



Original Research Article

Production and characteristic of sesame proteins

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ABSTRACT

Sesame seed (*Sesamum indicum* L.) is an important oil seed crop. Most of the sesame seeds are used for extraction of oil. Sesame meal is by-product after oil extraction. The meal is a good source of protein and usually used as animal feed. The aim of this research is to study the production and characteristic of proteins from different varieties of sesame seeds, including black, white and red seed. Three sesame protein concentrates (SPC) namely, black (BSPC), red (RSPC) and white, (WSPC) were prepared from defatted sesame meals by alkali solution at pH 10 and isoelectric precipitation at pH 4. All SPC samples were characterized in term of yield, protein content, molecular weight and denaturation temperature. Results showed that the percent yield of BSPC, RSPC and WSPC were 41.14%, 41.90% and 40.82%, respectively. The protein contents of BSPC, RSPC and WSPC were 80.38%, 79.85% and 80.92%, respectively. Results from molecular weight profile using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) revealed that SPC samples had protein bands with molecular weights during 42.5 and 83.32 kDa under non-reducing condition. Under reducing condition, the SDS-PAGE pattern showed that the SPC samples had molecular weight ranging from approximately 12.21 to 39.19 kDa. DSC analysis revealed no detectable endothermic peak.

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INTRODUCTION

Sesame (*Sesamum indicum* L.) belonging to the order Tubiflorae, family Pedaliaceae, is an important and ancient oil-yielding crop cultivated for its flavorsome. It is also an edible seed and has high quality oil (Pathak *et al.*, 2014). The seed has been called as "Queen of oil seeds" because of its high oils yield and its quality (Gandhi and Srivastava, 2007). The total world production was about 4 million metric tonnes of sesame seeds in 2011. Thailand was producer of sesame seed about 48,840 metric tonnes sesame seeds in 2011 (FAOSTAT, 2011) and sesame has become one of potentially important crops of Thailand. Sesame seeds are commonly used as a raw material for oil extraction. They have oil content of between 48 - 55%; as a result, they have become one of the main sources of edible oil. It is also a good source of protein, yielding between 20% and 25% protein depending on the variety (Cano-Medina *et al.*, 2011). Sesame seeds are also very used full for body as they are digestive, rejuvenative, anti-aging and rich in vitamins E, A and B complex and minerals such as calcium, phosphorus, iron, copper, magnesium, zinc and potassium (Bukya and Vijayakumar, 2013). The sesame meal is by product after oil extraction. It is usually used for animal feed. It is a good source of nutrition, containing approximately 50% protein. This meal has high potential for use as a protein source or as an ingredient in the food industry (Onsaard *et al.*, 2010). The sesame protein can be prepared using alkaline extraction and after the sesame oil extraction, the supernatant is centrifuged, and pH of the solution is adjusted to the isoelectric point to obtain protein precipitation (Zhao *et al.*, 2012). VegeTable protein isolation processes include an acidic or alkaline solubilization step, in order to obtain products that contain over 90% protein (López *et al.*, 2003). The used of protein isolates has increased in the food industry because of different factors such as high protein level, good functionality, and low content of anti-nutritional factors (Abugoch *et al.*, 2008). Sesame proteins have been classified into four classes of protein based on Osborne sequential extraction and different solubility (Onsaard, 2012). Most of the proteins presented in sesame seeds are storage proteins found as albumins (8.9%), globulins (67.3%), prolamins (1.3%) and glutelins (6.9%) on the basis of their solubility (Orruño and Morgan, 2005). SDS-PAGE is considered to be a practical and dependable method because seed storage proteins are extremely sovereign of environmental fluctuation (Akbar *et al.*, 2012). The aim of this research is to study the production and characteristic of proteins from different varieties of sesame seeds. The seeds vary in color with three main colors: black, white and red, all sesame cultivars mainly grown and developed from Ubon Ratchathani Field Crops Research Center, Thailand.

MATERIALS AND METHODS

Materials

The three varieties of sesame seed (black, white and red) used as a source of sesame protein throughout this research were purchased from Ubon Ratchathani Field Crops Research Centre, Thailand. Initially, seeds were cleaned to remove dirt, foreign matter, stubble, mold, rot and other infestation and then, they were washed with water and sundried. All other chemicals used were of analytical grade.

Methods

Preparation of defatted sesame flour

The sesame seeds were extracted by hydraulic press. Sesame meals were obtained from this extraction after that sesame meals were prepared by grounding in the blender. Defatted sesame flours (DSF) were prepared following the method by Inyang and Iduh (1996). The meals were dried in vacuum oven (Binder, VD115, Germany) at 50°C for one hour and then had finely sieved, later were defatted with hexane at a ratio of 1:6 (w/v) under constant shaking (Innova, New Brunswick Scientific, USA) at 220xg for one hour. The hexane was changed three times and then decanted and removed in a hot-air oven at 60°C for one hour. The DSF obtained had a final fat content of less than 2%. The DSF was grounded again to pass through 75 mesh and kept in vacuum containers at 4°C prior to using.

Preparation of sesame protein concentrates

Sesame protein concentrate (SPC) samples were prepared using the modified method described by Gandhi and Srivastava (2007). DSF was mixed with water at a ratio of 1:10 (w/v). The pH of the suspended sesame meal was adjusted to pH values ranging from 7 to 10 using 2.0 M NaOH, continuously stirred with a magnetic stirrer for 1 hour and centrifuged at 2,822 xg for 15 min. The soluble phases were adjusted to pH 4.5 using 0.1 or 1.0 M HCl, which led to the precipitation of protein. The suspensions were centrifuged at 2,822xg for 15 min, after which the precipitates were collected and weighed. The precipitates were neutralized to pH 7.0 using 0.1 or 1.0 M NaOH and then freeze-dried and used for protein content assay by the Kjeldahl method.

Proximate composition analysis

The moisture, crude protein, crude fat and ash of sesame meal, DSF and SPC were analyzed by using official methods by AOAC (1995).

Gel electrophoresis (SDS-PAGE)

Molecular weight patterns of SPC samples were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). SDS-PAGE was carried out with 12% (w/v) polyacrylamide gels with or without 2-mercaptoethanol (2-ME) in a Mini-Protean II unit (Bio-Rad Hercules, CA). SPC samples were solubilized to a final concentration of 0.5 mg/mL in the ProeoGel Tris-Acetate sample buffer. Pipet 5 µl of protein sample was loaded in each well and the electrophoresis was conducted at a constant current of 200 Voltages. After electrophoresis, the samples were stained with Coomassie brilliant blue and destained with acetic acid: methanol: water (10:40:50 v/v/v) for an hour. Protein markers ranging from 10 to 225 kDa, were used as standard (USB molecular biology reagents and biochemical, Cleveland, Ohio 44128, U.S.A.).

Thermal properties

Differential scanning calorimeter (DSC) was used to determine thermal denaturation characteristics of sesame proteins concentrates. Sample of 2.5 mg was mixed with phosphate buffer pH 7. Aluminum container has sealed and kept at room temperature for one hour to equilibrate the sample with phosphate buffer. The sample was heated in DSC from 30°C to 120°C at the rate of 10°C /min. The phosphate buffer pH 7 (10 mg) was used as a reference. Denaturation temperature (Td) and denaturation enthalpy (ΔH) was calculated by STAR[®] thermal analysis.

RESULTS AND DISCUSSION

Proximate composition and protein yield of sesame proteins concentrate

The chemical compositions of three sesame protein concentrates (black; BSPP, white; WSPC and red; RSPC) are presented in Table 1. It can be observed that all results are very similar. The percent yield of BSPP, RSPC and WSPC were 41.14%, 41.90% and 40.82%, respectively. The moisture content of all three DSM samples was not different, while the other three SPC samples have low moisture content after precipitation.

protein analysis. The SDS-PAGE profiles of BSPP, WSPC and RSPC are shown in Figure 1. The SDS-PAGE pattern revealed that SPC samples had protein bands with molecular weights during 42 and 83 kDa under non-reducing condition. In the presence or absence of the reducing agent, β -mercaptoethanol (2-ME), shows the protein constituents of the three sesame varieties are similar in Figure 2. On the other hand, the SPC samples are under reducing condition, the SDS-PAGE pattern showed that the SPC samples had protein bands with molecular weights during 12 to 39 kDa. These results suggested that all sesame proteins of all sesame varieties consisted of some polypeptides linked disulfide bonds.

Table 1 Chemical composition of defatted sesame meals and sesame proteins concentrate

Chemical composition	Defatted sesame meal			Sesame protein concentrate		
	Black	White	Red	Black	White	Red
Moisture (%)	4.04 ± 0.02 ^b	4.16 ± 0.02 ^a	4.09 ± 0.01 ^{ab}	1.60 ± 0.64 ⁿ	1.75 ± 0.05 ⁿ	1.78 ± 0.01 ⁿ
Protein (%)	39.40 ± 0.02 ^b	40.04 ± 0.17 ^a	37.24 ± 0.69 ^c	80.38 ± 0.18 ⁿ	80.92 ± 0.42 ⁿ	79.85 ± 0.44 ⁿ
Fat (%)	3.46 ± 0.11 ^b	3.72 ± 0.14 ^a	4.15 ± 0.07 ^a	1.82 ± 0.04 ^a	1.97 ± 0.02 ^b	2.03 ± 0.04 ^b
Ash (%)	11.71 ± 0.50 ⁿ	11.69 ± 0.23 ⁿ	11.87 ± 0.20 ⁿ	4.21 ± 0.15 ⁿ	4.38 ± 0.13 ⁿ	3.88 ± 0.33 ⁿ
Carbohydrate *	41.89 ± 0.52 ^b	40.95 ± 0.05 ^c	39.61 ± 1.17 ^a	12.00 ± 0.71 ^{ab}	10.99 ± 0.33 ^b	12.77 ± 0.08 ^a

Values are given as mean ± SD from triplicate determination

* Estimated by difference

(^{a, b, c}) In a column, mean values followed by the same superscript are not significantly different at $p < 0.05$ (DMRT).

The low moisture content is an index of stability, quality, shelf life and also high yields (Nweke *et al.*, 2011). Protein contents of BSPP (80.38%), WSPC (80.92%) and RSPC (79.85%) samples were higher than those of defatted sesame meal samples. These results showed that the protein content of three sesames were not significantly different. The high protein content can be used to supplement low protein flours from cereal that it used for infant feeding (Ranganayaki *et al.*, 2012). The SPC samples were shown that fat contents have low which ranged from 1.82%-2.03%. Ash contents of DSM were ranged 11.69%-11.87 %, while SPC were ranging 3.88% - 4.38%. The soaking of seeds causes a loss of ash content and the presence of ash is a reflection of inorganic matter in a food sample (Inyang and Ekanem 1996; Adebowale *et al.*, 2010). Carbohydrate contents of SPCs were quite low and at a range between 10.99 %-12.77 %. Different content could be a reflection of varieties and/or genetic makeup of the seed (Adebowale *et al.*, 2010). The composition of the sesame seed depends on genetic, environment factors, variety, cultivation, climate, ripening stage, the harvesting time of the seeds and the analytical method used (Kinman and Stark, 1954; Salunkhe *et al.*, 1992).

Thermal properties

Denaturation temperature (Td) and denaturation enthalpy (ΔH) were not shown in the results. DSC analysis revealed no detectable endothermic peak. This result may suggest that protein isolate was denatured due to its extreme fat extraction by using hexane.

Gel electrophoresis (SDS-PAGE)

Proteins were separated by means of SDS-PAGE, as well as 2-D gel electrophoresis, and either stained with Coomassie blue for total

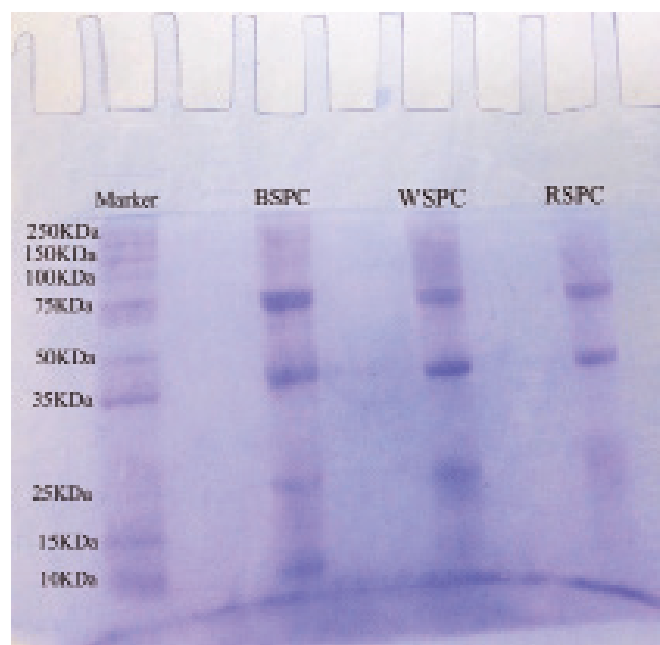


Figure 1 SDS-PAGE patterns of sesame protein concentrate under non-reducing conditions of protein molecular weight standard marker (Marker), black sesame protein concentrate (BSPP), white sesame protein concentrate (WSPC) and red sesame protein concentrate (RSPC).

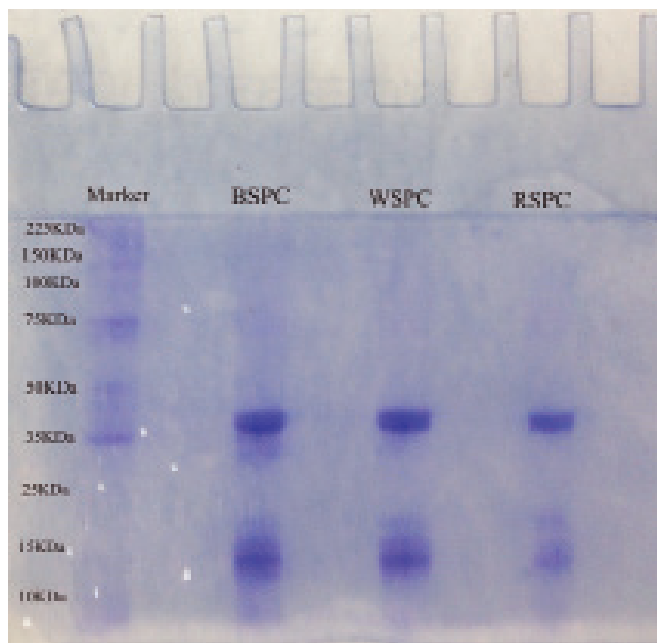


Figure 2 SDS-PAGE patterns of sesame protein concentrate under reducing conditions of protein molecular weight standards marker (Marker), black sesame protein concentrate (BSPC), white sesame protein concentrate (WSPC) and red sesame protein concentrate (RSPC)

CONCLUSION

Three sesame protein concentrates were prepared from different sesame meals (black, red and white). The percent yield of SPC samples had shown that in BSPC (41.14%), RSPC (41.90%) and WSPC (40.82%). The protein contents of SPC samples were 79.85%-80.92%. The SPC samples had protein bands with molecular weights during 42 and 83 kDa under non-reducing condition and molecular weights during 12 to 39 kDa with reducing condition. Therefore, these sesame meals can be used as a protein source ingredient in the food industry.

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