Original paper

Stable Mixed Beverage is Produced from Walnut Milk and Raw Soymilk by Homogenization with Subsequent Heating

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Received September 17, 2013 ; Accepted February 24, 2014

Walnut milk, which is generally produced in the presence of stabilizers by homogenization, is being increasingly consumed by people in China, due to its good nutritional and functional properties. In this study, the mechanism behind the poor dispersion stability of walnut milk without stabilizer has been clarified as caused by heat-induced large aggregate formation from walnut oil bodies and protein. It was found that addition of raw soymilk into walnut milk could result in the stable mixed beverage by homogenization with subsequent heating. This was because raw soymilk played roles on decreasing the particle size of walnut oil bodies during the homogenization, and preventing the large aggregate formation from walnut oil bodies and protein during the subsequent heat treatment. Amino acid analysis showed that the stable mixed beverage contained more lysine than the walnut milk. In addition, the stable mixed beverage had higher levels of protein content (about 2%) than the commercial walnut milk (commonly < 1%) in China. Therefore, this study was meaningful for the convenient and value-added utilization of walnut.

Keywords: walnut milk, soymilk, mixed beverage, walnut oil bodies, walnut protein

Introduction

Walnut is one of the mostly cultivated tree nuts in the world, including southern Europe, northern Africa, eastern Asia, the USA and western South America (Martinez *et al.*, 2010), and China is the leading producer. It is consumed due to its nutritional, healthy and sensory attributes. It has been reported that walnut exhibits greater antioxidant capacity than any other nuts (Arranz *et al.*, 2008) and its oil has a perfect balance of n-6 and n-3 polyunsaturated fatty acids (4:1) (Simopoulos, 2002). The consumption of walnut can lower plasma total- and low-density lipoprotein cholesterol (Lavedrine *et al.*, 1999; Banel and Hu, 2009), and incidences of cardiovascular diseases (Simopoulos, 2002). In addition, the intake of walnut has been proved to effectively reduce inflammation and improve arterial functions (Ros *et al.*, 2004; Tapsell *et al.*, 2004).

The most popular walnut products are made from toasted nuts.

However, not many people may be motivated to consume the toasted walnut all the times due to the inconvenience of the shell removal. Therefore, the walnut products, convenient to the consumers, are prioritized by the food industry. Some food scientists have tried to produce walnut-containing meat products (Cofrades *et al.*, 2004; Ayo *et al.*, 2005). Nevertheless, the most popular way is the production of walnut milk beverage (Gharibzahedi *et al.*, 2012; Luo *et al.*, 2010; Yu *et al.*, 2010). But poor dispersion stability of walnut milk is a key limiting factor. Until now, the mechanism behind the poor dispersion stability is unclear. Commonly, homogenization in the presence of various stabilizers (xanthan, sodium carboxymethyl cellulose, monoglyceride, sucrose ester, sodium caseinate, arabian gum and so on) is a well-used strategy to produce stable walnut milk beverage (Gharibzahedi *et al.*, 2012; Luo *et al.*, 2010; Yu *et al.*, 2010). However, the beverages with no or few additives are

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preferred by consumers nowadays.

Walnut contains 62–74% of oil (Martinez *et al.*, 2010) with an average value of 69%. It has been reported that the oil in plant seed exists as organelles called oil bodies, which consist of a core of triglyceride matrix coated by one monolayer of phospholipids embedded with oil body intrinsic oleosin proteins (Huang, 1992). Gallier *et al.* (2013) reported that walnut oil bodies were in a particle size range of 1–30 μm . In addition, walnut contains 18–24% of protein, of which glutelin (70%) is the major component, followed by globulin (18%), albumin (7%) and prolamin (5%) (Sze-Tao and Sathe, 2000). Generally, walnut milk is obtained by grinding unshelled and toasted walnut and subsequently filtering. It has been found that walnut milk separates into three fractions (floating, supernatant and precipitate) within a short time of standing (about 30 min). This behavior is considered to correlate with the large size of walnut oil bodies (Gallier *et al.*, 2013) and the poor aqueous solubility of walnut protein (especially glutelin). This speculation was systematically examined in this study.

Nowadays, few additives, high protein and mixed beverages are three important aspects in beverage production. For example, some mixed beverages containing soybean ingredients have been produced by several food scientists (Omueti *et al.*, 2000; Pottera *et al.*, 2007). In this study, raw soymilk (soybean water extract) was likewise selected to make a stable mixed beverage with walnut milk basing on several reasons as follows: soybean products has many nutritional and functional properties (FDA, 1999); soybean contains many components (i.e., phospholipid, saponin and β -conglycinin) with good emulsifying activities, which would be able to stabilize the walnut oil bodies and protein (Ren *et al.*, 2009; Wagner and Gueguen, 1999); the high lysine content of soybean protein would supplement the lysine of walnut milk (the lysine is the first limiting amino acid in walnut protein) (Sze-Tao and Sathe, 2000).

Materials and Methods

Materials Unshelled walnuts Lvling 1 were kindly supplied by Lvling Hebei Co., Ltd. (Hebei Province, China). The split and discolored ones were removed, and the good ones were stored at 4°C until use. Heinong 54 soybeans were kindly supplied by the Northeast Soybean Research Institute (Heilongjiang Province, China) and stored at 4°C until use.

Walnut milk preparation Unshelled walnuts were put into 1% NaOH (w/w) solution, and kept in a 90°C water bath for 10 min. Then they were washed with cold water to remove seed coat, and kept washing until the water pH became neutral. The walnuts were then toasted at 120°C for 20 min. The toasted nuts (100 g) were put into de-ionized water (1/10, w/v) and ground in a Waring Commercial Blender (Philips, HR 2870/00) for 3 min. The homogenate was filtrated through a 150-mesh sieve to obtain filter (termed walnut milk; protein, 1.6%; oil, 4.3%) and residue (walnut

okara). The walnut okara was collected and freeze-dried. The protein content of the freeze-dried okara determined by micro-Kjeldahl method was 22% (w/w, dry basis). Ten milligrams of the freeze-dried okara was dispersed into 1 mL of SDS-PAGE sample buffer and vigorously mixed. Then, twenty microliters of β -mercaptoethanol was added and heated in boiling water bath for 3 min. After centrifugation (10,000 g, 1 min), the supernatant was retained and used for SDS-PAGE.

Walnut milk treated by three methods Three different treatments were applied to the original walnut milk. The first was heating the walnut milk at 120°C for 10 min. The second was a two-stage homogenization (40 MPa at each stage). The last one was a two-stage homogenization (40 MPa at each stage) followed by heating at 120°C for 10 min.

Mixtures of raw soymilk and walnut milk Twenty grams of soybeans were soaked in de-ionized (DI) water at 4°C for 18 h. The soaking water was discarded and fresh DI water was added to make the total weight of 200 g. This was ground in a Waring Commercial Blender (Philips, HR 2870/00) for 3 min. The homogenate was filtrated through a 150-mesh sieve and the filter was termed raw soymilk (protein, 2.3%; oil, 0.9%). Ninety grams of raw soymilk and 90 g of walnut milk were mixed well and the mixture was subjected to the above mentioned treatments.

Observation by microscope Ten microliters of sample was placed on the microscope slide, and covered by a cover glass. It was observed at a magnification of 400 $\times$ by a microscope (Model CS, Carton Optical Industries Co. Ltd., Tokyo, Japan) attached to a digital camera (Eos Kiss X2, Canon Inc., Tokyo, Japan).

The extraction of walnut oil bodies Forty five grams of sucrose was added into 180 g of walnut milk and mixed well (20% sucrose, w/w). It was then divided into three beakers and pH adjusted to 7, 9 and 11 by 1 M or 2 M NaOH. An aliquot (40 g) of each sample was centrifuged at 25,000 g for 30 min. The floating fractions were carefully collected and put into separate beakers. Into each, 36 g of sucrose solution (20%, w/w) was added. They were dispersed well, correspondingly readjusted to pH 7, 9 and 11, and centrifuged again under the same conditions above. Floating fractions were collected, put into three separate clean beakers. Then, 4.5 mL of DI water was added to each and dispersed well by magnetic stirring. To determine solid and protein contents, 1 mL and 2.5 mL of oil body dispersions were respectively used. The residual dispersion, defatted by diethyl ether (Tzen *et al.*, 1993), was used to examine the protein composition by SDS-PAGE. The oil bodies in the mixtures of walnut milk and raw soymilk (homogenization; homogenization with subsequent heating) were extracted by the same method above without pH adjustment.

SDS-PAGE SDS-PAGE was conducted by the method of Laemmli (1970) with the concentrations of the stacking and running gels being 5% and 12.5%, respectively. A 0.5 mL of sample (2 mg protein/mL) was mixed with 0.5 mL of sample buffer (0.05 M Tris-HCl, pH 6.8; 1% SDS; 0.01% bromophenol blue;

30% glycerol) containing 2% (v/v) β -mercaptoethanol. This was heated in boiling water bath for 3 min and cooled with tap water. Ten microliters were loaded into sample well and electrophoresed.

Particle size distribution determination Particle size distribution was determined by a particle size analyzer (Microtrac S3500, Microtrac., USA). DI water was used as the dispersion solvent.

Walnut protein dispersibility in mixtures of walnut milk and raw soymilk obtained by homogenization with subsequent heating A series of mixtures of walnut milk/raw soymilk (135 g/45 g, 120 g/60 g, 90 g/90 g, 60 g/120 g, 45 g/135 g) were prepared and all were treated by the two-stage homogenization (40 MPa at each stage) with subsequent heating (120°C, 10 min). These mixtures contained 1.20–0.40% of walnut protein and 0.58–1.73% of soybean protein. After cooled in tap water, each mixture was separated into three fractions by centrifugation (4,000 g, 30 min): floating, supernatant, and precipitate, and each supernatant was carefully collected by syringe. The protein contents of these supernatants, raw soymilk and walnut milk were determined by micro-Kjeldahl method. All soymilk protein was assumed to remain in supernatant. Therefore, the walnut protein in supernatant could be calculated, and the walnut protein dispersibility in mixture was expressed as follows:

$$\text{The walnut protein dispersibility in mixture} = (\text{walnut protein in supernatant}) / (\text{walnut protein in mixture}) \times 100\% \quad \text{.....Eq. 1}$$

Amino Acid Analysis Two mixtures of walnut milk and raw soymilk (90 g/90 g; 120 g/60 g) were prepared, and subjected to the homogenization with subsequent heating as described above. In addition, walnut milk and raw soymilk were also treated by the same method. They were then freeze-dried and used for amino acid analysis.

The freeze-dried samples were hydrolyzed in 6 N HCl (110°C, 24 h). The amino acid composition was determined by an automatic amino acid analyzer (Agilent 1100, Santa Clara, CA) by precolumn online derivatization with *O*-phthalaldehyde.

Results and Discussion

Poor dispersion stability of walnut milk About 80% of walnut protein was found to be dispersed in the walnut milk, and about 20% remained in the okara. The protein compositions of the walnut milk and okara were examined by reducing SDS-PAGE. Fig. 1 shows that the protein composition of walnut milk is the same as that of okara with a molecular weight (MW) range of 12–55 kDa. The major walnut proteins have MW ranges of around 30–35 kDa and 18–22 kDa, agreeing with the results by Mao and Hua (2012). These results revealed that most of walnut protein (about 80%), including albumin, globulin, glutelin and prolamin, were dispersed into walnut milk although glutelin and prolamin were known to have poor aqueous solubility. Walnut milk was observed by

microscope. It is found that there are oil droplets of different sizes (Fig. 2a), which are considered as walnut oil bodies. Fig. 3a shows that particle size distribution of walnut milk is nearly a monomodal type in a range of 1–30 μ m, agreeing with the results by Gallier *et al.* (2013).

When the walnut milk was left to stand for about 30 min, large amounts of floating layer and precipitate were formed. It was suggested that the floating layer was mainly comprised of walnut oil bodies while the precipitate mainly consisted of walnut protein, especially glutelin. Then the walnut milk was mixed well and treated by three methods: 1) heating; 2) homogenization; 3) homogenization with subsequent heating. Fig. 3a shows that heating increases the particle size of walnut milk; surprisingly, homogenization also increases the particle size of walnut milk; homogenization with subsequent heating increases the particle size more obviously. Their microscope results are shown in Fig. 2a, indicating that large oil droplets remain in the walnut milk after heating and tend to form the large aggregates of oil droplets. After homogenization, the large oil droplets become smaller, but they show a tendency to flocculate. After the homogenization with subsequent heating, flocculation occurs in the walnut milk. These results greatly agree with those presented in Fig. 3a. In addition, it was found that larger amounts of floating layer and smaller amounts of precipitates were formed in the heated walnut milks (heating; homogenization with subsequent heating) after about 30 min standing compared to the unheated one, clearly indicating that heating could induce the interaction between walnut oil bodies and protein. Thus, it was suggested that the heat-induced large aggregate formation (Fig. 2a) from walnut oil bodies and protein was the main reason for the poor dispersion stability of walnut milk.

Walnut oil bodies Plant seed oil bodies consist of a lipid core that is coated by a monolayer of phospholipids embedded with oleosin proteins (Huang, 1992). However, oil bodies can also get

Fig. 1. Protein profiles of walnut milk and okara by reducing SDS-PAGE. Lane 1, marker; lane 2, walnut milk; lane 3, walnut okara.

(a)

(b)

Fig. 2. (a) Microscope images of walnut milk obtained by: 1) heating; 2) homogenization; 3) homogenization with subsequent heating. (b) The microscope images of walnut milk and raw soymilk mixture treated by: 1) heating; 2) homogenization; 3) homogenization with subsequent heating. The ratio of raw soymilk/walnut milk is 1/1 (w/w). Bars in all images are 50 μm.

(a)

(b)

Fig. 3. (a) Particle size distributions of walnut milk in Fig. 2a: 1) walnut milk; 2) heated walnut milk; 3) homogenized walnut milk; 4) homogenized and heated walnut milk. (b) The particle size distributions of mixtures in Fig. 2b: 1) mixture of walnut milk and raw soymilk; 2) heated mixture; 3) homogenized mixture; 4) homogenized and heated mixture. Soymilk is obtained by homogenization with subsequent heating. The ratio of raw soymilk/walnut milk is 1/1 (w/w).

bound to the seed protein after the seed's microstructure is disrupted. For example, many proteins were found to be bound to oil bodies after soaked soybean was ground in water (Guo *et al.*, 1997). It was considered that the walnut oil bodies were also bound to walnut protein during grinding. Chen and Ono (2010) reported that more and more bound proteins were released from soybean oil

bodies with increasing aqueous pH, and almost all were released at $\text{pH} \geq 11$. Therefore, the effect of pH on walnut protein, which might have been bound to walnut oil bodies, was examined. Fig. 4 shows that the protein compositions of the pH 7, 9 and 11 extracted walnut oil bodies are almost the same as that of walnut milk. Generally, the MWs of plant seed oil body oleosins are in a range

of 16–24 kDa (Tzen *et al.*, 1990). Therefore, it was considered that the bands indicated by three arrows (Fig. 4) might be comprised of walnut protein and oleosin. Table 1 shows that the amounts of protein in the pH 7, 9 and 11 extracted walnut oil bodies are 7.13, 7.05 and 7.00 g/100 g oil body (dry basis), respectively. Studies have shown that plant seed oil bodies contain 0.6–3.5% of oleosin in dry basis (Tzen *et al.*, 1993). These clearly revealed that some walnut protein was strongly bound to walnut oil bodies even at pH 11, showing a different trend from soybean oil bodies, which might be resulted from the treatment of toasting (120°C, 20 min) of walnut. Table 1 also shows that the protein amount of oil bodies extracted from homogenized walnut milk increases to 8.02 g/100 g oil body and to 16.39 g/100 g oil body by further heating, indicating that heating indeed induces the interaction between walnut protein and oil bodies.

Mixture of raw soymilk and walnut milk It is known that raw soymilk contains some components (i.e., saponin, phospholipids

and β -conglycinin) with good emulsifying activities. Therefore, raw soymilk was mixed with walnut milk, and treated by the three methods above. It was found that heating alone could not stabilize the mixture and large amount of floating layer was formed after about 30 min of standing, which should be resulted from the large size of walnut oil bodies (Fig. 2b). A stable mixture was obtained by homogenization, and more especially with subsequent heating. However, Fig. 2b shows that the homogenized mixture has a weak tendency to flocculate, while the homogenized and heated mixture has a better dispersibility (Fig. 2b and 3b). Oil bodies were extracted from these two mixtures and their determined protein contents were 6.32 and 9.31 g/100 g oil body (Table 1). Fig. 5 shows that oil bodies extracted from the former (lane 4) mainly contain walnut protein, while oil bodies extracted from the latter (lane 5) contain soybean protein (especially the α' , α and β subunits of β -conglycinin) as well as walnut protein. Therefore, it was suggested that the amphiphathic molecules (i.e., phospholipids and saponin) of raw soymilk acted as emulsifiers to decrease the particle size of walnut oil bodies in the homogenized mixture, and the soybean protein, especially β -conglycinin, got bound to walnut oil bodies when the mixture was further heated. It was considered that the bound soybean protein (especially β -conglycinin) could possibly prevent the large aggregate formation from walnut oil bodies and protein, which could be used to explain why the particle size distribution of the homogenized and heated mixture shift to smaller size compared to the homogenized mixture. This was reasonable owing to the facts that: visible aggregates were formed from both of soybean whey protein and glycinin by heating, but in the presence of β -conglycinin, the formation of visible aggregates could efficiently be prevented (Ren *et al.*, 2009; Guo *et al.*, 2012).

It is well known that homogenizing and/or heating is beneficial for the stabilization of soymilk. As such, the data about raw soymilk treated by the three methods (heating; homogenization; homogenization with subsequent heating) are not shown in this study. Cruz *et al.* (2007) reported that the particle size of soymilk obtained by homogenizing first and then heating decreased compared to the original soymilk. According to the research by Nik *et al.* (2009), the heated soymilk showed smaller particle size than unheated soymilk. Other than the combined treatment of

Fig. 4. Protein compositions of pH 7, 9 and 11 extracted walnut oil bodies. Lane 1, marker; lane 2, walnut milk; lanes 3 and 4, the supernatant and precipitate obtained from walnut milk treated by centrifugation (25,000 g, 30 min); lanes 5–7, walnut oil bodies extracted from walnut milk by pH 7, 9 and 11, respectively. The protein bands indicated by three arrows might be comprised of walnut protein and oleosin.

Table 1. Protein amounts of extracted oil bodies. The ratio of walnut milk/raw soymilk is 1/1 (w/w).

Origin of oil bodies	Protein amounts (g/100 g oil body, dry basis)
pH 7 walnut milk	7.13 ± 0.08
pH 9 walnut milk	7.05 ± 0.07
pH 11 walnut milk	7.00 ± 0.04
Homogenized walnut milk	8.02 ± 0.12
Homogenized and heated walnut milk	16.39 ± 0.08
Homogenized mixture of raw soymilk and walnut milk	6.32 ± 0.04
Homogenized and heated mixture of raw soymilk and walnut milk	9.31 ± 0.24

homogenizing first and then heating, the combined treatment of heating first and then homogenizing might be another method for stabilizing the mixture of raw soymilk and walnut milk, but this was not conducted in this study because the process would be unrealistic by the standard practice in the beverage industry.

Walnut protein The sections above focused on the dispersion stability of walnut oil bodies. In this section, the dispersion stability of walnut protein was examined. The mixtures of walnut milk/raw soymilk (135 g/45 g, 120 g/60 g, 90 g/90 g, 60 g/120 g and 45 g/135 g) were homogenized and subsequently heated. They contained 1.20–0.40% and 0.58–1.73% of walnut and soybean protein, respectively. The walnut milk was used as control and treated in the same manner as the mixtures. It was found that all mixtures were stable, while large amounts of floating layer and nearly no precipitate were formed in the control. After centrifugation (4,000 g, 30 min), small amounts of floating layer and precipitate were formed in the mixtures, and they gradually decreased further with increasing soymilk. Fig. 6 shows that only about 35% of walnut protein remains in the supernatant of the control, revealing that about 65% of walnut protein is bound to walnut oil bodies by heating. The percentage of walnut protein remaining in supernatant increased with increasing soymilk, and the maximum value of about 90% was achieved when 60 g of walnut milk was mixed with 120 g of raw soymilk.

In order to examine the dilution effect induced by raw soymilk,

ninety grams of walnut milk was mixed well with 90 g of raw soymilk or DI water. They were treated by homogenization with subsequent heating as described above. After centrifugation (4,000 g, 30 min), it was found that there was about 85% of walnut protein remaining in the supernatant of the homogenized and heated mixture of walnut milk and raw soymilk, while there was only about 22% of walnut protein remaining in the supernatant of the homogenized and heated walnut milk diluted with DI water. This clearly revealed that the stable mixture was resulted from the raw soymilk, but not its dilution effect.

As stated above, 35% of walnut protein remained in the supernatant of walnut milk (control), and 22% of walnut protein remained in the supernatant of the diluted walnut milk. Generally, the former value should be smaller than the latter one due to the dilution effect. The reason for this was unclear in this study, which might be resulted from experimental error or some other reasons. Fortunately, this did not give bad effects on the fundamental results in this study.

The amino acid compositions of mixtures It has been reported that lysine is the first limiting amino acid in walnut protein (Sze-Tao and Sathe, 2000), and soybean protein is limiting in sulfur-containing amino acids (methionine and cysteine) (Zezulka and Calloway, 1976). Table 2 shows that soymilk protein contains obviously more lysine than walnut milk protein, while walnut milk protein possesses more methionine and cysteine than soymilk

Fig. 5. Protein compositions of oil bodies extracted from the mixture of walnut milk and raw soymilk (homogenized; homogenized and subsequently heated). The ratio of raw soymilk/walnut milk is 1/1 (w/w). Lane 1, marker; lane 2, oil bodies extracted from the homogenized walnut milk; lane 3, oil bodies extracted from the homogenized and heated walnut milk; lane 4, oil bodies extracted from the homogenized mixture of walnut milk and raw soymilk; lane 5, oil bodies extracted from the homogenized and heated mixture of walnut milk and raw soymilk; lane 6, soymilk; lane 7, oil bodies extracted from walnut milk. Walnut milk is used as control. α' , α and β are the subunits of β -conglycinin; A3 and A are acidic polypeptides of glycinin, while B is basic polypeptides of glycinin.

Fig. 6. Walnut protein dispersibility in the homogenized and heated mixtures of walnut milk and raw soymilk (135 g/45 g, 120 g/60 g, 90 g/90 g, 60 g/120 g and 45 g/135 g). All soymilk protein is assumed to remain in supernatant after centrifugation (4,000 g, 30 min), and walnut milk is used as control.

Table 2. Amino acid compositions of soymilk, walnut milk and their mixed beverages, and all obtained by homogenization with subsequent heating.

	Soy milk (mg/g protein)	Walnut milk (mg/g protein)	Walnut milk/ soymilk (1/1) (mg/g protein)	Walnut milk/ soymilk (2/1) (mg/g protein)
Asx	116.81	103.03	109.16	112.54
Glx	214.61	208.85	212.79	208.85
Ser	44.88	48.19	49.19	48.95
His	27.26	25.83	26.22	24.62
Gly	43.91	46.28	44.09	45.03
Thr	36.53	36.36	37.03	38.03
Arg	73.54	147.53	112.28	100.68
Ala	40.64	46.03	42.90	45.12
Tyr	27.21	22.46	21.61	24.04
Cys	1.71	2.57	2.37	2.49
Val	51.20	52.63	50.80	53.90
Met	9.32	12.60	10.81	11.29
Phe	52.87	51.11	52.46	54.98
Ile	49.15	44.67	46.06	47.78
Leu	76.63	79.38	78.59	79.17
Lys	60.13	24.05	49.01	43.91
Pro	74.78	48.42	59.74	49.33

protein. When mixtures of walnut milk/soymilk were made in the ratios of 1/1 and 2/1 (w/w), it was found that they could supplement each other on methionine/cysteine and lysine. Therefore, it was asserted that the production of mixed beverage from soybean and walnut was not only good at the dispersion stability of beverage, but also the nutritional quality of beverage.

Conclusions

This study, on one hand, has clarified that the poor dispersion stability of walnut milk is resulted from the heat-induced large aggregate formation from walnut oil bodies and protein. And homogenization, which is a popular and very important method for stabilizing beverages, is not adequate to stabilize walnut milk. On the other hand, it has been found that homogenization followed by heating can result in a stable mixed beverage if raw soymilk and walnut milk are used. This is so, because raw soymilk can decrease the particle size of walnut oil bodies during homogenization, and can effectively prevent large aggregate formation from walnut oil bodies and protein when the homogenized mixture is subsequently heated. When the walnut milk was mixed with raw soymilk at a ratio of 1/2 (w/w), about 90% of walnut protein remained in supernatant after centrifugation (4,000 g, 30 min), signifying a recommendable ratio for better walnut protein dispersibility. The amino acid analysis showed that the mixed beverage could contain more lysine than walnut milk alone. Therefore, this study is considered very meaningful for the convenient and value-added utilization of walnut, because the mixed beverage has better dispersion stability, higher levels of protein content (about 2%),

and more lysine than walnut milk.

Acknowledgements We gratefully acknowledge the financial support received from Natural Science Foundation of China (31301496), 863 Program (Hi-tech research and development program of China) (2013AA102204-3), Fundamental Research Fund of Ministry of Education (2050205), Natural Science Foundation of China (21276107), the “12th five-year plan” National Key Technology R & D program of China (2012BAD34B04-1), and a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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