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Bayberry (*Myrica rubra* Sieb. et Zucc.) kernel: A new protein source

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ABSTRACT

Bayberry (*Myrica rubra* Sieb. et Zucc.) kernel protein isolate (BKPI) was isolated from bayberry kernel defatted flour (BKDF) by isoelectric precipitation. BKPI was evaluated for chemical composition and selected functional properties with defatted kernel flour as reference. BKPI contained over 90% dry weight (DW) of protein versus 60.5% DW of protein in BKDF. It possessed a well-balanced amino acid composition according to the FAO/WHO reference except for a low content of lysine. BKPI had a solubility profile similar to that of BKDF, with minimum solubility observed at pH 4.0 and maximum solubility at pH 12.0. BKPI exhibited minimum foaming capacity (FC) (31.1%) and maximum foaming stability (FS) (72.7%) at pH 4.0. Minimum emulsifying capacity (EC) and emulsifying stability (ES) of BKPI and BKDF were observed at pH 4.0. BKPI had a least gelation concentration of (LGC) of 6% (w/v) at pH 4.0. Results indicated that bayberry kernel has potential to be exploited as a new protein source in China.

1. Introduction

The proteins from oilseed can be incorporated into foods to impart nutritive value and functional properties (Moure, Sineiro, Domínguez, & Parajó, 2006). Many studies on the characteristics and functional properties of proteins from various oilseeds, such as soybean (Arrese, Sorgentini, Wagner, & Añón, 1991; Sorgentini & Wagner, 2002), peanut (Cherry, 1990; El-Zalaki, Gomaa, & Abdel-Rahman, 1995), rapeseed (Pedroche et al., 2004), sunflower (Kabirullah & Wills, 1982; Lin, Humbert, & Sosulski, 1974), pumpkin seed (Lazos, 1992), and almond kernel (Sze-Tao & Sathe, 2000), have been reported.

Bayberry (*Myrica rubra* Sieb. et Zucc.) is native to eastern Asia, mainly to China; it belongs to the *Myricaceae* (Chen, Xu, & Zhang, 2004). In China, it is cultivated mainly south of the Yangtze River, and Zhejiang is the top area producing bayberry fruit, with an annual production over 300,000 tons (data provided by Zhejiang Agricultural Office). Bayberry fruit is a small drupe and composed of a fleshy pericarp comprising individual segments and a hard endocarp protecting a single kernel (Miao & Wang, 1987). Besides being consumed fresh, the fruit are also processed into juice or concentrate juice, while the seeds are thrown away. The amount of bayberry seeds, which comprise 10% or more by weight of the bayberry fruits remaining after being processed, is quite large. These wasted seed kernels are excellent sources of proteins (25–28%) and oils (62–68%) (Chen, Xu, & Xia, 2004; Cheng, Ye, Chen, Liu, & Zhou, 2008), and have high potential to be exploited as a new oilseed source in China.

During the bayberry seed kernel oil extraction process, defatted flour (as a by-product) may be a vegetable protein source with huge exploitation potential. At present, no information on the characteristics and functional properties of this protein product is available. In order to evaluate its potential for food use, a study was done on the chemical compositions and selected processing functional properties (e.g., solubility, water- and oil-holding capacity, foaming and emulsifying properties and gelation) of defatted flour and protein isolate derived from bayberry seed kernel.

2. Materials and methods

2.1. Material

One batch of sun-dried bayberry seeds used for this study was collected from a fruit winery in Zhejiang Province, China. The seeds were shelled by cracking with a small hammer and the seed hull was manually removed to obtain the kernels. The kernels were ground and extracted with hexane in a Soxhlet extractor for 9 h to remove the fat. The defatted flour was air-dried at ambient temperature and ground again to pass through an 80 mesh screen. The fine flour was packaged in black polyethylene bags and stored in a refrigerator at 4 °C prior to analysis. All the chemicals and solvents used in the experiments were of analytical grade.

2.2. Preparation of protein isolate

Dispersions of bayberry kernel defatted flour (BKDF) (5%, w/v) in distilled water were adjusted to pH 10.0 with 1 N NaOH at 30 °C,

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stirred for 1 h and centrifuged at 5000*g* for 20 min. In order to obtain increased yields, the extraction and centrifugation procedures were repeated twice on the residues. The extracts were combined and the pH was adjusted to 4.0 with 1 N HCl to precipitate the proteins. The precipitate was recovered by centrifugation at 5000*g* for 20 min, followed by removal of the supernatant by decantation. Protein curd was washed twice with distilled water and centrifuged at 5000*g* for 15 min. Then the washed precipitate was freeze-dried as defatted bayberry kernel protein isolates (BKPI).

2.3. Proximate composition

Moisture, ash, fat, crude protein ($N \times 6.25$) and crude fibre contents were determined according to the standard Association of Official Analytical Chemists (AOAC, 1990) methods.

2.4. Amino acids profile

Amino acids were determined using a Sycom S-433D automatic amino acid analyser (Sykam, Eresing, Germany). Hydrolysis of the flour samples (100 mg) was performed in 6 N HCl (10 ml) at 110 °C for 24 h under nitrogen atmosphere. Identification and quantification of amino acids were achieved by comparing the retention times of the peaks with those of standards. Trytophan content was colorimetrically determined by the method of Miller (1967) after alkaline hydrolysis with a spectrophotometer (UV-2450; Shimadzu, Tokyo, Japan). Amino acid composition was expressed as grammes of amino acid (AA) per 100 g of protein. Chemical scores of amino acids were calculated using the FAO/WHO (1991) reference pattern.

2.5. Functional properties

2.5.1. Nitrogen solubility

Protein solubility was determined in a pH range from 2.0 to 12.0, as described by Were, Hettiarachchy, and Kalapathy (1997). Sample (100 mg), for each pH, was dispersed in 20 ml of distilled water whose pH had been adjusted to a specific value using either 0.1 N HCl or NaOH. The slurries were agitated for 1 h at ambient temperature using a magnetic stirrer, and then centrifuged at 8000*g* for 20 min. Nitrogen content in the supernatant was determined by the micro-Kjeldahl method (AOAC, 1990). The percentage of soluble protein was calculated as follows: solubility (%) = (amount of nitrogen in the supernatant)/(amount of nitrogen in the sample) × 100.

2.5.2. Water and oil absorption capacities

One gramme of sample was mixed with 10 ml of distilled water or olive oil for 30 s. The samples were then allowed to stand at ambient temperature (30 ± 2 °C) for 30 min, and then centrifuged at 5000*g* for 30 min. The volume of supernatant was recorded. Water absorption capacity (WAC) was expressed as g of water held per g of protein sample. Oil absorption capacity (OAC) was expressed as g of oil held per g of protein sample. The density of the olive oil was 0.9105 g/ml.

2.5.3. Foaming capacity (FC) and foam stability (FS)

The foaming capacity and stability were evaluated in a pH range from 2.0 to 10.0. A 100 ml sample of 2.0% (w/v) suspension was homogenised vigorously using a FSH-2 homogeniser (Honghua, China) at 2000 rpm for 2 min. The volume of foam after whipping was taken as the foam capacity. Volume of foam of after standing (30 ± 2 °C) for 1 h was expressed as foam stability.

2.5.4. Emulsifying capacity (EC) and emulsion stability (ES)

Samples of 5 ml of 2.0% (w/v) solution, adjusted to a specific pH range from 2.0 to 10.0, were homogenised with 5 ml of olive oil in a calibrated centrifuge tube, using a FSH-2 homogeniser (Honghua, China) at 2000 rpm for 1 min. The emulsions were centrifuged at 3000*g* for 5 min. The ratio of the height of the emulsion layer to the total height of the contents in the tube was calculated as the emulsion activity. To determine the emulsion stability, the prepared emulsions were heated at 80 °C for 30 min, cooled at ambient temperature and then centrifuged at 3000*g* for 5 min. The emulsion stability, expressed in percentage, was calculated as the ratio of the height of the emulsion layer to the total height of the tube content.

2.5.5. Least gelation concentration (LGC)

Gelation properties were investigated as described by Adebowale and Lawal (2003). Protein isolate suspensions of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20% (w/v) were prepared in distilled water. Ten millilitres of each of the prepared dispersions were transferred into a test tube, and heated for 1 h in boiling water, followed by rapid cooling under cold running water. The test tubes were further cooled at 4 °C for 2 h. The least gelation concentration (LGC) was recorded as the concentration when the sample did not slip or fall from the inverted test tube. Effect of pH was conducted on the sample at various concentrations by adjusting the pH to the desired value from 2.0 to 10.0. LGC was recorded as described above. All the experiments were conducted in triplicate.

2.6. Statistics

One way analysis of variance (ANOVA), with multiple range significant difference (LSD) test ($P < 0.05$), was carried out using SPSS13.0.

3. Results and discussion

3.1. Chemical compositions

The moisture, fat, protein, crude fibre and ash contents of defatted bayberry kernel flour (BKDF) and bayberry kernel protein isolate (BKPI) are shown in Table 1. Protein content (91.6% DW) of BKPI was much higher than that of BKDF (60.5% DW). Ash content (1.25% DW) in BKPI was much lower than that in BKDF (9.38% DW). Crude fibre was discarded in the supernatant after protein precipitation. Results indicated that some non-protein components, such as sugars and fibre can be removed during the alkaline extraction and precipitation process.

Amino acid compositions of BKDF and BKPI are presented in Table 2, and their concentrations of essential amino acids were comparable to the FAO/WHO (1991). The amino acid profile of BKPI was similar to that of BKDF. Total essential amino acids contents in BKDF and BKPI were 46.6 and 46.4%, respectively. The nutritive value of dietary proteins is determined by the pattern and quantity of essential amino acids. Chemical scores of methionine + cystine, isoleucine, leucine, histidine, threonine, tryptophan and tyrosine + phenylalanine in BKDF and BKPI were higher or almost

Table 1
Chemical compositions of BKDF and BKPI[a]

Composition	BKDF	BKPI
Dry matter (%)	90.3 ± 1.53	96.7 ± 0.67
Protein (%)	60.5 ± 1.08	91.6 ± 1.92
Lipid (%)	3.50 ± 0.02	0.82 ± 0.00
Ash (%)	9.38 ± 0.67	1.25 ± 0.03
Crude fiber (%)	2.24 ± 0.23	n.d.

n.d.: not detected.
[a] Means ± standard deviation of triplicate determinations, and expressed on dry weight.

Table 2
Amino acid profile (g AA/100 g protein) of BKDF and BKPI[a]

Amino acid	BKDF	BKPI	FAO/WHO (1991)
Asp	8.26	10.2	
Thr	3.36 (0.99)[b]	3.63 (1.07)	3.4
Ser	3.82	2.75	
Glu	24.6	24.0	
Gly	5.11	5.23	
Ala	3.5	3.47	
Val	3.47 (0.99)	3.14 (0.90)	3.5
Cys	2.27	2.09	
Met	5.22	5.18	
Met + Cys	7.49 (3.00)	7.27 (2.91)	2.5
Iso	2.8	2.19	2.8
Leu	6.89 (1.04)	6.81 (1.03)	6.6
Tyr	3.02	3.12	
Phe	3.55	4.19	
Phe + Tyr	6.37 (1.01)	6.31 (1.00)	6.3
His	3.07 (1.62)	3.25 (1.71)	1.9
Lys	2.03 (0.35)	1.81 (0.31)	5.8
Arg	15.0	14.8	
Pro	2.96	2.68	
Try	1.27	1.42	1.1
Total EAA[c]	46.6	46.4	

[a] All values are means of duplicate determinations.
[b] Figures in parentheses indicate aminoacids scores, which are the result of dividing grammes of EEA in 100 g test protein by grammes of EEA in 100 g FAO/WHO (1991) reference pattern.
[c] EAA: essential amino acids.

equivalent to the FAO/WHO requirement pattern. Results indicated that BKDF and BKPI possessed good quality because of well-balanced amino acid composition, except for lysine. In general, the protein of bayberry kernel contained relatively high levels of glutamic acid (24.0–24.6%), arginine (14.8–15.0%) and aspartic acid (8.26–10.2%) and was rich in the sulfur amino acids (7.27–7.49%), but limiting in lysine (1.81–2.03%). The low lysine content in proteins can be complemented when these proteins are consumed in conjunction with other foods containing high amounts of lysine.

3.2. Functional properties

Nitrogen solubility curves for BKDF and BKPI were pH-dependent as depicted in Fig. 1, and U-shaped patterns were observed. Nitrogen solubility is a good option to determine the pH value for extraction. Minimum nitrogen solubility of BKDF (18.3%) occurred at pH 4.0, which might be the region of the isoelectric point, and solubility increased at more acid and alkaline pH values. The profile of BKPI also showed a decreasing solubility with increasing pH until it reached minimum solubility (4.3%) at the isoelectric point (pH 4.0), followed by increase in solubility with increasing pH values. Protein has no net charge at the isoelectric point. However, protein acquires net positive or negative charges at highly acidic or alkaline pH values, which enhances the repulsive interactions and thereby increases the solubility of protein (Singh, Kaur, & Sandhu, 2005). Protein solubility (4.3–52.0%) of BKPI at acidic and neutral pH (pH 2–7) is lower than that of BKDF (18.3–57.7%), because certain proteins, soluble at low pH, are not extracted during the preparation of protein isolate. However, protein solubility (74.1–94.0%) of BKPI at basic pH (pH 8–12) is higher than that of the original flour (65.5–86.8%), because protein solubility in the flour is referred to total protein, a part of which is unextractable.

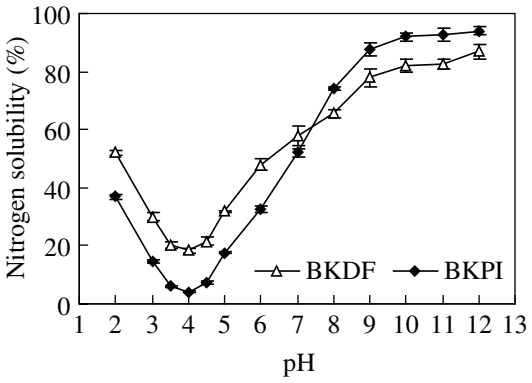

Fig. 1. Effect of pH on the nitrogen solubilities of BKDF and BKPI.

The water and oil absorption capacities of BKDF and BKPI were shown in Table 3. Results showed that the ability of protein isolate to bind water and oil is significantly impaired when compared to the original flour ($P < 0.05$). Various treatments (e.g., stirring velocity, pH, and temperature) in the preparation of protein isolate may change the protein structure, resulting in lower water and oil absorption power. The water absorption capacity (WAC) of BKDF (3.0 g/g) was similar to the WAC of pumpkin seed kernel flour (El-Adawy & Taha, 2001), and higher than that of peach kernel flour (Rahma & Abd El-Aal, 1988). The WAC of BKPI (2.2 g/g) was similar to that found in those protein concentrates and protein isolates derived from L. lablab (El-Adawy, Rahma, El-Bedawey, & Gafar, 2001), much lower than those of protein isolates from *P.calcaratus, D. lablaband, and P. angularis* (Chau, Cheung, & Wong, 1997), and higher than the WAC observed in *B. carinata* protein isolates (Pedroche et al., 2004). The oil absorption capacity (OAC) of BKDF (2.8 g/g) was similar to that of *B. carinata* flours reported by Mahajan, Bhardwaj, and Dua (1999), and quite higher than those reported for V. unguiculata flours (Prinyawiwatkul, Beuchat, McWatters, & Philips, 1997). The OAC of BKPI (1.8 g/g) was much lower than that reported for *P. calcaratus, D. lablab* and *P. angularis* (Chau et al., 1997) and similar to that of *L. angustifolius* protein isolate (Lqari, Vioque, Pedroche, & Millán, 2002).

Table 3
Water/oil absorption capacities, foaming and emulsion properties of BKPI and BKDF[a]

Parameters	BKDF	BKPI
Water absorption capacity (g/g)	3.0 ± 0.2	2.2 ± 0.1
Oil absorption capacity (g/g)	2.8 ± 0.1	1.8 ± 0.2
Foaming capacity (%)		
pH 2.0	17.3 ± 2.5	52.6 ± 3.1
pH 4.0	11.2 ± 1.4	31.1 ± 2.8
pH 6.0	15.6 ± 3.0	47.4 ± 3.2
pH 8.0	20.7 ± 3.9	67.2 ± 4.2
pH 10.0	28.7 ± 2.7	110.4 ± 4.6
Foaming stability (%)		
pH 2.0	0.0 ± 0.0	58.2 ± 1.3
pH 4.0	0.0 ± 0.0	72.7 ± 2.9
pH 6.0	0.0 ± 0.0	56.0 ± 2.8
pH 8.0	0.0 ± 0.0	37.1 ± 1.1
pH 10.0	0.0 ± 0.0	24.0 ± 3.5
Emulsion capacity (%)		
pH 2.0	42.0 ± 2.1	53.2 ± 4.1
pH 4.0	36.0 ± 1.4	22.0 ± 2.4
pH 6.0	42.7 ± 2.5	48.7 ± 3.0
pH 8.0	47.3 ± 3.4	66.6 ± 1.4
pH 10.0	52.0 ± 4.9	83.0 ± 5.1
Emulsion stability (%)		
pH 2.0	81.5 ± 3.2	78.5 ± 4.1
pH 4.0	44.0 ± 1.6	42.3 ± 2.1
pH 6.0	82.3 ± 2.0	84.0 ± 1.5
pH 8.0	83.0 ± 3.4	86.9 ± 1.6
pH 10.0	91.5 ± 2.7	94.6 ± 2.9

[a] Means ± standard deviation of triplicate determinations.

Foaming capacity (FC) of BKDF and BKPI was affected by pH, and the trends for FC and BKDF and BKPI were similar (Table 3). FC profiles at different pH (2.0–10.0) levels for BKDF and BKPI had values of 11.2–28.7% and 31.1–110.4%, respectively. The lowest FC was recorded at pH 4.0, where the protein molecules are in more compact form at the isoelectric point range than at other values. BKPI had significantly higher ($P < 0.05$) FC than had BKDF, with the highest value of 110.4% at pH 10.0. Results showed that the profile of FC against pH was more or less similar to the profile of nitrogen solubility. As shown in Table 3, the foaming stability (FS) of BKDF was weaker than that of BKPI. After whipping and standing for 1 h at room temperature, all the foam of BKDF disappeared, while 24.0–72.7% of foam of BKPI remained. Maximum FS (72.7%) of BKPI was recorded at pH 4 after standing for 60 min at room temperature, while foam stability decreased as the pH of the protein solution increased or decreased further. It is possible that, in the region of the isoelectric point, an adequate balance between attractive and repulsive forces in proteins exists, which helps to form the foams and causes maximum foam stability (Martínez-Flores et al., 2006).

Proteins possess both hydrophilic and hydrophobic properties and thereby proteins can interact with both water and oil in a food system. Emulsifying capacities (EC) of BKDF and BKPI were also pH-dependent (Table 3), and the same trends were observed. EC profiles at different pH (2.0–10.0) levels for BKDF and BKPI had values of 36.0–52.0% and 22.0–83.0%, respectively. EC for BKDF and BKPI increased when distant from their isoelectric point. The lowest EC was observed at pH 4.0, where proteins are the lowest in solubility and lack electrostatic repulsive forces, leading to reduction in emulsion formation. As shown in Table 3, emulsifying stability (ES) of BKDF and BKPI had similar trends. The minimum ES values were also observed at pH 4.0, with values of 44.0%, 42.3%, respectively. However, at other pHs (pH 2.0, 6.0–10.0), BKDF and BKPI were found to form more steady emulsions, with values of 78.5–94.6%. Low ES at the isoelectric pH may be attributed to lack of electrostatic repulsive interactions among particles, which promote flocculation and coalescence (Lawal, 2004).

Effects of concentration and pH on gelation capacity are displayed in Table 4. This property was not only a function of protein concentration but also related to pH. For gelation, lower least gelation concentration (LGC) means better gelation capacity (Akintayo, Oshodi, & Esuoso, 1999). Concentration (10%, w/v) was required to form a gel when protein solution was prepared at pH 2.0. Reduction in LGC was observed at pH 4.0 as the value was reduced to 6% (w/v). Marked increase in LGC, from 12 to 18% (w/v), was observed as the pH of the protein solution increased further from 4.0 to 10.0. The gelation of the BKPI was best at pH 4.0, where proteins have no net charge, which enhances the protein–protein interactions and increases gelation.

Table 4
Effects of concentration and pH on the gelation capacity of BKPI

Concentration (%)	pH 2.0	pH 4.0	pH 6.0	pH 8.0	pH10.0
2	liquid	liquid	liquid	liquid	liquid
4	liquid	liquid	liquid	liquid	liquid
6	liquid	gel	liquid	liquid	liquid
8	viscous	gel	viscous	liquid	liquid
10	gel	gel	viscous	viscous	viscous
12	gel	gel	gel	viscous	viscous
14	gel	gel	gel	gel	viscous
16	gel	gel	gel	gel	viscous
18	gel	gel	gel	gel	gel
20	gel	gel	gel	gel	gel
LGC	10	6	12	14	18

LGC: least gelation concentration.

4. Conclusion

In China, bayberry (*Myrica rubra* Sieb. et Zucc.) seed kernel has a high potential, to be exploited, as a new oilseed source. The alkaline-protein isolates from the defatted flour had high protein contents and possessed good quality because of their well-balanced amino acid composition, except for lysine, according to the FAO/WHO reference. They had good functional properties, such as water/oil absorption capacities, nitrogen solubility, foaming activity and stability, emulsifying activity and emulsifying stability, and gelation. The data obtained from this study could provide a guide to determine parameter values for use in further studies on functionality of protein products from bayberry kernel in different food systems.

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