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Chemical composition and functional properties of three soy processing by-products (soy hull, okara and molasses)

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RESEARCH ARTICLE

Abstract

Three soy processing by-products, soy hull, okara, and molasses, were analysed for their amino acids, fatty acids, dietary fibre, and other chemical compounds. Their digestibility and odour variance were also quantified. Okara had the greatest content of protein (306.1 g/kg) and amino acids (340.6 g/kg dry matter; DM), while soy hull had the highest amount of dietary fibre (546.7 g/kg) and extractable pectin (47.4 g galacturonic acid equivalents/kg DM), and molasses possessed the most abundant total phenolics, resulting in its highest radical scavenging activity. The high unsaturated fatty acids presented in soy by-products (accounting for 73.1-82.3% of total lipids) indicated their favourable physiological functions. In simulated digestion test, the by-products generally underwent fast digestion within the first 30 min in gastric digestion step, and then slowed down. However, okara was still digested rapidly in the stimulated intestinal digestion stage. Electronic nose was able to clearly discriminate the odour differences among the three soy by-products.

Keywords: chemical composition, digestibility, odour characteristics, radical scavenging activity, soy processing by-products

1. Introduction

Soy products are favourable nutritional sources which can provide basic nutrients and various potential functional substances. During the processing of soy products, large quantities of residues or by-products are produced, which are rich in nutritional and bioactive substances. Directly discarding these materials would not only cause environmental problems, but also lead to large amounts of resource wastage.

Okara is a by-product of soymilk, soy protein isolate and tofu processes. Raw okara is white yellowish puree containing insoluble parts of soybeans. Approximately 8×10^5 tons of okara are produced from the tofu industry in Japan every year, and about 3.1×10^5 and 2.8×10^6 tons in Korea and China, respectively (Li *et al.*, 2012). Okara contains high amount of protein, lipids, dietary fibre and minerals, and is also a potential source of natural antioxidant (Jimenez-Escrig *et al.*, 2010). Soy hull is a major by-product generated before extraction of soy oil with a US annual production in excess of 9.1×10^5 tons (Sessa, 2004). Karr-Lilienthal *et al.* (2005) reported that soy hull mainly contains cellulose, hemicellulose, and acidic polysaccharides, and suggested soy hull as commercial source of pectin or fibre. Soy molasses is a concentrated liquid obtained in the production of protein-concentrate soy meal extracted with ethanol, and mainly consists of carbohydrates, lipids and protein with the most abundant carbohydrates being sucrose, raffinose and stachyose (Siqueira *et al.*, 2008).

Presently, the by-products of soy processing are generally used in the animal feed industry. However, they might be inexpensive sources of value-added products. Wiboonsirikul *et al.* (2013) pointed out that extracts from okara exhibit high radical scavenging activity and suppressive activity to the autoxidation of linoleic acid. Replacing 30% of coconut with wet okara was found to increase fibre content and decrease fat content of a coconut-based snack, with the overall sensory score being significantly higher than that

of the control sample (Radocaj and Dimic, 2013). A 1.5 and 4% substitution of okara flour to meat in frankfurter displayed no significant differences in the physical (colour, texture, and emulsion stability) and chemical (pH and proximate composition) properties from those without okara flour (Grizotto *et al.*, 2012). Soy hull coated on frying batter could reduce fat uptake during deep fat frying. With increased soy hull content, the inner crust structure showed improved cellular integrity (Kim *et al.*, 2008). Soy molasses could be used as a combined nitrogen and carbon-source for microbial growth to produce single cell protein, exopolysaccharides, and sophorolipids (Gao *et al.*, 2012).

Amino acid, fatty acid, pectin and dietary fibre are the important bioactive components in soy by-products. Their fractions represent different physiological functionalities, thus critical for developing specific applications of the by-products. Besides, the digestion abilities of different soy by-products may vary that directly relate to the processing conditions. Unfortunately, few studies have compared the above constituents in different soy processing by-products. Comprehensively analysing the functional components of the soy processing by-products could help clarify their potential functionality, thus providing useful information for further industrial applications. Therefore, the objectives of this study were: (1) to investigate the types and contents of pectin, dietary fibre, amino acid, and fatty acid of three soy processing by-products; (2) to evaluate the antioxidant capacity of certain functional components; and (3) to analyse *in vitro* digestibility and odour differences of three soy processing by-products.

2. Materials and methods

Soy hull, fresh okara and soy molasses were donated by the Wilmar International (Shanghai) Co., Ltd. (Shanghai, China). Samples were freeze dried, milled to pass a 40-mesh screen, packaged in the polyethylene bags, and stored in a desiccator at 25 °C until used. Trypsin from bovine pancreas (≥10,000 BAEE U/mg protein) was obtained from Sigma-Aldrich (Shanghai) Trading Co. Ltd. (Shanghai, China). Pepsin (≥1,200 U/g) and all other chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Analysis of basic chemical component

Basic chemical components were determined according to the methods of Iqbal *et al.* (2006). Crude protein was determined by Kjeldahl method and calculated using the conversion factor of 6.25. Crude lipid was determined by Soxhlet extraction using hexane as solvent. Ash was obtained using a muffle furnace at 550 °C for 4 h. Data were expressed as g/kg dry matter (DM).

Analysis of soluble sugar

One gram of dried sample was extracted in an ultrasonic unit (Model S-10H; Zealway Instrument Inc., Xiamen, China) with 50 ml of 80% ethanol for 15 min at 25 °C for three times (Deng *et al.*, 2011). The extraction was centrifuged at 10,000×*g* for 10 min. The combined supernatant was then evaporated at 50 °C under vacuum (Model RE-52AA; Yuhua Instruments Co., Henan, China) to remove solvent and diluted to 50 ml with deionised water. One ml of the water solution was mixed thoroughly with 2 ml of 75% H_2SO_4 and 4 ml of anthrone reagent (0.5 mg anthrone in 250 ml 75% H_2SO_4), and incubated at 100 °C for 15 min. After cooling to room temperature, the absorbance of the sample or glucose standard was measured at 578 nm (UV-1800; Shimadzu Co., Ltd., Kyoto, Japan). Results were expressed as g glucose (GLU)/kg DM.

Analysis of total phenolic content

Five gram of dried sample was extracted once with acetone containing 0.1 ml/l HCl and then twice with 700 ml/l aqueous acetone containing 0.1 ml/l HCl under ultrasonication (Model S-10H; Zealway Instrument Inc.) for 30 min (Deng *et al.*, 2011). The mixture was centrifuged at 4,000×*g* for 5 min, and the combined supernatant was evaporated at 40 °C under vacuum to remove organic solvent. The extract was diluted to 50 ml with deionised water and the total phenolic content (TPC) was measured using Folin-Ciocalteau (FC) reagent (Deng *et al.*, 2011). A 1.5 mL of diluted extract or gallic acid standard was mixed with 7.5 ml of distilled water and 0.5 ml of FC reagent, and allowed to stand at 25 °C for 10 min. The solutions were then mixed with 3 ml of 20% (w/v) Na_2CO_3 and placed in water bath at 40 °C for 20 min. Then samples were immediately cooled to room temperature and the absorbance was measured at 765 nm (UV-1800; Shimadzu Co., Ltd.). TPC was calculated as g of gallic acid equivalents/kg DM.

Analysis of amino acids

Amino acid profiles were analysed using a L-8900 automatic amino acid analyser (Hitachi, Ltd., Kyoto, Japan) based on the method by Liu *et al.* (2013) with some modifications. Ten mg of dried sample was mixed with 10 ml of 6 M HCl and hydrolysed at 110±1 °C for 22 h and then cooled to ambient temperature. The hydrolysate was filtered and diluted to 50 ml using distilled water. One ml of diluted filtrate was dried and dissolved in 2 ml of distilled water, and the process was repeated twice. The solution was then dried and the residue was dissolved in 1 ml of citrate buffer (pH 2.2). Finally, 20 µl of the sample was injected into the amino acid analyser for quantitation. The amino acid content was expressed as g/kg DM.

Analysis of fatty acids

Briefly, known quantity of dried sample (3 g for soy hull, 1 g for okara, and 2 g for molasses) was mixed with 100 mg pyrogallic acid, 5 mg/ml triundecanoin solution, 95% ethanol, and 8.3 mol/l HCl in order, and then incubated for 40 min at 80 °C. Subsequently, the mixture was extracted using diethyl ether, and the upper ester layer was refluxed at 80 °C with addition of 8 ml of 2% NaOH. The treated fatty acid methyl esters were then extracted with n-hexane. One µl of the upper layer was injected into a GC-2010 (Shimadzu Co., Ltd.) gas chromatography system equipped with a capillary column (60 m × 25 mm, 0.25 µm) detected with a FID detector. Parameters for gas chromatography analysis were: nitrogen carrier gas at a flow rate of 1.1 ml/min, split ratio of 50:1; injector temperature of 270 °C, FID detector temperature of 280 °C; temperature programme: initial 130 °C for 1 min, increasing to 170 °C at 6.5 °C/min, then raising to 215 °C at 2.75 °C/min and maintained for 12 min. Finally, the temperature was increased to 230 °C at 4 °C/min and held for 3 min. The fatty acid concentration was expressed as % in DM.

Analysis of extractable pectin

Water soluble pectin (WSP), chelator soluble pectin (CSP), and hydroxide soluble pectin (HSP) were quantified according to the method by Deng *et al.* (2011). For extraction, 1.5 g of soy hull and okara and 3.0 g of soy molasses were used. Briefly, powdered samples were homogenised in distilled water (powder:water = 1:20) for 10 min, filtrated to obtain WSP, and the retentate was collected for subsequent extraction. WSP was precipitated by adding 95% ethanol to the filtrate (filtrate: 95% ethanol = 1:5) and allowing to stand overnight at 4 °C. Water extracted residue was first boiling with 95% ethanol for 10 min, the resulting residue was successively extracted three times with 50 ml of 20 mM Na_2-EDTA (pH=8.0), and CSP was obtained as Na_2-EDTA filtrate. The residue from the Na_2-EDTA extraction was at last extracted with 50 ml of 50 mM NaOH for 15 min at 25 °C and the filtrate was collected for measuring HSP. The results of pectin content were expressed as g galacturonic acid equivalents (GUAE)/kg DM.

Analysis of dietary fibre

Dietary fibre (DF) contained soluble (SDF) and insoluble dietary fibre (IDF) fractions, and was determined by the AOAC 994.13 procedure (AOAC, 2007) modified by Deng *et al.* (2011). SDF was composed of neutral sugars (NSs) and uronic acids (UAs), and IDF was the total of NSs, UAs and klason lignin (KL). In brief, 1.0 g of defatted sample was treated with protease (P-5459) in 0.05 M phosphate buffer (pH 7.5) at 60 °C for 30 min and then centrifuged. The supernatant was used for determining SDF, and the residue was used for measuring IDF. SDF fraction was dialysed in 1 l of distilled water for 36 h and the water was changed at each 8 h. The dialysed SDF was then freeze-dried and hydrolysed in 72% sulfuric acid at 121 °C for 1 h. NSs was quantified with spectrometric assay by anthrone method as D-glucose equivalent and UAs was determined using galacturonic acid as standard. The sample to be tested was mixed with 98% H_2SO_4 and boric acid-sodium chloride solution, and was incubated at 70 °C for 40 min. The mixture was then blended with 3,5-dimethyphenol-glacial acetic acid, and the absorbance was measured at 400 and 450 nm, respectively. IDF fraction was first hydrolysed with 72% sulfuric acid at 30 °C for 1 h, and then followed at 121 °C for 1 h. The mixture was filtrated, and the filtrate was used for measuring NSs and UAs, while the residue was dried at 105 °C for 16 h and considered as KL. All the data were displayed as % in DM.

Analysis of radical scavenging activity

Radical scavenging activity (RSA) was determined using 1,1-diphenyl-2-picryhydrazyl (DPPH) assay (Deng *et al.*, 2011) based on ascorbic acid equivalent. Briefly, 0.5 ml of diluted extract or ascorbic acid standard solution (0, 0.01, 0.02, 0.03, 0.04 mg/ml) was mixed thoroughly with 3 ml of 0.09 mg/ml DPPH methanol reagent. After standing in dark at room temperature for 10 min, the absorbance was determined by a UV-1800 spectrophotometer (Shimadzu Co., Ltd.) at 517 nm. RSA was expressed as g ascorbic acid equivalents (AAE)/kg DM.

In vitro digestibility test

A two-step *in vitro* digestibility test was used to evaluate protein and carbohydrate digestion (Yegani *et al.*, 2013). Five gram of sample was weighed into a 500 ml flask with addition of 100 ml 0.1 M phosphate buffer. Subsequently, 0.2 M HCl was added to reduce the pH to 2. Then, 0.05 g of porcine pepsin was added and chloramphenicol (0.5 ml) was used to minimise the effects of bacterial fermentation. The flask was swirled thoroughly and incubated in a shaking water bath (100 rpm, 37 °C) for 2 h. Digested mixtures were taken out at 30, 60, and 120 min, respectively, and the quantity of nitrogen released and the amount of reducing sugar were measured. After simulated gastric digestion phase, the flask was removed and 0.6 M NaOH was added to raise the pH to 7.0. Consequently, the flask contents were combined with 0.05 g of porcine pancreatin containing amylase, lipase, and protease, and swirled thoroughly. The flask was incubated in the shaking water bath for another 2 h. Analysis was the same as the first phase to simulate the post-gastric phase of digestion.

The amount of nitrogen (N) release was determined by the TCA-NSI method (Tang, 2007). Ten ml of the digested mixture was mixed with 10 ml of 10% (w/v) trichloroacetic acid (TCA) and then centrifuged at 8,000×*g* for 30 min

to obtain the precipitate. After being washed with 10 ml of TCA (10%, w/v), the precipitate was centrifuged again under the same conditions. The N content of the samples was determined by the Kjeldahl method (Iqbal *et al.*, 2006) using the conversion factor of 6.25.

Reducing sugars were estimated by the 3,5-dinitrosalicylic acid (DNS) method using D-glucose as a standard (Fu *et al.*, 2010). Briefly, 5 ml of the digested mixture was centrifuged at 13,000×*g* for 10 min. One ml of the properly diluted supernatant was mixed thoroughly with 1 ml of DNS reagent. After boiling for 15 min, the mixture was blended with 1 ml of 40% (w/v) potassium sodium tartrate solution and allowed to stand at room temperature for 10 min. Absorbance at 540 nm was measured on a UV-1800 spectrophotometer (Shimadzu Co., Ltd.).

Odour evaluation

An electronic nose (iNose 2000; Labservice Analytica, Bologna, Italy) was used to discriminate odour of three soy processing by-products. Five g of dried sample was collected in a headspace bottle and placed at 25 °C overnight. The equilibrated headspace was then delivered into the electronic nose sensor cell for analysis. Multivariate analysis methods play an important role in differentiating sample, and principle component analysis (PCA) procedure in e-nose system was used to process the results.

Statistical analysis

Three replications were carried out for each sample and each parameter, and the results were expressed as mean values. Analysis of variance for the data was carried out using one-way ANOVA. Significant mean differences (P<0.05) were determined by the Duncan's multiple range tests using SAS software (version 13.0; Statistical Package for the Social Sciences Inc., Chicago, IL, USA).

3. Results and discussions

Basic chemical constituents

Overall, okara had the highest crude protein content, which was 1.3 times higher than soy hull and 3.9 times higher than molasses (Table 1). The crude fat content of okara was 168.0 g/kg, similar to the fat content of normal Chinese soy seeds ranging from 161 to 242 g/kg (Dong and Qu, 2012). The fat content in soy hull, however, was the lowest (P<0.05) indicating that fat was mainly concentrated in the cotyledon and embryo segments. Considering ash content, the value of molasses was at least 2 times more than those of the other two samples reflecting its high mineral content (Table 1). As seen in Table 1, the soluble sugar content in molasses was 237.5 g GLU/kg DM, lower than the value reported by Siqueira *et al.* (2008) using high performance liquid chromatography method. A possible explanation is that high concentration ethanol used in this study could not completely extract monosaccharide and oligosaccharide. Soy hull contained soluble polysaccharides that contributed to its soluble sugar content (32.9 g GLU/kg DM). TPC was significantly different among the three samples (Table 1), with TPC of molasses 7.5 times higher than that of soy hull and almost 20 times more than that of okara.

Amino acids

Okara has the greatest concentration of total amino acids (340.62 g/kg DM) (Table 2), and the value in molasses was the lowest (55.95 g/kg DM). Valencia *et al.* (2009) detected 17 different amino acids in soya bean meal and the total content was 195 g/kg DM sample. However, Frikha *et al.* (2012) reported that the amino acid contents of the soy meals from USA, Brazil and Argentina were about 530, 550 and 520 g/kg DM, respectively. As is evident in Table 2, phenylalanine was the most abundant essential amino acid, accounting for 19.2, 24.0 and 32.3% of the total essential amino acids in soy hull, okara and molasses, respectively. Furthermore, tryptophan was the only essential amino acid

Table 1. Basic chemical constituents of three soy processing by-products.[1]

	Protein (g/kg DM)[2]	Fat (g/kg DM)	Ash (g/kg DM)	Soluble sugar (g GLU/kg DM)	Total phenolic content (g GAE/kg DM)
Hull	133.9±1.6^{b}	45.8±12.0^{c}	47.6±0.7^{b}	32.9±1.4^{b}	1.6±0.0^{b}
Okara	306.1±4.9^{a}	168.0±21.2^{a}	36.0±0.4^{b}	4.1±0.1^{c}	0.6±0.0^{c}
Molasses	61.9±0.5^{c}	83.3±13.6^{b}	116.4±15.2^{a}	237.5±5.4^{a}	12.1±0.7^{a}

[1] Data are expressed as mean ± standard deviation (n=3). Values followed by different superscript letters in the same column are significantly different (P<0.05).
[2] DM = dry matter.

Table 2. Amino acid profile of three soy processing by-products.[1]

Amino acids (g/kg DM)[2]	Hull	Okara	Molasses
Aspartic acid	7.72±0.13^{deB}	20.41±0.29^{efgA}	3.21±0.01^{fC}
Threonine	7.14±0.20^{eB}	19.46±0.22^{fA}	1.84±0.00^{hC}
Serine	6.48±0.30^{efB}	16.30±0.11^{hA}	1.30±0.01^{ijC}
Glutamic acid	8.90±0.27^{cB}	27.90±0.42^{bA}	5.01±0.05^{cC}
Proline	7.81±0.67^{deB}	21.50±0.48^{eA}	2.20±0.14^{gC}
Glycine	4.28±0.16^{hB}	6.55±0.00^{jA}	0.85±0.00^{kC}
Alanine	5.10±0.07^{gB}	12.65±0.64^{iA}	2.20±0.09^{gC}
Cystine	12.40±1.00^{aB}	24.33±0.13^{cA}	7.59±0.44^{aC}
Valine	6.54±0.17^{efB}	17.25±0.26^{ghA}	1.57±0.07^{hiC}
Methionine	5.81±0.49^{fgB}	8.29±0.24^{jA}	3.63±0.13^{eC}
Isoleucine	7.72±0.10^{deB}	22.07±0.33^{deA}	1.48±0.02^{ijC}
Leucine	7.92±0.20d^{eB}	23.50±0.30^{cdA}	1.22±0.01^{jC}
Tyrosine	10.75±0.13^{bB}	23.74±0.11^{cdA}	4.58±0.09^{dC}
Phenylalanine	10.46±0.52^{bB}	35.21±0.86^{aA}	5.06±0.01^{cC}
Histidine	8.46±0.35^{cdB}	15.82±0.24^{hA}	5.42±0.35^{bC}
Lysine	8.71±0.15^{cB}	20.71±0.29^{efA}	0.87±0.02^{kC}
Arginine	8.09±0.49^{cdB}	24.90±3.11^{cA}	7.88±0.11^{aB}
Essential amino acids	54.31	146.51	15.68
Total amino acids	134.32	340.62	55.95

[1] Data are expressed as mean ± standard deviation (n=3). Values followed by different lowercase letters in the same column are significantly different (P<0.05) and the values followed by different capital letters in the same row are significantly different (P<0.05).
[2] DM = dry matter.

not detected in our samples. The ratio of essential to non-essential amino acids is important for overall efficiency of protein utilisation, and a lower value generally results in smaller nitrogen metabolism. The ratio of soy hull was 0.68, and was 0.75 for okara and 0.39 for molasses. According to the previous studies, this ratio was 0.62-0.67 in broad bean (Lisiewska *et al.*, 2007), 0.81 in lentil, and 0.99 in chickpea (Iqbal *et al.*, 2006). Hence, the protein utilisation in okara is more similar with common legumes. In addition, as okara is rich in essential amino acid (phenylalanine) and semi-essential amino acid (arginine), its amino acid composition is more reasonable, especially benefit to infants.

Fatty acids

Sixteen different fatty acids were detected (Table 3). Pentadecanoic acid and tricosanoic acid were not detected in okara, while eicosenoic acid was not detected in molasses. Unsaturated fatty acids primarily consisted of linoleic acid, oleic and α-linolenic acids, accounting for 73.1-82.3% of total lipids. The content of linoleic acid was particularly high in each soy processing by-product (ca. 50-56%). The major saturated fatty acid was palmitic acid, accounting for 21.1% of total fatty acids in molasses. The percentage content of the other fatty acids was very low, <0.5%. Li *et al.* (2012) indicated that linoleic acid is the most abundant fatty acid in okara (54.1%), then oleic acid (20.4%), palmitic acid (12.3%), and stearic acid (4.7%), which was comparable with our findings. As the fat content in okara is considerable (16.8%) and mainly composed of unsaturated fatty acids, okara can be used as a healthy alternative to lower down fat intake (Grizotto *et al.*, 2012).

Dietary fibre

Dietary fibre content was minimal in molasses, thus we limited our analyses to soy hull and okara (Table 4). Soy hull had total dietary fibre (TDF) content of 55%, and the IDF content was significantly higher than SDF level. For soy hull, SDF was mainly composed of UA (67.6% in SDF), indicating higher content of pectic polysaccharide forming cell wall materials. NS was the major fraction in IDF and the ratio of NS to UA was 9.94. It has been reported that the major polysaccharide of soy hull cell wall consists of acidic galactomannan fractions and xylan, and non-cellulosic polysaccharide composes of mannose and xylose (Karr-Lilienthal *et al.*, 2005), which resulted in a high NS content in IDF.

The concentration of UA in okara was similar to NS content in SDF (about 0.5%), but its value was significantly lower than the NS level in IDF (P<0.05). According to Redondo-

Table 3. Fatty acid profile of three soy processing by-products.[1]

Fatty acids (%)	Hull	Okara	Molasses
Myristic acid (C14:0)	0.21±0.04^{hjB}	0.12±0.01^{gA}	0.12±0.00^{ijB}
Pentadecanoic acid (C15:0)	0.12±0.00^{iA}	–[2]	0.07±0.00^{jB}
Palmitic acid (C16:0)	13.70±0.08^{cB}	12.55±0.04^{cC}	21.15±0.04^{bA}
Palmitoleic acid (C16:1)	0.16±0.00^{hijA}	0.12±0.00^{gB}	0.10±0.00^{ijC}
Margaric acid (C17:0)	0.28±0.00^{ghiA}	0.12±0.00^{gC}	0.15±0.00^{iB}
Stearic acid (C18:0)	5.20±0.04^{eA}	3.73±0.01^{eC}	3.95±0.03^{eB}
Trans oleic acid (C18:1N9T)	0.15±0.02^{ijA}	0.05±0.00^{gC}	0.11±0.00^{ijB}
Oleic acid (C18:1N9C)	18.95±0.12^{bA}	16.25±0.12^{bB}	9.86±0.02^{cC}
Trans linoleic acid (C18:2N6T)	0.12±0.00^{iB}	0.06±0.00^{gC}	0.22±0.001^{hA}
Linoleic acid (C18:2N6C)	49.55±0.20^{aC}	55.45±0.12^{aB}	56.00±0.08^{aA}
Peanut acid (C20:0)	0.43±0.01^{fA}	0.29±0.00^{fB}	0.13±0.00^{iC}
Eicosenoic acid (C20:1)	0.12±0.00^{iA}	0.10±0.00^{gB}	–
α-linolenic acid (C18:3N3)	10.10±0.00^{dB}	10.60±0.08^{dA}	7.22±0.02^{dC}
Behenic acid (C22:0)	0.36±0.01^{fgB}	0.35±0.00^{fC}	0.38±0.00^{fA}
Tricosanoic acid (C23:0)	0.14±0.00^{iB}	–	0.15±0.00^{iA}
Tetracosanoic acid (C24:0)	0.29±0.00^{ghA}	0.13±0.00^{gC}	0.28±0.00^{gB}

[1] Data are expressed as mean ± standard deviation (n=3). The amount of total fatty acid was 100% for each soy processing byproduct. Values followed by different lowercase letters in the same column are significantly different (P<0.05) and the values followed by different capital letters in the same row are significantly different (P<0.05).
[2] Not detected.

Table 4. Dietary fibre content of three soy processing by-products.[1]

	SDF (% DM)[2]		IDF (% DM)			TDF
	UAs	NSs	UAs	NSs	KL	
Hull	1.69±0.01^{d}	0.81±0.04^{e}	3.93±0.01^{c}	39.08±0.31^{a}	9.16±0.57^{b}	54.67
Okara	0.53±0.00^{d}	0.45±0.00^{d}	1.59±0.12^{c}	10.98±0.15^{b}	15.84±2.19^{a}	29.39

[1] Data are expressed as mean ± standard deviation (n=3). Values followed by different superscript letters in the same row are significantly different (P<0.05).
[2] DM = dry matter; SDF = soluble dietary fibre; IDF = insoluble dietary fibre; UAs = uronic acids; NS = neutral sugars; KL = Klason lignin; TDF = total dietary fibre.

Cuenca *et al.* (2008), glucose was the primary monomer in the IDF fraction of okara, indicating that the IDF comprised basically cellulose and hemicelluloses (Mateos-Aparicio *et al.*, 2010).

Klason lignin (KL) was another important IDF fraction in sample. From Table 4, there existed a considerable amount of KL in our samples, especially the ratio of KL to TDF was 54% in okara. The high content of KL was probably caused by protein and other compounds resistant to protease treatment.

Pectin

Pectin is a complex group of polysaccharides and can be divided into three different fractions. WSP is loosely bound to the cell wall through non-covalent and non-ionic bonds which can be dispersed in the liquid phase of the tissues. CSP consists mainly of ionically cross-linked pectin, while HSP contains pectic polymers that are predominantly linked to cell-wall polysaccharides by covalent ester bonds (Christiaens *et al.*, 2012). The amount of total extractable pectin (TEP) was the highest in soy hull (47.4 g GUAE/kg DM), while the content of TEP in okara was half that of the soy hull, and the amount was only 2.3 g GUAE/kg DM in molasses (Table 5).

Table 5. Pectin distributions of three soybean by-products.[1]

	Water soluble pectin (g GUAE/kg DM)[2]	Hydroxide soluble pectin (g GUAE/kg DM)	Chelator soluble pectin (g GUAE/kg DM)	Total extractable pectin (g GUAE/kg DM)
Hull	7.11±0.20^{cA}	8.37±0.72^{cA}	31.90±1.49^{bA}	47.37±1.38^{aA}
Okara	1.71±0.03^{dB}	8.42±0.08^{cA}	14.39±0.83^{bB}	24.52±0.69^{aB}
Molasses	0.65±0.01^{dC}	0.25±0.01^{cB}	1.42±0.09^{bC}	2.32±0.07^{aC}

[1] Data are expressed as mean ± standard deviation (n=3). Values followed by different lowercase letters in the same column are significantly different (P<0.05) and values followed by different capital letters in the same row are significantly different (P<0.05).
[2] GUAE = galacturonic acid equivalents; DM = dry matter.

Among the three fractions of TEP, the amount of CSP was the greatest, accounting for 67.3, 58.7 and 61.1% of the TEP for soy hull, oakara and molasses, respectively. The content of HSP in soy hull was slightly higher than that of WSP. However, the ratio of HSP and WSP in okara was almost 5, indicating strong intercellular adhesions in okara samples. Considering molasses, the concentration of WSP was significantly higher than HSP which suggested greater destruction of cellular.

The high amount of pectin in soy hull, particularly CSP content, indicates that the pectin in soy hull can be extracted as a good source of thickener and stabiliser.

Radical scavenging activity

RSA of SDF in soy hull was almost 20 times greater than that in okara (Table 6). It has been suggested that active substances for antioxidant in legume might be the products of the carbohydrate degradation (Wiboonsirikul *et al.*, 2013); therefore, the differences in RSA in SDF might be caused by the variances of monosaccharides in hydrolysates.

Considering the RSA of pectin, HSP possessed the lowest value and the value of molasses was only 0.01 mg AAE/kg DM. Although the RSA values of HSP in soy hull and okara were much higher than that in molasses (Figure 1), we did not detect RSA in the HSP of soy hull and okara, suggested that RSA of pectin was more related to the structures of pectin. RSA of CSP was generally greater than that of WSP which was probably due to the higher levels of CSP (Table 5). However, RSA of CSP in soy hull was 0.09 g AAE/kg DM, only 42.8% of the value for WSP. This further verified that RSA of pectin has less relation with the content.

As for total phenolics, RSA had a significant linear correlation with the contents of total phenolics in three soy by-products (R=0.9997).

In vitro digestion

As soy processing by-products are generally rich in protein (Table 1), we investigated the *in vitro* protein digestibility. TCA-soluble peptides are released rapidly during the initial 30 min with more than 30% of the total protein being released (Figure 1A). This was particularly true for molasses which released 67.6% of the total protein. The release rate slowed down at the later stage of stimulated gastric digestion with the consumption of pepsin, and the protein digestibility at 2 h was 55.9, 30.5 and 68.1% for soy hull, okara and molasses, respectively. Nakornpanom *et al.*

Table 6. Antiradical scavenging activity (RSA, g AAE/kg DM) of different frictions of soy processing by-products.[1]

	Soluble dietary fibre	Pectin			Total phenolics
		Water soluble pectin	Hydroxide soluble pectin	Chelator soluble pectin	
Hull	0.83±0.003^{aA}	0.21±0.002^{aC}	–[2]	0.09±0.012^{cD}	0.32±0.002^{bB}
Okara	0.04±0.003^{bC}	0.01±0.003^{cD}	–	0.33±0.011^{aA}	0.17±0.002^{cB}
Molasses	–	0.13±0.001^{bC}	0.01±0.000 ^{D}	0.24±0.005^{bB}	2.70±0.020^{aA}

[1] Data are expressed as mean ± standard deviation (n=3). Values followed by different lowercase letters in the same column are significant different (P<0.05) and the values followed by different capital letters in the same row are significantly different (P<0.05).
[2] Not detected.

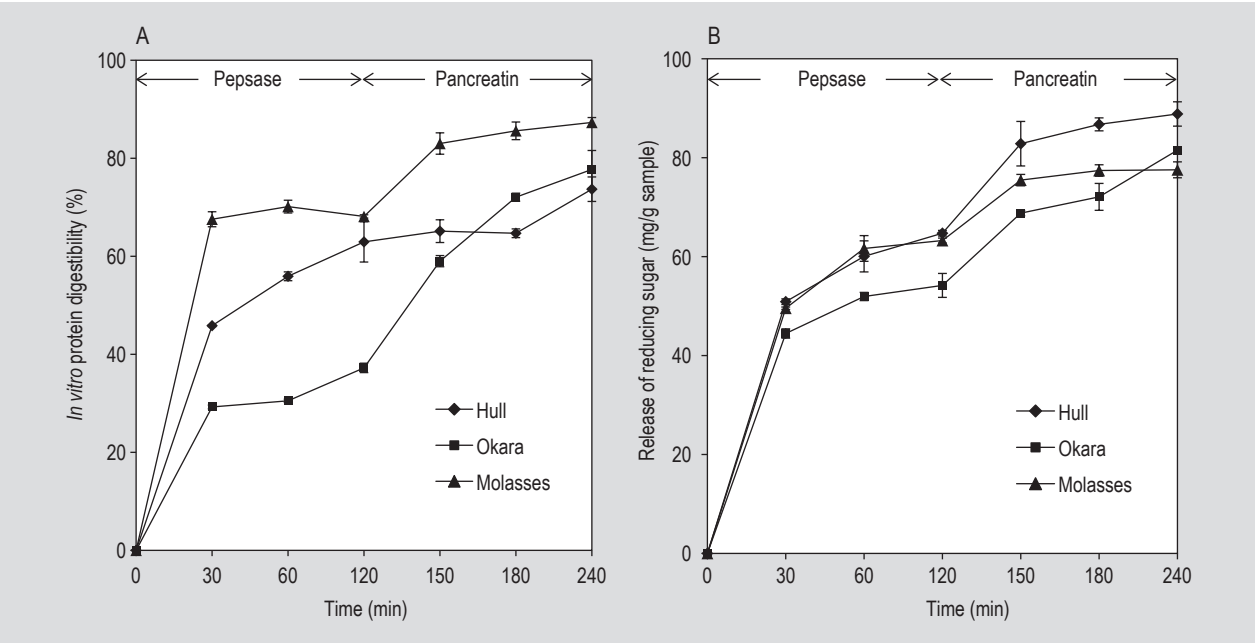

Figure 1. (A) *In vitro* protein digestibility and (B) release of reducing sugar of three soy processing by-products.

(2010) reported that there exists competitive adsorption of protein with soy polysaccharides against the original protein species under acidic conditions during digestion. This might account for less digestible protein (30.5%) be released in okara at the gastric digestion stage. At stimulated intestinal digestion stage, peptic digesta of soy hull and molasses appeared to release TCA-soluble peptides more slowly than that of the gastric digestion stage, the increase rates being 10.7 and 19.2% for 2 h. Okara protein, however, was still digested rapidly at this stage, increasing from 37.2 to 77.7%, which indicated the susceptibility of okara to pancreatin.

Soy products contain considerable amounts of digestible energy, providing carbohydrates that can be hydrolysed to reducing sugars, so we analysed the amount of reducing sugars released during the two step digestion process. The trends for release in the three soy by-products were similar (Figure 1B), there being two stages with rapid release within 30 min and followed by slow release. The released content at the end of gastric digestion ranged from 54.1 to 64.7%, and the data varied from 77.5 to 88.8% at the end of experiments. The digestibility of okara in the whole digestion phase is relatively low, which might be attributed to the high amount of antinutritional factors preserved after processing.

Odour discrimination by e-nose

The electronic nose is a fast and easy technique to distinguish the odour of a product without separation of the various constituents. As illustrated in Figure 2, the first component explained 97.3% of the variance, effectively reflecting the characteristics of each sample. The discrimination index (DI) was used as an assessment

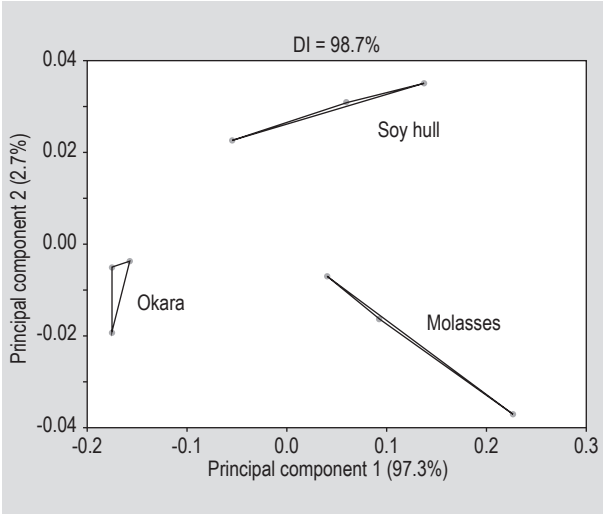

Figure 2. Distinguish of aroma for three soy processing by-products by electronic nose analysed by principle component analysis.

criterion for the discrimination ability of PCA. The DI value in this experiment was 98.7%, far greater than 80%, indicating that the discrimination result was ideal. Soy by-products contain substantial lipid susceptible to oxidative rancidity, and emitting undesirable beany flavour, which has a negative impacts on customer acceptance. As fatty oxidase is mainly distributed in soy skin or cotyledon close to the skin, the beany flavour was stronger in soy hull. Molasses possessed a typical sweet odour, markedly different from the odour compounds in normal soy products, which could explain for the high discrimination validity in this study.

4. Conclusions

This study reported the basic chemical composition and functional properties of three soy processing by-products. Overall, soy hull was rich in dietary fibre and pectin, making it an excellent source of functional substance; okara had high contents of protein and amino acids which had great potential as low-cost protein substitute; the amount of soluble sugars and total phenols was high in molasses, enabling its favourable antioxidant ability. All three soy processing by-products went through rapid and slow release stages during stimulated two-step digestion experiment. The beany flavour affects the consumption of soy products, and as electronic nose could conveniently distinguish the odour characteristics of three soy by-products, it provides a simple way to discriminate whether the beany flavour is eliminated in food production process. This information should be useful in finding most appropriate applications for the use of each of these soy processing by-products. More studies on these three soy by-products should be carried out in the future, such as the identification of the polysaccharide composition in dietary fibre, and odour compounds analysis by gas chromatography/mass spectrometry.

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