

ROLE OF TEMPEH FROM WEST SUMATRA AS A SOURCE OF VITAMIN B12 AND ANTIOXIDANT CAPACITY

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Abstract

Tempe is the most popular traditional fermented food in Indonesia that has high nutritional values such as high protein content high fatty acid content, vitamin B12, excellent digestibility, and good antioxidant content sources. Tempe also has low in anti-nutrition factors such as phytic acid, oligosaccharides. The purpose of this study is to compare traditional Tempe produced from West Sumatra, Indonesia, which is comparing the yellow and black soybeans, with a focus on antioxidant capacity. Six samples of Tempe were collected in Padang and Padang Panjang, Indonesia. Microorganism isolated from tempe samples were identified and the antioxidant capacity was determined using different antioxidant capacity assays, then Vitamin B12 determination in microbiological assay was performed. The result is included the mean of antioxidant of black soybeans = 0.008333 with a standard deviation of 0.0006429 and the mean of antioxidant of yellow soybeans = 0.008767 with a standard deviation of 0.0027465. In conclusion, it can be seen that the F count for antioxidants is 8647 with a probability of 0.042. Because of the probability value is < 0.05, then H₀ is rejected or the two population variances are different (heterogeneous). Six samples of Tempe were collected in Padang and Padang Panjang, Indonesia. Microorganism isolated from tempe samples were identified on basis of the key to general in Bergey's Manual of Determinative Bacteriology. The antioxidant capacity was determined using different antioxidant capacity assays described previously by Al-Duais et al. Vitamin B12 determination in microbiological assay was performed according to method of Strochecker and Henning (1963).

Keywords: Tempe, Vitamin B12, Antioxidant Capacity

1. Introduction

Tempe is the most popular traditional fermented food in Indonesia. The price of Tempe is affordable and Tempe has a pleasant taste. Not only that, a high nutritional value is also contained in Tempe due to its high protein content high fatty acid content, vitamin B12, excellent digestibility. Tempe also has low in anti-nutrition factors such as phytic acid, oligosaccharides, and good antioxidant content. Tempe is a traditional Indonesian food made from fermented soybeans. The manufacturing process involves the fermentation of soybeans using the fungus *Rhizopus oligosporus*.

The basic ingredient for making Tempe is soybeans. Soybeans become the basis of many foods from East Asia. There are two types of soybeans, those are black and yellow soybean. The black soybeans are a native variety that re-gained its popularity locally. The black soybeans have more nutritionally beneficial effect which had been proven [15]. Xu and Chang [8] indicated that black soybeans contain phenolic substances, tannins and isoflavones higher than the yellow variety and they also have higher antioxidant activity, as well. Meanwhile, In Indonesian markets, Tempe is mostly processed from imported yellow soybeans due to the availability of soybeans in the world market and the imbalance of high consumption and low production of soybeans in Indonesia.

The traditional process in the production of Tempe varies from place to place in Indonesia, i.e. the process is carried out in a slightly different way in every village. In general, the production of Tempe is carried out by cooking/boiled, soaking and dehulling soybeans, then the soybeans are cooked again, superficially dried and then inoculated with *Rhizopus* sp. In Indonesia, tempe production is generally done in family and small enterprises. More

over a starter is used for fermenting effect purpose bacteria which are common in the environment are contaminating the product at various stages of the tempe production. In the course of the soybeans fermentation by the fungus to Tempe, the contaminating factors are also growing in the growth of Tempe. Some bacteria which are growing together with the tempe fungus may play role in the traditional tempe fermentation, i.e. the role of producing vitamin B12 which may lead to the vitamin B12 formation in tempe

One of the very important stages is the soaking process of soybeans. During the soaking of soybeans lactic acid is taking place over lactic acid and in this way the pH is lowered down so that contaminating lots of pathogenic bacteria are suppressed in their growth. Before the soaking process, the soybeans are boiled traditionally so that superficially adhering living microorganisms are killed. After the soaking process, a second boiling process takes place. Some tempe manufactures neglect the first boiling process with the aim of saving money. This is in lower processing costs, but the quality of the Tempe produced may also be reduced. Up to date, there has been no report on comparing the antioxidant capacity of the black and yellow soybeans Tempe samples from West Sumatra. The purpose of this study is to compare traditional Tempe produced from West Sumatra, Indonesia, based on yellow and black soybeans with a focus on antioxidant capacity.

2. Method

Six samples of Tempe were collected in Padang and Padang Panjang, Indonesia. Microorganism isolated from tempe samples were identified on basis of the key to general in Bergey's Manual of Determinative Bacteriology. The antioxidant capacity was determined using different antioxidant capacity assays described previously by Al-Duais et al. Vitamin B12 determination in microbiological assay was performed according to method of Strochecker and Henning (1963).

Table 1. Different Soybeans Samples Collected From West Sumatera Showing The Environmental Conditions and Method Used for Tempe Production.

| Sample Number | Sample Type | Place of Origin | Environmental Conditions | Soybeans Processing |
|---------------|-------------|-----------------|--------------------------|---------------------|
| 1 | Black Tempe | Padang | 29-33 (Warm) | 1 x boiled |
| 2 | Soybean | Padang | 29-33 (Warm) | 1 x boiled |
| 3 | Soybean | Padang Panjang | 22-28 (Cold) | 2 x boiled |
| 4 | Black Tempe | Padang Panjang | 22-28 (Cold) | 2 x boiled |
| 5 | Black Tempe | Padang Panjang | 22-28 (Cold) | 1 x boiled |
| 6 | Soybean | Padang Panjang | 22-28 (Cold) | 1 x boiled |

Isolation of bacteria Bacteria tested Gram Test, Oxidase test, Catalase Test

BBL Oxi FERM Tube II

The following tests such as arginin, lysine, ana-gluc, lactose, N2, sucrose/saccharose, indole, xylose, aer-gluc, maltose, mannitol, phenil-alanin, harstoff/urea/uree, citrate, oksidaase, were done by using DB BBL TM Oxi/Ferm TM Tube II, Becton Dickinson GmbH.

Enteropluri Test

The purpose of Enteropluri Test is for the identification of Enterobacteriaceae gram negative and Oxydase negative. Enterobacteriaceae were used Enteropluri-Test, LIOFILCHEM Bacteriology Products, Via Scozia - Zona Ind.le

Vitamin B12 Test

Extracted Vitamin B12 was done with a microbiology assay. According to Okada et al, this assay is considered to distinguish between different forms of corrinoids, physiologically active vitamin B12, which can be used by human, and analogous forms, which cannot be so used. All vitamin B12 assays were executed in triplicate, each of them is analyzed 4 times. The maximal relative standard deviation was For the analysis, 1 g of tempe was ground with liquid nitrogen and mixed with 5 ml of 0.2 mol | -1 acetate buffer ph 4.5, 0.2 ml of potassium cyanide, and 30 ml of double distilled water. The samples are then homogenized for 15 min

and heated for 10 min at 121 C. After cooling 0.3 ml of 10 percent metaphosphoric acid solution were added, the mixture was cooled in ice water for 30 min, and the solution brought up to a volume of 50 ml. It was then centrifuged for 30 min at 38000 g, Sorval RC-5B, SS 34 rotor, Du Pont, Wilmington, DE, USA and the supernatant fluid was divided into two 20 ml portions. One portion was adjusted to pH 6.0 and the volume adjusted with double distilled water to 40 ml extract A. The other portion was adjusted pH 11-12 and heated at 121 C for 30 min. This procedure destroyed the physiologically active vitamin B12. Thereafter pH was adjusted to 6.0 and the extract was also adjusted to volume 40 ml. Both solutions were centrifuged again and supernatant fluids used for vitamin B12 determination in the microbiological assay which was performed according to the method of Strohecker and Henning 1963 with *Lactobacillus leichmanii* ATCC 7830 as test organism.

Microplate Fotometer

Antioxidant Tes

Extraction samples for antioxidant test

Extraction of legume samples By. Xu and Chang 2007. Legumes were grounded to pass a 60 mesh-sieve with an IKA all basic mill (IKA Works Inc., Wilmington, N.C., U.S.A.). A portion of 0.5 g of powder was extracted in a capped centrifuge tube with 5 mL of solvents, including acetone/water (50:50, v/v), acetone/water (80:20, v/v), acetone/water/acetic acid (70:29.5:0.5, v/v/v), ethanol/water (70:30, v/v), methanol/water (70:30, v/v), and absolute ethanol. The mixture was shaken at 300 rpm at ambient temperature on an orbital shaker for 3 h. The mixture was extracted for an additional in the dark overnight. The extracts were centrifuged by an Allegra 21R Centrifuge (Beckman Coulter Ltd, Palo Alto, Calif., U.S.A.) at 3000 RPM for 10 min, and the supernatants were removed into new tubes. Residues were extracted with 5 mL of the respective solvents for the 2nd time. Both extracts were combined and stored at 4 C in the dark until further analysis within 2 days. Extractions were performed in 3 replicates.

The tempe samples were extracted according to Xu and Chang [8]. Tempe were ground and a portion of 0.5 g of powder was extracted in a capped centrifuge tube with 5 mL of acetone:water (80:20, v/v). The mixture was shaken at 300 rpm at ambient temperature on an orbital shaker for 3 hours. The mixture was extracted for an additional in the dark overnight. The extracts were centrifuged by an Allegra 21R Centrifuge (Beckman Coulter Ltd, Palo Alto, Calif., U.S.A.) at 3000 rpm for 10 min, and the supernatants were removed into new tubes. Both extracts were combined and stored at 4 ° C in the dark until further analysis within 2 days.

HPLC

HPLC Conditions:

HPLC device: VWR Hitachi LaChrom ELITE

Column : Phenomenex Jupiter RP 18; 150 x 4,6 mm, 300 A

Flow : 0,7 mL/min

Injection volume: 99µL

Solvent A : H2O

Solvent B : Acetonitrile

Gradient : 0- 10 min 5% B

10-20 min 10% B

20-30 min 20% B

30-40min 30%B

40-50 min 100% B

50-55 min 5% B

Microplate Fotometer Spectofotometri

TEAC-Assay

ABTS stock solution: 55 mg/ 50mL in bi-distilled water (c= 1 mmol). (Has to be shield from light) .

K₂S₂O₈ stock solution 37.5 mg/ 100mL in bi-distilled water (0,14 mmol).

Radical-solution: use the same volumes of the ABTS and K₂S₂O₈ stock solution.(Has to be shieldfrom light). The solution has to incubate at room temperature for at least 14h. The solution can be used if the blank absorbance is 0,7±0,1 at 730 nm. Trolox stock solution: 26.5mg/ 100 mL in 5 mL ethanol and fill up to the mark with bi-distilledwater (c= 0,25 mmol).

Samples were diluted in acetone/MeOH; 500µL each. Samples were dried under nitrogen flow and then diluted in MeOH

Table 2. Concentration of standar in TEAC-Assay

| Sample | Sample Volume [µL] | Volume Solvent [µL] | Dilution Factor |
|--------|--------------------|---------------------|-----------------|
| 1 | 25 | 100 | 4 |
| 2 | 25 | 100 | 4 |
| 3 | 25 | 100 | 4 |
| 4 | 50 | 100 | 2 |
| 5 | 50 | 100 | 2 |
| 6 | 50 | 100 | 2 |

In a 96 well plate are 200 µL Radical Solution pipetted, then add 20 µL of each sample,calibration solution and blank to its well. The absorbance is read after 6 minutes at 760 nm.

3. Results and Discussion

Isolation of bacteria

Bacteria tested Five out of 17 isolates were identified as *Serratia liquefaciens*

Four isolates *Klebsiella pneumoniae* Two isolates *Acinobacter calcoaceticus*. Two isolates *Citobacter freundii*.

One *Enterobacter agglomerans*.

One isolates identified as *Escherichia coli*

One isolate *Pseudomonas aeruginosa*

one isolate closely resembled either *Pasteurella multocida* or *Mozarella spp* or *Pseudomonas spp*. We found only in sample no 3 : *Escherichia coli*

We found only in sample no 4: *Staphylococcus Aureus*

We found only in sample no 5: *Pasteurella* and *Pseudomonas aeruginosa*

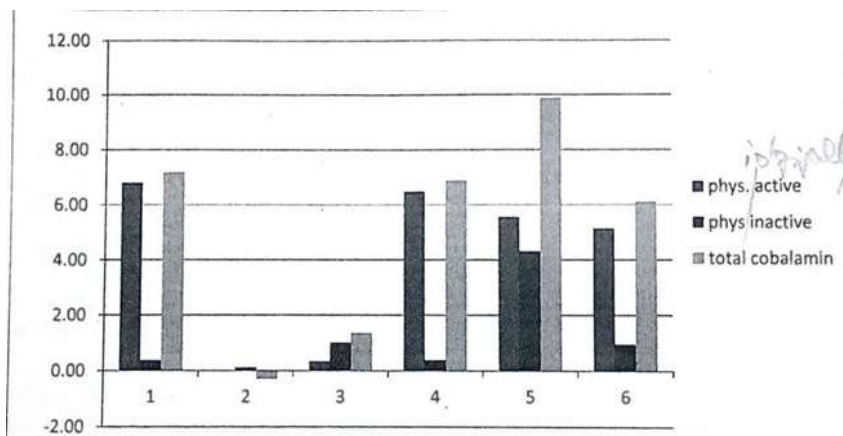
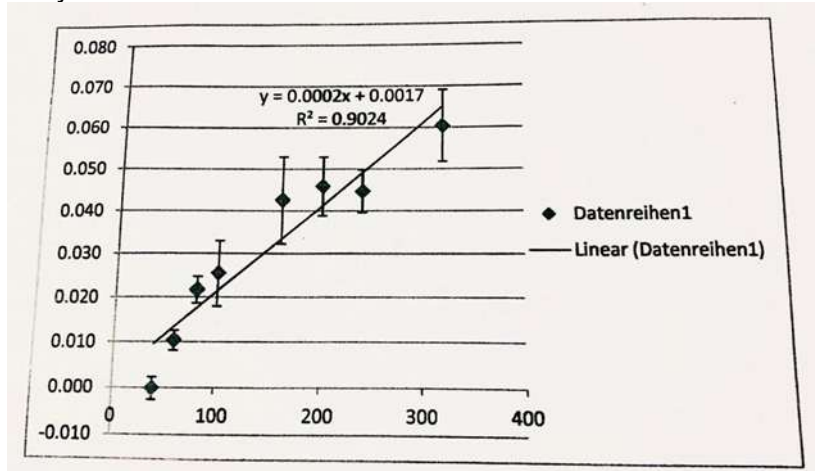
Rhizopus: 4 colonies in sample no 3 found in the third day

Tabel 3. Antioxidant capacity and Vitamin B12 content for the microbes isolated from thetempe produced from soybean collected from different origins

| Sample Number | Area | Boiling Times | Temperature in Celcius °C | Antioxidant capacity (mmol/g dried tempe) | Vitamin B12 In ng/g tempe |
|---------------|----------------|---------------|---------------------------|---|---------------------------|
| 1 | Padang | 1x | 29-33 | 0.0076 | 6.8 |
| 2 | Padang | 1x | 29-33 | 0.0056 | 0 |
| 3 | Padang Panjang | 2x | 22-28 | 0.0088 | 0.35 |
| 4 | Padang Panjang | 2x | 22-28 | 0.0102 | 6.48 |
| 5 | Padang Panjang | 2x | 22-28 | 0.0086 | 5.56 |
| 6 | Padang Panjang | 2x | 22-28 | 0.0105 | 5.14 |

Antioxidant Capacity

According to both types of different of soybeans Tempe, those resulted no significant differences amount of antioxidant capacity .The 2x boiled Tempe showed a higher amount of antioxidant capacity. Also, lower environmental temperature resulted a Tempe with higher antioxidant capacity



CASE: The data of soybeans' color on antioxidants
 Anti-oxidant content based on soybeans' color:

Significance level 5

Based on data processing obtained the following output: OUTPUT SPSS

Group Statistics

| | warna_kedele | N | Mean | Std. Deviation | Std. Error Mean |
|----------------------|--------------|---|---------|----------------|-----------------|
| antioksidan_kapasiti | hitam | 3 | .008333 | .0006429 | .0003712 |
| | kuning | 3 | .008767 | .0027465 | .0015857 |

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---------------------|-----------------------------|---|------|------------------------------|-------|-----------------|-----------------|-----------------------|---|----------|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| antoksidan_kapasiti | Equal variances assumed | 8.647 | .042 | -.266 | 4 | .803 | -.0004333 | .0016286 | -.0049550 | .0040883 |
| | Equal variances not assumed | | | -.266 | 2.219 | .813 | -.0004333 | .0016286 | -.0068189 | .0059522 |

Output Interpretation

First Part Output (Group Statistics)

In the first part, it presents a description of the analyzed variables, which include the mean (mean) antioxidant of black soybeans = 0.008333 with a standard deviation of 0.0006429 and an average antioxidant of yellow soybeans = 0.008767 with a standard deviation of 0.0027465.

Second Part Output (Independent Sample Test)

F . Test Analysis

Hypothesis:

H0 = Both variances of populations is same (homogenous)

H1 = Both variances of populations is not same (not homogenous)

Decision-making :

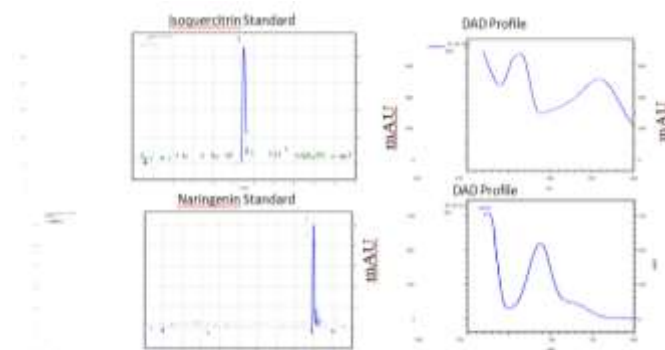
If the probability value > 0.05, then H0 is accepted

If the probability value < 0.05, then H0 is rejected

Decision:

It can be seen that the F count for antioxidants is 8647 with a probability of 0.042. Because the probability value is < 0.05, then H0 is rejected or the two population variances are different (heterogeneous)

HPLC result for Antioxidant



4. Conclusion

Based on the results, there was no significant difference between the two types of soybeans in terms of antioxidant capacity. Tempeh that was boiled twice showed a higher amount of antioxidant capacity. In addition, lower ambient temperature resulted in tempeh

with higher antioxidant capacity. The average antioxidant of black soybean = 0.008333 with a standard deviation of 0.0006429 and the average antioxidant of yellow soybean = 0.008767 with a standard deviation of 0.0027465. In conclusion, it can be seen that the F count for antioxidants is 8647 with a probability of 0.042. Since the probability value is <0.05, H₀ is rejected or both population variances are different (heterogeneous).

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