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In vitro evaluation of whole faba bean and its seed coat as a potential source of functional food components

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ABSTRACT

In vitro studies were conducted to evaluate the particular nutritional benefits of whole faba bean seed (WFB) and fava bean seed coat (FBSC). Total dietary fiber contents of WFB and FBSC were 27.5% and 82.3%, respectively. FBSC were contained much higher total phenolic substances, condensed tannins, and total antioxidant activity than WFB. Bile acid (BA)-binding capacities of *in vitro* digested samples and nutritionally important products produced by *in vitro* fermentation of digestion residues were also studied. The BA-binding capacities of WFB and FBSC were 1.94 and 37.50 μmol/100 mg, respectively. Total BA bound by FBSC was even higher than the positive standard cholestyramine. Lignin and other constituents of the Klason residue were found to influence BA-binding properties. Moreover, the extent of the *in vitro* fermentation process showed that, fermentability of FBSC residue was significantly lower than that of WFB residue. Overall, faba bean, especially its seed coat, has great potential as a functional food.

1. Introduction

Faba bean (*Vicia faba* L.), also known as broad bean, is an important nutritious food legume, especially in Middle Eastern Countries. Annual global production is more than 4 million tons, with China, Ethiopia, Australia, France, and Egypt the leading faba bean producers (FAOSTAT, 2016). Faba bean can be grown in multiple climatic zones. It also contains a high proportion of protein and significant amounts of complex carbohydrates with important health benefits. In addition, it is a good source of macro- and micro elements (Hacıseferoğulları, Gezer, Bahtiyarca, & Mengeş, 2003). It can be used both as livestock feed and human food. Faba beans are consumed in a variety of forms. Fresh faba bean can be consumed raw or cooked as a vegetable. However, most faba beans are consumed in dry form. Dry faba bean seed is used in variety of foods, such as Doubanjiang (spicy fermented bean paste), Janjetina s bižima (lamb with faba bean: a traditional Dalmatian dish), Maccu (traditional Italian soup), Medamis (stewed beans), Falafel (deep fried cotyledon paste with some vegetables and spices), Bissara (a paste prepared from faba bean cotyledon) and Nabet soup (boiled germinated beans) (as derived from

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information provided in Wikipedia). In addition, a spicy snack prepared from fried faba bean is popular in China, Malaysia, Colombia, Peru, Guatemala, Mexico, Gilan (a province in northern Iran) and Thailand (as derived from information provided in Wikipedia). The faba bean seed can be utilized with or without its seed coat. The whole seed has three basic parts, the seed coat, the cotyledons, and the embryo, constituting 15%, 84% and 1% of whole seed mass, respectively. Cotyledon is the main reserve of protein and carbohydrate. However, the seed coat is also generally high in functional food components, such as phenolic substances, dietary fiber and minerals. Therefore, utilization of the seed with its seed coat (as a whole seed) has additional nutritional benefits.

Numerous *in vitro* and *in vivo* studies have documented a correlation between the consumption of foods high in dietary fiber and phenolic substances and decreasing incidences of cardiovascular diseases and cancer, predominant causes of death in most regions of the world. In Turkey heart diseases account for about 43% of all deaths (Yücecan, 2001). It is reported that poor nutritional habits account for about 60% of cancers in males and 40% in females, and it is widely accepted that many types of cancer can be prevented by a proper diet (Yücecan, 2001). Further, functional food components have beneficial effects on many physiological functions, thereby reducing the risk of illness and improving well-being and general health (Yücecan, 2001).

It has also been shown that soluble and non-soluble dietary fibers can bind bile acids (BAs) while passing in small intestine without digestion. (Drzikova, Dongowski, Gebhardt, & Habel, 2005; Sayar, Jannink, & White, 2005). Bile acids are synthesized from destruction of cholesterol in liver and secreted into the intestine. It is estimated that approximately half of the cholesterol produced by the human body is used in the production of BAs (Larusso, 1993). Under normal conditions, approximately 90% of total BA secreted into the small intestine is absorbed and reused (Larusso, 1993). However, some food components prevent reabsorption of BAs in small intestine by binding them and thus trigger more cholesterol destruction by the liver. However, the mechanism of BA-binding to dietary fiber are still not well explained. Therefore, the BA-binding properties and mechanisms of different dietary fibers require further study.

Some dietary fiber components (beta-glucan, resistant starch, hemicellulose, etc.) that escape digestion in the small intestine become useful substrates for colonic flora. Colonic fermentation of particular dietary fiber components results in the production of gas and short-chain fatty acids (SCFAs) (Barry et al., 1995; Cummings, 1981). Production of SCFAs lowers the pH of the colonic environment, which may prevent the growth of harmful bacteria and help in the absorption of some minerals. Cummings (1981) proposed that certain SCFAs may defend against colon carcinogenesis. Additional benefits of colonic fermentation products were reviewed by Wong, De Souza, Kendall, Emam, and Jenkins (2006).

The aim of this study was to evaluate the potential of whole faba bean (WFB) and its seed coat as functional food components. The antioxidant capacities, phenolic contents, condensed tannin contents and *in vitro* BA-binding capacities of WFB and faba bean seed coat (FBSC) were determined. Additionally, the residues remaining after *in vitro* digestion were used as substrates for *in vitro* fermentation studies. The extent and the products of *in vitro* fermentation, as well as the depletion of the available carbohydrates during *in vitro* fermentation, were investigated.

The outcomes of this study will improve our knowledge on the nutritional benefits provided by faba bean and the impact of the seed coat on these benefits.

2. Material and methods

2.1. Materials

Faba bean (*Vicia faba* L.) was obtained from a legume processing company in Mersin, Turkey. Foreign materials and damaged grains were hand selected and removed before all experiments. Seed coats were manually separated from the seeds and seed coat thickness was measured by a digital micrometer (Mitutoyo Corp., Kawasaki, Japan), capable of measuring to the nearest 0.001 mm. The seed coat percentage of the grains was determined gravimetrically according to the method described by Giami (2001). The whole seeds and seed coats were ground in a centrifugal mill fitted with a 0.5 mm sieve. Flour samples were stored at −18 °C during the study.

2.2. Chemicals and reagents

Arabinose, lactulose, D(+)-xylose, sodium cholate, sodium deoxycholate, sodium glycocholate, sodium taurocholate, sodium chenodeoxycholate, DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteau's Phenol Reagent, gallic acid, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), (+)-catechin, vanillin, cholestyramine, cellulose, resazurin, human salivary alpha amylase (EC 3.2.1.1), porcine pepsin (EC 3.4.23.1) and pancreatin (from porcine pancreas, activity at least equivalent to 8× USP specifications) were supplied by Sigma (Sigma-Aldrich Corp., St. Louis, MO, USA). L-Cysteine, Brain Heart Infusion, acetic acid, propionic acid, valeric acid and butyric acid were purchased from Fluka (Sigma-Aldrich Corp.). All other chemical reagents used in this study were of analytical grade.

2.3. Proximate composition

Moisture, ash, total protein, and total lipid contents of WFB and FBSC flours were measured according to standard methods of the American Association of Cereal Chemists (AACC, 2000). The starch content in flour was analyzed by AACC Method 76-13 (AACC, 2000), using a Total Starch Kit (Megazyme Ltd, Co. Wicklow, Ireland) and total dietary fiber by AACC Method 32-21 (AACC, 2000) using a Total Dietary Fiber Kit (Megazyme). Lignin content (Klason lignin) was determined gravimetrically according to Theander, Aman, Westerlund, Andersson, and Pettersson (1995).

2.4. Analyses of antioxidant activity, phenolic substances, and condensed tannins

Antioxidant activity, phenolic substances and condensed tannins were measured in the methanolic-acetonic extracts of the samples (Perez-Jimenez & Saura-Calixto, 2005). The radical scavenging capacity of the samples was measured according to the DPPH method (Moure et al., 2000). Antioxidant activity is expressed as micromole trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) equivalents (TE) per gram of flour on dry basis (d.b.). Total phenolic substances were measured according to the Folin-Ciocalteau procedure (Heimler, Vignolini, Dini, & Romani, 2005) and the results are expressed as milligram gallic acid equivalents (GA) per gram of flour (d.b.). Total condensed tannin content was measured according to the method of Heimler et al. (2005) and results expressed as (+)-catechin equivalents (CE) per gram of flour (d.b.).

2.5. In vitro digestion and bile acid (BA) binding methods

In vitro digestion was conducted according to the method of Sayar et al. (2005). First, the samples were cooked in boiling water

for 20 min, then treated sequentially with human salivary alpha amylase, porcine pepsin, and pancreatin enzymes. A standard BA mixture (1.51 mM containing 31% cholate, 23% deoxycholate, 10% glycocholate, 12% taurocholate, and 4% chenodeoxycholate) was added before the pancreatic digestion step. At the end of the process, the *in vitro* digestion mixture was centrifuged and the supernatant used for BA-binding analyses. The digestion residue (insoluble part, pellets) was freeze-dried and used as the substrate for *in vitro* fermentation (Sayar, Jannink, & White, 2007).

Total digestibility of the samples was calculated by the equation

$$\text{Total digestibility, } \% = \frac{M_S - M_P}{M_S} \times 100 \quad (1)$$

where M_S is the initial dry weight (in grams) of sample for *in vitro* digestion and M_P is the dry weight (in grams) of the residue that could not be digested (sediment of centrifugation).

The amount of BA in the extract, which corresponds to the unbound BA, was analyzed using a Bile Acid Diagnostic Kit (Trinity Biotech PLC, Bray Co. Wicklow, Ireland) as described elsewhere (Sayar et al., 2005). Cellulose was included as a negative control with no BA-binding capacity and the anionic resin cholestyramine as a positive control in each set of assays. If necessary, the extracts were diluted to fall within the BA measurement range of the test kit.

2.6. In vitro fermentation method

In vitro fermentation studies were conducted under strict anaerobic conditions for a period of 24 h according to the method of Sayar et al. (2007). The anaerobic fermentation medium consisted of 3.7 g Brain Heart Infusion medium, 93 mL distilled water, 5 mL Na_2CO_3 (80 g/L), 2 mL L-cysteine sulfide (12.5 g/L) and 0.1 mL resazurin. The medium was autoclaved at 121 °C for 45 min prior to each fermentation trial (Zheng et al., 2003). The inoculum was prepared using fresh feces from three healthy volunteers who had not taken antibiotics for at least the past three months. The feces were collected in plastic bags, pooled, and homogenized with the sterile fermentation medium (ratio of feces to medium = 1:3). The mixture was then filtered through 4-layer cheesecloth. This inoculum was kept under continuous CO_2 flows during the experiments. *In vitro* fermentation of samples was carried out in serum bottles according to the method given in Sayar et al. (2007). Separate bottles were prepared for each time data point (6, 12, and 24 h) to prevent pressure loss and a break in anaerobicity during sampling (Sayar et al., 2007). In addition to samples, the completely fermentable substrate lactulose and blanks without substrate were also included in the *in vitro* fermentation studies (Zheng et al., 2003). Total gas production was measured by the overpressure in bottles using a digital manometer (Fisher Scientific, Pittsburgh, PA). Saturated mercury chloride solution (0.1 mL) was used to stop the fermentation. The slurry was then transferred to centrifuge tubes for pH measures and then centrifuged at 4000×*g* for 10 min. A 1-mL aliquot of the supernatant was taken for SCFA analysis. The fermentation residues (pellets) were freeze dried and used for the neutral sugar analysis.

The SCFAs (acetic, propionic, butyric and valeric acids) were analyzed as their silyl derivatives by gas chromatography (GC) according to the method of Schooley, Kubiak, and Evans (1985) using a non-polar column (BP 5; 60 m × 0.25 mm × 0.25 mm; SGE Europe Ltd., UK). 2-Ethylbutyric acid was used as the internal standard. Neutral sugar residues arising from acid hydrolysis were analyzed from the freeze-dried fermentation residues as their alditol acetates by GC using a polar column (BPX-70; 30 m × 0.25 mm × 0.25 mm; SGE Europe Ltd., UK), as previously described (Theander et al., 1995).

2.7. Statistical analysis

All analyses were performed in triplicate unless otherwise stated. All statistical tests were conducted using the SPSS package program (SPSS Version 12.0, SPSS Inc., IL). Differences among substrates were compared by the least significant difference (LSD) test with a probability level (α) of 0.05.

3. Results and discussion

3.1. Composition and physical properties of faba bean

The faba bean seeds used in the current study had an average hundred seed weight of 138.5 g and seed coat content of 15.4 g/100 g dry seed. Compared to most other food legume seeds, faba bean has a larger seed and a higher proportion of seed coat. The seed coat thickness was 0.16 mm, thicker than the seed coats of chickpea (0.09 mm; Sayar, Turhan, & Gunasekaran, 2001), lentil (0.03 mm; Tang & Sokhansanj, 1993) and lima bean (0.14 mm; Moraes et al., 2000). Table 1 summarizes the composition, total phenolic content, condensed tannin content, and antioxidant activity of WFB and FBSC. The FBSC was rich in dietary fiber but contained lower amounts of protein and lipid. There was also a trace amount of starch which was thought to be a residue from the endosperm during seed coat separation. Protein, lipid, ash and starch contents of WFB were comparable with values reported in a previous study (Petitot, Boyer, Minier, & Micard, 2010). Most of the lignin was concentrated in the seed coat.

3.2. Antioxidant activity and contents of total phenolics and condensed tannins

Antioxidant activity and contents of total phenolics and condensed tannins of WFB and FBSC flour methanolic-acetonic extracts are also shown in Table 1. Each was significantly higher in FBSC extracts compared to WFB extracts (total phenolic content: 21.5 mg GAE/g vs. 2.9 mg GAE/g; total condensed tannin content: 47.7 mg CE/g vs. 1.9 mg CE/g, antioxidant activity: 22.9 mg TE/g vs. 1.8 mg TE/g). Higher phenolic contents and antioxidant activities were also found in the seed coats of other legumes (Troszynska & Ciska, 2002). The antioxidant activity was also found to be higher in dark colored legume seeds compared to light colored seeds (Beninger & Hosfield, 2003). Total phenolic content of wheat and oat bran were determined by Perez-Jimenez and Saura-Calixto (2005) as 2.8 and 2.0 mg GAE/g, while antioxidant capacities were 1.1 and 0.9 mg TE/g, respectively. Thus, FBSC has substantially higher phenolic content and higher antioxidant activity than other legume seeds.

Table 1
Proximate composition, total phenolics, condensed tannins and antioxidant activity of whole faba bean (WFB) and faba bean seed coat (FBSC) flours.[1]

Component	WFB	FBSC
Protein, % (d.b.)	27.9 ± 0.1^a	5.0 ± 0.0^b
Lipid, % (d.b.)	1.2 ± 0.1^a	0.2 ± 0.1^b
Ash, % (d.b.)	3.4 ± 0.0^b	4.4 ± 0.1^a
Starch, % (d.b.)	46.3 ± 1.2^a	0.9 ± 0.2^b
Total dietary fiber, % (d.b.)	27.5 ± 3.9^a	82.3 ± 4.2^b
Lignin, % (d.b.)	2.6 ± 0.8^b	17.5 ± 1.3^a
Total phenolics, mg GAE/g	2.9 ± 0.3^b	21.5 ± 0.3^a
Total condensed tannin, mg CE/g	1.9 ± 0.9^b	47.7 ± 0.9^a
Antioxidant activity, mg TROLOX/g	1.8 ± 0.1^b	22.9 ± 0.7^a

[1] Values are means of n = 3 measurements ± standard deviation. Values within a raw followed by a common letter are not significantly different ($P > 0.05$). GAE: Gallic acid equivalent; CE: (+)-catechin equivalent; TE: TROLOX (*6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid*) equivalent.

3.3. In vitro digestion and bile acid (BA) binding tests

A variety of *in vitro* digestion methods have been developed with the general aim of simulating the human digestion system. As described in Sayar et al. (2005), the *in vitro* digestion procedure used in the current study has several advantages in terms of mimicking the human digestive system compared to others (Camire, Zhao, & Violette, 1993; Drzikova et al., 2005).

The total digestibility of WFB and FBSC were determined as 66.9% and 12.1%, respectively (Eq. (1)). Higher *in vitro* digestibility of WFB compared to FBSC is expected since most of the digestible components (e.g., protein, starch) are concentrated in the cotyledon. Studies performed using a similar enzyme combination determined overall digestibility values between 77% and 81% for oat flour (Sayar et al., 2005), 28% and 32% for wheat aleurone (Amrein, Graenicher, Arrigoni, & Amado, 2003), and around 60% for aleurone-rich wheat bran (Wood, Arrigoni, Miller, & Amadò, 2002). Therefore, WFB has lower digestibility than oat flour, and FBSC has lower digestibility than wheat bran and wheat aleurone. Lower digestibility of FBSC in this study compared to wheat bran or wheat aleurone can be explained by the higher amounts of dietary fiber and lignin in the seed coat (Table 1), which are known to be resistant to digestive enzymes.

Table 2 shows the BA-binding capacities of WFB and FBSC at the end of the *in vitro* digestion process. In these experiments, cellulose was used as a negative control because it does not bind BA (Wood et al., 2002) while cholestyramine, an anionic resin used commercially for reducing plasma cholesterol level, was used as a positive control. Cholestyramine is known to bind BA to anionic sites on the resin thus interrupting their enterohepatic recycling and increasing fecal BA excretion (Daggy, O'Connell, Jerdack, Stinson, & Setchell, 1997). Due to methodological differences across previous studies, BA-binding capacities relative to cholestyramine were compared (Sayar et al., 2005). Taking the BA-binding capacity of cholestyramine as 100%, binding capacities of FBSC and WFB were 282.6% and 14.6%, respectively. Unexpectedly, FBSC bound very high amounts of BA, higher than the results obtained in previous studies (Camire et al., 1993; Kahlon, Chapman, & Smith, 2007; Sayar et al., 2005) and even higher than the positive control cholestyramine. The BA-binding capacity of the samples was also calculated relative to total dietary fiber, Klason lignin, total phenolic content, and condensed tannin content (Table 2). Results showed that BA-binding was affected only by condensed tannin content. Relative BA-binding values on an equal condensed tannin content basis were not statistically different ($P > 0.05$) (Table 2). Previous studies have also examined the effects of specific food components on BA-binding. Examination of BA-binding by oat flour based on starch, protein, insoluble dietary fiber (IDF), soluble dietary fiber and pentosane compositions revealed that BA-binding was only influenced by IDF (Sayar et al., 2005). Other studies have also reported a correlation between BA-binding and IDF content (Drzikova et al., 2005; Eastwood & Hamilton, 1968; Kahlon & Woodruff, 2003; Story & Kritchevsky, 1976). Some of these studies also addressed the influence of lignin content or hemicellulose content on BA-binding (Eastwood & Hamilton, 1968; Story & Kritchevsky, 1976). A study by Funk, Grabber, Steinhart, and Bunzel (2008) assessing the influence of lignin on BA-binding capacity of maize dietary fiber found no significant difference between non-lignified and artificially lignified maize, thus disproving a major role for pure lignin in BA adsorption. However, acid-insoluble Klason residues (Klason lignin) contain not only lignin, but also tannins, cutin, and various proteinaceous products (Funk et al., 2008; Hatfield & Fukushima, 2005). Therefore, the correlation detected between BA-binding capacity and Klason lignin in previous studies may be due to these other constituents (Funk et al., 2008). The significant correlation between BA-binding and condensed tannin content found in present study also supports this conclusion.

3.4. In vitro fermentation tests and short chain fatty acids (SCFA)

In vitro digestion assays (Section 3.3) revealed that 33.1% of WFB and 87.9% of FBSC were not digested. Dietary fibers, oligosaccharides and polysaccharides that cannot be digested by the human digestive system are potential substrates for colonic flora, including cellulose, hemicellulose, beta-glucan, pectin, enzyme resistant starch, and some gums. In this study, the residues obtained from the *in vitro* digestion process were fermented under batch anaerobic conditions for 24 h and progress was monitored by measuring changes in pH, amount of gas generated, and the amount of SCFA produced, as a function of time.

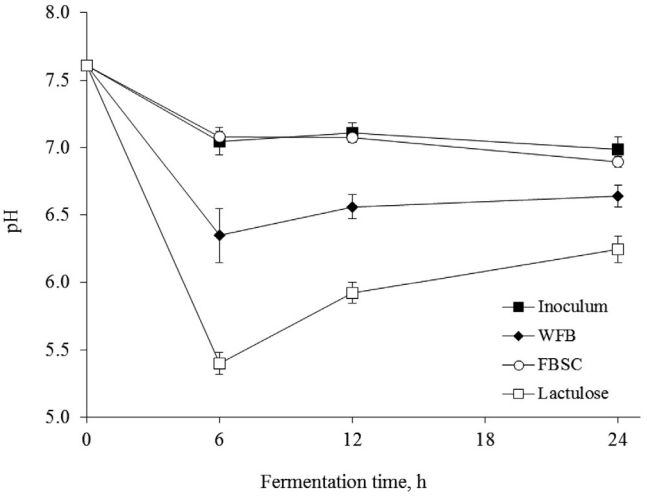
The highest pH decrease was obtained for lactulose (Fig. 1a). The change in pH during FBSC treatment was not significantly different from the inoculum (blank), possibly due to the limited fermentability of FBSC residue. There was a larger decrease in pH during WFB residue treatment, compared to FBSC treatment. A sharp decrease in pH was observed during the first 6 h, for all samples. After this sharp decrease, pH values increased modestly through to the end of the process. Similar trends have been reported for *in vitro* fermentation of oat flour, oat bran, and β-glucan (Sayar et al., 2007; Wood et al., 2002). The slight increase in pH after 6 h of fermentation can be explained by the formation of alkaline metabolites such as ammonia (Barry et al., 1995). The decrease in pH during fermentation was associated with the formation of SCFA. Lower pH in colon is generally preferred due to the health benefits (Samuelson, Nelson, & Nyhus, 1985).

Maximum gas formation was determined during the fermentation of the positive control, lactulose (Fig. 1b). There was a strong correlation ($R^2 = 0.88–0.98$) between the amount of gas formed and the total amount SCFA for all the substrates studied. The amounts of gas produced after 24 h fermentation were 19.2 mL/100 mg substrate for FBSC and 24.5 mL/100 mg substrate for WFB. *In vitro* fermentation of oat bran was found to produce around 25 mL gas/100 mg substrate, after 24 h fermentation (Wood et al., 2002), close to the value obtained for WFB substrate in this study.

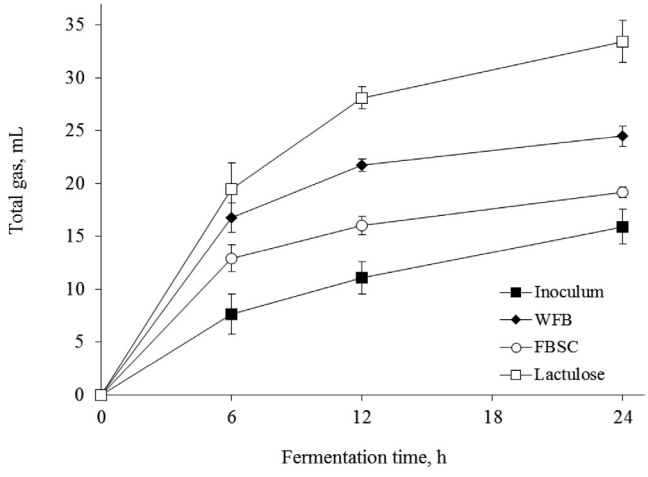
Table 2
Bile acid (BA) binding capacities calculated based on the composition of whole faba bean (WFB) and faba bean seed coat (FBSC) flours.[1]

Samples	Amount of BA bound				
	μmol/100 mg sample	μmol/100 mg total dietary fiber	μmol/100 mg Klason lignin	μmol/100 mg total phenolics	μmol/100 mg condensed tannins
WFB	1.94 ± 0.24^c (14.6)[2]	7.1 ± 0.1^b	77.7 ± 15.4^b	668.0 ± 13.7^b	1166.5 ± 477.6^a
FBSC	37.50 ± 3.08^a (282.6)	45.5 ± 1.4^a	214.2 ± 1.7^a	1743.1 ± 118.9^a	785.5 ± 49.8^a
Cholestyramine	13.27 ± 0.74^b (100.0)				
Cellulose	0.04 ± 0.02^d (0.3)				

[1] Values are means of n = 3 measurements ± standard deviation. Values within a column followed by a common letter are not significantly different ($P > 0.05$).
[2] Values given in the parenthesis are the relative BA-binding, which was calculated by assigning BA-binding to cholestyramine as 100%.



(a)



(b)

Fig. 1. Changes in pH (a) and total gas formation (b) during *in vitro* fermentation of whole faba bean (WFB), faba bean seed coat (FBSC) flour digestion residues and lactulose.

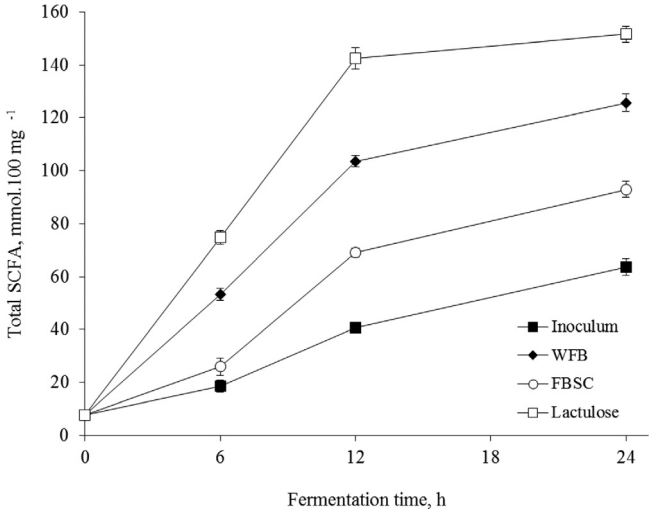


Fig. 2. Total short chain fatty acids formation during *in vitro* fermentation of whole faba bean (WFB), faba bean seed coat (FBSC) flour digestion residues and lactulose.

Table 3
The levels of acetic, propionic, butyric and valeric acids (μmol/100 mg substrate) determined in the fermentation medium.[1]

SCFA	Fermentation time, h	Inoculum	WFB	FBSC	Lactulose
Acetic	6	10.7 ± 0.6^{d}	30.0 ± 2.2^{b}	13.9 ± 1.4^{c}	42.2 ± 1.5^{a}
	12	19.6 ± 0.7^{d}	48.2 ± 1.1^{b}	34.3 ± 1.7^{c}	64.5 ± 0.8^{a}
	24	31.1 ± 1.4^{d}	56.9 ± 0.8^{b}	46.0 ± 1.6^{c}	68.5 ± 0.1^{a}
Propionic	6	3.0 ± 0.3^{d}	9.8 ± 1.1^{b}	4.9 ± 1.0^{c}	13.8 ± 0.5^{a}
	12	8.1 ± 1.6^{d}	20.1 ± 0.5^{b}	14.9 ± 0.0^{c}	28.5 ± 0.6^{a}
	24	11.2 ± 0.8^{d}	23.9 ± 0.9^{b}	17.2 ± 0.1^{c}	29.9 ± 1.8^{a}
Butyric	6	4.5 ± 0.7^{c}	12.7 ± 1.4^{b}	6.6 ± 1.7^{c}	17.7 ± 0.5^{a}
	12	11.6 ± 1.0^{d}	32.2 ± 1.6^{b}	22.9 ± 0.4^{c}	44.8 ± 0.9^{a}
	24	19.2 ± 0.5^{c}	36.1 ± 3.7^{b}	31.6 ± 1.7^{b}	45.0 ± 2.0^{a}
Valeric	6	0.3 ± 0.2^{c}	0.7 ± 0.1^{b}	0.4 ± 0.1^{c}	1.0 ± 0.0^{a}
	12	1.3 ± 0.6^{c}	3.1 ± 0.2^{b}	2.3 ± 0.4^{b}	4.6 ± 0.9^{a}
	24	2.1 ± 0.8^{c}	8.8 ± 0.8^{a}	6.2 ± 0.4^{b}	8.3 ± 2.0^{a}

[1] SCFA: Short chain fatty acids, WFB: Whole faba bean flour, FBSC: Faba bean seed coat. Values are means of n = 3 measurements ± standard deviation. Values within the same raw followed by a common letter are not significantly different ($P > 0.05$).

The production rates of different SCFAs during fermentation are given in Fig. 2. The rank order of SCFA production was acetic > butyric > propionic > valeric acids from all substrates (Table 3). Similar patterns of SCFA production were reported for the *in vitro* fermentation of different SDFs and IDFs (Karppinen, Liukkonen, Aura, Forssell, & Poutanen, 2000; Sayar et al., 2007). The SCFAs butyric acid and propionic acid in particular are known to have important health benefits. Propionic acid prevents hepatic cholesterol synthesis (Wright, Anderson, & Bridges, 1990), increases the amount of high-density lipoprotein, and decreases insulin level (Gustafsson, Asp, Hagander, & Nyman, 1993). Butyric acid is known as the basic energy resource for colonic epithelium and has colon cancer preventive properties (Amrein et al., 2003; Gustafsson et al., 1993). In the current study, the total proportions of propionic and butyric acids obtained after 24 h fermentation were slightly higher than 48% for both WFB and FBSC residues (Table 3). The proportions of combined propionic and butyric acid from the fermentation of grape seed, grape pomace, wheat bran, rye bran, oat bran, and inulin were reported to be in the range of 41%–49% (Amrein et al., 2003; Goni, Martin, & Saura-Calixto, 2005; Karppinen et al., 2000), close to the findings of this study.

In vitro fermentabilities of the WFB and FBSC relative to the blank and positive control lactulose were interpreted above in terms of the fermentation products. The results indicated that the fermentability of FBSC residue was comparatively lower than that of WFB residue. A possible reason for this lower fermentability is the carbohydrate composition of FBSC residue. Therefore, the neutral sugar composition of the substrates at the beginning (0 h) and throughout *in vitro* fermentation were determined (Fig. 3). Depletion rates of the three main neutral monosaccharides from WFB residue were higher than the rates observed from FBSC residue. Almost 70% of the glucose-containing polysaccharides in WFB residue were fermented, whereas only about 38% were fermented in FBSC residue. Differences in the total consumption and consumption rate of glucose indicated distinct glucose-containing polysaccharides in WFB and FBSC. Presumably, the polysaccharide in WFB residue was mainly resistant starch where that in FBSC residue was mainly cellulose. In the case of xylose- and arabinose-containing polysaccharides, the depletion rates were slightly lower for FBSC compared to WFB residue. This could be attributed to the distinct molecular structures of the

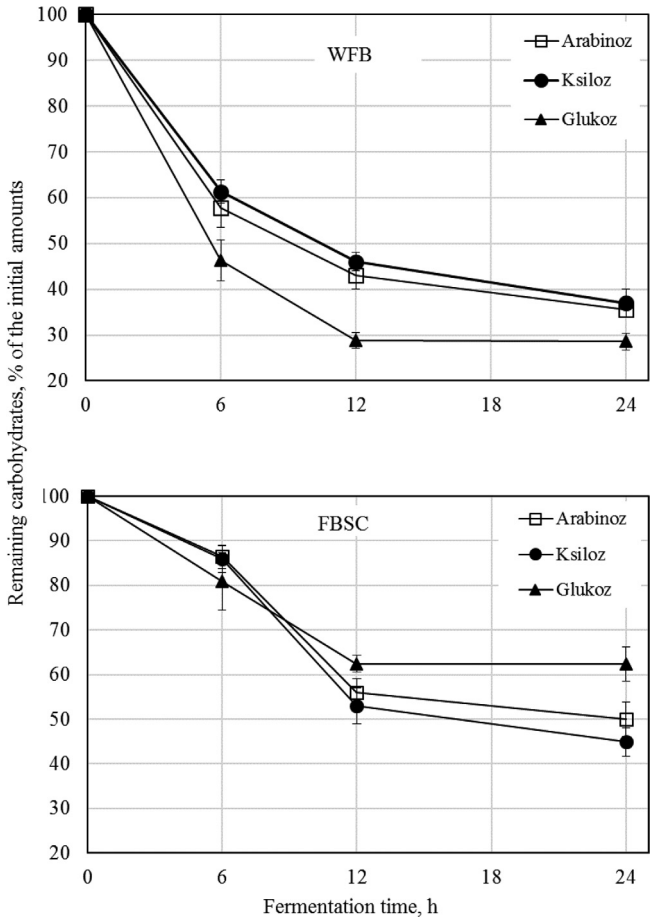


Fig. 3. Disappearance of the main neutral sugars (arabinose, xylose, glucose) during *in vitro* fermentation of whole faba bean (WFB) and faba bean seed coat (FBSC) flour digestion residues. Disappearance (percent) is expressed as percentage of the initial value.

arabinoxylans in FBSC, which may have lower susceptibility to microbial fermentation.

4. Conclusion

WFB and FBSC were evaluated for their potential as a functional food components. The thickness and the amount of seed coat are higher in faba bean than in other legumes. The antioxidant capacity, phenolic content, and condensed tannin content of FBSC found to be substantially higher than other legume seeds. *In vitro* BA-binding capacity of FBSC was very high, even higher than the positive control cholestyramine. High binding of BA cannot be attributed to the lignin content, but to other constituents of the Klason residue, like tannins, cutin, and proteinaceous products. *In vitro* fermentation of *in vitro* digestion residues revealed very low fermentability of the FBSC residue. Greater amounts of gas and SCFA formation from the fermentation of WFB residue were observed compared to FBSC residue. Overall evaluation of the results showed that faba bean is a potential source of functional food and its seed coat has great impact on these properties.

Conflicts of interest

There are no conflicts of interest in this study.

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