The effect of extrusion screw-speed on the water extractability and molecular weight distribution of arabinoxylans from defatted rice bran

Authors: Abdulmannan Fadel[*,†], Andrew Plunkett[*], Mohamed El mohtadi[†], Ayman M. Mahmoud[‡*], Yazan Ranneh[××], Jason Ashworth[†] and Weili Li [‡]

[*]Department of Food and Nutrition, School of Health Psychology and Social Care, Manchester Metropolitan University, Manchester M15 6BH, United Kingdom

[†]School of Healthcare Science, Faculty of Science and Engineering, Manchester Metropolitan Univeristy, Manchester M1 5GD, United Kingdom

[‡*]Department of Endocrinology, Diabetes and Nutrition, Center for Cardiovascular Research (CCR), Charité-University Medicine Berlin, Germany

[‡*]Permanent address: Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Salah Salim St., 62514, Beni-Suef, Egypt.

Tel.: +201144168280. E-mail: ayman.mahmoud@science.bsu.edu.eg

[××]Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Malaysia

[‡]Institute of Food Science & Innovation, University of Chester, Chester CH1 4BJ, United Kingdom

[*,†]Corresponding author: Abdulmannan Fadel

Email: man_fadel@hotmail.com

Telephone: +447878365500

Journal of Food Science and Technology (Short Communications)

Abstract

Arabinoxylans (AXs) are major dietary fibre in cereals. Recently, AXs have attracted a great deal of attention because of their biological activities. These activities have been suggested to be related to the content of low molecular weight (Mw) AXs, in particular those with a Mw below 32 kDa.

Rice bran is a rich source of AXs. However, water extraction of AXs is difficult and often gives low yield. Extrusion processing has been used to increase the solubility of cereal dietary fibre. The aim of this research was to study the effect of extrusion screw-speeds of 80 and 160 rpm on the extraction yield and Mw of water extractable AXs from rice bran. It was found that the extraction yield of AXs increased significantly ($P<0.05$) with an increase in screw speed and was accompanied by a significant decrease ($P<0.05$) in the Mw of AXs from extruded rice bran.

Keywords

- Rice bran, dietary fibre, water soluble arabinoxylans, extrusion technology, low molecular weight

Introduction

Extrusion cooking is an important food processing technology, especially for the production of cereal-based products (Vasanthan et al. 2002). It involves the cooking of expansive, moistened food materials in a tube by the application of high temperature, high shear forces and high pressure for a short time and results in structural modification by physiochemical changes and molecular re-distribution (Pawar et al. 2014; Thymi et al. 2005).

Several researchers have investigated the effects of extrusion on the composition of cereal-based products. Vasanthan et al. (2002) reported that extrusion could increase the soluble dietary fibre (SDF) in extruded barley flour. The increase of SDF content was from 5.62% in non-extruded barley flour to 7.24% in extruded barley flour under a barrel temperature of 140°C, screw-speed of 50 rpm and moisture content of 50%. The increase in SDF was explained by transglycosylation from non-dietary fibre components (i.e. starch) to SDF and transformation of insoluble dietary fibre (IDF) to SDF.

Recently, Daou and Zhang (2012) investigated the effect of extrusion cooking on rice bran dietary fibre solubility. A twin-screw extruder was used with a screw-speed of 90 rpm. They found that the content of soluble fibre increased from 5.9% in non-extruded samples to 6.8% in extruded samples, and concluded the increase in dietary fibre solubility may be due to the reduction in particle sizes of extruded samples. Another possible explanation for the increase in dietary fibre solubility may be the breakdown of covalent and non-covalent bonds in larger molecules due to the high temperature of the barrel (Rashid et al. 2015). Gualberto et al. (1997) showed a significant decrease in IDF in extruded dietary fibre of rice, oats and wheat but a significant increase in SDF in all samples.

Arabinoxylans (AXs) are major dietary fibres found in many cereals such as wheat and rice (Broekaert et al. 2011). Few researchers have investigated the effect of extrusion cooking on the solubility and molecular weight distribution of AXs. Santala et al. (2013) investigated the effect of extrusion cooking on water extractable arabinoxylan (WEAX) levels and molecular weight obtained from wheat bran. The extrusion of wheat bran increased the solubility of AXs from 1.7% to 2.5% when the water content decreased from 92% to 42-60% respectively under a fixed screw-speed at 65 rpm and fixed barrel temperature of 50 °C. Optimal WEAX levels and significantly lower Mw WEAX were obtained from extruded wheat bran at a water content of 48-60%.

The aim of this study was to determine the effect of extrusion on solubility and molecular weight distribution of AXs from rice bran. It has been reported that low molecular weight (30-50 kDa) AXs have immunomodulatory effects, both *in vivo* and *in vitro* (Ghoneum and Matsuura 2004). Our hypothesis is that lower molecular weight AXs would be observed through extrusion than with water extraction alone, thus highlighting extrusion as a valuable method to enrich AXs with most potential health benefits from cereals.

Materials and methods

Rice bran and chemicals

Ener-G, General Dietary Ltd (Surrey, UK) kindly provided rice bran. D-(+)-Xylose, dextrose (D-glucose) anhydrous, acetic acid (glacial), hydrochloric acid, phloroglucinol and ethanol were purchased from Sigma-Aldrich (Brøndby, Denmark) for the determination of xylose in rice bran. Eight pullulan (linear α-(1-4) glucans with no side chain) standards of varying molecular weights (ranging from 5-708 kDa) were purchased from Shodex (Shanghai, China) to characterise the Mw of AXs by SEC-

HPLC. Sodium nitrate ($NaNO_3$) and sodium, azide (NaN_3) were purchased from Sigma-Aldrich (Gillingham, UK) for the HPLC mobile phase.

Enzymes

Termamyl (α-amylase), type XII-A, A3403-1MU and proteinase, type XXIII, P4032 were purchased from Sigma-Aldrich (Brøndby, Denmark).

Fat extraction

Rice bran fats were extracted prior to extrusion using the method provided by Buchi (2016). Briefly, 10 g of rice bran was mixed with 40 ml of petroleum ether (Fisher Scientific, Loughborough) and transferring to an E-812/E-816 HE extraction unit (Buchi, Switzerland).

Extrusion pre-treatment

A Werner Pfleiderer Continua 37 co-rotating, self-wiping twin-screw extruder (Werner Pfleiderer, Stuttgart, Germany) was used for the extrusion pre-treatment of rice bran. The extruder had the following characteristics: a length to diameter ratio (L/D) of 27:1, screw-speeds (SS) of 80 rpm and 160 revolution per minute (rpm) and a feed rate of 10 kg/h. The barrel temperature was set at 80 to 140°C (feed end and die end respectively) with a fixed moisture content of 30% (w/w wet weight basis). Extruded samples were dried at 60°C for 12 hours.

Extraction of water-extractable AXs (WEAXs)

Li et al. (2013) method was used to extract the WEAXs. Rice bran samples (100 g) were extracted with 333 ml water, incubating in a shaking water bath (Precision SWB 15, ThermoScientific, London, UK) for two hours at 40°C. Samples were then centrifuged for 40 minutes at 6000 x g and supernatants transferred to Erlenmeyer flasks with pH adjusted to pH7 using (1M HCl) or (1M NaOH) before adding 400 ppm thermostable α-amylase (500 Units/mg) and incubating in a water bath at 91°C for 60 minutes. Samples were then cooled to room temperature and before removing protein with the addition of 400 ppm proteinase (3 Units/mg) at 50°C for 14h. The proteinase was then deactivated by placing samples in a boiling water bath for 10 minutes. Samples were allowed to cool to room temperature and centrifuged at 4600 x g for 20 minutes. Ethanol (70:30 v/v in distilled water) was added to supernatants at 4°C overnight. The precipitate that formed was recovered by centrifugation at 4600 x g for 20 minutes, discarding supernatant and retaining the residue. The residue was weighed before washing and vortexing twice with 20 ml absolute ethanol (minimum 99%). Finally, 20ml acetone was added and samples vortexed for one minute followed by centrifugation at 4600 x g for 20 minutes. The final precipitates were dried for 48 hours at 45°C in a drying oven before transferring to vacuum-sealed, food-grade bags using a Turbovac SB425 Vacuum Packer (Stockport, UK) and storing at 21°C until further analysis.

Water-extractable arabinoxylan (WEAX) determination

The percentage of xylose in extracts was determined using a phloroglucinol assay following the method described by Douglas (Douglas 1981). The absorbance of each sample was measured at 552 nm and 510 nm using a ThermoScientific GENESYS 10S Bio Spectrophotometer (London, UK). A xylose standard curve was constructed to determine the xylose content of rice bran samples, which was subsequently used to calculate the amount of AXs in rice bran extracts.

Determination of sugar composition of purified extracts by HPLC

The sugar composition of purified extracts was determined using a method adapted from Zheng et al. (2011). A Shimadzu LC-20 AB HPLC system, (Shimadzu

Corporation, Tokyo, Japan), equipped with a Refractive Index Detector (RID) 10A, SUPELGUARD Pb (5 cm × 4.6 mm) guard column (Phenomenex, Macclesfield, UK) and SUPELCOGEL Pb (30 cm × 7.8 mm) column (Ion exclusion separation mode) (Phenomenex, Macclesfield, UK) was used to determine the sugar content of samples. The column temperature, mobile phase and flow rate were 80°C, HPLC grade water and 0.5 ml/min respectively in an isocratic run**.** Different concentrations (0.25, 0.5, 0.75 and 1 mg/ml) of glucose, xylose, galactose and arabinose were prepared as standards to plot a series of calibration curves from which the amount of each sugar was calculated based upon the relative peak areas.

Molecular weight standard curve

Five pullulan standards ranging from 5-375 kDa were used to construct the standard curve. Standards were prepared at 0.5 mg/ml using mobile phase and left overnight at 5°C. All samples and standards were filtered through a 0.45 µm nylon membrane and transferred to 1 ml glass shell vials. To prepare the pullulan standard curve, the pullulan molecular weights were converted to log molecular weights before plotting against the retention time.

Determination of the molecular weight distribution of AXs by HPLC

Dry samples were prepared for analysis by dissolving 2 mg of each sample in 1 ml mobile phase and leaving overnight at 5°C. The mobile phase was prepared by dissolving 0.65 g NaN3 and 17g NaNO3 in 2000 ml HPLC-grade water.

The molecular weight distribution of AXs was determined using size exclusion chromatography. All samples were analysed using a Shimadzu LC-10 HPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a JASCO RI-2031 Refractive Index (RI) Detector (Jasco Corporation, Tokyo, Japan), and BioSep-SEC-S 4000 and

BioSep-SEC-S 3000 columns (Phenomenex, Macclesfield, UK). An isocratic run was used, with a flow rate of 0.6 ml/min (Li et al. 2013).

Statistics

Data were expressed as mean ± standard error of the mean (SEM). Significant differences between samples were determined by one-way analysis of variance ANOVA. A *P* value < 0.05 was considered statistically significant.

Results

The effect of extrusion on extraction of AXs from rice bran

The extrusion process had a positive effect on the extraction yield of AXs. An increase in extrusion screw-speed resulted in a significant increase in extraction yield. Total AXs present in samples were calculated using the xylose standard curve and the Ar/Xy ratio obtained by HPLC. Extrusion significantly ($P<0.05$) increased the percentage of water-extractable AX, from 0.58 % without extrusion to 1.22 % with an extrusion screw-speed of 80 rpm and to 1.62 % with an extrusion screw speed of 160 rpm (Table 1).

Table 1. Extraction yield of AXs (%) from rice bran (RB) samples extruded at different screw-speeds, 80 rpm (RB80) and 160 rpm (RB160)

Samples	Ar/Xyl	Total AX
RBW	1.55 ± 0.020	0.58 ± 0.082
RB80	1.46 ± 0.008	1.22 ± 0.09
RB160	1.40 ± 0.006	1.62 ± 0.07

The arabinose to xylose ratio (Ar/Xy) of extracts

Glucose, arabinose, galactose and xylose monosaccharides were identified in the purified AXs from rice bran (Figure 1).

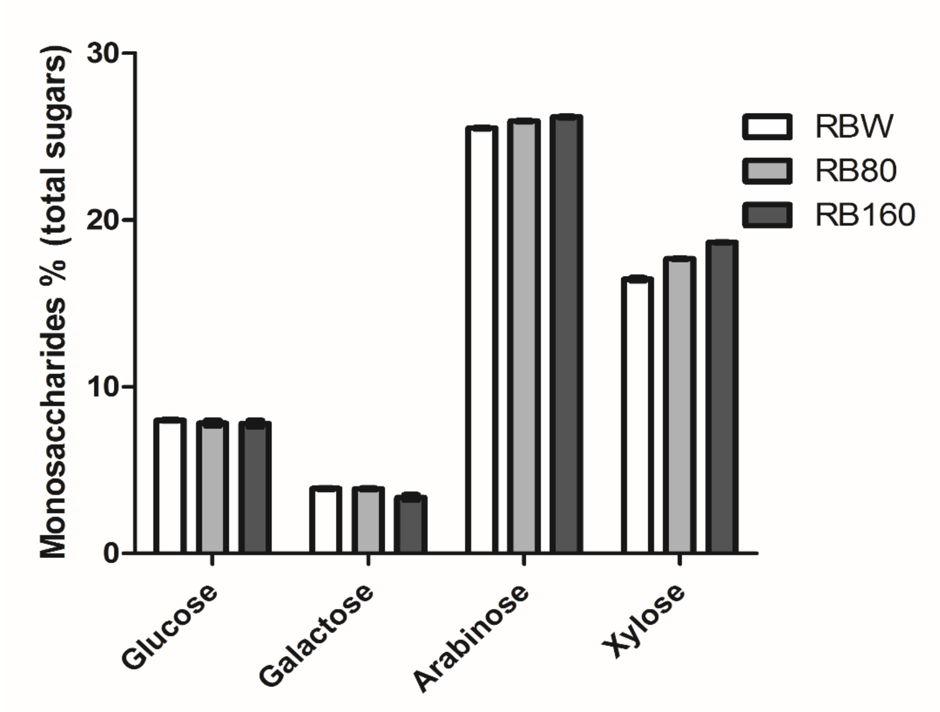

Figure 1. Sugar composition for purified AXs from rice bran (RB) obtained by water extraction alone (RBW) or via extrusion at 80 rpm (RB80) and 160 rpm (RB160).

The ratio of arabinose to xylose (Ar/Xy) decreased in rice bran samples as the extrusion screw-speed increased. The Ar/Xy ratio for water extracted AXs from un-extruded rice bran was 1.55 ± 0.2. The Ar/Xy ratios for extruded rice bran samples were 1.47 ± 0.008 and 1.40 ± 0.006 at screw speeds of 80 and 160 rpm respectively.

Molecular weight analysis of AXs using HPSEC

Pullulan standard curve construction

A standard curve was constructed using five pullulan standards (P5, P20, P100, P200 and P400) analysed by HPLC-SEC and used to determine the molecular weight and retention time of AXs in samples. The Mw of the five pullulan standards ranged between 5.9-375 kDa.

Molecular weight distribution of AXs

The Mw distribution of AXs from rice bran samples was characterized by HPLC-SEC. Table 2 illustrates the Mw range of AXs and percentage levels obtained.

Table 2. Mw distribution and Levels (%) of AXs extracted from rice bran (RB) by water extraction alone (RBW) or extrusion at 80 rpm (RB80) and 160 rpm (RB160)
Data are presented as mean ± SEM (n = 3). * indicates significant difference (*P*< 0.05) in levels of AXs compared to the RBW sample.

Areas	**Log Mw**	**Mw range (kDa)**	**%**		
			RB160	RB80	RBW
Area 1	2.90 - 3.2	0.79 - 1.58	18.24 ± 0.006 *	14.15 ± 0.08 *	11.42 ± 0.0057
Area 2	3.2 - 3.5	1.58 - 3.16	11.52 ± 0.08	16.13 ± 0.01 *	13.15 ± 0.01
Area 3	3.5 – 4	3.16 - 10	45.22 ± 0.73	47.09 ± 0.68	44.59 ± 0.76
Area 4	4 - 4.4	10 - 25.11	25.05 ± 0.027 *	22.63 ± 0.008 *	30.84 ± 0.01

Most notably, extrusion with a screw speed of 80 rpm (RB80) and 160 rpm (RB160) resulted in significantly (*P*<0.05) higher levels (14.15 ± 0.08 % and 18.24 ± 0.006 % respectively) of very low Mw (0.79-1.58 kDa) AXs compared to extraction without extrusion (RBW).

Discussion

Extraction rate of water-soluble AXs

In rice bran, WEAX is only around 0.9 % of the total AXs (Hashimoto et al. 1987). The low extractability of AXs could be due the large molecular weight of AXs (Saulnier et al. 2007) and to its ferulic acid (FA) contents (0.31-0.56 mg/g) (Michniewicz et al. 1990). FA side chains are esterified to some arabinose residues (Snelders et al. 2013), which form covalent/non-covalent bonds with the cell wall materials, thus decreasing the solubility of AXs in water. Jeon *et al*. (2014) stated that using extrusion cooking, as a pre-treatment is an efficient, environmentally friendly and low-cost process to increase the level of water extractable arabinoxylans in corn fibre. The results of this study confirm those of Jeon *et al*. (2014), showing an increase in the WEAX content of extruded rice bran with progressively increasing screw-speed, from 80 to 160 rpm. There was a 1.1-fold and 1.8-fold increase in WEAX content compared to the un-extruded rice bran sample as the extrusion screw-speed increased from 80 to 160 rpm respectively.

The increase in WEAX in extruded samples could be due to; the liberation of FA side chains, the softening of lignin and a reduction of MW by high mechanical shear forces. Holguín-Acuña *et al*. (2008) found that FA content increased from 0.2 mg/g in non-extruded maize bran to 2.5 mg/g in extruded maize bran. The increase in FA content might be the reason for the observed increase in solubility of AXs, since more AXs will be in contact with water as FA becomes liberated from side chains. Moreover, the increase in screw-speeds from 80 to 160 rpm may soften lignin (Yoo et al. 2012). AXs act as a glue between lignin and cellulose (Vermaas et al. 2015), therefore a high screw-speed will create high shear stress in the barrel which might soften the lignin

(Yoo et al. 2012), leading to greater exposure of AXs to water and a subsequent increase in solubility.

Molecular weight distribution of AXs from extruded/non-extruded rice bran

Molecular weight determinations for whole wheat AXs were reported to be within the ranges 56-65 kDa using gel permeation chromatography (Girhammar and Nair 1992) and 6-600 kDa for wheat endosperm using HPSEC (Li et al. 2013), the differences most likely arising from the type of wheat material used and the methodology applied for Mw determination. In this study, HPSEC was used to show the Mw of AXs from extruded/non-extruded rice-bran samples was between 0.79-25 kDa. Our findings fell within the lower end of the Mw range of AXs (0.6-500 kDa) previously reported from rice bran by Rose et al. (2009), suggesting HPSEC provides low Mw AXs and a narrower Mw range compared to the alkaline-hydrogen peroxide extraction used by Rose et al.

Table 2 demonstrates higher percentage levels of low Mw AXs from extruded rice bran samples compared with non-extruded samples. The increases in the percentage levels of low Mw AXs is probably due to the extrusion process, such as high shear forces and high temperatures resulting in depolymerisation of the fibre (Svanberg et al. 1995). It is also possible that extrusion cooking breaks down the glyosidic bonds resulting in depolymerisation of the cell wall material, thus reducing the Mw of AXs (Margareta and Nyman 2003).

Levels of very low Mw (0.79-1.58 kDa) AXs were significantly ($P<0.05$) increased in extruded compared to non-extruded rice bran samples. This could be related to the xylan backbone, which carries more arabinose side chains (Annison et al., 1995), which might be esterified by ferulic acids. It has been reported that extrusion breaks up FA side chains, thus reducing the Mw of AXs (Holguín-Acuña et al. 2008).

Conclusions

Higher percentage levels of low molecular weight AXs were obtained by twin-screw extrusion than by water extraction alone. These findings confirm that extrusion is suitable process to generate low molecular weight AXs for future studies investigating the immunomodulatory properties of AXs and may be practical solution to enrich AXs with most potential health benefits from cereals.

References

Annison G, Moughan P, Thomas D (1995) Nutritive activity of soluble rice bran arabinoxylans in broiler diets Br Poult Sci 36:479-488

Broekaert WF, Courtin CM, Verbeke K, Van de Wiele T, Verstraete W, Delcour JA (2011) Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides Crit Rev Food Sci Nutr 51:178-194

Büchi (2016) Extraction Unit E-812 / E-816 HE/ Technical data sheet. [online] [Accessed June 19th 2016] http://www.buchi.com/gb-en/products/extraction/extraction-unit-e-812-e-816

Daou C, Zhang H (2012) Study on functional properties of physically modified dietary fibres derived from defatted rice bran Journal of Agricultural Science 4:85

Douglas S (1981) A rapid method for the determination of pentosans in wheat flour Food Chem 7:139-145

Ghoneum M, Matsuura M (2004) Augmentation of macrphage phagocytosis by modified arabinoxylan rice bran (MGN-3/biobran) Int J Immunopathol Pharmacol 17:283-292

Girhammar U, Nair BM (1992) Certain physical properties of water soluble non-starch polysaccharides from wheat, rye, triticale, barley and oats Food Hydrocolloids 6:329-343

Gualberto D, Bergman C, Kazemzadeh M, Weber C (1997) Effect of extrusion processing on the soluble and insoluble fiber, and phytic acid contents of cereal brans Plant Foods Hum Nutr 51:187-198

Hashimoto S, Shogren M, Pomeranz Y (1987) Cereal pentosans: their estimation and significance. I. Pentosans in wheat and milled wheat products Cereal chemistry (USA)

Holguín-Acuña AL, Carvajal-Millán E, Santana-Rodríguez V, Rascón-Chu A, Márquez-Escalante JA, de León-Renova NEP, Gastelum-Franco G (2008) Maize bran/oat flour extruded breakfast cereal: A novel source of complex polysaccharides and an antioxidant Food Chem 111:654-657

Jeon S-J, Singkhornart S, Ryu G-H (2014) The Effect of Extrusion Conditions on Water-extractable Arabinoxylans from Corn Fiber Preventive nutrition and food science 19:124

Li W, Hu H, Wang Q, Brennan CS (2013) Molecular features of wheat endosperm arabinoxylan inclusion in functional bread Foods 2:225-237

Margareta E, Nyman G-L (2003) Importance of processing for physico-chemical and physiological properties of dietary fibre Proc Nutr Soc 62:187-192

Michniewicz J, Biliaderis C, Bushuk W (1990) Water-insoluble pentosans of wheat: composition and some physical properties Cereal Chem 67:434-439

Pawar S, Pardeshi I, Borkar P, Rajput M (2014) Optimization of process parameters of microwave puffed sorghum based ready-to-eat (RTE) food Journal of Ready to Eat Food 1:59-68

Rashid S, Rakha A, Anjum FM, Ahmed W, Sohail M (2015) Effects of extrusion cooking on the dietary fibre content and Water Solubility Index of wheat bran extrudates Int J Food Sci Technol 50:1533-1537

Rose DJ, Patterson JA, Hamaker BR (2009) Structural differences among alkali-soluble arabinoxylans from maize (Zea mays), rice (Oryza sativa), and wheat

(Triticum aestivum) brans influence human fecal fermentation profiles J Agric Food Chem 58:493-499
Santala O, Nordlund E, Poutanen K (2013) Use of an extruder for pre-mixing enhances xylanase action on wheat bran at low water content Bioresource technology 149:191-199
Saulnier L, Sado P-E, Branlard G, Charmet G, Guillon F (2007) Wheat arabinoxylans: exploiting variation in amount and composition to develop enhanced varieties Journal of Cereal Science 46:261-281
Snelders J, Dornez E, Delcour JA, Courtin CM (2013) Ferulic acid content and appearance determine the antioxidant capacity of arabinoxylanoligosaccharides J Agric Food Chem 61:10173-10182
Svanberg SM, Gustafsson KB, Suortti T, Nyman E-L (1995) Molecular weight distribution, measured by HPSEC, and viscosity of water-soluble dietary fiber in carrots following different types of processing J Agric Food Chem 43:2692-2697
Thymi S, Krokida M, Pappa A, Maroulis Z (2005) Structural properties of extruded corn starch Journal of food engineering 68:519-526
Vasanthan T, Gaosong J, Yeung J, Li J (2002) Dietary fiber profile of barley flour as affected by extrusion cooking Food Chem 77:35-40
Vermaas JV, Petridis L, Qi X, Schulz R, Lindner B, Smith JC (2015) Mechanism of lignin inhibition of enzymatic biomass deconstruction Biotechnology for biofuels 8:217
Yoo J, Alavi S, Vadlani P, Behnke KC (2012) Soybean hulls pretreated using thermo-mechanical extrusion—hydrolysis efficiency, fermentation inhibitors, and ethanol yield Appl Biochem Biotechnol 166:576-589
Zheng X, Li L, Wang X (2011) Molecular characterization of arabinoxylans from hull-less barley milling fractions Molecules 16:2743-2753