The effect of addition of turmeric (*Curcuma longa* L.) on the rancidity process of concentrate feed based on lactic acid bacteria fermentation during aerobic storage

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Abstract. This study aimed to determine the effect of the addition of turmeric (*Curcuma longa* L.) on rancidity process of concentrate feed based on lactic acid bacteria fermentation (CFLAB) in aerobic storage conditions. The treatments were the addition of turmeric extract, turmeric flour, and vitamin E in feed with levels of 0, 0.5, 1, 1.5, 2 and storage for 0, 10, 20, 30, 40 days with three replications. Antioxidant activity was carried out by 2,2-diphenyl-1-picrylhydrazyl analysis. The pH, free fatty acids numbers (FFA), and organoleptic analysis were determined in CFLAB quality during storage. DPPH analysis showed that turmeric extract and vitamin E had the best antioxidant activity. Addition of 2% turmeric extract, 2% turmeric flour, and 2% vitamin E lowered the pH. The addition of antioxidant compounds decreased FFA levels compared to controls except for the addition of vitamin E, but in terms of the percentage increase in FFA levels during storage, the addition of 1.5% turmeric flour gave the best results. Physical quality in the form of color, odor, texture, and fungus indicated that the quality of the CFLAB was able to keep in good condition due to the antioxidant sources addition.

1. Introduction
Concentrates based on lactic acid bacteria fermentation (CFLAB) are mostly composed of tofu by-products with high water content and raw rice bran and pollard containing fat. When stored in aerobic conditions it is prone to damage, one of which is rancidity. One of the disadvantages of raw rice bran is the instability of oil in raw rice bran which causes it to be very easily damaged. Winarno *et al.* [1] stated that material with high-fat content is damaged easily due to oxidation and hydrolysis during storage, especially open storage with direct exposure to air containing oxygen. Feed damage due to oxidation and hydrolysis causes rancidity, which can reduce quality feed. This oxidation or hydrolysis process usually can be prevented or minimized by adding ingredients or additives such as antioxidants [2]. Antioxidants are compounds that can inhibit oxidation reactions because this compound can break the chain of reactions inside oxidation or stabilize oxidized radicals.

Recently, the use of synthetic compounds as a source of antioxidants has been avoided. Even though it is effective, this synthetic compound is thought to cause undesirable effects for health, thus, the researchers have an attention to the use of natural antioxidants [3], i.e., turmeric rhizome. The compounds contained in turmeric rhizome are curcuminoid, which gives a yellow color of turmeric. Curcuminoid in the form of curcumin can protect materials from oxidative destruction [4].
The objective of this study was to determine the effect of the addition of turmeric (Curcuma longa L.) in minimalizing the rancidity process of concentrate feed based on lactic acid bacteria fermentation in aerobic storage conditions.

2. Material and methods

2.1. Material
Dry turmeric, which bought from a local market, then ground in the laboratory to get turmeric powder. Extract turmeric powder is prepared in the laboratory with an extraction method. The DPPH (2,2-diphenyl-1-picrylhydrazyl) and Vitamin E (alpha-tocopherol 95%) of ‘Sigma Aldrich’ brand purchased from sigma Aldrich online store, Singapore. Also, all the other chemicals used were of analytical grade and procured from E-Merck Ltd.

2.2. Sample preparation
Turmeric powder was prepared by peeled and washed and were sliced using a manual slicer and was heated for 24 hours in the oven with 50°C temperature, then the dried turmeric ground and stored in the dark place with 4°C temperature [5].

Extracted turmeric powder produced from dried turmeric powder with the following procedures: 20 g dried turmeric was added with 200 ml ethanol 95%, mixed with shaker in room temperature for three days until the extraction completed. The extract was filtered, and the ethanol evaporated, then turmeric extracted was stored in the dark place. The same extraction was done afterward with aquadest to replaced ethanol. The extract dried using vacuum rotary evaporator in 55°C temperature. Extract stored in 4°C temperature.

Concentrate feed based on lactic acids bacteria fermentation (CFLAB) is made from tofu by-products, raw rice bran, pollard, molasses, mineral premix, vitamin premix, and starter of lactic acid bacteria which is a collection of Biochemical Nutrition Laboratory with a dose of 10 g/1 kg (1%) fermentation. The mixture those feed as ration were incubated in anaerobic condition for 21 days.

The treatments carried out were the addition of a source of antioxidants with levels of 0, 0.5, 1, 1.5, and 2.0%. Storage time was carried out for 0, 10, 20, 30, and 40 days in aerobic condition, and each storage time have three replications.

2.3. Antioxidant activity determination
Antioxidant activity was carried out by 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis. The tests sample (50 μl) with various concentrations was added with 1 ml of DPPH 0.4 mm, and 3.950 ml of ethanol.

The mixture then mixed for 30 minutes. The solution then measured for its absorbance at a wavelength of 517 nm against the blank (of 50 μl of extract and 4,950 ml of ethanol). The control absorbance measurements consisted of 1.0 ml DPPH and 4 ml ethanol. The results obtained are by the formula. The pH value measured by dissolving 1 g sample to 10 ml of aquadest, then the pH was measured using a calibrated pH meter [6].

The free fatty acids (FFA) analysis was carried out using the alkaliometry titration method. One gram of sample and 25 ml of neutral alcohol were put into Erlenmeyer, then heated for boiling. After the sample was cold, PP as the indicator was added as much as 1 ml, then homogenized using vortex. The sample was titrated using 0.05 N NaOH until the color was pink and did not disappear for 30 seconds. Free fatty acid levels can be calculated using formula. Organoleptic test that observed were color, odor, texture, and fungus. Five organoleptic panelists give the score. The CFLAB organoleptic score assessment questionnaire is presented in Table 1.

2.4. Data analysis
The data were analyzed by analysis of variance complete and followed by Duncan’s Multiple Range Test (DMRT).
levels, and storage time and the interaction of the two factors had a significant effect (P < 0.05) on the bacteria fermentation (CFLAB) analysis.

The pH value of this experiment is presented in Table 1.

Organoleptic score assessment questionnaire for concentrate feed based on lactic acids bacteria fermentation during aerobic storage

Table 1. Organoleptic score assessment questionnaire for concentrate feed based on lactic acids bacteria fermentation (CFLAB) analysis

| Score | Color            | Odor       | Texture                | Fungus     |
|-------|------------------|------------|------------------------|------------|
| 1     | Black            | Rancid     | Slimy and clotted      | So many    |
| 2     | Blackish brown   | Neuroal    | Clotted                | Many       |
| 3     | Faded brown      | A little sour | A little clotted       | A lot      |
| 4     | Brown            | Sour       | Mesh, a little clotted | A little    |
| 5     | Yellowish-brown  | Fresh sour | Mesh, not clotted      | Nothing    |

3. Results and discussion

3.1. Antioxidant activity

The result of this experiment is presented in Table 2. Turmeric extract and vitamin E had the best antioxidant activity due to the smallest IC50 value of turmeric and vitamin E extract. This finding is in agreement with Morales-Gonzalez [7] who stated that the smaller the IC50 value, the more active the extract or fraction of the test as DPPH radical capture compounds or antioxidant compounds.

Table 2. Antioxidant activity determination of different antioxidants sources

| Antioxidant Sources | IC50 (mg/ml) |
|---------------------|--------------|
| Turmeric extract    | 0.275        |
| Turmeric flour      | 0.431        |
| Vitamin E           | 0.195        |

3.2. pH value

The pH value of this experiment is presented in Table 3. The types of antioxidant sources, antioxidant levels, and storage time and the interaction of the two factors had a significant effect (P < 0.05) on the pH value of CFLAB. During storage, the pH did not change at different storage period.

Table 3. The effect of addition of turmeric (Curcuma longa L.) on the pH value concentrate feed based on lactic acid bacteria fermentation during aerobic storage

| Antioxidant   | Level (%) | Storage Time (day) |
|---------------|-----------|--------------------|
|               | 0         | 10                 | 20 | 30 | 40 |
| Curcuma extract | 0.5       | 4.23<sup>gh</sup> | 4.23<sup>gh</sup> | 4.28<sup>hijkl</sup> | 4.27<sup>hijkl</sup> | 4.25<sup>ghijkl</sup> |
|               | 1         | 4.24<sup>g</sup>  | 4.21<sup>ef</sup> | 4.21<sup>ef</sup> | 4.22<sup>g</sup>  | 4.21<sup>ef</sup>  |
|               | 1.5       | 4.14<sup>d</sup>  | 4.14<sup>d</sup> | 4.14<sup>d</sup> | 4.15<sup>d</sup> | 4.13<sup>d</sup>  |
|               | 2         | 3.69<sup>a</sup>  | 3.73<sup>b</sup> | 3.76<sup>bc</sup> | 3.77<sup>bc</sup> | 3.75<sup>bc</sup>  |
| Mean          | 4.11      | 4.12               | 4.13               | 4.14               | 4.13               |
| Curcuma powder | 0.5       | 4.27<sup>ghij</sup> | 4.27<sup>ghij</sup> | 4.25<sup>ghijk</sup> | 4.27<sup>ghijkl</sup> | 4.26<sup>ghijk</sup> |
|               | 1         | 4.24<sup>ef</sup> | 4.21<sup>ef</sup> | 4.26<sup>ef</sup> | 4.26<sup>ef</sup> | 4.16<sup>c</sup>  |
|               | 1.5       | 4.13<sup>d</sup>  | 4.14<sup>d</sup> | 4.13<sup>d</sup> | 4.14<sup>d</sup> | 4.14<sup>d</sup>  |
|               | 2         | 3.75<sup>bc</sup> | 3.75<sup>bc</sup> | 3.8<sup>c</sup>   | 3.77<sup>bc</sup> | 3.8<sup>c</sup>   |
| Mean          | 4.11      | 4.14               | 4.14               | 4.15               | 4.12               |
| Vitamin E     | 0         | 4.28<sup>ij</sup> | 4.24<sup>gh</sup> | 4.24<sup>gh</sup> | 4.25<sup>ghij</sup> | 4.22<sup>ef</sup> |
|               | 0.5       | 4.28<sup>ij</sup> | 4.28<sup>ij</sup> | 4.28<sup>ij</sup> | 4.32<sup>e</sup>  | 4.24<sup>g</sup>  |
|               | 1         | 4.27<sup>ij</sup> | 4.25<sup>ghij</sup> | 4.26<sup>ghij</sup> | 4.25<sup>ghij</sup> | 4.2<sup>f</sup>  |
|               | 1.5       | 4.12<sup>d</sup>  | 4.13<sup>d</sup> | 4.14<sup>d</sup> | 4.14<sup>d</sup> | 4.15<sup>d</sup>  |
|               | 2         | 3.76<sup>b</sup>  | 3.77<sup>b</sup> | 3.76<sup>b</sup> | 3.81<sup>c</sup>  | 3.79<sup>bc</sup> |
| Mean          | 4.14      | 4.13               | 4.14               | 4.15               | 4.12               |
| Average storage time | 4.12   | 4.13               | 4.14               | 4.15               | 4.12               |
The lowest pH value was found in the addition of 2% turmeric extract, 2% turmeric flour, and 2% vitamin E with a range of pH values 3.6-3.7, while other additions of the treatment produce a pH about 4.2. The results showed that the higher the level of antioxidants given, the smaller the pH value; in other words, the greater decrease in the pH. Sandi et al. [8] states that silage pH can be categorized into four categories; very good (pH 3.2 to 4.2), good (pH 4.2 to 4.6), moderate (pH 4.5 to 4.8 ), and bad (pH> 4.8).

3.3. Free fatty acids
The FFA levels increased in all treatments from day 0 to day 30 (Table 4). The addition of turmeric extract with a concentration of 0, 0.5, 1, 1.5 and 2% increased the FFA levels from days 0 to 30 by 14.19, 14.56, 15.08, 18.76, and 19.53%, respectively.

The addition of turmeric flour increased FFA levels by 14.19, 15.90, 13.61, and 12.98%, respectively. Addition of vitamin E increased FFA levels by 12.41, 13.85, 12.33, 14.42 and 16.00%, respectively. The results indicated that storage in an open aerobic condition causes fat oxidation. Zhong and Shahidi [9] observed that the condition of products exposed to free air causes oxidation of fat by oxygen. The process of fat hydrolysis produces free fatty acids.

| Antioxidant Sources | Levels (%) | Day 0 | Day 30 | Increase of FFA (%) |
|---------------------|------------|------|-------|---------------------|
| Turmeric extract    | 0          | 14.02| 16.01 | 14.19               |
|                     | 0.5        | 13.13| 15.03 | 14.56               |
|                     | 1          | 11.89| 13.58 | 15.08               |
|                     | 1.5        | 9.11 | 11.14 | 18.76               |
|                     | 2          | 8.91 | 10.65 | 19.53               |
| Turmeric flour      | 0          | 14.02| 16.01 | 14.19               |
|                     | 0.5        | 11.88| 13.78 | 15.90               |
|                     | 1          | 11.81| 13.43 | 12.76               |
|                     | 1.5        | 9.76 | 11.02 | 13.61               |
|                     | 2          | 8.01 | 9.05  | 12.98               |
| Vitamin E           | 0          | 14.02| 16.01 | 14.19               |
|                     | 0.5        | 13.55| 15.54 | 14.69               |
|                     | 1          | 13.51| 15.42 | 14.14               |
|                     | 1.5        | 13.12| 15.11 | 15.17               |
|                     | 2          | 12.15| 14.46 | 19.01               |

3.4. Physical quality
Colors during storage on days 0, 10, 20, 30, and 40 were relatively the same and a reduction in color during storage was occurred. However, there was a difference at the beginning of storage, i.e., on day 0, in the vitamin E sample, it had a brown color, unlike turmeric extract and turmeric flour which had a yellowish-brown color.

The color of the samples was relatively good, and the decrease in color on the addition of turmeric extract and turmeric flour was better than the control. It proves that the addition of turmeric can maintain color in CFLAB, and this was in line with Utomo et al. [10] who stated that a right silage color was close to the original color.

The aroma of the sample with the addition of an antioxidant source at the beginning of storage was good, with a distinctive sour aroma and sweet aroma, and decreased until the 20th day into an acidic aroma. Starting from the 30th day the aroma gradually became slightly acidic. Open storage decreased aroma due to air during storage. Maharani [11] stated that product aroma decreases with increasing
storage time. Materials tend to be more rancid with the long storage time. The condition significantly decreased until it reached a rancid smell in the 8th week.

All treatments produced good texture quality with no lumps at the beginning of storage until the 30th day, until a small amount of clot occurs on day 40. This clot indicated that the quality of CFLAB was good and the addition of sources of antioxidants could maintain texture during storage. According to Siregar [12], the good feed fermentation had a clear texture resembling fresh and did not clot.

The presence of fungi during storage 40 in all treatments showed the same results, namely the formation of a little fungus on day 30. Judging from the physical quality score CFLAB was classified as good quality, and the addition of sources of antioxidants maintained CFLAB quality during storage by no growing fungi at the end of storage.

4. Conclusion

Addition of antioxidant sources inhibits the rancidity process during aerobic storage. The DPPH analysis showed that turmeric extract and vitamin E had the best antioxidant activity. The pH value indicated that the more levels of additional antioxidant sources, the lower the pH achieved until the end of storage. In this case, the treatment of adding 2% turmeric extract, 2% turmeric flour, and 2% vitamin E have the best results with the lowest pH value during storage. The addition of antioxidant compounds decreased FFA levels except for the addition of vitamin E, but in terms of the percentage increase in FFA levels during storage, the addition of 1.5% turmeric flour gave the best results (lower than controls). The results of physical quality in the form of color, odor, texture, and fungus, indicated that the quality of the feed lasting well with the addition of sources of antioxidants in all additions to the source of antioxidants.

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