

# 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.*, *Zygosaccharomyces 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 standar 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 Insitute (Iletri), 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  $\pm 30^{\circ}\text{C}$ . 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

dw = dry weight

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)
<i>R. oligosporus</i> flour form (2 day fermentation)	57.29 c	48.9 b	32.5 c
<i>R. oligosporus</i> flour form (3 day fermentation)	57.16 c	52.2 a	35.2b
<i>A. sojae</i> pure culture (3 day fermentation)	60.60 a	51.3 a	41.0a
<i>A. sojae</i> flour form (3 day fermentation)	59.33 b	51.8 a	41.5 a
Lsd (5 %)	1.11	1.34	1.30
Cv (%)	1.19	1.64	2.16

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 *kaji* were significantly different between treatments with the highest protein content of the *kaji* mould fermentation using *R. oligosporus* mould and *A. S ojae* 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 *kaji* 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 *kaji* 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 *kaji* fermentation. The highest protein content of *kaji* 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.

#### References

- [1] Meryana E 2012 The best black seeded soybean in the world are in Indonesia (in Bahasa Indonesia) <http://bisniskeuangan.kompas.com/read/2012/07/26/21322949/Kedelai.Hitam.Terbaik.Dunia.Ada.di.Indonesia> (Accessed on March 18<sup>th</sup> 2014)
- [2] Ginting E, Antarlina SS and Widowati S 2009 Improved variety for food industry raw materials (in Bahasa Indonesia) *J. Penelitian dan Pengembangan Pertanian* **28** (3) 79-87
- [3] Iletri 2016 Descriptions of legumes and tubers improved varieties (in Bahasa Indonesia) Balitkabi Malang p 171
- [4] Antarlina SS and Ginting E 1998 Supplementation of Some Legumes on the Manufactures of Soysauce (in Bahasa Indonesia) p 147-156 *Pros Seminar Nasional Teknologi Pangan dan Gizi* ed S. Rahardjo, Marseno DW and Supartono W Yogyakarta 15 Desember 1998 PATPI Yogyakarta-PAU Pangan dan Gizi UGM-Fakultas Teknologi Pertanian UGM.
- [5] Purwoko T and Handajani NS 2007 Protein content of soysauce without moromi fermentation result *Rhizopus oryzae* and *R. Oligosporus* fermentation (in Bahasa Indonesia) *Biodiversitas* **8** (2) 223–227
- [6] Septiani Y 2004 Study of carbohydrate, fat, and protein content on soysauce from tempeh (Essay) (in Bahasa Indonesia) Surakarta: FMIPA UNS
- [7] Agency for National Standarization 1999 National Standart for soysauce (in Bahasa Indonesia) SNI 01-3543-1999 Badan Standarisasi Nasional Jakarta p 7
- [8] Rahayu ES 1985 Hydrolysis of soy protein by *Aspergillus sojae*, *Aspergillus oryzae* dan *Rhizopus oligosporus*(in Bahasa Indonesia) Tesis S2 Program Studi Ilmu dan Teknologi Pangan, Fakultas Pasca Sarjana UGM Yogyakarta

- [9] Ginting E and Adie MM 2007 Physicochemical properties of five black seeded soybean genotypes and the quality of soysauce produced (in Bahasa Indonesia) p 495-510 *In* Harnowo D, Rahmianna AA, Suharsono, Adie MM, Rozi F, Subandi and Makarim AK (eds) *The Improvement of Nuts and Tubers Production to Support Food Self Sufficiency* (in Bahasa Indonesia) Puslitbang Tanaman Pangan Bogor
- [10] Susanto T and Saneto B 1994 *Agricultural Processing Technology* (in Bahasa Indonesia) PT Bina Ilmu Surabaya
- [11] Agency for National Standardization 1995 National Standard for soybean seed (in Bahasa Indonesia) SNI 01-3543-1995 Badan Standarisasi Nasional Jakarta 7 p
- [12] Antarlina SS, Utomo JS, Ginting E and Nikkuni S 2002 Evaluation of Indonesian soybean varieties for food processing p 58-68 *In* Rahmianna AA and Nikkuni S (eds) *Soybean Production and Post Harvest Technology for Innovation in Indonesia. Proc. of RILET-JIRCAS Workshop on Soybean Research* Malang 28 September 2000
- [13] Kumar V, Rani A, Solanki S and Hussain SM 2006 Influence of growing environment on the biochemical composition and physical characteristics of soybean seed *J. Food Com. Anal.* **19** 188–195
- [14] Rotundo JL and Westgate ME 2009 Meta-analysis of environmental effects on soybean seed composition *Field Crop Res.* **110** 147–156
- [15] Tanaka Y, Watanabe J and Mogi Y 2012 Monitoring of the microbial communities involved in the soysauce manufacturing process by PCR-denaturing gradient gel electrophoresis *Food Microbiology* **31** 100–106
- [16] Astuti AF and Wardani AK 2016 Effect of Tofu Slurry Sweet Sauce Fermentation on Its Physical, Chemical and Organoleptic Quality (in Bahasa Indonesia) *Jurnal Pangan dan Agroindustri* **47** (1) 27–83