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The Development of Yogurt Powder is High in Minerals, Rich in Antioxidants from Tempeh as a Synbiotic Drink

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A B S T R A C T

The habit of consuming ultra-processed foods with high calories and low in nutrients increases the prevalence of obesity and mineral deficiencies, such as calcium (Ca), magnesium (Mg), and iron (Fe). The purpose of this study was to determine the content of Ca, Mg, and Fe and antioxidant activity in synbiotic tempeh yogurt powder. This study consisted of three treatments, with the addition of tempeh as much as 0% (YT 0%), 50% (YT 50%), and 95% (YT 95%). The mineral content was analyzed by the permanganometric titration method, while antioxidant activity used the 2,2-diphenyl-1-picrylhidrazyl (DPPH) method. The addition of tempeh in the manufacture of yogurt powder to the content of Ca, Mg, and Fe was significantly different in all treatment groups with a value of ($p<0.05$). The best treatment group with the highest mineral content in YT (0%) and YT (95%) with values of 895.53 + 0.04 and 858.83 + 0.01 mg (Ca), 78.83 + 0.01 and 72.20 + 0.00 mg (Mg) as well as 25.87 + 0.28 and 25.85 + 0.28 mg (Fe) were significantly different from YT (50%). However, the highest antioxidant content in the YT treatment (50%) was 78.12+0.10%. The addition of tempeh in the manufacture of yogurt powder is very significantly different from the value ($p<0.001$) of antioxidant content. The development of yogurt powder high in minerals and rich in antioxidants from tempeh as a synbiotic drink can be an alternative in the management of obesity.

Kata kunci:

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A B S T R A K

Kebiasaan mengkonsumsi makanan ultra-olahan berkalori tinggi dan rendah zat gizi, meningkatkan prevalensi obesitas dan defisiensi mineral, seperti: kalsium (Ca), magnesium (Mg) dan zat besi (Fe). Tujuan penelitian ini, untuk mengetahui kandungan Ca, Mg, dan Fe dan aktivitas antioksidan dalam bubuk yogurt tempe sinbiotik. Penelitian ini terdiri dari tiga perlakuan, dengan penambahan tempe sebanyak 0% (YT 0%), 50% (YT 50%) dan 95% (YT 95%). Kandungan mineral dianalisis dengan metode titrasi permanganometri, sedangkan aktivitas antioksidan menggunakan metode 2,2-diphenyl-1-picrylhidrazyl (DPPH). Penambahan tempe dalam pembuatan bubuk yogurt terhadap kandungan Ca, Mg, dan Fe secara signifikan beda nyata pada semua kelompok perlakuan dengan nilai ($p<0,05$). Kelompok perlakuan terbaik dengan kandungan mineral tertinggi pada YT (0%) dan YT (95%) dengan nilai 895.53 $\pm$ 0.04 dan 858.83 $\pm$ 0.01 mg (Ca), 78.83 $\pm$ 0.01 dan 72.20 $\pm$ 0.00 mg (Mg) serta 25.87 $\pm$ 0.28 dan 25.85 $\pm$ 0.28 mg (Fe) secara signifikan beda nyata dengan YT (50%). Namun, kandungan antioksidan tertinggi pada perlakuan YT (50%) sebesar 78.12$\pm$0.10%. Penambahan tempe dalam pembuatan bubuk yogurt sangat signifikan beda nyata dengan nilai ($p<0,001$) terhadap kandungan

antioksidan. Pengembangan bubuk yogurt tinggi mineral dan kaya antioksidan dari tempe sebagai minuman sinbiotik dapat menjadi alternatif dalam manajemen obesitas

INTRODUCTION

A sedentary lifestyle changes the occurrence of globalization, especially in food systems that offer ultra-processed foods and drinks at low prices (Popkin et al., 2020), high-calories, low nutritious significantly increase the prevalence of obesity (Astrup & Bügel, 2019). The prevalence of obesity globally has doubled over the past 30 years, while in Indonesia it continues to increase, reaching 21.8%, even at the risk of triggering diabetes mellitus and metabolic disorders (Mohammadi-Sartang et al., 2018, Ministry of Health in Indonesia, 2018). Previous research has suggested certain micronutrient deficiencies, such as Ca, Mg, and Fe in many people with obese nutritional status due to poor nutritional intake (Astrup & Bügel, 2019).

Nutritional interventions that aim as preventive and curative in a diseases, need to follow the development of food. While the current trend is powdered drinks containing antioxidant compounds (Suratno et al., 2014). Yogurt includes nutrient-dense fermented dairy products (proteins, vitamins, and minerals), contains antioxidants, beneficial living microorganisms, is relatively inexpensive, serves as a carrier of probiotic and prebiotic bacteria and is recommended as part of a healthy diet that is now starting to be introduced to the wider community (Al-baarri et al., 2016, Gomez-Gallego et al., 2018). It is known that milk is a drink with high functional value (Al-Baarri et al., 2010) and is widely produced into yogurt. But in addition to milk, soy is also one of the vegetable ingredients in yogurt making (Moore et al., 2018).

Soybean consumption in Indonesia is as much as 60% in the form of tempeh (Astawan et al., 2018). Tempeh is a traditional food native to Indonesia from soy fermentation with *Rhizopus spp* inoculation as a highly nutritious vegetable food and as a source of probiotics with a minimum containing live bacteria as much as 10^6 CFU / ml in the medium agar (Kadar et al., 2020, Magdalena et al., 2022). The fermentation process of soybeans into tempeh causes the bioconversion of nutrients and bioactive components that play a role in the treatment of obesity, in the form of proteins, peptides, antioxidants, and isoflavones (Astawan et al., 2018; Gadde et al., 2018; Proestos, 2018). In addition, it contains micronutrients such as Ca, Mg (Ariani & Angwar, 2018), Fe (Ministry of Health in indonesia, 2018), and can improve active physiological abilities (Astawan et al., 2018). Tempeh also contains a prebiotic in the form of *Oligosaccaride* (Hijová et al., 2019). Tempeh contains probiotics as well as prebiotics, tempeh can be categorized as a synbiotic product.

Making synbiotic tempeh yogurt powder can increase shelf life, easy storage and maintain nutritional and probiotic content (Sahib et al., 2020). In addition, it has the potential as an alternative to powdered drinks that are practical and rich in benefits. Currently there is no data related to mineral content and antioxidant activity in the synbiotic tempeh yogurt powder. So the purpose of this study is to find out the content of Ca, Mg, and Fe as well as the antioxidant activity in the synbiotic tempeh yogurt powder.

METHOD

The design in this study was experimental with a completely randomized design. There were three treatments with different additions of tempeh as much as 0% (YT 0%), 50% (YT 50%), and 95% (YT 95%) of the total liquid volume. The manufacture of synbiotic tempeh yogurt powder is carried out at the Integrated UPT Food Technology Laboratory of Diponegoro University and the Catholic University Laboratory. Meanwhile, mineral and antioxidant analysis were carried out at the Chemmix Pratama Laboratory, Yogyakarta.

Materials and methods

The ingredients in making synbiotic tempeh yogurt powder in the form of fresh tempeh which has an expiration date label and *low fat* UHT milk (Ultramilk, PT Ultrajaya Milk Industry Tbk), comes from supermarkets in the Semarang region, and Fujicco's yogurt starter culture (Caspian Sea) comes from Japan. The chemicals used: HNO_3, $KMnO_4$ 0.1 N, Na_2HPO 10%, NH_4OH, and DPPH are obtained from the Chemmix Pratama Laboratory, Yogyakarta. Analysis of mineral content using permanganometric titration tools. Tools for the analysis of antioxidant activity in the form of spectrophotometers (*Specord* 210 *Plus*, *Analytic Jena*).

Yogurt starter

The starter of tempeh yogurt powder is made by adding inoculum as much as 3 g to 50 ml of low-fat UHT milk and incubated for 24 hours at room temperature 18-38^{0}C as F1 in accordance with the procedure carried out by Al-barrii el al (2016) with minor modifications. Liquid starter (working culture*)* as F2 which will be used in the manufacture of tempeh yogurt can be made by adding 50 ml of F1 to 500 ml of pasteurized milk and incubated for 24 hours at room temperature 18-38^{0}C (Al-baarri et al., 2016).

Synbiotic tempeh yogurt powder

The manufacture of synbiotic tempeh yogurt powder begins with the manufacture of liquid synbiotic tempeh yogurt. The first step is done by making tempeh juice. Tempeh in blancing for 5 minutes at a temperature of 90^{0}C, then add water in a ratio of 1: 5, carrying out melting, filtering until obtained tempeh juice (Ayuningtyas et al., 2018). The addition of tempeh juice is 0%, 50%, and 95% of the total volume. Then pasteurized at a temperature of 72-85^{0}C for 5 minutes, then cooled until the temperature reaches 37^{0}C and inoculation with a working culture of 5% in aseptic conditions. The next step is the incubation process at room temperature 18-38^{0}C for 48 hours, and ends with a cooling process at a temperature of 5^{0}C to stop the fermentation process (Al-baarri et al., 2016; Bintari & Parman, 2019). Liquid synbiotic tempeh yogurt is dried by the freeze drier method, then grinded with a low-speed

blender for one minute, and sifted with a sieve of 48 mesh (Astawan et al., 2016).

Analysis of mineral content and antioxidant activity

Analysis of mineral content Ca, Mg, and Fe using permanganometric titration (Slamet & Bambang, 2002). Antioxidant analysis was conducted based on Lee (2020) using the DPPH method. Dissolves 100 µL DPPH in 96% pure ethanol. Then take 1 ml of DPPH and add 1 ml of synbiotic beverages and 3 ml of 96% ethanol. Next homogenize and store it in a dark room at room temperature, for 10 minutes (Lee et al., 2020). Measurements using a spectrophotometer (UV-1601, Shimadzu Co, Kyoto, Japan) at a wavelength of 517 nm. The analysis is carried out with three repetitions.

Data Analysis

The experiment was conducted three times. Results are presented in the form of mean±SD. The data were analyzed with an analysis of variance (ANOVA), followed by *Duncan's Multiple Range Test* (DMRT) using SPSS 21 (IBM, Chicago) and said to be significant if the $p<0.05$.

RESULT AND DISCUSSION

An assessment of 75 informed consent documents in the

Mineral content of synbiotic tempeh yogurt powder

Deficiencies in certain minerals such as calcium, magnesium, and iron occur in many people with obesity nutritional status due to poor nutritional intake with easy access to ultra-processed foods and beverages (Astrup & Bügel, 2019). Even the prevalence of obesity globally has doubled, which is a serious problem that needs to be found a solution (Mohammadi-Sartang et al., 2018). The development of yogurt powder is high in minerals and rich in antioxidants from tempeh as a synbiotic drink can be an alternative. The composition of the material refers to Bintari's research (2019) with slight modifications can be seen in Table 1 (Bintari & Parman, 2019).

Table 1
Composition of Synbiotic Tempeh Yogurt Powder (1000 ml)

Material	Concentration of addition of tempeh juice		
	YT (0%)	YT (50%)	YT (95%)
Tempeh juice (ml)	0	500	950
UHT Milk (ml)	950	450	0
Starter (ml)	50	50	50
Sum	1000	1000	1000

YT (0%): *yogurt* with an additional tempeh 0%, YT (50%) : *yogurt* with an additional tempeh 50%, YT (95%) : *yogurt* with an additional tempeh 95%.

Tempeh as a nutrient-dense food in the form of minerals and vitamins that are easily absorbed at affordable prices and become one of the favorite foods of the People of Indonesia (Ariani & Angwar, 2018). The potential of good tempeh nutritional content can be used as a local food to prevent various diseases and overcome nutritional problems, including obesity (Astawan et al., 2018). The addition of tempeh in the manufacture of yogurt powder to the mineral content (Ca, Mg, and Fe) was significantly different in all treatment groups with values ($p < 0.05$) with the ANOVA test. The best treatment groups with the highest mineral content in YT (0%) and YT (95%) with values of 895.53±0.04 and 858.83±0.01 mg / 100g (Ca), 78.83±0.01 and 72.20±0.00 mg / 100g (Mg) and 25.87±0.28 and 25.85±0.28 (Fe) are significantly different from YT (50%). This shows that tempeh yogurt with vegetable ingredients can be an alternative to yogurt from animal ingredients because in YT (0%) and YT (95%) it is no real difference from the value ($p>0.05$), can be seen in Table 2.

Table 2
Mineral Content of Synbiotic Tempeh Yogurt Powder

Mineral Content mg/100 g	Treatment		
	YT (0%)	YT (50%)	YT (95%)
Calcium	895.53 ± 0.04[a]	691.40 ± 0.02[b]	858.83 ± 0.01[a]
Magnesium	78.83 ± 0.01[a]	51.87 ± 0.01[b]	72.20 ± 0.00[a]
Iron	25.87 ± 0.28[a]	21.41 ± 0.57[b]	25.85 ± 0.28[a]

YT (0%): yogurt with an additional tempeh 0%, YT (50%): yogurt with an additional tempeh 50%, YT (95%) : yogurt with an additional tempeh 95%. Each data is obtained from three repetitions and presented in mean ± SD. Values with the same superscript in the same row do not differ markedly ($p>0.05$).

YT (0%) is a yogurt powder based on milk that is the treatment group with the highest mineral content. It is well known that milk is a nutrient-dense drink with a variety of easily absorbed essential minerals (Gorska-Warsewicz et al., 2019). YT (95%) also has a high mineral content that is no different from YT (0%). Recent evidence suggests that the addition of tempeh powder to yogurt may add nutritional value (Bintari & Parman, 2019). Fermentation as an enzymatic and microbial way of processing food can increase food security, lower anti-nutritional substances, and extend shelf life. The fermentation process can increase the availability of minerals, with the production of phytosanity enzymes that degrade phytic acid while increasing the content of Ca, Mg, and Fe and increasing mineral bioavability (Samtiya et al., 2021). The results showed an increase in ca content by 53.4%, Mg by 43.41%, and Fe by 59.56% after the fermentation process in soybean sprouts that contributed to an increase in dissolved minerals fermented (Bahaciu et al., 2018).

Antioxidant activity of synbiotic tempeh yogurt powder

Research began to be done a lot to find out the benefits and mechanisms of flavonoid compounds to improve health. Various food ingredients are widely researched flavonoid compounds. Even some flavonoid compounds have been available in supplement form as nutritional therapies (Mahan & Raymond, 2016). Isoflavones in soybeans are the main flavonoids that have antioxidant potential that is able to bind to free radicals (de Souza de Azevedo et al., 2020; Magee & McCann, 2019). The main ingredients of making tempeh yogurt powder in the form of tempeh containing antioxidants in the form of genistein in isoflavones have benefits in cells, improve glucose and fat metabolism, protect β-pancreatic cells, and can lose weight (Yulifianti et al., 2018).

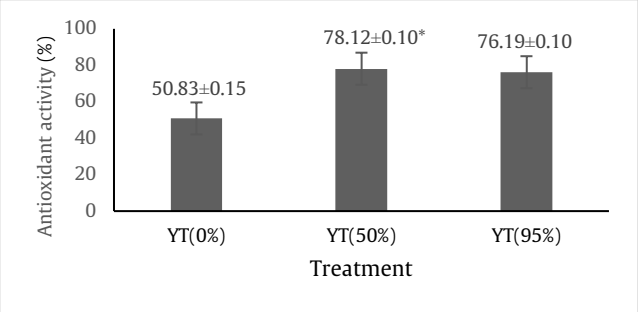

Figure 1. Average Antioxidant Activity of Synbiotic Tempeh Yogurt Powder

YT (0%): yogurt with an additional tempeh 0%, YT (50%): yogurt with an additional tempeh 50%, YT (95%): yogurt with an additional tempeh 95%. Each data is obtained from three repetitions and presented in mean±SD. * p<0.05.

Based on the results of the study that can be seen in Figure 1 shows that the highest antioxidant activity in the tempeh yogurt powder with YT treatment (50%) of 78.12±0.10%. The addition of tempeh in the manufacture of tempeh yogurt powder is very significant in contrast to the value (p<0.001). YT treatment (50%) is the best treatment with a composition of 50% tempeh, 45% milk, and 5% yogurt starter. Milk and tempeh are high in protein. During the fermentation process, the protein and exopolysaccharides produced can act as antioxidants (Uranga & Keller, 2019). This shows that YT (50%) has higher antioxidant activity due to the synergistic effect between milk and tempeh. Based on the results of research related to tempeh yogurt with an additional 50% tempeh in 100g contains antioxidant activity of 41.94% (Bintari & Parman, 2019).

The fermentation process in the presence of lactic acid bacteria, can degrade proteins contained in milk and tempeh into amino acids, while oligosaccharides in tempeh and lactose in milk are converted into lactic acid, and isoflavone compounds are hydrolyzed into aglicons so as to increase antioxidant activity (Arkan et al., 2022). Increased lactic acid content in yogurt with the addition of tempeh flour indicates that tempeh provides additional nutrients that can be used by lactic acid bacteria to grow well (Bintari & Parman, 2019).

It is well known, that BAL can serve to suppress the capacity of bacteria to reduce antinutrient factors such as phytic acid, increase phenolic compounds as antioxidants, and increase certain nutrients such as micronutrients that function in various metabolic reactions in the body. In addition, BAL fermentation can increase *the nutraceutical* value of food, relatively cheap, and can increase the organoleptic value of fermented foods (Rollán et al., 2019). According to Durazzo (2015) antioxidant activity can increase in soy-based beverages because there is a process of cooking and fermentation (Durazzo et al., 2015). The addition of soy to milk yogurt can also increase its antioxidant content (Bintari & Parman, 2019).

CONCLUSIONS AND SUGGESTIONS

The addition of different tempeh in yogurt powder significantly affects the increase in mineral content Ca, Mg, and Fe as well as antioxidant activity. The developer of yogurt powder, high in minerals and rich in antioxidants from tempeh as a synbiotic drink can be an alternative in the management of obesity and preventive for metabolic disorders that can trigger various degenerative diseases. However, there is a need for preclinical studies to confirm this mechanism.

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Ethical Considerations

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Conflict of Interest Statement

The author stated there was no conflict of interest.

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