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The Manufacturing nanofibrils of black soybean and testing of cross polarizers

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Abstract

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Black soybean has a high protein content, has a more savory taste, contains antioxidants, can lower cholesterol levels, improve digestion and other benefits for the health of the human body. Black soybean is generally used as an ingredient of soy sauce, but can be processed into various types of food such as tempeh, tofu, milk, tauco and black soybean flour. Black soybean flour contains high protein so it has the potential to be a protein isolate.

The purpose of this study was to process black soybeans into soy protein isolate and convert to nanofibrils. Black soybeans was converted into soy flour using hammer mill and then five time defatted by hexane solution. Soybean meal without lipid was processed into soy protein isolate.

The material content in the form of black soybean seeds is 100%, the material content decreases during the process of isolation to 97% in the form of soybean meal, 63.05% in the form of defatted black soybean flour and 25.43% in the form of black soybean protein; consisting of 0.28% fat, 22.51% protein, 0.52% ash and 2.12% carbohydrates. The final protein content of black soybean protein in this study was about 88.49% on a dry basis so that the resulting protein can be classified as soy protein isolate because its value is close to 90%. Nanofibrils can be observed with a cross polarizer. Nanofibrils produced is long and branched in a diameter of several nanometers, obtained by heating the SPI suspension at pH 2.0 for 16 h.

Keywords: black soybean; cross polarizer; defatted; isolation; nanofibrils; soy protein isolate
Abbreviations: SPC – soy protein concentrate; SPI – soy protein isolate; HMP – high methoxyl pectin; SBM – soybean meal; TEM – transmission electron microscopy

Introduction

Black soybean is a nutritious legume that is high in proteins, phenolic acids, dietary fiber, essential amino acids, vitamins, minerals, flavones (Kumar et al., 2022), anthocyanin (Lee et al., 2020), and isoflavones (Asan et al., 2019). Black soybean is a type of grain known by the Latin name *Glycine max* (L) Merrit. Black soybeans are spread in Japan, Korea, Indonesia, India, Australia and America. Black soybeans can be processed into soy sauce and various types of food such as: tempeh, tofu, soy milk, tauco, traditional medicine (Lee et al., 2020; Asan et al., 2019), and black soybean flour. The advantage of black soybeans compared to yellow soybeans is that they have a more savory taste because the glutamic acid in black soybeans is higher than that of yellow soybeans.

Black soybeans have many advantages in terms of body health, such as being an alternative source of main vegetable protein nutrition and various other benefits. Anthocyanin in black soybean skin is a potential antioxidant to prevent premature oxidation and degenerative diseases. Black soybeans contain compounds that protect body cells from free radicals that cause cancer and premature aging. Black soybeans also contain isoflavones. The content of isoflavones and anthocyanins in black soybean skin can improve lipid

profiles, namely lowering cholesterol, triglycerides and also reducing blood pressure. Anthocyanins from black soybean skin can inhibit the oxidation of LDL cholesterol. Where the oxidation of cholesterol is the beginning of the formation of plaque in blood vessels that trigger high blood pressure and coronary heart disease. Black soybean contains substances needed by the body such as macro nutrients (carbohydrates, fats and proteins), micro nutrients (vitamins A, B, C and K), as well as various minerals (calcium, magnesium, phosphorus, sodium, potassium, iron, and zinc). Fiber in Black Soybean can facilitate the body's digestive system which drains toxic substances out of the body and is also a natural ingredient to prevent diarrhea. Peptide found in soybeans can boost the immune system to fight various disease attacks.

Black soybean is one of the superior varieties of non-GMO. This black soybean has seed pods weighing 9 g to 10 g per 100 seeds. The protein content of black soybeans is 39.09%, higher than imported soybeans, which is 37.84%. Meanwhile, processing soybeans into food ingredients such as soy flour, soy protein concentrate (SPC), soy protein isolate (SPI) and textured protein is still very limited. Soy protein isolate is reported to be able to form nanofibrils (Warji et al., 2017; Warji et al., 2018; Warji et al., 2019; Warji et al., 2020; Warji et al., 2021; Purwanti et al., 2018), producing film (Paglione et al., 2019), and as a layer to maintain the quality of cut fruit (Han et al., 2023). Nanofibrils made from soy protein isolate can function as emulsifiers, microcapsules wall-forming materials and functional foods (Warji et al., 2017). Black soy protein isolate has potential as nanofibrils from soybeans and health functions for the body. So it is important to conduct research on the process of making soy protein isolate from black soybeans as raw material for black soybean protein isolate nanofibrils, and convert soy protein isolates (SPI) to nanofibrils. The purpose of this study is to process black soybeans into soy protein isolates (SPI) and convert to nanofibrils.

Materials and Methods

Materials

The ingredients used are high methoxyl pectin (HMP) with methoxylation degrees of 70 to 75% and black soybeans. The chemicals used were aqua bides, tris (hydroxymethyl) aminomethane, 2-Mercaptoethanol, n-hexane, n-hexadecane and 37% HCl. This research was conducted at the Postharvest and Bioprocess Laboratory, Agricultural Engineering Department, Faculty of Agriculture, University of Lampung, Indonesia. In March to September 2023.

Methods

Proteins were isolated based on the method of Warji et al. (2017). Soybean meal (SBM) is made from milling black soybeans using a hummer mill. The soybean meal was then defatted five times with hexane. After that, the suspension was filtered using filter paper to separate the defatted soybean meal with hexane (Figure 1). The defatted soybean meal was dried at room temperature.

Proteins of black soybean were isolated by dissolving the defatted soybean meal in buffer solution (Figure 2). The solution was stirred at room temperature followed by centrifugation. The precipitate was washed using aqua bides and centrifuged at each washing step. The precipitate was then redissolved in aqua bides and adjusted to pH 8 using a tris solution. This suspension was stirred overnight and adjusted to pH 8 and then freeze-dried.

Proximate analysis

The analysis of the proximate composition of black soybeans, soybean meal, defatted soybean meal and the resulting SPI was carried out by several methods. Carbohydrate content was determined based on all other fractions obtained by analysis of the proximate composition. Crude protein was determined using the method of Kjeldahl. Fat content,

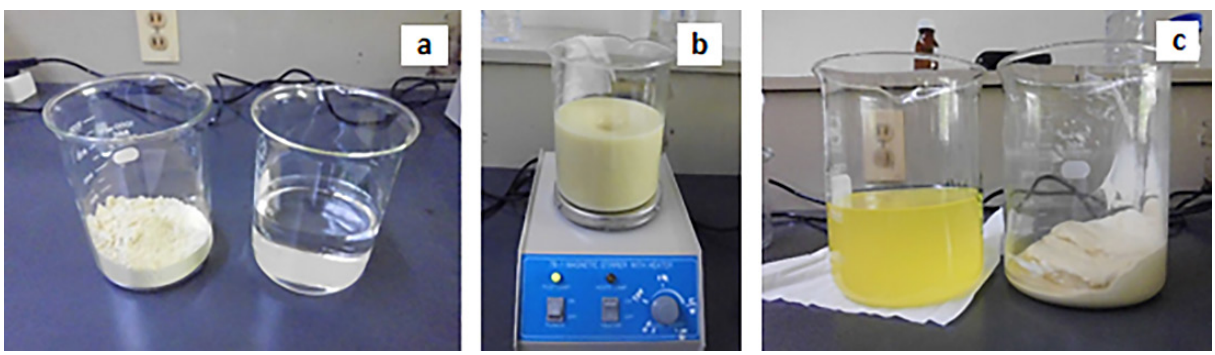


Fig. 1. (a) Preparing SBM and n-hexane before defatting; (b) Defatting process stirring; (c) Defatting process soybean meal and fat separated

Fig. 2. (a) Isolation process separating coarse fractions; (b) Isolation process precipitation; (c) Isolation process SPI harvesting

moisture content, and ash content were determined using the standard methods of AOAC.

Manufacturing of Nanofibrils

The prepared protein solution is an SPI solution based on the Warji et al. (2017) and Warji et al. (2020). SPI 2% solution was prepared by dissolving the protein in aqua bides. The protein solution was stirred overnight to get a perfect solution. After that, the pH of the solution was adjusted to 2 using 6M HCl solution. The protein solution at this pH was then heated in a water heater at 80°C (solution temperature) while stirring for 16 h. The result is a solution containing protein fibrils (nanofibrils).

Nanofibril detection using a cross polarizer

The test object is placed in the test object space which is between two polarized films installed at a 90° cross which are illuminated from an artificial polychromatic light source and observed from the direction of the observation room. Images of test objects can also be saved or documented using a photographic device or recorder placed in the room where the image is taken.

Transmission Electron Microscopy (TEM)

TEM is used to visualize nanofibrils proteins. The TEM method follows the method of Warji et al. (2017). The nanofibrils prepared as previously described were diluted to 0.05% by weight of protein in HCl solution pH 2. A drop of the diluted sample was placed on a 5 nm thick carbon film on a copper grid (400 mesh). The excess is removed from the copper grid, after 15 s using a piece of filter paper. Electron micrographs were prepared using a TEM (G2F20, FEI TechnaiTM, USA) operated at 120 kV.

Results and Discussion

Black soybean SPI

Soybean flour is produced from the flouring process using a hammer mill and the removal of the black epidermis. Non-fat soy flour is produced from the defatting process of soy flour. Isolate protein is produced from the process of isolation soy flour without fat. Figures 3a, b and c show the black soybean seeds, soybean meal and the resulting soy protein. Figure 4 presents the proximate compositions of black soybeans, soybean meal, defatted soybean meal and black soybean protein on a dry basis. Meanwhile, the compositions on a wet basis is presented in Table 1. Soy protein has a lighter color than soybean meal and soybean seeds, because the fat has been removed during the process of isolation (Figure 3).

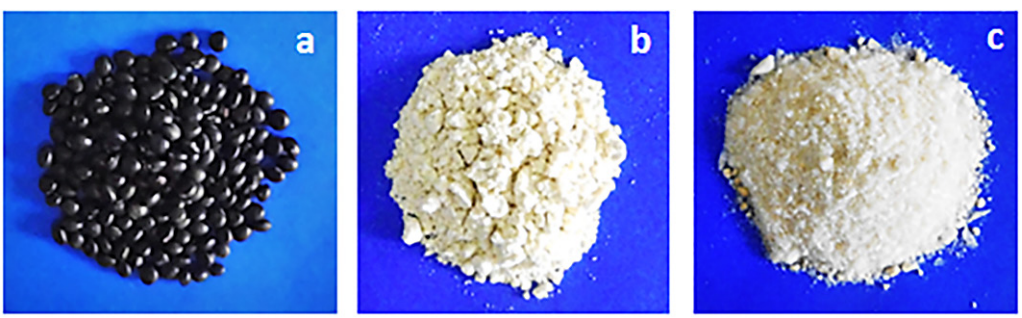


Fig. 3. (a) Black soybeans seeds; (b) Black soybeans flour; (c) Black soybeans SPI

Table 1. Proximate analysis of black soybean meal and SPI (wet basis)

Components	Soybean meal	SPI	Codex standard of SPI
Water content, %	9.06±0.13	5.52±0.52	≤ 10
Protein, %	38.82±0,11	83.61±0.74	≥ 90
Ash, %	491±0,45	1.93±0.74	≤ 8
Fat, %	16.74±3,31	1.05±0.18	–
Carbohydrates, %	30.48±2,93	7.89±0.34	–

The composition of soybeans that decreased during the isolation process is showed in Figure 4. The content of material in the form of soybean seeds was 100%, the content of material decreased during the process of isolation to 97% in the form of soybean meal, 63.05% in the form of defatted soybean meal and 25.43% in the form of black soybean protein. The protein content of black soybean in black soybeans decreased slightly when black soybeans were milled and sieved (from 42.69% to 41.40%). The fat content of black soybean decreased sharply from 17.86% in soybean meal to 1.03% in defatted soybean meal. The protein content of back soybean decreased sharply after the process of defatting, which was 36.92% and finally became 22.51% in the form of black soybean protein. Carbohydrate content also decreased sharply, from 20.94% in defatted soybean meal to 2.12% in black soybean protein. Protein isolation from black soybeans produced 25.43% black soybean protein consisting of 0.28% fat, 0.52% ash 22.51 % protein and 2.12% carbohydrates (dry basis).

Variations in protein content are closely related to the isolation method. Protein content varies from 82% to 88% obtained Akkermans et al. (2007) based on the same method, using Grobogan soybean raw material with a protein content of 40.56% obtained 90% (Warji et al., 2017). Meanwhile, protein content of 92.46% of soybean hypocotyl was obtained which was defatted using CO_2 supercritical Lu et al. (2016). And obtained a protein content of about 83% using conventional alkaline extraction (Yu et al., 2007). The protein content of black soybeans is thought to be increased by improving the protein isolation process. Improvements to the isolation process were carried out in the defatting process so that the fat involved was very low, the filtering process to separate carbohydrates more thoroughly so that fewer carbohydrates were carried and the precipitation process for harvesting black soybean protein was more accurate so that more SPI of black soybeans was produced.

Black soybean SPI Nanofibrils

SPI solutions at pH 2 before and after heating are presented in Figures 5a and b, where both look similar. The presence of SPI nanofibrils in the solution can be observed by placing the solution between the cross polarizers as shown in Figures 5c and d, where in the Figure the SPI solution before being heated shows the presence of fibers or fibrils (Figure 5c) while when heated it shows the presence of fibrils (Figure 5d). Nanofibrils can be observed with a cross polarizer. A cross polarizer is a pair of polarized optics arranged perpendicularly (90°) and there is a distance between the two optics as a sample and observed towards natural light (Warji et al., 2017).

The morphology of the SPI solution and nanofibrils is presented in Figures 6a and b. Unheated SPI solution did not

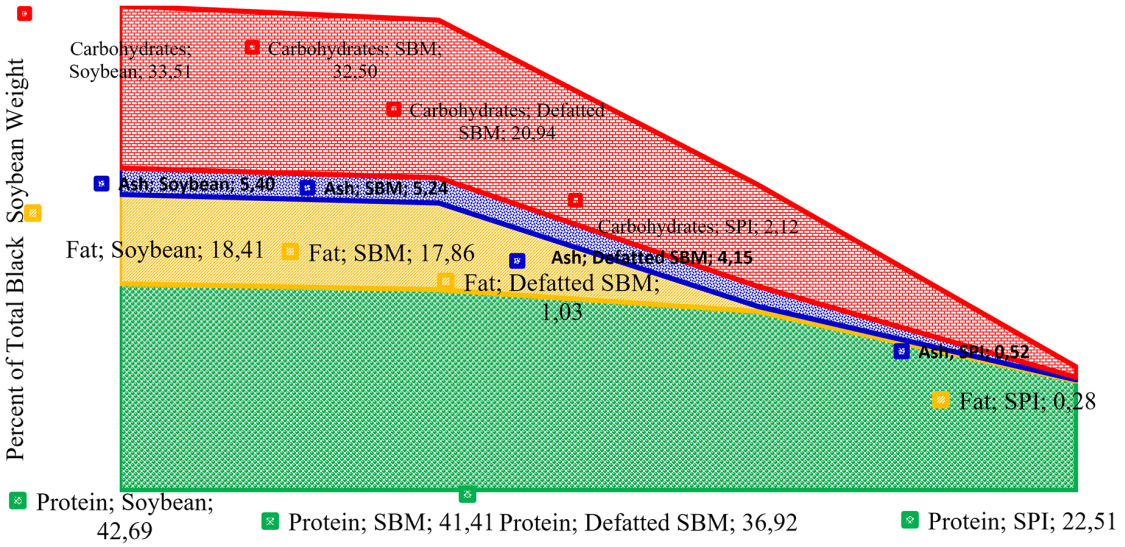


Fig. 4. Proximate composition (dry basis) of black soybean, SBM, defatted SBM and black soybean protein isolate. ( ) protein, ( ) fat, ( ) ash and ( ) carbohydrates

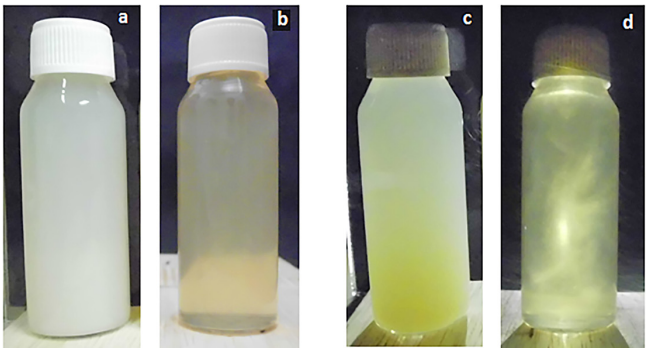
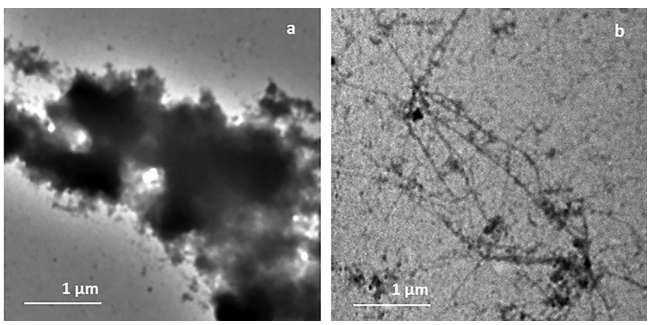


Fig. 5. Suspension of SPI from black soybean (a and c) and solutions containing nanofibrils made of SPI from black soybean (b and d). The pictures of c and d were taken by placing the solutions in between a cross-polariser

form nanofibrils, only visible clumps like clouds, while 2% SPI nanofibril solution heated at pH 2 for a long time could form long but curved and branched nanofibrils, as reported by Warji et al. (2017) and Akkermans et al. (2007). As a comparison with the same process, WPI nanofibrils form long and straight nanofibrils with a diameter of several nanometers, as reported by Sagis et al. (2008) and Rossier-Miranda et al. (2010). There are stains on the SPI nanofibril images, it is suspected that the SPI nanofibrils still contain relatively high SPI fats and carbohydrates.



a) **b)**

Fig. 6. TEM pictures of (a) SPI suspension and (b) nanofibrils made of SPI from black soybean

Conclusions

Black soybeans can produce 22.51% soy protein, which is only about 52.75% of the black soybean protein content of 42.69% (dry basis). Process optimization is needed to increase yield because half of soy protein content is still wasted during the isolation process. Based on the results of the isolation that the protein content of 88.49%, close to 90% so that black soybean protein can be classified as SPI. Black soybean SPI can be converted into branched and curved protein nanofibrils with a diameter of several nanometers.

Conflict of interest

The authors declare no conflict of interest.

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