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Flour made from cacao-soybean tempeh fermented using soybean tempeh inoculum

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Abstract

Raw cacao beans are non-premium cacao without cacao pulp fermentation. This raw bean still contains enough protein and polyphenol contents for functional food. In this study, cacao bean was used to substitute soybeans in tempeh fermentation. The soybean-cacao tempeh, called tekao tempeh, was then processed to make tekao flour. The aim of this research was to evaluate the characteristics of the flour made from soybean:cacao tempeh. The tekao flour was made from tempeh with a soybean:cacao proportion of 70:30. The tekao flour had an antioxidant activity of 48.1% and a soluble protein content of 444.7 $mg.L^{-1}$. The water- and oil-holding capacities of tekao flour were 240.4% and 107.2%, respectively. The bulk density, angle of repose, and particle size were 0.427 $mg.L^{-1}$, 44.9°, and 546 µm, respectively. The water content and water activity were 7.3% and 0.55% w/w, respectively. Based on the flour characteristics, tekao flour made from 70:30 of soybean:cacao bean tempeh has the potential to be functional food considering its antioxidant properties and other characteristics.

Keywords: unfermented cacao beans; cacao tempeh; flour; tekao.

Practical Application: The low-grade cacao bean is not an export commodity. As a substitute for soybean in tempeh fermentation, it gives the advantage that it is less expensive and still has nutritional value. Transforming bean into tempeh and then flour generates flour products with higher protein content and antioxidant activity than the common wheat flour. The flour can be stored longer for the next application. The properties are suitable to make baked goods and other foods. Additionally, the brown color of tempeh flour can be an advantage in coloring food with a natural hue.

1. INTRODUCTION

Cacao bean is a seed from cacao plant (*Theobroma cacao* L.). Cacao beans can be considered one of the most important Indonesian commodities. Indonesia has cocoa tree plantations covering up to 1.5 million hectares, spreading across Sulawesi, North Sumatera, West Sumatera, East Kalimantan, Papua, and West Java. The highest production was from Center Sulawesi (Badan Pusat Statistik, 2021). Cacao beans for export purposes are of premium quality. The bean is large from good varieties of plants, such as Foratero, Criollo, and Trinitario. For fine cocoa production, the premium cacao bean must be fermented properly before it is dried. This premium bean can be sold at a higher price than non-fermented bean (Hayatudin et al., 2020).

Non-premium cacao bean or bulk cacao is a non-fermented or lower-grade cacao bean. The non-premium bean is easier to be found in the market nowadays because low-grade bean has not been used for fine cocoa production. Another reason is that the farmers prefer to sell them without any fermentation process. Many factors influence the success of a fermentation process to produce fine cacao. In addition, the fermentation requires large quantities of cacao beans for each batch, whereas many farmers are unable to harvest large quantities of cocoa beans at each harvest because their land is not large enough (Hayatudin et al., 2020; Manalu, 2018). During the COVID-19 pandemic, a lot of cacao beans were harvested, but market demand was low.

Raw cacao beans still have high nutritional value for food products. It contains approximately 20% protein, 50% carbohydrates, 15% fat, various minerals, vitamins, pigments, polyphenols (Hernani et al., 2019), 3.6% essential amino acids (Fang et al., 2020), and other compounds important for health. Raw cacao beans contain antioxidant agents, such as polyphenols (Cempaka et al., 2021). Cacao beans are commonly fermented to produce a distinctive cocoa aroma. Fermentation of cacao beans occurred on cacao pulp by microbes naturally found on cacao fruit carp. Yeast, lactic acid bacteria, and acetic acid bacteria work together on the pulp-producing acids which then break the seed skin and ferment the compounds inside (Díaz-muñoz et al., 2020). Non-fermented cacao is rarely eaten directly, but it is used as a substitute in the cacao industry.

Another bean-fermented food in Indonesia is tempeh. Tempeh is known as a nutritious food, which is fermented from soybean. In the traditional process of tempeh, soybean is fermented by *Rhizopus* sp., and then various bacteria will grow on it. This process makes soybean tempeh more nutritious because proteins and other compounds are more digestible, and certain vitamins are produced. *Rhizopus oligosporus* can

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increase the content of isoflavones during soybean tempeh fermentation (Dwiatmaka et al., 2022). Therefore, it is interesting to conduct further studies on the potential of cacao tempeh as a functional food.

Based on the soybean fermentation process, cacao beans can be processed in the same way. If successful, this cocoa tempeh innovation has the potential to be one of the cacao diversification products of low-grade quality cacao beans. Furthermore, cacao beans can be expected to substitute soybean in tempeh. Due to competition for land use and profits, soybean productivity declines by 3.01% in 2021 (Handayani et al., 2018; Harsono et al., 2021). Therefore, the diversification of products from other raw beans is also important.

This research focused on investigating the fermentation of cacao beans using soybean tempeh inoculum. The chemical compounds of soybeans are partly the same as other common beans, such as cacao beans. To some extent, it might be different. Macronutrients in cacao beans are available for fungus growth. However, the chemical and physical structures of other seeds might influence the growth of the fungus. The fermentation process of cacao bean might influence the antioxidant activity of the bean, such as in the natural cacao process, showing the reduction of polyphenols in beans (Peña-correa et al., 2022). Therefore, the composition of soybean and cacao bean was investigated in this research to maintain high-soluble protein content and antioxidant activity. The cacao tempeh would be expanded for its use by developing cacao tempeh flour for further use. Flour is easier to store and process into various products.

The aim of this research was to evaluate the characteristics of the flour made from tempeh of soybean substituted with cacao bean.

2. MATERIALS AND METHODS

The materials used in this research were soybean var. Devon I (BALITKABI) (Putri, 2021), raw-bulk cacao, and soybean tempeh inoculum (Prima), 2,2-diphenyl-1-picrylhydrazyl, sodium hydroxide, methanol P.A., Folin-Ciocalteu solution, sodium tartaric, $CuSO_4.5H_2O$, and Na_2CO_3.

2.1. Fermentation of soybean and cacao bean using soybean tempeh inoculum

The soybeans were washed and soaked in water for 9 h at room temperature. After removing the peel, the seeds were then steamed for 15 min. Pre-treatment of raw cacao beans was carried out as follows: husks and pulp were removed from the seed. The seed was then washed with streaming water, chopped coarsely, and steamed for 40 min (Ahnan-winarno et al., 2021). Steamed soybeans and cacao seeds were mixed according to the ratios of 100:0, 70:30, 60:40, 50:50, 40:60, and 30:70. The seeds were then inoculated with tempeh inoculum as much as 2% of the initial weight of the seeds' mixture. The inoculated seeds were put into plastic bags, sealed, and then perforated randomly. The incubation of the package was carried out in an incubator at 32°C for 48 h.

2.2. The process of making cacao tempeh flour and tekao flour

The process of making tempeh and cacao tempeh flour was performed according to Dwiatmaka et al. (2022) with a slight modification. Tempeh was cut into small pieces and dried in a fluidized bed dryer at 45°C for 4 h. Dry tempeh was then ground using food and sieved using 70 mesh. The flour obtained from soybean tempeh was hereinafter referred to as tempeh flour, while the one obtained from soybean-cacao bean was called Tekao flour.

2.3. Determination of soluble protein content

The soluble protein content of tempeh and tempeh flour was determined according to the Lowry method (Lowry et al., 1951). Bovine serum albumin was used for standard protein content.

2.4. Antioxidant activity

Before the assay, all samples were extracted in methanol according to the methods developed by Mohammed et al. (2022). Scavenging activity was performed to determine the antioxidant activity using DPPH according to the method developed by Barus et al. (2019) without sonication. The antioxidant activity of samples was determined as inhibition activity using the Equation 1:

$$\text{Inhibition activity (\%)} = [(A_{517}\ \text{control} - A_{517}\ \text{sample}) / (A_{517}\ \text{control})] \times 100\% \quad (1)$$

2.5. Determination of water- and oil-absorption capacity

The water- and oil-absorption capacity of the sample was performed as follows. The flour sample was weighted (W0) in a centrifuge tube. The total weight of the sample and tube was counted as W1. Then, the flour was added and immersed in demineralized water or corn oil for 18 h. The suspension was centrifuged at 2,000 rpm for 20 min. The tube containing the sediment was weighed after the discarding of the supernatant (W2). The absorption capacity of cocoa tempeh flour was calculated by the Equation 2:

$$\text{Water-holding capacity (WHC) and Oil-holding capacity (OHC)} = [(W2-W1)/W0] \times 100\% \quad (2)$$

where:

W0: the weight of samples (g);

W1: the weight of centrifuge tube plus samples (g);

W2: the weight of centrifuge tube plus sediment.

2.6. Determination of water content and water activity

Water activity was measured using an AW meter. The water content of the flour was determined using the dried oven at a temperature of 105±3°C for 1 h in a porcelain crucible. The water content of the flour sample was calculated as follows (Equation 3):

$$\text{Water content (\%)} = [(W1-W2)/(W1-W0)] \times 100\% \quad (3)$$

Where:

W0: the weight of crucible;

W1: the weight of crucible plus sample;

W2: the weight of crucible plus sample after the drying process.

2.7. Determination of particle size, bulk density, and angle of repose

The size of the flour particle was measured under a light microscope using a micrometer scale. Bulk density was determined according to the method developed by Aditi and Arivuchudar (2018) with minor modifications. The sample was weighed and taken in a measuring tube. Bulk density was calculated as follows (Equation 4):

$$\text{Bulk density } (g.mL^{-1}) = \text{sample mass (g)/sample volume (mL)} \quad (4)$$

The angle of repose was measured according to the method developed by Macho et al. (2020). Flour flowed smoothly through a funnel set 7 cm above a flat surface until the angle of mass peak did not change. The diameter of peak (d) and height of peak (H) were measured. The angle of repose was calculated as Equation 5:

$$\text{angle of repose } (°) = \arctan [H/(d/2)] \quad (5)$$

2.8. Statistical analysis

The differences between parameters were analyzed statistically using the *Duncan multiple range test*.

3. RESULTS AND DISCUSSION

3.1. Soybean and soybean-cacao bean tempeh

Several tempehs containing various compositions of soybean-cacao bean were successfully made. All the tempeh made in this study was mature after 48 h of fermentation (Figure 1). The ratios of soybean and cacao bean were 100/0, 70/30, 60/40, 50/50, 40/60, and 30/70% w/w. The cacao bean remained dark brown after 48 h of tempeh fermentation, and it seemed that the mold of *Rhizopus* sp. only grew on the surface of the cacao bean (Figure 1).

3.1.1. Soluble protein content and antioxidant activity of tempeh

The result showed that the greater the cacao contents, the lower the scavenging activity and the soluble protein content in tempeh (Figure 2). Based on the antioxidant activity and protein content, the best tempeh composition was the tempeh

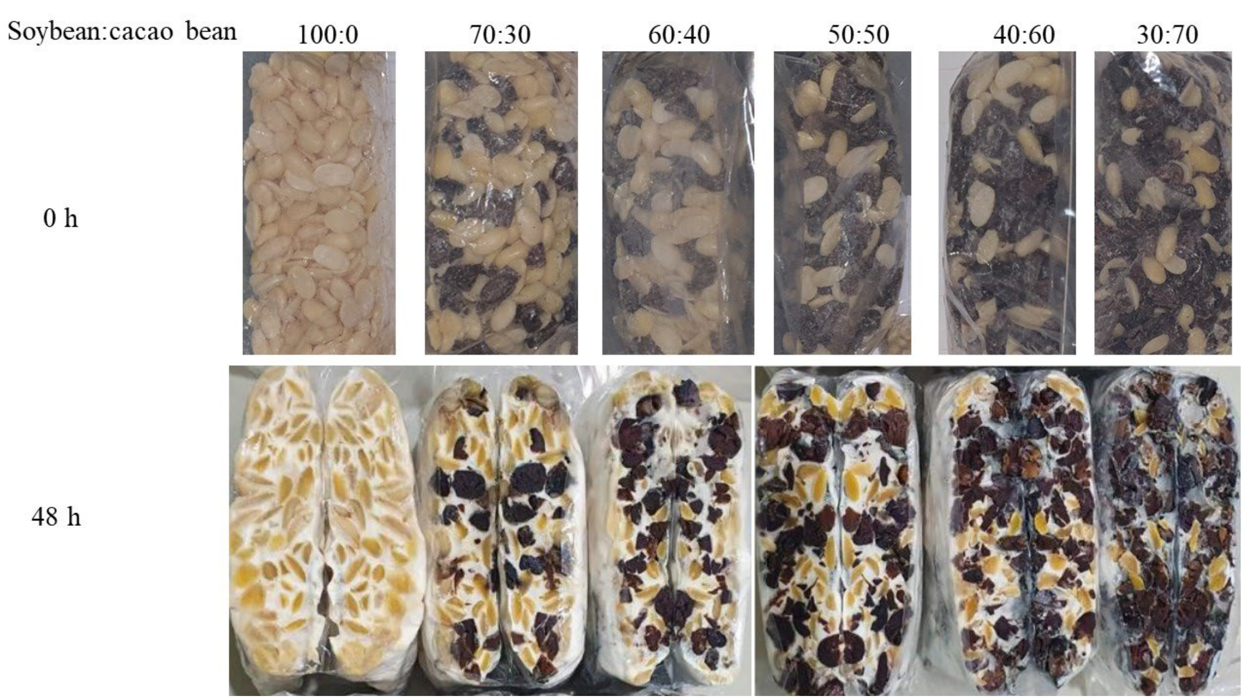

Figure 1. Fermentation of soybean and soybean/cacao bean using soybean tempeh inoculum. Fermentation was carried on at room temperature (~30°C) for 48 h. On day 0, the beans were inoculated with the usual inoculum for soybean tempeh (top row image). On day 2, tempeh was inoculated for 48 h (bottom row picture). The dark brown part was the pieces of cocoa `

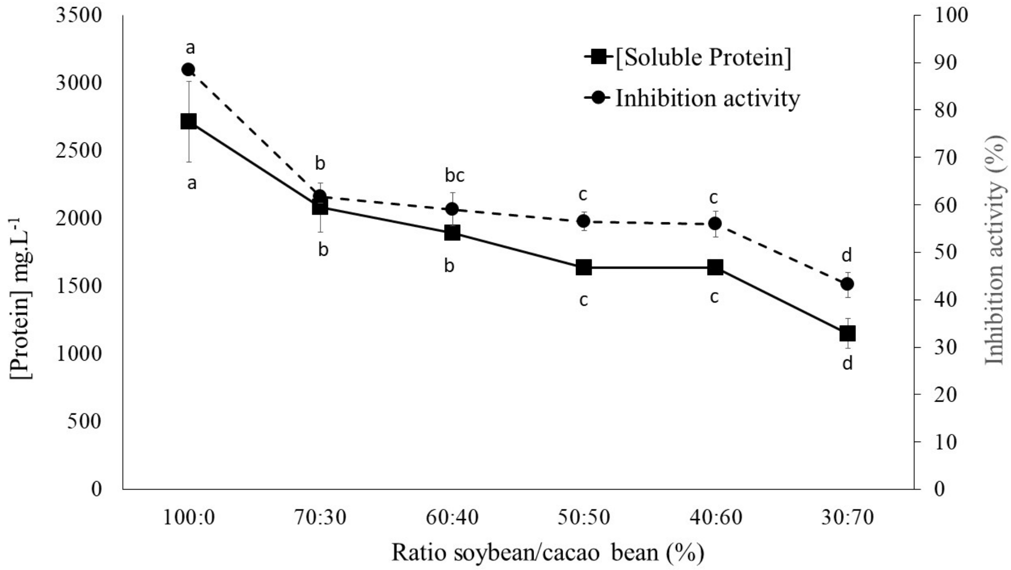

Figure 2. Soluble protein content and antioxidant activity of tempeh consist of soybean, cacao bean, or soybean/cacao bean. The best cacao content was 30% mixed with 70% of soybean, based on soluble protein content and antioxidant activity.

with 70% soybean and 30% cacao bean. Soybean tempeh had the highest protein content and antioxidant activity. This indicates that a large portion of soluble protein and the antioxidant agent came from soybeans. The differences might be caused by the steaming duration of seeds, i.e., 15 min for soybean and 40 min for cacao bean.

Based on the tempeh nutrition, we sort out the best composition of soybean/cocoa bean using the effectivity index of treatment. In this calculation, the priorities of characters of tempeh were scavenging activity and soluble protein contents which each scored 0.5. The best composition of tempeh-containing cacao beans was 70:30 w/w of soybean/cacao bean. It contained higher protein content and scavenging activity among other soybean-cacao bean tempehs. The 70:30 composition was then chosen for making the flour of tempeh soybean/cacao. Hereinafter, the tempeh of this composition was referred to as tekao tempeh.

3.2. Characteristics of tekao flour

The tekao flour was made from the soy-/cacao-bean tempeh with the proportion of 70:30. The tempeh was dried using the fluidized bed dryer method, ground using a food processor, and filtered using a 70-mesh sieve. This flour was called tekao flour, whereas flour derived from soybean tempeh is referred to as tempeh flour. The characteristics of tekao flour are described below.

3.2.1. Antioxidant activity of tempeh and tempeh flour

Antioxidant activity assay showed that all the tempeh flour still contained scavenging activity (Figure 3). There were no significant differences in this activity between soybean tempeh flour made in this study (Figure 3B) and commercial tempeh flour. Although the tekao flour had lower activity than the soybean tempeh flour, it had larger activity than the wheat flour. Compared to fresh tempeh, the scavenging activity of the tempeh and tekao flour decreased. This might be caused by the drying process of all tempeh.

3.2.2. Soluble protein content of tempeh and tekao flour

The soluble protein concentration of fresh tempeh was higher than that of tempeh flour. The flouring process might affect the denaturation of soluble protein. The soybean tempeh contained higher soluble protein content compared to tekao (Figure 3). Soybeans are known to contain higher soluble protein as much as 37–39% (Wijewardana et al., 2019), whereas, in cocoa beans, it ranges between 10 and 15% w/w (Muñoz et al., 2020).

3.2.3. Water absorption and oil absorption of tempeh flour

The data in Table 1 indicate that tempeh flour had a higher capacity to absorb water compared to tekao flour, but the oil-absorption capacity of both flours was similar (Table 1). The difference in water-absorption capacity between both flours might be due to the differences in their chemical contents. The water-absorption capacity of flour will increase with increasing fiber and starch contents (Lovegrove et al., 2020) and soluble protein content (Arivuchudar, 2018). The oil-absorption capacity of both flours was not different, indicating that the two flours might have similar hydrophobicity. Hydrophobicity was related to hydrophobic groups of surface proteins (Ettoumi & Chibane, 2015) to hold and retain oil (Chen et al., 2017). Oil absorption can also be affected by starch in tempeh and tekao flour (Habeebrakuman et al., 2019).

3.2.4. Water content and water activity of tempeh and tekao flour

Water content and water activity are important parameters to determine the quality and shelf life of a product. The tekao

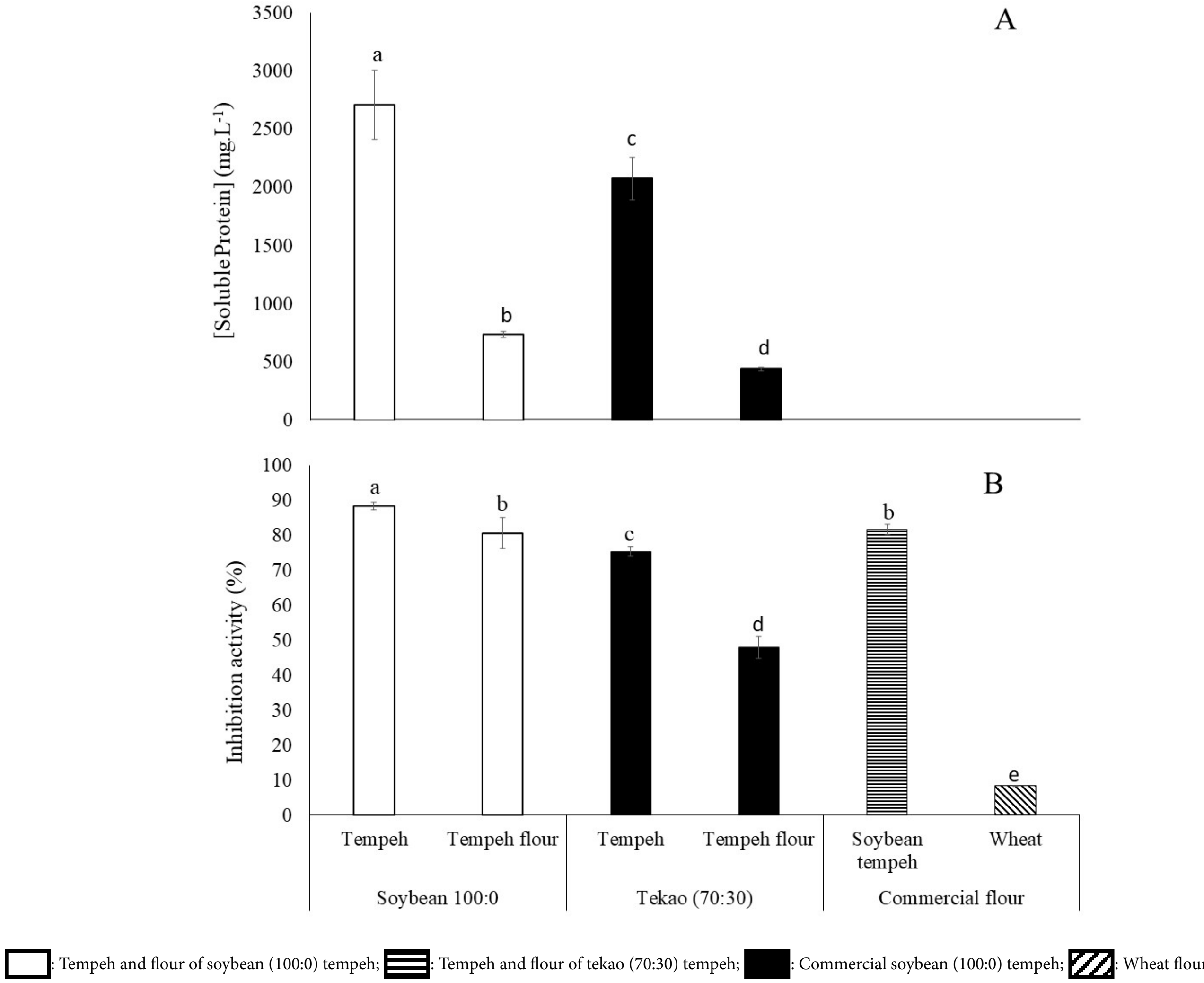

Figure 3. Protein contents and antioxidant activity of tempehs and tempehs flours, and wheat flour. The flour contained less protein and scavenging activity compared to its original tempehs.

Table 1. Several characteristics of tempeh and tekao flours*.

Characteristics	Tempeh flour	Tekao flour
Water absorption (%)	303.6^b±2.6	240.4^a±1.4
Oil absorption (%)	106.7^a±1.5	107.2^a±1.9
Water content (% w/w)	10.8^b±0.1	7.3^a±0.1
Water activity (aw)	0.57^b±0.004	0.55^a±0.007
Particle size (μm)	633^a±58.6	546^a±34.1
Bulk density (g.mL^{-1})	0.422^a±0.013	0.427^a±0.009
Angle of repose (°)	42.6^a±1.8	44.9^a±0.3

*Different letters indicate significant differences (p-value<0.05).

flour contained less water than tempeh flour (Table 1). The water-absorption capacity of tempeh flour was 303.6%, which was higher than the capacity of tekao flour, resulting in higher water content. Both flours had water activity less than 0.65, which preserves the flours from fungal growth. Low water activity will be more beneficial for product shelf life (Abdullah et al., 2000; Saha, 2020). Based on this result, tekao flour had lower water activity, which indicated that tekao flour might have a longer shelf life than tempeh flour. Both flours had water content and water activity that met the provisions of the Indonesian National Standard (SNI3751:2009) regarding wheat flour.

3.2.5. Particle size, bulk density, and angle of repose of tempeh and tekao flours

Both tempeh and tekao flours had similar particle size, bulk density, and angle of repose (Table 1), and these results were consistent with those of a previous study (Astawan et al., 2016). In this study, the particle size of tekao flour was smaller (546 μm) than tempeh flour (633 μm). However, the angle of repose was larger (44.9°) than tempeh flour (42.6°). Referred to wheat flour, which is 212μm, the particle size of both flours tends to be larger than flour in general, according to SNI 3,751:2009. Bulk density and angle of repose are affected by the particle size. The smaller the particle size, the higher the bulk angle (Macho et al., 2020).

The protein content and antioxidant activity of tempeh decrease as the cacao content increases. The decrease in antioxidant activity in cacao beans tempeh or the flours might be related to the heating process and fungal activity. In seed pre-treatment, steamer duration was longer for cacao beans than for soybean. The antioxidant agents in cacao beans and soybean are mostly contributed by the polyphenol contents of beans, whose compounds are unstable to heat (Indiarto et al., 2019; Oracz & Nebesny, 2016) and reduced during the processing and fermentation process (Dwiatmaka et al., 2022). Cacao bean fermentation by Rhizopus inoculum might not be performed well as the bean is harder than soybean. Further treatment is needed to facilitate the complete fermentation of cacao beans.

Although tekao flour has lower antioxidant and protein content than soybean tempeh flour, there are several reasons why it has the prospect to be developed in the future. First, a part of the cacao could substitute for soybean. The availability of raw cacao was higher than soybean. The second reason is that tekao flour has higher protein content and antioxidant activitycompared to wheat flour. The water-holding capacity and oil-holding capacity are quite high, which is suitable for the preparation of bakery products (Ettoumi & Chibane, 2015) and meat (Chen et al., 2017), and the fortification of bakery products.

The tekao flour has potential as a functional food due to its high protein content and antioxidant activity. The brown color of tekao tempeh flour can be an advantage in coloring food with a natural hue. This can be appealing to consumers who are looking for food products that are minimally processed and made with natural ingredients. Additionally, the brown color of tempeh flour can help add visual appeal to baked goods and other foods, making them more attractive to consumers. However, the brown color of the flour can be off-putting to some people and may limit its acceptance as a certain food ingredient. To address this, some studies may evolve to lighten the color of the flour while maintaining its nutritional benefits.

4. CONCLUSION

The tempeh flour made from 70:30 of soybean:cacao bean tempeh has the potential to be functional food considering its antioxidant properties and other characteristics. The tekao flour had an antioxidant activity of 48.1% and soluble protein content of 444.7 $mg.L^{-1}$. The water-holding and oil-holding capacities of tekao flour were 240.4% and 107.2%, respectively. The bulk density, angle of repose, and particle size were 0.427 $mg.L^{-1}$, 44.9^{o}, and 546 μm, respectively. Water content and water activity were 7.3% and 0.55% w/w, respectively.

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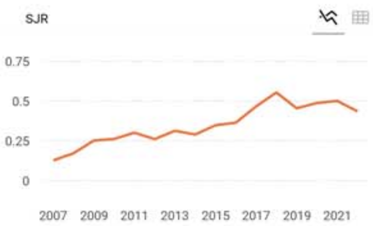

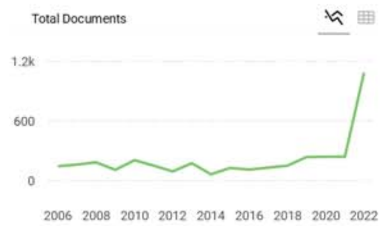

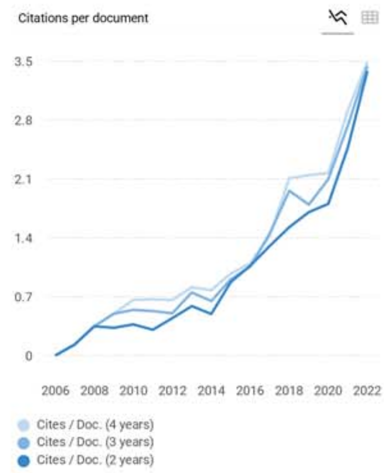

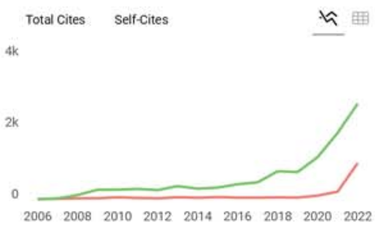

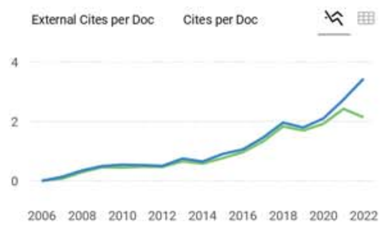

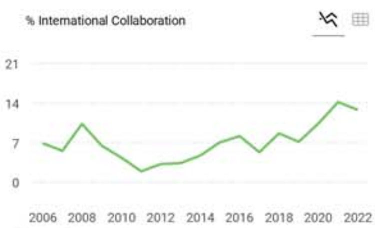

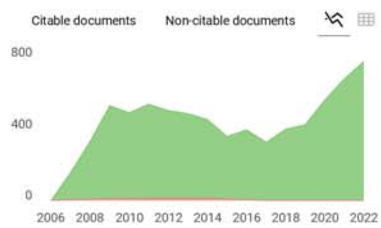

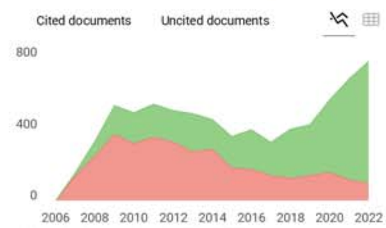

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P **Padma Chidambaram** 3 years ago

Hello,

I am Padma Chidambaram from Bangalore, India. I returned from the USA a couple of years back. I worked for many multinationals and am now making a big career change.

I plan to establish a startup to produce zero calorie sweetener from sugarcane and yeast fermentation to produce Reb M.

Would your organization please let me know which professor I should approach/email to support me on this project.

Looking forward to hearing from you.

With best regards,
Padma Chidambaram

reply

B **Blityilza Carbajal** 2 years ago

Hola Padma Chidambaram, mi nombre es blityilza Steffi Carbajal Nuñez soy del Perú, soy egresada de la carrera de industrias alimentarias, actualmente estoy elaborando una trabajo de tesis relacionado a su proyecto de negocio, si desea alguna ayuda podria brindarle información, aunque no soy una experta. saludos

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