Characterization of GABA (gamma-aminobutyric acid) levels in some fermented food in Indonesia

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Abstract. Indonesia has a huge wealth of local fermented products. These local food products have the potential to be developed into functional food that has added value and can contribute to public health in order to prevent disease. GABA (gamma-aminobutyric acid) is a prospective amino acid component that is widely found in fermented products from local food. GABA is alleged to have the functional capacity to build the immune system, improve the respiratory system, benefit diabetics, improve brain performance, prevent cancer and tumors and other health effects. The purpose of this research activity is to characterize the levels of GABA in several commercial fermented foods in Indonesia. The methodology used in this research is to identify and characterize commercial fermented food and to analyze the proximate levels and levels of GABA contained therein. The analysis was carried out using the spectrophotometric method. Based on the analysis results, several commercial fermented foods in Indonesia contain varying levels of GABA. The increased use of MSG increases the GABA levels to a certain level. This research deserves to be further researched and developed in relation to the optimization of the extraction process and the implementation of the use of other additives to increase GABA levels.

1. Introduction

Indonesia has a huge wealth of local fermented products. The fermentation product is very prospective to contain potential active components. These local food products have the potential to be developed into functional food that has added value and can contribute to public health in order to prevent disease. Based on the Global Market Forecast, there is an increase in functional food that has increased between 2017-2022, where for food and beverage products by 7.9 and for supplement products by 7.6 (CAGR%) (Gutierrez et al, 2020). One of the functional foods that contains GABA (Gama Amino Butyric Acid).

One of these potentials is that it contains GABA (gamma amino butyric acid) and functional components and other macro components that are known to have the capacity to play a role in increasing body immunity. Gamma Amino Butyric Acid or GABA is a non-protein amino acid with a general structure of H₂N(CH₂)₂CO₂H (Anju et al., 2014) found in plants (Roberts, 2007), lactic acid bacteria (Kook & Cho, 2013) animals (Luscher & Keller, 2004) and free food. In Mammalia, GABA is the main neurotransmitter of the central nervous system (Kittler & Moss, 2003), acting as a synaptic
inhibitor (Gottlieb, 1988) by inducing GABAA receptors, causing postynaptic membrane hyperpolarization (Zimmermann, 1993; Luscher & Keller, 2004).

Apart from the central nervous system, GABA is also present in high concentrations in pancreatic cells along with insulin but in different vesicles (Franklin & Wollheim, 2004). Pancreatic GABA is associated with diabetes in terms of maintaining glucose homeostasis (Taneera et al., 2012) and involves insulin and glucagon in its regulation (Wang et al., 2013). Apart from being potential antidiabetic, GABA is known to have the ability to lower blood pressure in mice (Hayakawa et al., 2004) and humans (Noguchi et al., 2007) (Indrowati et al, 2015).

Food products that contain lots of GABA composition include fermented sheep's milk (Minervini et al, 2009); processed cheese from several strains (Lacrox et al 2013); cheese from microbial strains from Italy (Siragua et al. 2009); skim milk fermentation with L. Helveticus bacteria (Sun et al. 2009); fermented milk (Inoue et al 2003); fermented milk of L. Plantarum and LAB (Nojati et al, 2013); fermented low-fat milk with LAB (Hayakawa et al 2004). The types of products and their preparations produce quite varied concentrations of GABA. The purpose of this research activity is to characterize the levels of GABA in several commercial fermented foods in Indonesia.

2. Methodology

The research activity was carried out at the Laboratory of the Center for Agricultural Post-Preparation Research from September to December 2020. The materials used included: tempeh, oncom, tauco, yoghurt, yakult and cheese. The analytical materials used include chemicals for analysis of proximate levels and GABA levels. The equipment used includes: scales, blender, heater, distillatory, oven, spectrophotometer, Soxstec and other glass ware equipment.

The research stages included the analysis of proximate levels and GABA levels from commercial samples. Furthermore, the process of adding GMS to the tempe sample was carried out with the addition ratio (0; 0.4%; 0.8%; 1.2%; 1.6%) percent of the fresh soybean material, which was subsequently identified sequentially as samples of A-E tempeh. Based on the modification results, the proximate levels and GABA levels were analyzed. Analysis of proximate levels is based on AOAC 1998. While the analysis method for GABA levels is as stated in Watchararparpaiboon et al. (2010) with the modifications listed below.

2.1 GABA Level Analysis

The sample extract was 100 µl plus 0.2 M of borate buffer as much as 200 µl and 0.6% phenol reagent as much as 1 ml. The solution is then added with 400 µl of 0.75% sodium hypochlorite, then boiled for 10 minutes at 100°C. After heating, the samples were immediately cooled for 10 minutes. The absorbance of the solution was measured with a UV-visible spectrophotometer at a wavelength of 630 nm. The measurement results are converted to the standard GABA curve. GABA standard curve is used for GABA standard solution by making 0 dilution series; 0.1 mg / ml; 0.2 mg / ml; 0.3 mg / ml; 0.4 mg / ml; 0.5 mg / ml; 0.6 mg / ml.

3. Result and Discussion

3.1 Proximate Analysis of Commercial Samples

Some of the fermented products sold commercially have their proximate content analyzed, which consists of moisture, ash, fat, protein and carbohydrates, which are analyzed with acra by different.

Based on the results of the analysis of the proximate levels of the products of tauco, tempeh, oncom, yakult, yoghurt and cheese as shown in Table 1 below.

| Sample | Moisture Content (%) | Ash Content (%) | Fat Content (%) | Protein Content (%) | Carbohydrate Content (%) | Energy (cal) |
|--------|----------------------|----------------|----------------|---------------------|--------------------------|-------------|
| Tauco  | 65,41                | 3,71           | 0,01           | 11,14               | 19,72                    | 123,56      |
| Tempeh | 68,13                | 0,42           | 1,60           | 17,07               | 12,78                    | 133,79      |
| Oncom  | 76,02                | 0,75           | 0,82           | 6,91                | 15,50                    | 97,70       |
| Yakult | -                    | -              | 0,00           | 2,00                | 4,00                     | 50,00       |
The proximate content of several fermented food products varies widely. For beverage products, the main component is water. Meanwhile, in food products the water content is relatively lower than that of beverages. Tauco and tempeh contain higher levels of protein than the other samples. The water content of this fermented food sample has a high level because it is a fresh sample that has not been dried. Astawan et al (2013) analyzed the moisture content of tempe ranging from 57.98 to 61.42% both from local soybeans and GMOs.

3.2 GABA Levels Analysis in Commercial Samples

One way to identify the active components contained in fermented products is by analyzing the levels of GABA in these food products. Based on the results of the analysis of GABA levels found in commercial sample products as shown in Table 2 below.

| Sample                      | GABA Content (ppm) |
|-----------------------------|--------------------|
| Tempeh                      | 168,58             |
| Oncom                       | 136,92             |
| Tauco                       | 220,96             |
| Yoghurt Cimory (mixed fruit)| 257,63             |
| Yoghurt KIN (Blue Berry)    | 196,75             |
| Yakult                      | 186,92             |
| Keju                        | 193,50             |

GABA levels in fermented products vary widely. Like cheddar cheese using probiotic microbes, it also has higher GABA levels, namely 6773.5 mg / kg (Wang et al. 2010). Meanwhile, fermented milk from Tibet actually does not contain GABA levels (Sun et al. 2009). Fellow fermented milk also has different levels of GABA. The types of microbes used for the fermentation process affect the quality and quantity of GABA produced.

3.3 Tempeh Proximate Level Analysis

To determine the effect of the addition of GMS content in one of the fermentation products, namely by analyzing the proximate levels of the tempe product. The variation in the addition of GMS concentrations to tempe products resulted in different proximate levels (water, ash, fat, protein and carbohydrates) as shown in Table 3.

| Sample      | Moisture Content (%) | Ash Content (%) | Fat Content (%) | Protein Content (%) | Carbohydrate Content (%) | Energy (ccal) |
|-------------|----------------------|----------------|-----------------|---------------------|--------------------------|---------------|
| Tempeh      | 67,83b               | 0,71a          | 2,67c           | 15,51b              | 13,63c                   | 137,23b       |
| Tempeh B    | 67,54a               | 0,65a          | 3,36a           | 15,74b              | 12,71b                   | 144,06a       |
| Tempeh C    | 67,54a               | 0,85a          | 1,69c           | 17,27a              | 12,23b                   | 133,20a       |
| Tempeh D    | 65,78b               | 0,94a          | 1,81c           | 18,70a              | 12,78b                   | 142,15ab      |
| Tempeh E    | 66,83ab              | 0,60b          | 1,75b           | 17,51b              | 13,90b                   | 141,39ab      |

Note: numbers followed by different letters show significantly different at 95% significance level

Based on the results of the analysis of proximate levels for tempeh samples with the addition of GMS levels of 0 to 1.6%, it produces different values for moisture, ash, fat, protein, carbohydrate and energy content. The water content of tempe in this sample is much greater than that of the tempe produced by Adtawan et al. (2013) which ranged from 57.98 to 61.42%. The differences in the types
of soybeans and the implementation of the treatment also affected the proximate levels of the resulting tempeh.

3.4 Analysis of Tempe GABA Levels
To determine the effect of the use of GMS concentration on GABA levels, an analysis of its effect was carried out on tempe products. Based on the results of the analysis, the concentration of the addition of GMS content to the resulting GABA content was shown in Table 4 below.

| Sample     | GABA Content (ppm) |
|------------|---------------------|
| Tempeh A   | 117.67              |
| Tempeh B   | 126.04              |
| Tempeh C   | 511.15              |
| Tempeh D   | 148.54              |
| Tempeh E   | 175.98              |

Note: numbers followed by different letters show significantly different at 95% significance level

The addition of GMS provides an increase in GABA levels in the resulting tempe products. This is related to the concentration of GMS added. An increase in GABA levels occurred in tempe products with the addition of 0.8% GMS concentration. The increase in the GMS concentration, which is getting higher, actually results in a decrease in GABA levels in the resulting tempeh. This is as the result of research by Lu et al. (2009), where the addition of MSG will provide an increase in GABA levels at a certain concentration and will decrease with increasing MSG concentration excessively. Furthermore, Lu et al. (2009) also showed that the combination of MSG concentration and incubation time affected the levels of GABA produced.

4. Conclusion
The different types of fermented products affect the proximate levels produced. The treatment of increasing the number of concentrations of GMS levels affects the quantity of proximate levels produced. The GABA content of several fermented products has various values because it really depends on the raw materials used. The difference in GMS levels also affects the levels of GABA produced in Tempe products. An increase in the GMS concentration of 0.8% resulted in higher GABA levels compared to other treatments in tempeh. Based on the results of this analysis, it is very important to investigate the effect of GABA levels on the functional activity of the product and the prospects for further commercialization.

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