h	8 h	12 h	24 h	48 h	96 h	168 h	impact milling	jet milling
AX extractability (% of PSH AX)	13.2	15.7	20.3	34.5	46.0	73.4	89.9	94.5	98.4	13.4	14.6
structural characteristics											
A/X	0.20	0.22	0.23	0.27	0.29	0.29	0.31	0.38	0.42	0.21	0.22
apparent peak MM (kDa)[a]	216	289	258	207	68.0	32.4	21.5	18.7	10.8	256	270
physico-chemical properties											
η_{int} (dL/g)	22.4	16.4	13.7	7.5	5.6	3.8	1.8	0.3	0.2	19.8	16.4
water-holding capacity (mL/g dm)	31.3	29.3	27.5	24.2	20.3	11.7	5.3	4.7	4.4	31.0	26.7
particle size parameters											
d_{50} (μm) [b]	160.9	95.0	47.7	10.6	8.7	ND[d]	6.0	ND	ND	64.4	21.2
dispersion index [c]	2.0	2.1	2.0	3.4	3.1	ND	3.5	ND	ND	2.3	2.3

[a] Apparent peak MM, determined by HPSEC. [b] d_{50}, 50% of the overall particles showed a size less than this value (μm). [c] The particle size dispersion index was determined as: $(d_{90} - d_{10})/d_{50}$, with d_{90}, d_{50}, and d_{10} representing the particle size limit that included 90, 50, and 10% of the particles in the distribution, respectively. [d] Not determined.

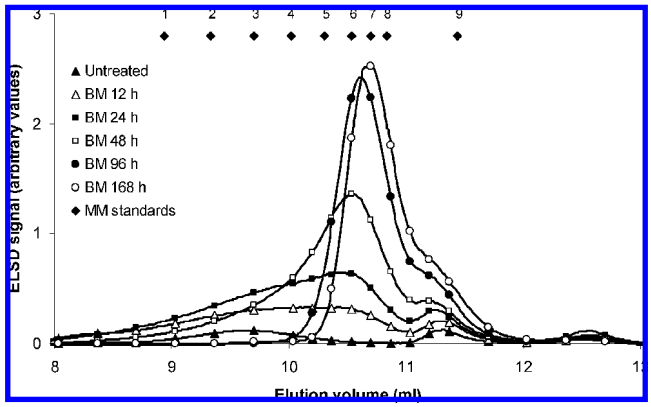

Figure 3. Influence of ball milling on MM. Curves show MM distributions for untreated PSH and ball-milled PSH (12, 24, 48, 96, and 168 h; 500 rpm; 30% jar volume capacity). MM markers from the left to the right are 788 (1), 404 (2), 212 (3), 112 (4), 47.3 (5), 22.8 (6), 11.8 (7), 5.9 (8), and 0.18 kDa (9).

to impact- and jet-milled PSH AX was looked for in the PSH particle size. Untreated PSH contained two populations of particles (**Figure 4**). The major part (91%) consisted of particles larger than 26 μm, with an average particle size of 210 μm. The other particles were smaller (<26 μm) and had an average size of 9.0 μm. Ball milling resulted in a shift of the particle size distribution toward smaller particle sizes. The d_{50} (the particle size limit that includes 50% of the particles in the distribution) decreased from 160.9 μm for the untreated sample down to 95.0 μm after 2 h ball milling and 47.7 μm after 4 h (**Table 2**). After 48 h of treatment, the d_{50} was reduced to 6.0 μm. The particle size dispersion index of 48 h ball-milled PSH was 1.8 times that of untreated PSH (3.5 and 2.0 for 48 h ball-milled and untreated PSH, respectively). Thus, ball milling resulted in a more polydisperse particle size distribution. Particle size distributions also showed that, after ball milling, PSH still contained two populations of particles. PSH ball milled for 48 h contained 19% of particles smaller than 2.0 μm (average particle size = 1.4 μm), while the major part of the particles had an average size of 6.6 μm.

Impact or jet milling had an effect on the particle size distribution comparable to that of between 2 and 4 h or 4 and 8 h of ball milling PSH, respectively (**Figure 4**). The d_{50} of impact-milled PSH was 64.4 μm, which was 2.5 times lower than that of untreated PSH (160.9 μm) and between the values for 2 (95.0 μm) and 4 h (47.7 μm) ball-milled PSH (**Table 2**).

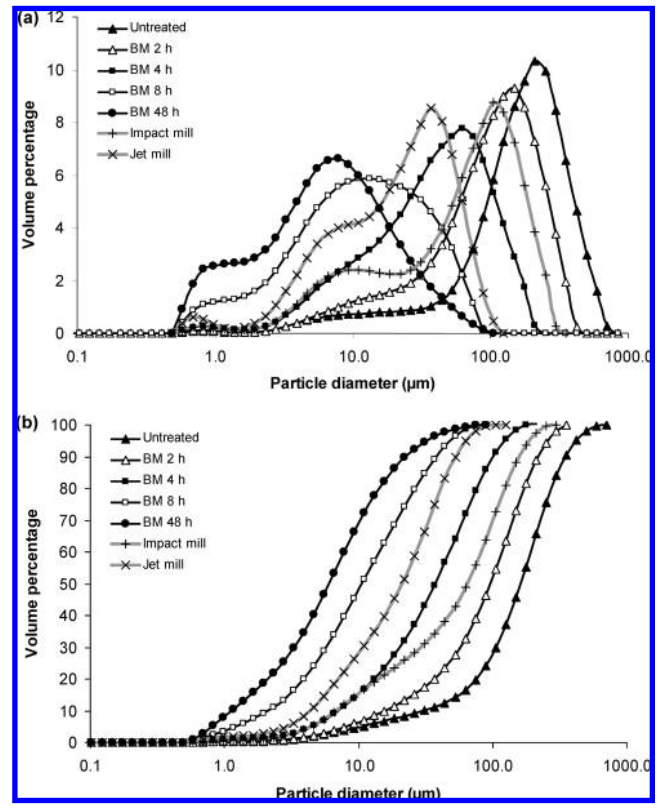

Figure 4. Influence of milling on particle size distribution of PSH. Curves show the amount of particles (volume %) as a function of particle diameter (μm) for untreated PSH, ball-milled PSH (2, 4, 8, and 48 h; 500 rpm; 30% capacity), impact-milled PSH, and jet-milled PSH. (**a**) Particle size distribution curves and (**b**) cumulative particle size distributions.

The d_{50} of jet-milled PSH was 21.2 μm, which was 7.6 times lower than that of untreated PSH and between the values for 4 (47.7 μm) and 8 h (10.6 μm) ball-milled PSH. The particle size dispersion indices of impact- and jet-milled PSH were similar (2.3).

DISCUSSION

The present study investigated the potential of ball milling for production of AX fragments with potential for physiological activity. The effects of ball, impact, and jet milling on the properties of PSH and PSH AX were compared.

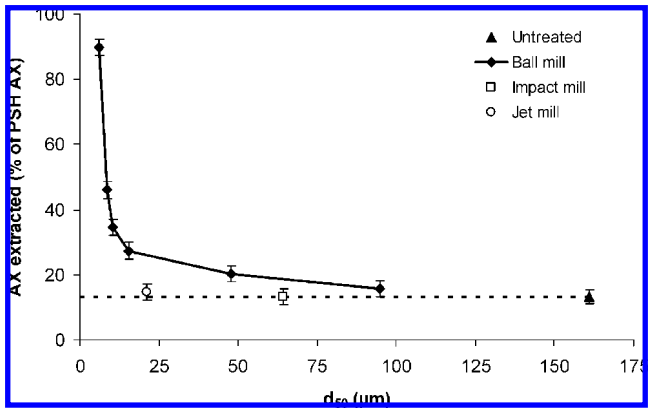

Figure 5. Plot of PSH AX extractability (expressed as % of total PSH AX content) against PSH particle size (d_{50}, expressed in μm) for untreated, impact-milled, jet-milled, and ball-milled PSH (2, 4, 6, 8, 12, and 48 h; 500 rpm; 30% jar volume capacity).

All three milling techniques shifted the PSH particle size distribution patterns toward smaller particle sizes. The average particle size of jet-milled PSH was in the range of those of different ball-milled PSH samples, in line with earlier results of Chau et al. (*27*) for carrot insoluble fiber. Concurrently, ball milling but not impact or jet milling increased the water extractability of PSH AX. Enhanced solubility by ball mill treatment has already been observed for cassava (*21, 22*) and wheat starches (*23, 24*) and for fruit and carrot insoluble fiber (*27, 28*). The cold water solubility (at 40 °C) of cassava starch increased from only 1% up to 94% after 2 h of treatment (*22*). **Figure 5**, in which the PSH WE-AX level is plotted against the d_{50} of the starting materials, indicates that ball-milled PSH AX are more extractable than impact- or jet-milled PSH AX in the same d_{50} range. This indicates that particle size reduction was not causally linked to the increased extractabilities and suggests that ball milling affects PSH particles and their constituent molecules differently than impact and jet milling. In contrast, Chau et al. (*29*) observed a small but similar increase in the water extractability of fruit insoluble fiber after either ball milling (10 h) or jet milling.

Of the three milling techniques tested, only ball milling lowered the apparent peak MM of the solubilized PSH AX with increasing milling time. Ball milling has already earlier been reported to break covalent bonds, and, as a result, to decrease the degree of polymerization (DP) and the molecular size of maize (*19*) and wheat starches (*23*) and of pectin (*26*). Wheat starch amylose was affected only after severe milling, while amylopectin was converted to low MM fragments more easily (*23*). In the case of maize starch, molecular size reduction occurred together with the formation of short chain fragments (DP 3−10), while the level of oligosaccharides with DP 10 to 20 decreased significantly (*19*). Van Deventer-Schriemer and Pilnik (*26*) showed pectin molecule degradation to smaller DP and MM (83% reduction after 48 h of treatment).

Ball milling strongly impacted both the water-holding capacity of PSH and the viscosity of extracts from ball-milled PSH. Other authors reported a decreased viscosity for solutions of ball-milled pectin (*26*) and ball-milled cassava starch (*22*). For the latter, apparent viscosity decreased with increasing milling time, and the decrease was generally largest in the initial milling period (*22*).

Worth mentioning is that the efficiency of a laboratory scale ball mill is substantially lower than that of an industrial ball mill. With the latter, much shorter milling times could suffice to obtain the same high AX extractabilities and corresponding structural and physicochemical characteristics. In conclusion, ball milling changed PSH from a strong water holding, and, therefore, unmanageable material, into an easy to handle and interesting source of soluble dietary fiber.

ABBREVIATIONS USED

AX, arabinoxylan; A/X ratio, arabinose to xylose ratio; DP, degree of polymerization; MM, molecular mass; η_{int}, intrinsic viscosity; η_{rel}, relative viscosity; η_{sp}, specific viscosity; PSH, psyllium seed husk; WE-AX, water-extractable arabinoxylan.

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