Proteolytic activity of selected moulds in the first fermentation of black-seeded soysauce

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Abstract. Black-seeded soybean is preferred as normally it has higher protein content and would give black colour to the soyfiltrate for making soysauce. Mould is usually used in the first fermentation of soysauce making to prepare koji with high soluble protein as a media for the subsequent bacteria fermentation in brine solution. Black-seeded soybean of Detam 1 variety was used as the soysauce ingredient. The trial was a randomized complete design with four replicates. The treatments were (1) Rhizopus oligosporus starter (in flour form) with two day-fermentation, (2) similar R. oligosporus starter with three-day fermentation, (3) Aspergillus sojae (pure culture) with three-day fermentation and (4) A. sojae in flour form with three-day fermentation. The black-seeded soybean had 100-grain weight of 11.7 g, high protein content of 42.5% dw and fat content of 14.9% dw. Koji prepared using R. oligosporus starter with two-day fermentation had the lowest protein content (48.9% dw). Both starters of A. sojae culture and flour gave the highest soluble protein content of koji (41.0-41.5% dw), followed by R. oligosporus starter with 3 day-fermentation (35.2% dw). Whilst the lowest value was noted in koji prepared using R. oligosporus starter with two day-fermentation (30.8% dw). This suggests that both A. sojae starters had similar proteolytic activity and higher than that of R. oligosporus starter. In terms of practical application and maintenance of the mould viability by soysauce processors, the use of A. sojae flour starter with three-day fermentation is suggested.

Keywords: fermentation, mould, protein, soybean.

1. Introduction

Soybean consumption in Indonesia leading to increasing the total national soybean demand in 2012 amounted to 2.2 million tons. The biggest portion of the used of soybean for food or tofu and tempeh produced (83.7%), while the rest of soybeans are used in soysauce, tauco industry, and others (14.7%), seeds (1.2 %), and feed (0.4%) [1]. Soysauce is one of soybean fermentation food products that can be derived from either yellow or black-seeded soybean.

Black-seeded soybean is preferred by the industry as a raw material of soysauce because it can give a natural colour on soysauce produced [2]. In order to meet the black-seeded soybeans demand as raw material for soysauce industry, a number of soybean improved varieties with large seed sizes had been released, namely Detam-1, Detam-2, Detam-3 Prida, and Detam-4 Prida which contain high protein (> 40% db) [3] in addition to Merapi, Cikuray, and Mallika varieties, which have small seed sizes. The high of protein content of soybean seed is essential for soysauce making as it would give high protein content in the soysauce produced [4].
Fermentation on soysauce preparation consists of two phases, namely mould fermentation or koji fermentation (solid stage fermentation) and fermentation in a salt solution (brine fermentation), called moromi. Moulds that usually used in solid fermentation are Aspergillus spp. and Rhizopus spp. [5]. Koji fermentation takes 3-5 days, then koji was dried and soaked in brine solution of 20-30% for 14-28 days [6]. Microorganisms that are tolerant to high salt concentration, like Hansenula spp., Zygosacharomices spp., and Lactobacillus spp. are active in the brine fermentation. Afterwards, moromi is blended with spices and sugar and boiled up to particular viscosity to obtain sweet soysauce. In general, the protein content of soysauce sold in the market were below the minimum level (2.5%) of national standard quality for sweet soysauce [7]. However, some studies showed that soysauce preparation without brine/moromi fermentation gave higher protein content relative to soysauce produced through moromi fermentation [5,6]. This reflects that first or mould fermentation (koji) play an important role in dictating the protein content of soysauce produced.

Protein content of koji is considerably influenced by the mould growth during fermentation [6]. Aspergillus spp. and Rhizopus spp. have different proteolytic activity in hydrolizing insoluble protein complex into peptides and amino acids [8]. The starter of Rhizopus oligosporus is commonly available in the market as it is widely used for tempeh fermentation. However, the starter of Aspergillus spp. is not available in the market and should be obtained from particular institution, like university, research institute or soysauce manufacturer. Therefore, this study was performed to identify effective level of concentration and proper length of fermentation both starters of Rhizopus oligosporus and Aspergillus sojae were used for koji fermentation through the protein content and soluble protein produced using a black-seeded soybean variety.

2. Materials and methods

The study was performed at the food chemistry and technology laboratory of Indonesian Legumes and Tuber Crops Research Institute (Ietri), Malang in April 2013. Materials used in this study are black-seeded improved variety of soybean namely Detam-1 and moulds namely Rhizopus oligosporus starter (flour form) produced by the Indonesian Institute of Sciences (LIPI) and Aspergillus sojae starter (pure culture and flour form) from the food & nutrition culture collection, PAU, Gadjah Mada University. The trial was a randomized complete design with four replicates. The treatments were (1) Rhizopus oligosporus starter (in flour form) with two day-fermentation, (2) similar R. oligosporus starter with three-day fermentation, (3) Aspergillus sojae (pure culture) with three-day fermentation and (4) A. sojae in flour form with three day fermentation.

Making of koji were done by soybean seeds (each 200 g) soaked for 8 hours, then washed and boiled at 100°C for 2 hours. Soybean seed drained and cooled to a temperature of ±30°C. Furthermore, the soybean seed inoculated with moulds R. oligosporus for two days and three days fermentation, and inoculated with a pure culture of A. sojae and A. sojae in flour form for three days fermentation, respectively. Observations included physical (100 grain weight) and chemical characteristics of soybean seed (moisture, ash, protein, and fat content), protein and soluble protein contents of koji produced.

3. Results and discussions

3.1. The weight of 100 grain and chemical properties of black-seeded soybean

Improved variety of soybean namely Detam-1 used in this study belong black seed coat with a large grain weight of 11.56 g/100 grain. According to Susanto and Saneto [10], soybean with 100-grain weight around 8-10 g is grouped as small seeded, medium for 10-13 g and large seeded for >13 g. Detam-1 variety is classified as black soybean variety released large seed [3].

Detam-1 had moisture content of 7.47%. All soybean seeds had moisture contents below the maximum level established by the national standard for soybean seed [11] that was <13%. Detam-1 variety had ash content of 5.48% dw (Table 1). Ash represents the mineral content of soybean seed, particularly phosphorus, calcium and iron, where the mineral content was different between varieties and growth environment [12].
Detam–1 variety had a higher protein content of 42.48 % dw (Table 1), the these values of protein content were comparable to those reported by Ginting and Adie [9] in Cikuray variety and some promising lines prepared of soysauce. Detam-1 variety used in this experiment had a lower protein content of similar variety in the description improved varieties of 45.36 % dw [3], this can be caused by differences in maturity, soil fertility, and climate, as well as fertilization and irrigation method [9,13,14]. Protein content is an essential quality trait in soybean as it positively correlated with the protein and soluble protein of koji produced [2].

Table 1. 100 grain weight and chemical properties of Detam-1 variety

| Variety | 100 grain weight (g) | Moisture content (%) | Ash content (% dw) | Protein content (% dw) | Fat content (% dw) |
|---------|----------------------|----------------------|--------------------|------------------------|--------------------|
| Detam-1 | 11.56 | 7.47 | 5.48 | 42.48 | 14.90 |

Fat content of black-seeded soybean, Detam-1 variety of 14.90% dw (Table 1). These value of fat content suggesting similar with the studied by Ginting and Adie [9] on the same variety, which ranged from 12.9 to 14.8% dw, but lower than Cikuray variety (black-seeded soybean), studied by Antarlina et al.[12] of 19.0% dw.

3.2. Characteristic of soybean koji

The main purpose of fermentation (koji fermentation) in the making of soysauce is hydrolyze the protein, starch, and fat in soybean seeds with enzymes produced by moulds into simpler components that can be used the next active microorganisms (bacteria and yeast) in fermentation (moromi fermentation). The results showed moisture content of koji was significantly different between treatments using the mould R. oligosporus in the flour form and A. sojae in the pure culture and flour form, for two days and three days fermentation. The koji from fermented by A. sojae in the pure culture showed the high of moisture content of 60.60% (Table 2), than the moisture content of koji using mould of A. oryzae for 40 hours fermentation, studied by Tanaka [14] that ranged from 20 to 35%, because there are using different mould in the fermentation process.

Table 2. Moisture, protein, dan soluble protein of koji

| Type of mould | Moisture content (%) | Protein content (% dw) | Soluble protein (% dw) |
|---------------|----------------------|------------------------|------------------------|
| R. oligosporus flour form (2 day fermentation) | 57.29 c | 48.9 b | 32.5 c |
| R. oligosporus flour form (3 day fermentation) | 57.16 c | 52.2 a | 35.2b |
| A. sojae pure culture (3 day fermentation) | 60.60 a | 51.3 a | 41.0a |
| A. sojae flour form (3 day fermentation) | 59.33 b | 51.8 a | 41.5 a |

Lsd: Least significant difference, values followed by different letters are significantly different at P< 0.05
Cv: Coefficient variation
dw: dry weight

Observations in this study included measuring protein content and soluble protein. Total protein is measured of nitrogen total (N) content in the sample. Whereas, soluble protein is oligopeptides easily
absorbed by the digestive system [5]. Protein levels of koji were significantly different between treatments with the highest protein content of the koji mould fermentation using R. oligosporus mould and A. S sojae mould for three days fermentation, of 51.3% dw and 51.8% dw, respectively (Table 2). Protein content is one of the parameters of soysauce quality. Protein content in soysauce product is affected by the protein content of koji and moromi fermentation [9]. Increasing the amount of microbial mass during the fermentation process can also increase the protein content of the analysis. Increasing the amount of mass of microbes will cause the content of fermentation products so that the protein content is a reflection of the mass amount of cells [16].

The result showed, soluble protein levels was significantly different among four treatments with the highest levels of soluble protein that was in the koji with A. sojae both of pure culture and flour form, 41.5 % and 41.0 %, respectively (Table 2). Soybean seed after undergoing a process of fermentation by moulds have increased levels of protein, because the activity of protein degradation by protease enzyme was higher than the consumption of amino acids by fungi for further growth. Rahayu [8] reported that the proteolytic activity of A. oryzae and A. sojae (observed on the amount of soluble N) from the first to the third day of fermentation is much higher (about 1.5 times) compared with R. oligosporus. This suggests that soy protein content is also influenced by the type of microorganisms in fermentation. This shows the proteolytic activity of R. oligosporus lower than A. sojae. The results of proteolytic activity was soluble protein and amino acids. During the first fermentation the amount of soluble nitrogen (N) ranged between 50 to 70 % of the total N and the number was increased to 72-82% during 2 months of fermentation [8].

4. Conclusion
Protein and soluble protein can be parameter of the quality of soysauce produced black-seeded soybean which can also be determined from the protein produced from koji fermentation. The highest protein content of koji fermentation using A. sojae starter flour form followed by A. sojae pure culture which values 51.8% and 51.3% dw, respectively. Similarly, the soluble protein content of values were 41.5 % and 41.0% dw, respectively.

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