PUBLICATION IV

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Enzymatic modification and particle size reduction of wheat bran improves the mechanical properties and structure of bran-enriched expanded extrudates

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KEY WORDS

Extrusion; wheat bran; particle size; enzymatic modification

ABBREVIATIONS

AX, arabinoxylan; C_i, crispiness index; DF, dietary fibre; DM, dry matter; ER, expansion rate; F_{cr}, crushing force; F_{max}, maximum point of the force-deformation curve; IDF, insoluble dietary fibre; SDF, soluble dietary fibre; WEAX, water extractable arabinoxylan; WHC, water holding capacity.

ABSTRACT

The aim of this study was to examine enzymatic modification of wheat bran, performed in a low-moisture process, and the reduction of bran particle size as means of improving the technological performance of wheat bran in expanded extrudates. Modification of bran by hydrolytic enzymes increased the crispiness and decreased the hardness and piece density of extrudates containing wheat bran and endosperm rye flour in 20:80 ratio. These improvements correlated ($P < 0.01$ or 0.05) with an increased content of water extractable arabinoxylan and decreased water holding capacity of the bran, as well as with increased longitudinal expansion of the extrudates. Furthermore, bran with a fine average particle size (84 µm) produced extrudates with improved mechanical properties and higher radial expansion than coarse bran (particle size 702 µm). The impact of bran particle size was also observed in the cellular structure of the extrudates as differences in cell size and homogeneity. The bran drying method, oven or freeze drying after enzymatic modification, did not have a major impact on the properties of the extrudates. The study showed that the functionality of wheat bran in extrusion can be improved by enzymatic modification using a low-water process and by reduction of bran particle size.

1. INTRODUCTION

Extrusion technology is a versatile option for the production of different types of ready-to-eat snacks with puffed structure and cooked characteristics appealing to consumers (Brennan, 2013). During the past 10–15 years, consumers have become more health conscious and are increasingly demanding tasty snack products which satisfy their hunger and yet are low in fat, rich in dietary fibre (DF) and preferably fortified with vitamins and minerals (Brennan, 2013). Wheat bran is a good source of DF and contains a relatively high amount of protein and phytochemicals. However, as recently reviewed by Robin et al. (2012) and Sozer and Poutanen (2013), increasing the amount of DF or bran in extrusion formulations typically causes deterioration of the textural properties of the product, e.g. by increasing the density and hardness and decreasing the expansion volume and crispiness of the product. Extrudate expansion, which is crucial for the formation of the appetizing and crispy textures, is governed by a complex series of events, in which starch plays a major role (Moraru and Kokini, 2003). The impact of DF on the texture of extruded products is generally considered to depend mainly on its interactions with starch and on its effects on the mechanistic steps of expansion, i.e. starch transformation, nucleation of bubbles, extrudate swell, growth of bubbles, and bubble collapse (Moraru and Kokini, 2003; Robin et al., 2012). Particularly insoluble DF (IDF) has been reported to be detrimental to the extrudate characteristics (Robin et al., 2012). The adverse effects of IDF in extrusion have been related to the changes they cause in the rheological properties of the batter melt and in the amount of free water available for starch transformations and expansion, as well as to the physical disruption of the continuous starch matrix and gas cell walls by the fibre particles (Pai et al., 2009; Moraru and Kokini, 2003; Robin et al., 2012).

Different strategies have been studied as means to improve the technological performance of DF ingredients in extruded products. Decreasing DF ingredient particle size has been reported to increase the expansion of extrudates containing DF (Lue et al., 1991; Blake, 2006; Alam et al., 2013). However, particle size reduction has not improved expansion when the size differences or addition levels are low (Robin et al., 2011b; Blake, 2006; Alam et al., 2013). It has also been reported that soluble DF (SDF), such as pectin, inulin or guar gum, generally performs better than fibres that are mostly insoluble (IDF), e.g. wheat bran (Yanniotis et al., 2007; Brennan et al., 2008), and it has been suggested that increasing the solubility of DF prior to extrusion could be a means to improve the functionality of DF in extruded products (Robin et al., 2012). However, only few studies have examined this possibility. Pai et al. (2009) showed that increasing the SDF content of corn bran with concomitant reduction of IDF by a chemical treatment resulted in higher expansion as compared to untreated bran. The improved expansion was related to favourable changes in melt viscosity and better interaction of SDF with starch (Pai et al., 2009).

Modification of bran by enzymatic processing has been shown to facilitate the addition of bran, and thus DF, to food products, and the beneficial effects of these processes have often been related to the transformation of IDF to SDF (Lebesi and Tzia, 2012; Coda et al., 2014). However, to our best knowledge enzymatically modified bran has not previously been studied as an ingredient in extrudates. Enzymatic treatments are typically preformed in high water content, which is not economical especially if the modified ingredient should be dried prior to its subsequent use in a low moisture process such as extrusion. On the other hand, enzymatic processing at low water content, i.e. high consistency, typically causes reduction of enzyme action and requires a substantial amount of energy for mixing. It has been shown that efficient xylanase action on wheat bran at low water content and without continuous mixing can be accomplished by the use of an extruder-aided pre-mixing process (Santala et al., 2013a).

In the current study the enzymatic degradation of wheat bran was investigated as a means of improving the quality of bran-supplemented endosperm flour-based expanded extrudates. The aims were 1) to study the impact of different variations of the extruder-aided low-moisture enzymatic treatment and the subsequent drying step on the physicochemical properties of wheat bran of two different particle sizes, and thereafter 2) to study how the physicochemical properties of the modified bran ingredient and the reduction of bran particle size affect the macro- and microstructure and mechanical properties of bran-supplemented expanded extrudates.

2. MATERIALS AND METHODS

2.1. Cereal raw materials

Commercial wheat bran (Fazer Mill & Mixes, Lahti, Finland) was ground by TurboRotor technology (Mahltechnik Görgens GmbH, Dormagen, Germany) to two different levels of fineness so that the mean particle sizes were 702 µm (hereafter referred as coarse bran) and 84 µm (fine bran). The grinding process did not contain any sieving or fractionation steps, thus the fine and coarse bran were composed of the same bran raw material. The high air throughput and short residence times used in the grinding technology ensured that the product temperature remained below 45 °C, thus avoiding the heat damage often associated with intensive grinding treatments. The total and water soluble DF contents were 49.9 and 6.7% in the coarse and 48.0 and 8.2% in the fine bran, respectively. Arabinoxylan content was 20.5% (coarse) and 20.6% (fine) and starch content was 16.7 (coarse) and 16.5% (fine).

Rye endosperm flour (Helsinki mills ltd. Järvenpää, Finland) was used as a base material for the expanded extrudates. The total and soluble DF contents were 11.8% and 9.6%, respectively, and the starch content was 84.7%.

2.2. Enzyme preparations

Commercial hydrolytic enzymes, Depol 761P (Biocatalysts Ltd, Cardiff, UK), a xylanase preparation derived from *Bacillus subtilis*, and Veron CP (AB Enzymes GmbH, Darmstadt, Germany), a cellulolytic enzyme preparation with hemicellulase side activities from *Trichoderma reesei*, were used either individually or in combination for the bran treatments. The activity profile of Depol 761P, endoxylanase 28,660 nkat/g, polygalacturonase 1,317 nkat/g, β-glucanase 1,625 nkat/g, α-amylase 44 nkat/g, and β-xylosidase 2 nkat/g, was previously reported by Santala et al. (2013b). The activity profile of Veron CP, determined by the methods described by Santala et al. (2013b), was as follows: endoglucanase 18974 nkat/g, cellulase (filter paper as substrate) 53 filter paper units/g, β-glucanase 75760 nkat/g, xylanase 14610 nkat/g, β-xylosidase 257 nkat/g, polygalacturonase 8469 nkat/g, mannanase 3022 nkat/g, α-arabinosidase 530 nkat/g, β-glucosidase 528 nkat/g and α-amylase 94 nkat/g. The preparation was free from ferulic acid esterase and proteinase. Enzyme preparations were dosed according to their xylanase activity at 200 nkat/g bran dry matter (DM) (treatments with Depol 761P) or 100 nkat/g (treatments with Veron CP, corresponding to an endoglucanase activity of 130 nkat/g). For the treatments with a combination of the enzymes the dosages as xylanase activity were 200 nkat Depol 761P and 100 nkat Veron CP /g bran DM.

2.3. Production of enzymatically modified bran

Bran modification was performed by extrusion-aided enzyme treatment as described by Santala et al. (2013a). 450 g of bran with an initial moisture content of 10.7% (coarse) or 5.5% (fine) was first mixed with the enzyme preparation(s) (in powder form) and pre-

conditioned to a moisture content of 20% by adding water slowly while mixing (speed setting 2) with a Kenwood KM300 mixer (Kenwood Ltd., Havant, UK) with a K-shaped blade for 2 min. Pre-conditioning was also performed for the blank treatments (i.e. without enzyme addition). The pre-conditioned bran mixture was transferred to the feeding unit (a co-rotating twin screw feeder, K-Tron Soder, Niederlenz, Switzerland) of a twin screw extruder (APV MPF 19/25, Baker Perkins Group Ltd, Peterborough, UK) within 20 min and fed to the extruder at a rate of 26 g/min. The screw configuration is presented in Fig. 1. The barrel temperature was 50 °C and the screw speed was 65 rpm. Water was pumped to the beginning of the barrel at an appropriate rate in order to obtain moisture contents of 48±1% in the bran mixture. Bran mixture was collected continuously from the die exit (diameter 3 mm) and the collected material was transferred every 2 minutes either to incubation (at 50°C for 4 h in sealed containers) or to drying, which was performed either in an oven (samples spread on metal trays and dried with air circulation at 50°C for 18–20 h) or by freezing the sample in liquid nitrogen for subsequent freeze drying. The incubated samples were also immediately dried by oven drying or by freeze drying. The dried samples were ground in a mill (Hosokawa Alpine, 100 UPZ, Retsch GmbH, Germany) with two different settings (coarse bran samples with sieve size 0.5 mm and rotor speed 6000 rpm; fine bran samples with a 0.3 mm sieve and 18 000 rpm) in order to maintain the different particle sizes of the two bran types (coarse and fine).

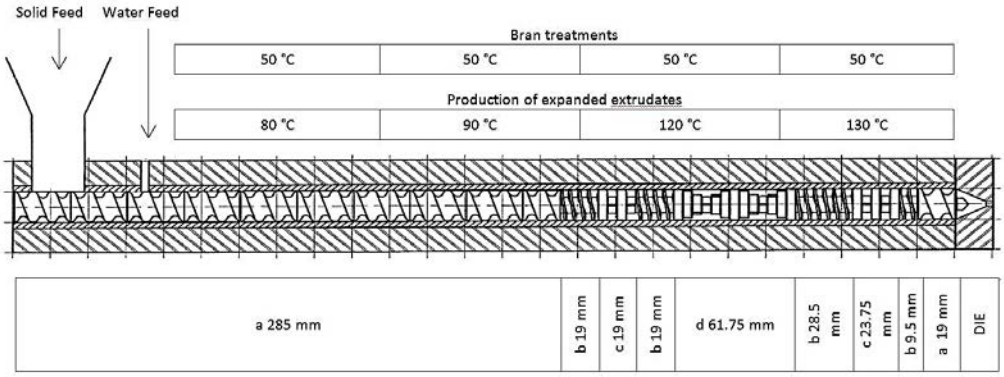

Fig. 1. Screw configuration of the extruder and the temperature profiles used in the experiments. The letters indicate the type of each screw element. Feed screw (a), lead discharge screw (b), mixing paddles at 90º (c), mixing paddles at 30º (d).

2.4. Production of expanded extrudates

Bran samples were mixed with rye endosperm flour in 20:80 ratio on a dry matter basis in a Kenwood KM300 mixer (Kenwood Ltd., Havant, UK) with a K-shaped blade for 4 min. The same extruder, feeder and die were used as for the bran treatments. The feed rate was calibrated separately for each bran ingredient-flour mixture to a level of 60 g/min. Extrusion parameters were selected on the basis of pre-trials performed with the flour base material in order to obtain maximum expansion. Temperatures at the barrel zones 1 to 4 were set at 80, 90, 120 and 130 °C (Fig. 1) and the screw speed was 450 rpm. The water feed rate was adjusted on the basis of the moisture content of the feed material to attain a moisture content of 16%. The extrudates were collected in trays and dried at 50°C for 30 min in an oven dryer with air circulation. The samples were stored at 14°C in sealed pouches. The extrusions were performed in duplicate.

2.5. Analysis of chemical composition and physicochemical properties

The moisture contents of the bran samples were analysed gravimetrically by oven drying samples of 1–2 g at 130 °C for 1 h. Total and soluble DF contents were analysed by AOAC method no. 2009.01 (McCleary et al., 2013) and starch by the Megazyme method (McCleary et al., 1994).

For the analysis of water extractable AX, 3.2 g of bran was mixed with 32 ml of ice-cold distilled water and shaken with glass beads for 15 min at 4°C. After centrifugation, the supernatant was boiled in a water bath for 20 minutes and recentrifuged. The supernatant was stored at -20 °C until analysis. The contents of AX and pentose monosaccharides in the water extracts (hereafter referred to as water extractable AX, WEAX) were determined by a colorimetric phloroglucinol method (Douglas, 1981) using xylose as a standard. For the quantification of total AX, 0.1 g of bran was mixed with 5 ml of 0.5 M H2SO4 and boiled for 30 min and centrifuged, followed by the colorimetric determination (Douglas, 1981).

The median particle size of the modified brans and the untreated fine bran was determined by laser light diffraction in a Beckman Coulter LS230 particle size analyser (Coulter Corporation, Miami, USA) after dispersing the samples in 95% ethanol using magnetic stirring for 1–2 min. Due to the limited analysis range (up to 2000 µm) of the Beckman Coulter analyser, the particle size of the untreated coarse bran was determined from dry bran dispersion in a Mastersizer 3000 apparatus (Malvern, Worcestershire, UK). Median particle sizes were calculated from the volumetric distribution of the particles using the Fraunhofer optical model.

Water holding capacity (WHC) was determined in a Baumann apparatus as described previously (Santala et al., 2013b), using a sample size of 50 mg and measurement time of 30 min.

2.6. Analysis of the expanded extrudates

For the analysis of macrostructural parameters, the extrudates were cut into 20 pieces each of 5 cm length using a band saw (Scheppach, Germany). Expansion rate (ER), specific length and piece density of each sample were calculated as described by Alam et al. (2013).

Mechanical properties of the extrudates were measured by applying uniaxial compression using a texture analyser (TA.XT plus, Stable Micro Systems Ltd., United Kingdom) containing a 30 kg load cell and a 25 mm aluminium probe under 70% strain with a test speed of 1 mm/s. All extrudates were cut into 10 mm pieces (radial section) and equilibrated at 43 % relative humidity (RH) at 21 °C prior to analysis. Measurements were performed for 20 replicates. Exponent software version 6.0.7.0 (Stable Micro Systems Ltd., United Kingdom) was used to obtain values for the calculation of the hardness indicators F_{max} (the maximum point of the force-deformation curve) and crushing force (F_{cr}), i.e. average puncturing force (van Hecke et al., 1998), and crispiness index (C_i), which was calculated by the following equation (Heidenreich et al., 2004):

$$C_i = \frac{L_N}{A \times F_{mean}}$$

where L_N is the normalized curve length (length of actual curve/F_{max}), A is the area under the force-deformation curve and F_{mean} is the sum of the actual force values in the data file divided by the number of data points.

For the stereomicroscope imaging of the radial cross-sections of the extrudates, the samples were cut into 10 mm pieces and examined under a SteREO Discovery.V8 stereomicroscope with an Achromat S 0.5x objective (Carl Zeiss MicroImaging GmbH,

Göttingen, Germany) and imaged using a DP-25 single chip colour CCD camera (Olympus Life Science Europa GmbH, Hamburg, Germany) and the Cell^P imaging software (Olympus).

For the analysis of total and soluble DF contents, the extrudates were ground in a laboratory mill with a 0.5 mm sieve and analysed by AOAC method no. 2009.01 (McCleary et al., 2013).

2.7. Statistical analyses

All bran treatments and extrusions were made in duplicate, and the physicochemical properties of each sample were analysed at least in duplicate. Thus all the results of the physicochemical properties were calculated as means of at least four analysis results. The parameters of macrostructure and mechanical properties were calculated as means of 35–40 results. Data were subjected to analysis of variance using IBM SPSS Statistics 21 (IBM Corporation, Somers, NY, USA), and significant differences ($P < 0.05$) between individual means were identified by the Tukey's test. Correlations between the different variables were determined by subjecting the mean values of each bran sample and the corresponding bran extrudate to the 2-tailed Pearson's bivariate correlation analysis.

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties of the untreated and modified brans

The current study explored extrusion-aided enzymatic degradation and reduction of wheat bran particle size as means of improving the quality of bran-supplemented endosperm flour-based expanded extrudates. Coarse and fine wheat bran were treated either without enzymes followed by direct freeze or oven drying, or with added xylanase enzyme preparation (Depol 761P) followed by 4 h incubation and drying. In order to gain better understanding on the impacts of the use of incubation and different types of enzymes, the fine bran was additionally treated with different combinations of the process parameters and by two different enzyme preparations, Depol 761P and Veron CP, and their combination.

All treatments caused significant reduction in the mean particle size of the coarse bran from 702 to 205–318 µm, whereas the particle size of the fine bran was reduced less, from 84 to 45–69 µm (Table 1). The use of an extruder for pre-mixing of bran and enzymes was studied previously without any drying or regrinding steps (Santala et al., 2013a). It was found that the particle size of the bran decreased during the extrusion mixing, and that the reduction was more severe in the case of coarse bran (from *ca*. 900 to 600 µm) than in case of fine bran (from 84 to 76 µm). In the current study, the particle size reductions were greater, presumably due to the regrinding after drying. The grinding was performed with two different intensities (mild and severe) in order to maintain the different particle sizes of the two bran types (coarse and fine). The reduction of the particle size of the treated brans did not depend significantly on the processing variables used (the use of enzymes, use of incubation vs. direct drying, and the drying method).

Table 1. Properties of the untreated and modified (0 h = treated without incubation, 4 h = treated with 4 h incubation, OD = oven dried, FD = freeze dried) coarse and fine bran ingredients. The results are expressed as means (n=4–6). Values marked with different letters within the results are significantly different ($P < 0.05$). For WEAX content, the statistical analysis was performed separately for samples treated with and without added enzyme preparations (Depol 761P and Veron CP). For median particle size, the statistical analysis was performed separately for fine and coarse bran, and thus the letters indicate significant difference ($P < 0.05$) within each bran type.

			WEAX (% bran DM)		**Median particle size (µm)**		**WHC (g water /g bran DM)**	
			Coarse	Ultrafine	Coarse	Ultrafine	Coarse	Ultrafine
Untreated bran			0.5 a	0.8 b	702 a	84 a	3.7 a	3.3 b
Treated with no added enzymes	0 h	OD	1.4 de	1.6 e	279 b	68 ab	3.0 c	3.1 bc
	0 h	FD	1.1 c	1.3 cd	318 b	61 ab	3.7 a	3.1 bc
	4 h	OD	-	2.7 f	-	69 ab	-	3.0 c
Treated with Depol 761P	0 h	OD	-	4.2 g	-	70 ab	-	2.6 d
	4 h	OD	4.8 h	5.6 i	205 c	52 ab	2.5 d	2.4 d
	4 h	FD	4.9 h	5.7 i	285 b	45 b	3.1 bc	2.4 d
Treated with Veron CP	4 h	OD	-	4.3 g	-	62 ab	-	2.4 d
Treated with Veron CP + Depol 761P	4 h	OD	-	6.2 j	-	57 ab	-	2.4 d

AX is the main DF component of wheat, and solubilisation of AX was assayed by measuring the content of WEAX in bran. Treatment without enzymes with direct drying increased the WEAX content of coarse and fine bran from the initial of 0.5–0.8% to 1.1–1.6% of bran DM (Table 1), indicating that some AX was solubilised already during the extrusion process, probably due to the shear exerted on the bran mixture. It has also previously been reported that mechanical work input may cause the degradation of DF components (Ralet et al., 1990; Hemery et al., 2011; Santala et al., 2013b). When the fine bran sample was further incubated the WEAX content increased to 2.7%, indicating the action of endogenous bran enzymes since there was no shear during the stationary incubation. With Depol 761P enzyme preparation, significant increase in the WEAX content (to 4.2%) occurred already without incubation, indicating that the added enzymes started to act immediately during mixing in the extruder, despite the relatively low water content used (48%). When incubated with Depol, the WEAX content further increased during the incubation, and it was higher in the fine bran (5.6–5.7%) than in the corresponding coarse bran sample (4.8–4.9%). This can be explained by the increase in the surface area of the bran due to the reduction of particle size, which has also previously been reported to make the substrate more accessible to enzymes (Niemi et al., 2012).

The fine bran treated with the combination of Veron CP and Depol 761P enzyme preparations had a slightly higher WEAX content (6.2%) than the brans treated with Depol (5.7%) or Veron (4.3%) alone. This was obviously due to the different doses of endoxylanase and other enzyme activities in the treatments. Bran treatments with Depol 761P contained mainly endoxylanase (200 nkat/g bran), whereas treatment with Veron CP contained only 100 nkat/g endoxylanase and additionally 130 nkat/g endoglucanase and 465 nkat/g β-glucanase, as well as higher levels of other side activities. The enzyme dose was highest in the combination treatment, since it contained both enzyme preparations dosed at the same level as in the

individual enzyme treatments. The use of multiple hydrolytic enzyme activities is generally considered beneficial in the degradation and solubilisation of bran, due to the synergistic action of different enzymes specific for certain cell wall components (Faulds and Williamson, 1995). In the current study, however, instead of studying the synergistic action of the enzymes, the dosages were selected with the aim of obtaining brans with different levels of AX degradation, in order to elucidate the impact of AX solubilisation on the functionality of bran in extrusion.

WHC of the untreated coarse bran (3.7 g water/g bran DM) was higher than that of the untreated fine bran (3.3 g/g). Treatment without enzymes with direct oven drying reduced the WHC of both brans to 3.0–3.1 g/g, and the use of enzymes reduced WHC further (Table 1). Degradation of AX apparently decreased the WHC of WB, since water unextractable AX is capable of binding more water than WEAX (Courtin and Delcour, 2002). Indeed, a significant ($P < 0.01$) negative correlation (-0.864) was found between WEAX content and WHC (Table 2). Particle size is also known to affect the hydration properties of bran (Noort et al., 2010; Santala et al., 2013b), and a significant ($P < 0.01$) positive correlation (0.666) between particle size and WHC was also found in the current study.

Table 2. Pearson's correlation matrix for physicochemical properties of the bran ingredients and the macrostructural and mechanical properties of the bran-supplemented extrudates.

	WHC	**Particle size**	**Expansion rate**	**Specific length**	**Piece density**	**F_{max}**	**Crushing force**	**Crispiness index**
WEAX	-0.864**	-0.454	0.127	0.709**	-0.617*	-0.828**	-0.799**	0.898**
WHC	1	0.666**	-0.355	-0.710**	0.775**	0.844**	0.858**	-0.866**
Particle size		1	-0.682**	-0.355	0.755**	0.604*	0.636*	-0.608*
Expansion rate			1	-0.045	-0.655*	-0.250	-0.319	0.285
Specific length				1	-0.716**	-0.824**	-0.808**	0.747**
Piece density					1	0.807**	0.852**	-0.764**
F_{max}						1	0.958**	-0.935**
Crushing force							1	-0.927**
Crispiness index								1

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

3.2. Impact of bran properties on the structure of bran-supplemented extrudates

The addition of untreated brans caused a significant decrease in the volumetric expansion of the extrudates, indicated by an increase in the piece density when the untreated coarse (169 kg/m^3) or fine bran (155 kg/m^3) was used as compared to the control extrudate without bran (130 kg/m^3) (Table 3). Expansion of extrudates occurs in both radial and longitudinal directions, and thus overall expansion is governed by both phenomena. As expected, the addition of bran caused a

reduction in the radial expansion of the extrudates, indicated by a reduction in the ER of the extrudates with untreated bran (ER 354–390%) as compared to that of the control extrudate without bran (452%). The specific length of the extrudates increased from that of the control (54 m/kg) when untreated coarse (68 m/kg) or fine (62 m/kg) bran was added (Table 3). In agreement, many sources have reported that radial expansion of cereal extrudates decreases and longitudinal expansion increases in the presence of IDF (Lue et al., 1991; Jin et al., 1995; Robin et al., 2011; Brennan et al., 2008). This has been attributed to the alignment of fibres in the direction of flow (Karkle et al., 2012; Moraru and Kokini, 2003).

Table 3. Macrostructural parameters of the extrudates with and without modified (0 h = treated without incubation, 4 h = treated with 4 h incubation, OD = oven dried, FD = freeze dried) coarse and fine bran ingredients. The results are expressed as means (n = 35–40). Values marked with different letters within the same parameter are significantly different ($P < 0.05$).

			Expansion rate (%)		**Specific length (m/kg)**		**Piece density (kg/m^3)**	
Control (no bran)			452 a		54 a		130 c	
			Coarse	Fine	Coarse	Fine	Coarse	Fine
Untreated bran			354 gh	390 bcd	68 c	62 b	169 a	155 b
Treated with no added enzymes	0 h	OD	371 ef	401 b	78 efg	79 efgh	132 c	113 e
	0 h	FD	351 h	404 b	74 de	69 cd	155 b	126 cd
	4 h	OD	-	371ef	-	82 ghi	-	128 cd
Treated with Depol 761P	0 h	OD	-	399 bc	-	83 ghi	-	108 e
	4 h	OD	355 gh	404 b	84 hij	83 ghi	136 c	106 e
	4 h	FD	367 fg	384 cde	80 fgh	74 ef	133 c	129 c
Treated with Veron CP	4 h	OD	-	372 ef	-	89 j	-	116 de
Treated with Veron CP +Depol 761P	4 h	OD	-	379 def	-	86 ij	-	116 de

The ER decreased less when untreated bran of fine particle size was used (ER 390%) as compared to the use of coarse bran (354%). Similarly, when modified brans were used the ER was always higher in the fine bran extrudates (371–404%) as compared to those of the corresponding coarse bran extrudates (351–371%) (Table 3). Indeed, there was a significant ($P < 0.01$) negative correlation between ER and bran particle size (-0.682) and a significant positive correlation between piece density and particle size (0.755) (Table 2). Smaller particle size has also previously been reported to favour radial expansion of rye bran extrudates (Alam et al. 2013) and corn meal extrudates containing sugar beet fibre (Lue et al., 1991) or corn bran (Blake, 2006). It has been suggested that coarse particles may cause early rupture of gas cells before their optimal expansion, or that reduction of particle size may improve expansion by providing more nucleation sites, and thus more air cells, than coarse fibre particles (Lue et al., 1991). Alam et al. (2013) reported that reduction of rye bran particle size from 440 µm to 28 µm improved expansion, and increased extrudate porosity and the average cell size. In the current study, bran particle size also affected the cellular structure as observed from the radial cross-section images obtained by stereomicroscopy (Fig. 2). In the samples with the coarse bran, the cells were small and the large bran particles were clearly visible, whereas in the samples with the fine bran, the cell size distribution was less homogeneous due to the

presence of some large cells, and the bran particles were less visible. It was also noted that particle size and WHC of the bran were significantly correlated (Table 2), and it is possible that the effect of fibre particle size on expansion and cellular structure was not only related to their physical dimensions, but also to their different hydration properties and their impact on melt rheology, as previously suggested by Sozer and Poutanen (2013).

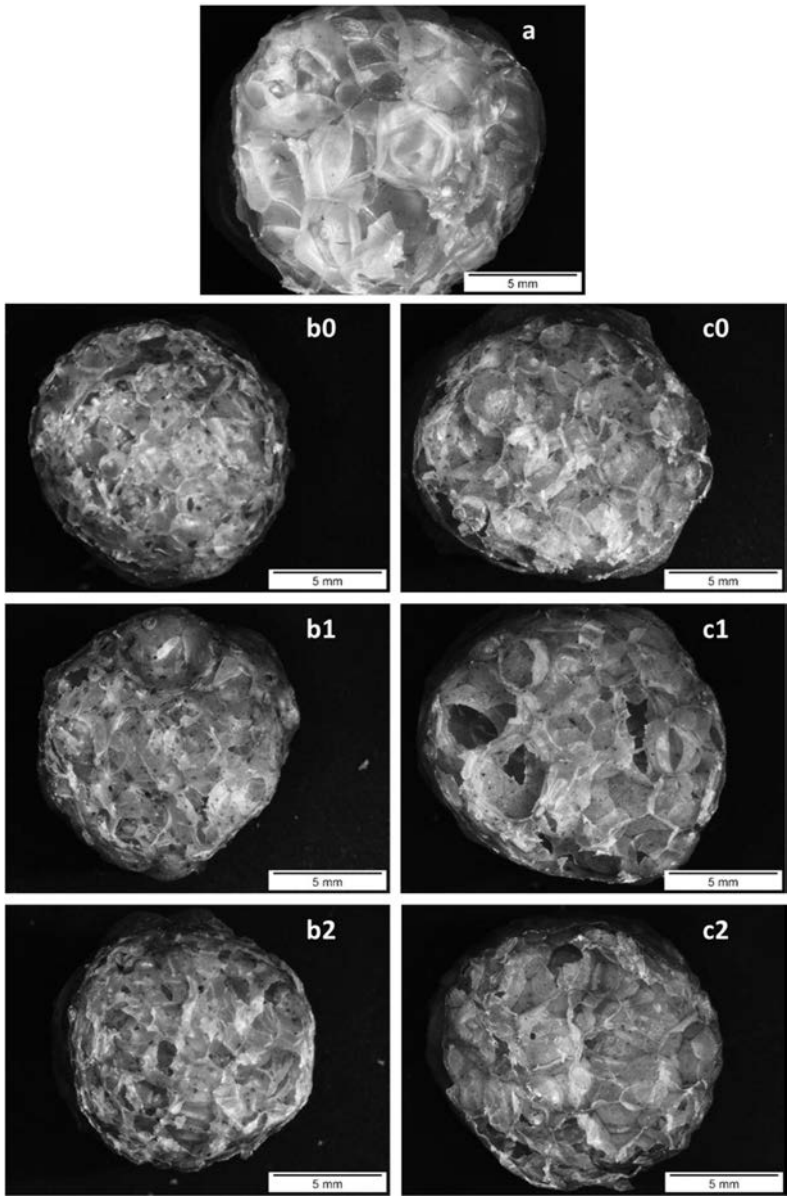

Fig. 2. Stereomicroscope images of radial sections of the extrudates. Control extrudate with no added bran (a), extrudates supplemented with 20% of untreated coarse bran (b0), coarse bran treated with no added enzymes and direct oven drying (b1), coarse bran incubated 4 h with Depol 716P enzyme preparation (b2), untreated fine bran (c0), fine bran treated with no added enzymes and direct oven drying (c1), fine bran incubated 4 h with Depol 716P enzyme preparation (c2).

The use of enzymatically modified brans produced extrudates with equal or lower piece density than that of the control (Table 3). The lowest piece densities were obtained with the enzyme-treated (Depol 761P, Veron CP or their combination) oven dried fine bran (106–116 kg/m^3). Radial expansion remained generally almost unchanged when modified brans were used as compared to the use of untreated brans (Table 3), whereas the specific length increased with modified brans, especially when treated with enzymes (74–89 m/kg), as compared to the specific length of the untreated bran extrudates (62–68 m/kg). A significant negative correlation was observed between piece density and specific length (-0.716) (Table 4). The results indicate that the higher volumetric expansion (lower piece density) of the extrudates with enzymatically modified brans was caused at least partly by increased longitudinal expansion.

Table 4. Mechanical properties of the extrudates with and without modified (0 h = treated without incubation, 4 h = treated with 4 h incubation, OD = oven dried, FD = freeze dried) coarse and fine bran ingredients. The results are expressed as means (n=35–40). Values marked with different letters within the same parameter are significantly different ($P < 0.05$).

			Crushing force (N)		**Hardness (N)**		**Crispiness index (×10^3)**	
Control (no bran)			18.3c		36.4ab		5.8bc	
			Coarse	Fine	Coarse	Fine	Coarse	Fine
Untreated bran			22.5a	21.8 ab	39.4 a	38.4 a	3.4 a	3.9 a
Treated with no added enzymes	0 h	OD	16.6 cd	15.3 def	30.2 cd	30.2 cd	6.3 cd	7.8 de
	0 h	FD	20.6 b	18.3 c	35.8 ab	32.8 bc	4.2 ab	5.8 bc
	4 h	OD	-	15.1 def	-	23.8 fg	-	10.8 gh
Treated with Depol 761P	0 h	OD	-	16.3 de	-	26.8 def	-	9.7 fg
	4 h	OD	15.4 def	13.2 g	26.1 ef	22.5 g	8.4 ef	12.1 hi
	4 h	FD	15.9 de	13.8 fg	28.1 de	25.6 efg	9.6 fg	12.9 i
Treated with Veron CP	4 h	OD	-	14.6 efg	-	27.4 def	-	10.7 gh
Treated with Veron CP +Depol 761P	4 h	OD	-	14.8 defg	-	25.6 efg	-	11.7 hi

Melt viscosity and the level of available water are considered important factors affecting expansion, and the effects of IDF and SDF on expansion have also been related to their effects on these properties (Robin et al., 2011; Moraru and Kokini, 2003). Pai et al. (2009) related the superior performance of alkali-solubilised corn bran on the degree of expansion to the reduction in melt viscosity. They concluded that in order to obtain good extrudate expansion, viscosity should be low enough to allow easy melt stretching, bubble growth and expansion, but high enough to withstand the stretching forces and prevent bubble collapse (Pai et al., 2009). Lobato et al. (2011) reported that addition of inulin to oat bran-supplemented soy flour resulted in improved expansion due to better melt flow properties. In the current study, the correlation analysis showed that specific length was significantly ($P < 0.01$) correlated with both WEAX content (0.709) and WHC (-0.710) (Table 2). It seems likely that the increase in longitudinal expansion was caused by altered rheological properties of the melt due to increase in WEAX content and/or by an increasing level of available water in the system due to reduced WHC. WEAX is known to affect viscosity depending on its molecular weight (Courtin and Delcour, 2002). Furthermore, Robin et al. (2011) reported that addition

of wheat bran resulted in increase in water activity and decrease in the glass transition temperature (T_g) of the melt, which would decrease the starch viscosity at constant temperature (Robin et al. 2011). Thus, it is also possible that in the current study, the increase in the level of available water (decrease in bran WHC) might also have promoted longitudinal expansion due to decreased melt viscosity by reducing melt T_g. However, the above-mentioned possible mechanisms behind the observed effects of bran WHC and WEAX content on expansion remain to be tested experimentally.

It has previously been reported that SDF generally produces higher radial expansion than IDF (Pai et al., 2009; Yanniotis et al., 2007; Brennan et al., 2008), but in the current study no correlation was observed between radial expansion and WEAX content of the bran (Table 2). In accordance, clear differences were not observed when comparing the radial cross-section images of the samples with treated brans to those of the corresponding untreated brans (Fig. 2). This could be due to the relatively low differences in the chemical composition of the flour-bran mixtures in the current study as compared to those of the previous studies. As analysed from selected extrudates (untreated fine and untreated coarse bran extrudates, and extrudates with oven dried fine bran treated with all different process variations), the content of IDF in the extrudates varied between 8.3 and 9.7% and the content of SDF (including oligosaccharides) between 6.5 and 7.9% (data not shown). Thus, the differences in the contents of IDF and SDF were relatively small in the flour-bran mixtures. For example, in the study of Pai et al. (2009), who studied the impact of alkali-solubilized corn bran on extrusion, the differences in SDF content ranged from 1.6 to 64%, whereas other studies have mainly compared the impacts of addition of bran to those of oligosaccharides or gums with no IDF and essentially different chemical composition from that of the bran (Brennan et al., 2008; Yanniotis et al., 2007). The total DF content in all extrudates was between 15.8 and 16.4% of the bran DM (data not shown), indicating that the bran treatments had only minor or no impact on the total DF content of the bran.

3.3. Impact of bran properties on the mechanical parameters of bran-supplemented extrudates

In the current study, the maximum point of the force-deformation curve (F_{max}) and crushing force (F_{cr}) were selected to indicate the hardness of the extrudates. The addition of untreated brans did not significantly change the F_{max} of the extrudates (39.4 N with coarse and 38.4 N with fine bran) as compared to the control extrudate without bran (36.4 N), whereas the F_{cr} of the extrudates increased when untreated coarse (22.5 N) or fine (21.8 N) bran was added, as compared to the control extrudate (18.3 N) (Table 4). This indicates that although the bran addition did not change the force needed to create the initial crack (F_{max}), it made the products more difficult to break down (F_{cr}). However, when the treated brans were used, the hardness generally decreased below that of the control as shown by both indicators (Table 4). Treatment of bran without enzymes with direct oven drying already reduced F_{max} (30.2 N with both bran types) and F_{cr} (16.6 N coarse and 15.3 N fine). F_{max} was further reduced when the fine bran was incubated for 4 h (23.8 N) and when the brans were incubated with Depol 761P (26.1–28.2 N coarse and 22.5–25.6 N fine). The use of Veron CP enzyme preparation produced slightly harder extrudates (F_{max} 27.4 N) than the use of Depol 761P (22.5 N) or the combination of the two enzyme preparations (25.6 N). The extrudates with fine bran were generally less hard than the corresponding coarse bran extrudates (Table 4), as also reported previously for rye bran (Alam et al., 2013).

Crispiness has been defined as a combination of auditory and vibratory sensations occurring in the mouth, but it can also be indicated by instrumental measurements (Heidenreich et al., 2004). The crispiness index (C_i) decreased from that of the control (5.8×10^3) when untreated coarse (3.4×10^3) or fine bran (3.9×10^3) was added (Table 4). Treatment

without enzymes with direct oven drying increased C_i with both brans (6.3×10^3 coarse and 7.8 fi$\times10^3$ fine). C_i was further increased when the fine bran was incubated (10.8×10^3), or when Depol 761P enzyme preparation was used, even without incubation (9.7×10^3). The results showed that bran treatment with enzymes produced more crispy extrudates than untreated bran or bran treated without enzymes with direct drying. C_i values obtained with the use of Veron CP alone (10.7×10^3) or in combination with Depol 761P (11.7×10^3) did not differ significantly from the values obtained with the use of Depol 761P alone (12.1×10^3) (Table 4).

Hardness and crispiness of cereal extrudates are mainly determined by their cellular structure, formed during the expansion of the extrudate, and by the phase properties and composition of the solid matrix (Moraru and Kokini, 2003). In the current study, a significant ($P < 0.01$) positive correlation was observed between improved mechanical properties (decreased hardness and increased crispiness) and decreased piece density and increased specific length (Table 4). Decreased hardness and increased crispiness also correlated ($P < 0.01$) with increased WEAX content and decreased WHC of bran, as well as with fine bran particle size ($P < 0.05$) (Table 4). Particle size also decreased during the bran treatments, but the properties of the extrudates prepared with untreated fine bran with mean particle size of 84 µm were significantly inferior to those of the extrudates made with modified coarse brans with particle size of 205–318 µm. Thus it can be concluded that the reduction of bran particle size after the bran treatments was not the primary reason for improved mechanical properties of the extrudates with modified bran. Rather, the extrudates with modified brans had improved mechanical properties probably due to the effects of the increased WEAX content and decreased WHC of the brans on the extrudate expansion, observed as decreased piece density and increased longitudinal expansion. The mechanical properties might also have improved due to possible changes in the strength of the solid matrix by the altered matrix composition (solubilisation of bran AX). It has been reported that addition of DF increases the hardness of expanded extrudates due to higher cell density and lower cell diameter (Karkle et al., 2012; Yanniotis et al., 2007; Jin et al., 1995; Robin et al., 2011). In the current study, the samples with coarse bran had more homogenous and smaller cell size than the samples with fine bran, as visually observed from the stereomicroscopy images (Fig. 2). However, the reduced hardness and increased crispiness of the extrudates with enzymatically modified brans was not clearly reflected in the cellular structure.

3.4. Impact of bran drying method on the properties of the modified brans and on the bran-supplemented expanded extrudates

In the treatment process brans were dried either by freeze drying or by oven drying. Freeze drying is known to cause minimal structural damage to the products, whereas oven drying is significantly less expensive. When bran was treated without enzymes, the content of WEAX was higher in the oven dried brans (1.4% WEAX in coarse and 1.6% in fine) than in the freeze dried brans (1.1% coarse and 1.3% fine). This was probably due to the action of the bran endogenous enzymes in the beginning of the oven drying. The oven drying was performed at relatively mild temperature (50 °C), enabling the continuation of the enzymatic action until the point where the moisture content was too low to support enzymatic action, whereas the use of liquid nitrogen for the samples that were freeze dried obviously stopped the enzyme reactions immediately. However, when the brans were treated with Depol 761P, the drying method did not have a significant impact on the level of WEAX, probably due to the high enzyme action already during the extruder-mixing, which probably obscured the possible impact of the short continuation of the enzyme action in the oven.

The WHC of the coarse bran was significantly lower after oven drying (3.0 without enzymes and 2.5 with Depol 761P) than after freeze drying (3.7 without enzymes and 3.1 with Depol 761P), both with and without the use of enzymes (Table 1). This can be explained by

the fact that forced heated air dehydration (oven drying) can cause collapse of the structure of DF (Massiot and Renard, 1997), whereas freeze drying preserves their structural features, resulting in higher porosity that allows more water entrapment. However, the drying method did not have an impact on the WHC of the fine bran, probably because it already underwent significant structural collapse due to severe grinding conditions both before and after the treatments.

In the case of the bran-supplemented expanded extrudates, it was noted that oven dried brans produced generally slightly lower piece densities and in case of fine bran also higher specific lengths than the freeze dried samples (Tables 3 and 4). When the brans were treated without enzymes, the oven dried brans also produced slightly less hard (F_{cr} 15.3–16.6 N) and crispier (C_i 6.3–7.8) extrudates than freeze dried brans (F_{cr} 18.3–20.6 N and C_i 4.2–5.8). By contrast, in the Depol 761P treatments with incubation, the drying method did not have a significant impact on the mechanical properties of the extrudates. The differences in the extrudates due to the drying method can be attributed to the slightly different impacts of the drying methods (or lack of impact, as in the case of the Depol 761P treatments) on the WEAX content and WHC of the brans. In addition, on the basis of visual observations, the drying method also affected the colour of the bran ingredients and the expanded extrudates. The oven dried brans were darker in colour, probably due to Maillard or other browning reactions occurring during the oven drying.

4. CONCLUSIONS

Modification of bran by hydrolytic enzymes in a low-moisture process increased the crispiness and reduced the hardness and density of the bran-supplemented expanded extrudates. These improvements correlated with increased level of WEAX and decreased WHC of the bran. Bran ingredient with a fine particle size produced extrudates with better texture and higher radial expansion than coarse bran. Particle size was also decreased by the enzymatic bran treatment, but the results showed that the reduction of bran particle size after the bran treatments was not the primary reason for improved mechanical properties of the extrudates with modified bran. Instead, extrudate texture and structure is proposed to have been improved due to the effects of the altered WEAX content and WHC of the enzymatically treated brans on the extrudate expansion and/or on the properties of the solid matrix. The precise mechanism behind the observed positive effects of enzymatic bran modification on the expansion and mechanical properties of the extrudates remains to be further elucidated.

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