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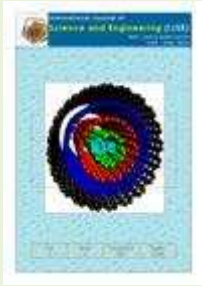

# Analysis of Chemical and Microbial Changes During Storage of Overripe Tempeh Powder as Seasoning Material

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***Abstract*** - Tempeh and other soy-derived products are historically and currently some of the most important foods in the Asian region where diets remain predominantly plant-based. Overripe tempeh (*tempe semangit*) is a term used for over-fermented tempeh with pungent odor and darkening appearance commonly used in Javanese cuisine. Unique taste and odor of overripe tempeh lead to the exploration of its potencies as condiment, which may add the nutritional, safety and economic values of tempeh. In this research, overripe tempeh is made into powder for better appearance and availability. Oven drying at 60°C and freeze drying were applied to the overripe tempeh until it reached moisture content below 5%, followed by subsequent crushing into powder using electric grinding machine. As seasoning material, the tempeh powder and overripe tempeh powder were then analyzed for their stability. Observations in chemical and microbial changes during storage were also applied to selected product during storage. Parameters that were observed during the research are: moisture content, protein content, acid content, total microbial count and total coliform count. Oven dried overripe tempeh (S60) has higher moisture content but lower in acid content, total microbial count and total coliform compared to freeze dried overripe tempeh (SFD). In addition, subjective profiling analysis was done as the product was intended to be used as seasoning material.

**Keywords -** freeze drying; oven drying; overripe tempeh; seasoning material

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## I. INTRODUCTION

Overripe tempeh (*tempe semangit)* is over fermented tempeh in which natural tempeh mould no longer can survive as bacterial fermentation takes over. The tempeh has pungent odor because of hydrolysed fat, resulting in the name *tempe semangit*. The word *semangit* derive from Javanese language '*sangit*' that usually explain the unpleasant odor. The overripe tempeh has grey-brown color, pungent odor and softer texture compared to normal tempeh. Overripe tempeh is usually fermented 2 to 5 days longer than normal tempeh. This kind of tempeh is frequently used in Javanese household as condiments, ingredients, and snack (Yudianto, 1997). Normally, individuals who like to use overripe tempeh as seasoning make their own by letting the normal tempeh continue the fermentation. This overripe tempeh has very short shelf life and not always on sales.

The aim of this research is to develop overripe tempeh powder, extending the shelf life of overripe tempeh and make it available at any time and to present overripe tempeh seasoning in more appealing looks compared to its original form.

## II. MATERIALS AND METHODS

*Materials and equipments*

Major ingredients for this research are yellow soybean that was obtained from soybean supplier in Ciputat, Tangerang, Indonesia. The tempeh starter (Raprima, Indonesia) was bought at Rumah Tempe Indonesia, Bogor, Indonesia.

The chemical material that was used in this research are NaOH (Merck, Germany), phenolphthalein, Bovine Serum Albumin (Sigma, USA), Folin-Ciocalteu's phenol reagent (Merck, Germany), Biuret Reagent, Plate Count Agar (Merck, Germany), Violet Red Bile Agar (Merck, Germany), and Sodium Chloride (Oxoid).

The equipment that was used in this research are conventional oven (Ariston, Australia), freeze dryer (Heto PowerDry PL9000), moisture analyzer (Sartorius), and GENESYS 10S UV-Vis spectrophotometer (Thermo Scientific, USA).

*Production of tempeh*

Soybeans were soaked overnight and cooked to soften the beans. Subsequently, it was left overnight in soaking water to allow the development of lactic acid fermentation. The acid fermented bean was then washed, peeled, and mixed with tempeh starter and then fermented for 3 days for fresh tempeh and 5 days for overripe tempeh.

*Drying and powdering*

Fresh tempeh and overripe tempeh were dried using oven at 60°C and freeze dryer at -80°C to reach moisture content below 5% then grinded into powder. All samples then labelled: F60 for fresh tempeh that dried with oven at 60°C, S60 for overripe tempeh that dried with oven at 60°C, FFD for for fresh tempeh that dried with freeze dryer at -80°C and SFD for overripe tempeh that dried with freeze dryer at -80°C.

*Storage analysis*

Samples were stored for 19 days in metalized pouches and analysed every 2 days starting from Day 9 for its moisture content, titratable acid (measured as lactic acid), protein content, total microbial count, and total coliform.

**Moisture content analysis.** The analysis was done with gravimetric method using Sartorius Moisture Analyzer. Each sample (1 gram) was heated at 105°C for 30 minutes, and then the moisture content on the display was recorded.

**Titratable acid analysis (measured as lactic acid)**. The analysis was done using base titration (SNI 01 – 2891 – 1992). Each sample (2.5 grams) was diluted in 50 ml aquadest. Diluted sample (10 ml) were taken and added with 10 drops of phenolphthalein. The diluted samples titrated with sodium hydroxide 0.1N.

**Protein content analysis.** The analysis was done using Lowry Method (Lowry et al, 1951). The sample was prepared by diluting 0.3 gram of powdered tempeh in 200 ml aquadest then filtrated using filter paper. Diluted sample (0.2 ml) taken to test tubes then added with 1 ml of Biuret reagent. Shook the test tubes with vortex shaker then leaved it at room temperature for 10 minutes. Next, 0.05 ml of Folin C reagent was added and samples were shook again with vortex shaker and once again kept at room temperature for 30 minutes. The mixture subsequently poured to cuvette and read using spectrophotometer with wavelength 650 nm, with the cuvettes of $H_2O$ that has been treated the same as the blank. The absorbance of each solution was obtained from the spectrophotometer then it can be determined by comparing to Bovine Serum Albumin (BSA) Standard.

**Total microbial number and total coliform number analysis.** Both analyses were done for every 2 days from Day 9 using method that is referred to AOAC 1990. All samples were diluted with sterile 8.5% sodium chloride as solvent then 1 ml of each samples were placed into petridishes and poured with total plate count agar for total microbial number and violet red bile agar for total coliform number.

## III. RESULTS AND DISCUSSIONS

Moisture content is important in food, too much of it could damage the life of food and increase the risk of microbial growth. Reducing water is a method that can be used in order to prolong their shelf lives. From Figure 1, it can be seen that samples dried using oven have more moisture than samples from freeze drying. During oven drying, moisture content is slowly evaporates to the outer layer of samples. If it is too fast, it can cause case hardening where the outer layer is harden and the moisture cannot escaped. This might happen to the samples and cause the moisture content of samples in oven drying is higher than freeze drying. Freeze drying involves crystallization of water in ice crystals, which subsequently sublimate, thus leaving a porous dried product (Voda et al, 2012), hence the moisture can evaporates through the porous.

Moisture content increased as the food will absorb moisture from surrounding air over the time. As expected, the moisture content in all samples increased over the time. After 9 days of storage, moisture content in freeze dried samples (FFD and SFD) were still under 5% while oven dried samples (F60 and S60) had more than 6% moisture content (Figure 1) The data shows the advantage freeze drying in preventing moisture absorbance of powder products.

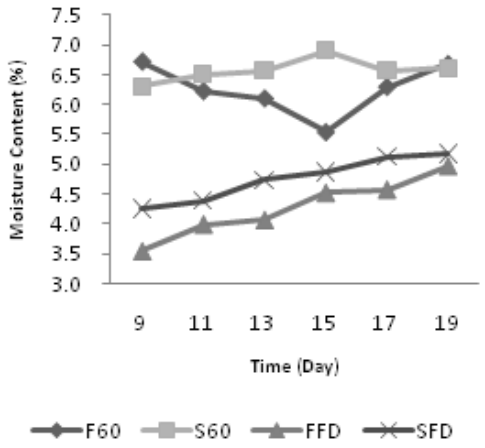


Figure 1. Changes in moisture content of tempeh and overripe tempeh powder

Acid value indicates the amount of lactic acid that produced by Lactic Acid Bacteria (LAB). In tempeh making, LAB plays important role because it helps to lower the pH for the starter (*R. oligosporus*) to grow as it grows well at pH range of 3.4 to 6 (Olesen et al, 2000) and also prevents pathogen to grow (Nout and Rombouts, 1990). Lactic acid is increase over time as seen on Figure 2. This is expected as the LAB produce lactic acid over time. The data showed that lactic acid production in freeze dried samples were higher than in oven dried samples.

Furthermore, protein analysis was done. Tempeh is naturally abundance in protein. As seen on Figure 3, the concentration of protein is fluctuated. Lowry method is chosen as it is sensitive to low concentration protein (Lowry et al., 1951) but there are a range of substance that would interfere with this assay such as amino acid (Lucarini and Kilikian, 1999). Although it appear that protein content fluctuated during storage, but statistically it does not have significant different (data not shown). Therefore, the protein content during storage was stable.

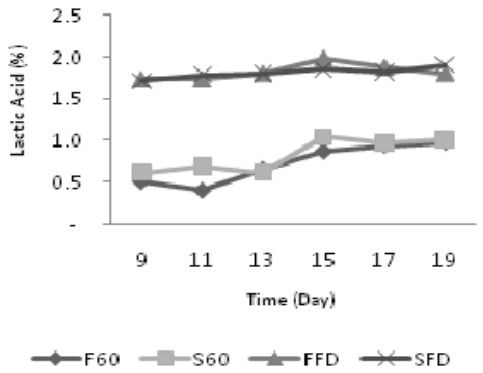


Figure 2. Changes in lactic acid of tempeh and overripe tempeh powder

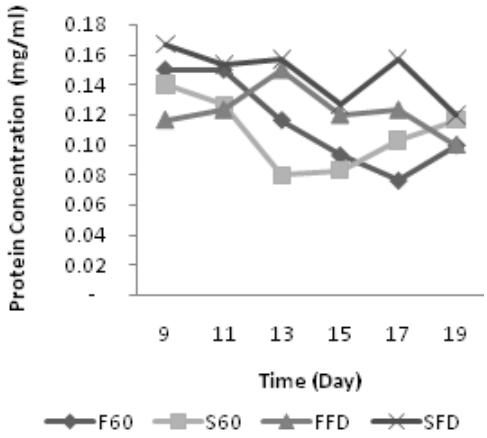


Figure 3. Changes in protein concentration of tempeh and overripe tempeh powder

Tempeh is a fermentation product, hence it can be expected that the product contain many type and numbers of microorganism. The aim of microbiological analysis is to evaluate the safety of the product. As seen on Figure 4, fresh tempeh powder showed lower total microbial number than overripe tempeh powder of respective method. In cake form, overripe tempeh has higher total microbial number than fresh tempeh (data not shown). Total microbial number in freeze dried is higher than oven drying.

Overripe tempeh is a result of bacteria fermentation that takes over the mould fermentation. From Figure 4 and 5, samples that were dried using oven have lower microbial number than samples that are processed using freeze dryer.

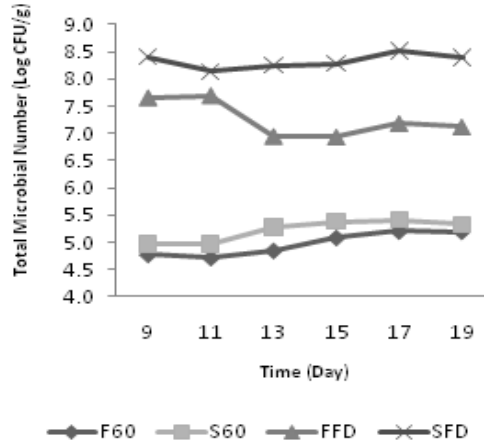


Figure 4. Total microbial number of tempeh and overripe tempeh powder

Morgan et al. (2006) explain that freeze dried is preferred method to transport and stored microorganism, as lack of heat treatment will cause the microorganism to stay in dormant stage and reactivated in the desired environment condition. Hence, total microbial numbers in freeze dried samples were greater than oven dried sample. Total coliform number from overripe tempeh powder showed lower number compared to the fresh tempeh powder of respective drying method. There was possibility that bacterial growth in later fermentation stage of overripe tempeh contributed in the suppression of coliform growth.

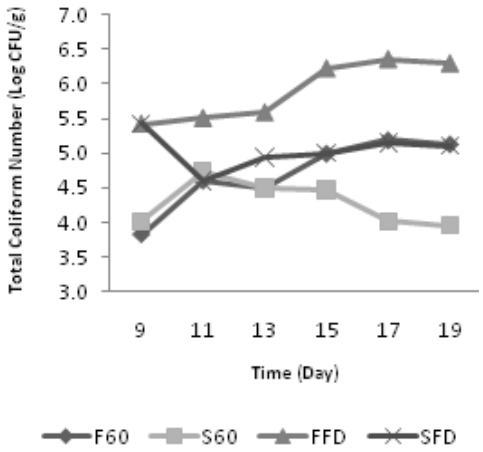


Fig. 5. Total coliform number of tempeh and overripe tempeh powder

All data from storage analysis were analyzed using ANOVA and it shows there is no significant different, therefore the all the samples were stable.

Since this product would be used as seasoning material, therefore subjective profiling analysis were done. Three parameters used for this analysis; intensity of taste, aroma and colour. As seen on Table 1 below, overripe tempeh powder samples (S60 and SFD) have higher intensity of taste. SFD has the highest intensity of aroma compared to other samples. Freeze dried tempeh powder samples (FFD and SFD) have lighter colour compared to oven dried tempeh powder samples (F60 and S60). Overripe tempeh powder samples (S60 and SFD) have darker colour than fresh tempeh powder samples (F60 and FFD). In cake form, overripe tempeh has darker colour than fresh tempeh.

Table 1. Subjective profiling analysis

| Tempeh Powder | Intensity of Taste | Intensity of Aroma | Intensity of Colour |
|---|---|---|---|
| F60 | Weak | Weak | Light |
| S60 | Strong | Medium | Darkest |
| FFD | Weak | Medium | Lightest |
| SFD | Strong | Strong | Light |

In the end of 19 days observation, all the powder samples were compared for their visual and sensorial characteristic (Table 1). Freeze drying seems to be more beneficial in preserving aroma and colour in the production of overripe tempeh powder.

## IV. CONLUSIONS

Oven dried samples have higher moisture content compared to freeze dried samples. In contrast, the lactic acid in freeze dried samples is higher than oven dried samples. Total Microbial Number on overripe tempeh powder samples is higher that fresh tempeh powder samples while total coliform number in overripe tempeh samples is lower that fresh tempeh powder samples. From the subjective profiling analysis, it is found that SFD has strong taste and aroma and potentially could be readily available product for overripe tempeh powder.

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