Evaluation of thermo-mechanical and microbial-facilitated processing on the chemical composition of soybean meal

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Abstract. A laboratory experiment was conducted to examine the effects of microbial fermentation using Bacillus subtilis and Aspergillus oryzae on the chemical composition of a commercial soybean meal (SBM). Five quadruplicate samples of SBM were subjected to four treatments with one batch serves as a control. The treatments were steam conditioning treatment (P1) where the other three groups were further fermented with B. subtilis (P2), A. oryzae (P3), and the combination of B. subtilis + A. oryzae (P4). The results showed that bacterial and fungal inoculation increased crude protein (CP) content when compared to control (p<0.05). In addition, fiber fractions including neutral detergent fiber (NDF) and acid detergent fiber (ADF) were concomitantly decreased with fermentation (p<0.05). In this study, no significant difference was observed on CP and NDF content with heating treatment (P1, p>0.05). However, this treatment decreased ADF content (p<0.05). Ether extract (EE), ash, non-fibrous carbohydrates (NFC), and total phosphorus contents were not affected by the treatments. To conclude, fermentation either with bacterial or fungal inoculants was effective to improve the chemical composition of SBM as indicated by increasing CP and decreasing fiber contents of SBM.

1. Introduction

Soybean meal (SBM), comprising 20–35% of ingredients in poultry formulated feed, is the primary source of protein. Although it is widely believed that plant protein structure from soybean meal is well digested by pigs and poultry, variabilities on growth performance have been reported in some studies associated with the quality of SBM [1]. The variability is often associated with several naturally antinutritional factors (ANFs) such as trypsin inhibitor (TI), tannins, phytic acid, and allergenic proteins present in SBM that have a detrimental effect on productive performance, primarily in chickens and pigs [2]. The presence of ANFs decreases the biological value of SBM and is subjected to some negative health consequences such as malabsorption syndrome, diarrhea, and growth depression due to hypersensitivity to intestinal morphology [3]. In addition, phytic acid is strongly related to nutrient metabolism as it can impair protein and energy utilization when available in a high amount. To some extent, it is well known that the protein and amino acids of SBM is not fully digested by chickens. Small portions of undigested protein components are undesirable because they contribute to the pathogenic
microbial growth in the hindgut, leading to the incidences of several intestinal diseases such as coccidiosis and necrotic enteritis [4].

Regarding this fact, many efforts have been made to address the drawbacks such as by fermentation process. Microbial fermentations are widely known to efficiently improve nutritional contents as well as eliminate ANFs in soybean. The enhancement of nutrient bioavailability promotes to increase in the performance of most monogastric and ruminant animals. However, our meta-analysis (data not published yet) suggested that the efficacy of microorganisms varies depending primarily on the strain. For instance, fungal fermentation with *Aspergillus oryzae* was reported to increase peptides content by 62.5% while reduced trypsin inhibitor by >80% [5]. Similarly, *B. stearothermophilus* fermentation increased amino acids concentration while decreased fiber fraction as well as trypsin inhibitor [6] although no change in protein content was also reported. In addition, substantial increases were also reported from fermentation with Bacillus subtilis on SBM nutrient contents [7,8] with various degrees of improvement.

On-farm, several positive evidences are available regarding the growth-promoting potential of using fermented SBM. Supplementation of SBM fermented with a combination of bacterial and fungal inoculants was demonstrated to enhance the cecal bacterial community in broiler chickens by suppressing some pathogenic bacteria and elevating the abundance of beneficial bacteria. They also highlighted that fermented SBM significantly increased daily gain, immunity, and production efficiency in broiler chickens [9]. Other experiments also reported that partial replacing of SBM with fermented SBM increased ADG and enhance digestive enzymes activities and intestinal morphology in all growing periods of broiler chickens [10,11]. To our knowledge, no decline effect was reported on the nutritional profile of plant protein sources. Nevertheless, the magnitude among inoculants varied depending on the type, strain, and processing method. So far, little information is available regarding a combination treatment with thermo-mechanical treatment and fermentation, are subsequent treatment facilitating further enhancement on the nutrient profile of SBM. Therefore, the present experiment aimed to evaluate the effects of microbial fermentation using *Bacillus subtilis* and *Aspergillus oryzae* with subsequent thermo-mechanical treatment on the chemical composition of a commercial soybean meal.

2. Materials and methods

2.1. Microbial inoculant preparation

*Bacillus subtilis* (B. subtilis) and *Aspergillus oryzae* (A. oryzae) were obtained from the collection of microbiology laboratory, Center for Food and Nutrition Studies – Universitas Gadjah Mada (CFNS UGM). The B. subtilis was isolated from the healthy intestinal tract of broiler chickens fed corn-SBM based. The pure strain of *B. subtilis* was prepared in a LB solid medium formulated using 10 g tryptone, 5 yeast extract, 5 g NaCl, and 20 g agar at 37°C for 24 h considering that the maximum growth of the strain was obtained at this time. The culture medium was suspended in LB liquid medium to contain 5×10⁹ CFU/g cell density as an inoculant for solid-state fermentation.

The fungal strain of *A. oryzae* was previously isolated from an Indonesian fermented soybean (tempeh). The fungi were cultivated in potato dextrose agar (PT Merck Chemicals and Life Sciences, Germany) at room temperature for 7 days to harvest their spores [12]. The spores obtained were suspended in liquid media (saline buffer solution) containing 0.05% NaCl. Enumeration of the spores was conducted in Neubauer chamber and the quantity was expressed as colony forming units (CFU) per gram. The final suspension used as inoculant contained 5×10⁷ CFU/g.

2.2. Solid-state fermentation procedures

The SBM containing 48% crude protein (CP) and 91% DM was purchased from a commercial poultry shop in Surakarta, Indonesia which was imported from Brazil. The ingredient was finely ground with a 1 mm screen using a mini hammer mill (Thomas Model 4 Wiley® Mill 2012; Thomas Scientific, Swedesboro, NJ, USA). Grounded SBM was sterilized at 121°C for 15 min using a laboratory autoclave. The sterile SBM was mixed with 150 mL of distilled water containing either *B. subtilis* or *A. oryzae*
inoculants at ratio 1:10, allowing the substrate to contain at least $1\times10^8$ CFU/ g and $1\times10^6$ CFU/ g of the respective inoculant. Following the inoculation, the samples were incubated at 25°C for 24 h for *A. subtilis* (BSBM) and 72 h for *A. oryzae* (ASBM), respectively.

Furthermore, two steps of fermentation was also conducted using the BSBM sample whereas further fermented using *A. oryzae* inoculant for 72 h (BA-SBM) [3]. After fermentations were completed, all samples were processed using mechanical treatment of steam conditioning in an autoclave at 121°C for 15 min. the fermentation was conducted in a static aerobic condition. Control treatments including SBM without steam conditioning and fermentation (CON) and with only steam conditioning (SC) were provided, giving a total of five treatment units with triplicate. The fermented FSBM was lyophilized for chemical nutrients analysis.

2.3. Chemical analyses

Analyses for chemical composition of all treatment groups were performed, including proximate, fiber fraction (Neutral detergent fiber, NDF; Acid detergent fiber, ADF), amino acids, and antinutritional contents (trypsin inhibitor, phytic acid), and phosphorus content. The dry matter (DM), organic matter (OM), CP, crude fiber (CF), ether extract (EE), and ash composition were determined using AOAC procedures [13]. The DM content was determined by oven-dried at 105°C until achieve a constant weight (#973.18; AOAC, 2005) while ash and OM contents were calculated after combustion at 550 °C (#942.05; AOAC, 2005). The CP content was calculated using Kjeldahl methods by multiplying the N content with 6.25 (#984.13; AOAC, 2005), and the EE content using ether extraction (#920.39; AOAC, 2005). The NDF and ADF content was determined following the methods by Van Soest et al. [14].

2.4. Statistical analysis

The treatment units consist of COB, SC, BSBM, ASBM, and BA-SBM represented five independent units for all variables analyzed where all groups were prepared in triplicate. Data were subjected to one-way ANOVA using GLM procedure of SAS (SAS Studio 3.8, University Edition, 2018) considering the treatment units as fixed effect and replication as a random effect. Comparison of the least-square means among treatments was performed using Tukey's HSD test when a significant effect was detected at $p<0.05$.

3. Results and discussion

Results of the proximate analysis, fiber contents, and total phosphorus content of experimental treatments are presented in Table 1. Crude protein was higher for SBM fermented with *B. subtilis*, *A. oryzae*, and their combination ($p<0.05$) compared with control while treatment with heat pressure did not influence the CP content. However, there was no significant difference between inoculants. Available literature support this finding where higher nutrient contents were reported as influenced by a solid state fermentation than that of conventional soybean meal. Most previous research has demonstrated an improvement even better than this result. Indeed, the nutrient composition after fermentation varied based on the characteristics of bacterial or fungal strains and also largely depends on the nature of ingredients (SBM). In term of bacterial strains, the different ability of microbial strains to improve the nutritive value of SBM has been well documented. Previous studies have reported that inoculation with *B. subtilis* and Lactobacillus plantarum resulted in CP increased by 58.4% and 37.1%, respectively [2]. Other studies reported an increase in CP content from 42 to 44.6% in a solid stated fermentation using *B. subtilis*, *candida utilis*, and *E. faecalis* [15]. Few different (+5.9% in CP) were found in SBM fermented with *A. oryzae* [10].

Increasing CP content in SBM is expected because it could be attributed to the enzyme activity produced by microbes used as inoculants. Aspergillus is known for its ability to produce various enzymes such as protease, amylase, pectinase, hemicellulose, etc. [16]. In addition, some bacteria especially Bacillus were reported to synthesis cellulase, xylanase, phytase, and glucanase enzymes. These exogenous enzymes not only utilize protein as their substrate, but also carbohydrates especially complexes non soluble polysaccharides (NSP) [5]. Therefore, the concomitant decreased in NDF and
ADF contents in this study occurred especially on SBM treated with microbial inoculants (BSBM, ASBM, and BA-SBM, Table 1). Non-starch polysaccharide (NSP)-degrading enzymes aforementioned contributed to breaking down the insoluble fiber fraction in SBM and it was in agreement with the previous study [3]. Such a decrease in fiber fraction also occurred in other ingredients such as rapeseed meal and canola meal [2].

Table 1. Chemical composition and amino acids contents of experimental groups

| Nutritional contents       | Treatments               | SEM | p-value |
|----------------------------|--------------------------|-----|---------|
|                            | CON                      | SC  | BSBM    | ASBM    | BA-SBM   |     |
| Crude protein              | 47.61<sup>a</sup>        | 48.32<sup>a</sup> | 49.56<sup>b</sup> | 49.61<sup>b</sup> | 51.28<sup>a</sup> | 4.10 | <0.05 |
| Ether extract              | 5.13                     | 5.01 | 4.73    | 4.96    | 4.79     | 0.42 | >0.05 |
| Ash                        | 2.08                     | 1.97 | 1.92    | 1.88    | 1.79     | 0.14 | >0.05 |
| Neutral detergent fiber    | 15.14<sup>a</sup>        | 14.83<sup>a</sup> | 13.22<sup>b</sup> | 13.06<sup>b</sup> | 11.54<sup>c</sup> | 0.92 | <0.05 |
| Acid detergent fiber       | 8.32                     | 7.44 | 7.49    | 7.39    | 7.61     | 0.61 | >0.05 |
| Non-fibrous carbohydrates  | 26.04                    | 25.87 | 26.57   | 26.49   | 26.60    | 3.26 | >0.05 |
| Total Phosphorus           | 0.83                     | 0.81 | 0.85    | 0.78    | 0.81     | 0.02 | >0.05 |
| Gross energy, MJ/kg*       | 18.12                    | 18.42 | 18.18   | 18.03   | 18.85    | 1.51 | >0.05 |

Note: CON= control; BSBM = SBM fermented using B. subtilis; ASBM = SBM fermented with A. oryzae for 72 h; BA-SBM = SBM fermented in two-steps fermentation using B. subtilis and A. oryzae for 24 h and 72 h, respectively; SC = SBM subjected to steam conditioning treatment without fermentation.

Other benefits from solid-state fermentation are that other metabolites such as organic acid are produced. These metabolites play an important role to enhance gut health and integrity thus can improve the growth performance of animals. Numerous studies were reporting a modulating effects on bacterial population and growth performance of broiler chickens as influenced by fermented SBM supplementation [3,6,9,17]. Another benefit that resulted from a combination treatment of thermo-mechanical and fermentation was also demonstrated on protein content and digestibility [18]. During fermentation, B. subtilis and A. oryzae produced proteolytic enzymes that could break down and eliminate some important ANFs such as antigenic proteins [3]. The advantage of solid stated fermentation when compared to conventional fermentation is the microbes could grow well in solid materials under a supportive environment. It is also believed to be more applicable and economical because it used low-cost substrates and materials with the non-complicated process [2]. Discrepancies might have occurred among experiments because the solid-stated fermentation process is largely determined by many factors set during the experiment such as initial moisture, temperature, pH, particle size, media composition, mixing, inoculum density, and strain, as well as operating system [19].

4. Conclusion
In this study, B. subtilis and A. oryzae were used as inoculants to improve the nutritional value and bioactivity of SBM following solid-stated fermentation and thermo-mechanical processing methods. Altogether, combination treatment using thermo-mechanical and fermentation processes as described in this study could be a promising strategy for enhancing the nutritional value of soybean meal as indicated from the increase in CP content as well as the decrease in fiber fraction contents.

Acknowledgment
The authors thank the Institute of Research and Community Service of Universitas Sebelas Maret (LPPM UNS) for the financial support through the excellence applied research scheme (PUT-UNS; contract no. 261/UN27.22/HK.07.00/2021) of the 2021 fiscal year.

References
[1] Moughan P J, Ravindran V and Sorbara J O B 2014 Poult. Sci. 93 2400–10
[2] Olukomaiya O, Fernando C, Mreddy R, Li X and Sultanbawa Y 2019 Anim. Nutr. 5 319–30
[3] Shi C, Zhang Y, Lu Z and Wang Y 2017 J. Anim. Sci. Biotechnol. 8 1–9
[4] Bryan D D L S, Abbott D A and Classen H L 2019 Poult. Sci. 98 4815–28
[5] Hong K J, Lee C H and Sung W K 2004 J. Med. Food. 7 430–5
[6] Wu P, Golly M K, Guo Y, Ma H, He R, Luo X, Luo S, Zhang C, Zhang L and Zhu J 2020 *Anim. Feed Sci. Technol.* **269** 114616

[7] Dai C, Ma H, He R, Huang L, Zhu S, Ding Q and Luo L 2017 *LWT - Food Sci. Technol.* **86** 1–7

[8] Seo S H and Cho S J 2016 *LWT - Food Sci. Technol.* **70** 208–12

[9] Li Y, Guo B, Wu Z, Wang W, Li C, Liu G and Cai H 2020 *Animals* **10** 1098

[10] Feng J, Liu X, Xu Z R, Liu Y Y and Lu Y P 2007 *Anim. Feed Sci. Technol.* **134** 235–42

[11] Feng J, Liu X, Xu Z R, Wang Y Z and Liu J X 2007 *Poult. Sci.* **86** 1149–54

[12] Teng D, Gao M, Yang Y, Liu B, Tian Z and Wang J 2012 *Biocatal. Agric. Biotechnol.* **1** 32–8

[13] AOAC 2005 *Official Methods of Analysis* 18th ed (Arlington, US: Association of Analytical Chemists)

[14] Van Soest P V, Robertson J and Lewis B 1991 *Journal of Dairy Science* **74** 3583–97

[15] Hu Y, Wang Y, Li A, Wang Z, Zhang X, Yun T, Qiu L and Yin Y 2016 *Food Agric. Immunol.* **27** 182–93

[16] Mathivanan R, Selvaraj P and Nanjappan K 2006 *Int. J. Poult. Sci.* **5** 868–72

[17] Soumeh E A, Mohebodini H, Toghyani M, Shabani A, Ashayerizadeh A and Jazi V 2019 *Poult. Sci.* **98** 6797–807

[18] Nu M A T, Lupatsch I, Zannatta J S, Schulze H and Zijlstra R T 2020 *J. Anim. Sci.* **98** skaa224

[19] Renge V C, Khedkar S V and Nandurkar N R 2012 *Sci. Rev. Chem. Commun.* **2** 585–90