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Nutritional profile and potential of tempeh flour and date seed flour as mixed complementary foods for chronic malnourished children

Profil zat gizi dan potensi tepung tempe dan tepung biji kurma sebagai bahan makanan campuran untuk anak malnutrisi kronik

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Abstract

Malnutrition has been increasingly linked to imbalances in the composition of gut microbiota. Disruptions in the microbiota can impair nutrient absorption, promote inflammation, and affect immune function, contributing to the development and persistence of malnutrition. Therefore, food intake that can maintain gut microbiota, such as tempeh and dates, is required. The purpose of this study was to characterize the nutrient content of tempeh and date seed flour. This study used an experimental design conducted from August to October 2024. The samples included tempeh and date seed flours boiled for 10 (A1) and 20 min (A2). The flour was processed using PRTPP BRIN. Data were analyzed using a one-way ANOVA test and a post-hoc Duncan test. The results showed a significant difference in the nutritional content of tempeh and date seed flours (p<0,01). Tempeh flour contained 48,11% protein. In contrast, date seed flour had a high dietary fiber content of 82,13% for A1 and 77,59% for A2. In conclusion, both flours have their own nutritional content, namely as a source of protein (tempeh flour) fiber (date seed flour). These findings suggest that tempeh and date seed flour have potential as complementary foods to support the gut microbiota and improve nutritional intake in malnourished individuals.

Keywords: Date seed flour, malnutrition, mixed complementary food, tempeh flour

Abstrak

Malnutrisi semakin dikaitkan dengan ketidakseimbangan dalam komposisi mikrobiota. Ketidakseimbangan mikrobiota dapat mengganggu penyerapan nutrisi, meningkatkan peradangan, dan mempengaruhi fungsi imun, yang berkontribusi pada perkembangan malnutrisi. Oleh karena itu, asupan makanan yang dapat menjaga mikrobiota saluran cerna, seperti tepung tempe dan tepung biji kurma, diperlukan. Tujuan dari penelitian ini adalah untuk mengkarakterisasi kandungan zat gizi tepung tempe dan tepung biji kurma. Penelitian ini menggunakan desain eksperimental yang dilakukan dari bulan Agustus hingga Oktober 2024. Sampel yang digunakan adalah tepung tempe dan tepung biji kurma yang direbus selama 10 menit (A1) dan 20 menit (A2). Tepung tersebut diproses di PRTPP BRIN. Data dianalisis menggunakan uji ANOVA dan uji lanjut Duncan. Hasil penelitian menunjukkan bahwa terdapat perbedaan signifikan dalam kandungan gizi antara tepung tempe dan tepung biji kurma (p<0,01). Tepung tempe mengandung 48,11% protein. Sebaliknya, tepung biji kurma memiliki kandungan serat pangan yang tinggi, yaitu 82,13% untuk A1 dan 77,59% untuk A2. Sebagai kesimpulan, kedua tepung ini memiliki kandungan nutrisi yang berbeda, yaitu sebagai sumber protein (tepung tempe) dan serat (tepung biji kurma). Temuan ini menunjukkan bahwa tepung tempe dan tepung biji kurma memiliki potensi untuk dikombinasikan sebagai bahan makanan campuran yang dapat digunakan dalam perbaikan mikrobiota saluran cerna dan meningkatkan asupan gizi pada individu yang mengalami malnutrisi.

Kata Kunci: Bahan makanan campuran, tepung biji kurma, tepung tempe

Introduction

In the last decade, studies on malnutrition in children under five years of age have often focused on its association with gut microbiota composition. A study from *Malnutrition and the Consequences for Child Health and Development* (MAL-ED) conducted across seven sites in different countries showed that subclinical enteropathogen infections, particularly *Shigella*, *enteroaggregative Escherichia coli*, *Campylobacter*, and *Giardia*, are associated with impaired linear growth that persists until the ages of 2 to 5 years (Rogawski et al., 2018). Earlier studies in Indonesia examining the link between microbiota and stunting revealed that stunted children had a higher population of the Firmicutes phylum and a lower population of the Bacteroidetes phylum than normal children (Surono et al., 2021). Comparable studies have also demonstrated that stunted children exhibit reduced beneficial bacteria and increased pathogenic bacteria levels (Ratnayani, Hegar, et al., 2024). A study involving elementary school children in West Lombok found that stunted children had lower levels of Lactobacillus and Bifidobacteria but higher levels of Enterobacter and E. coli (Helmyati et al., 2017).

These findings highlight how changes in microbiota composition may negatively affect the nutritional status of malnourished children by disrupting the production of short-chain fatty acids (SCFAs). The gut microbiota ferment nutrients that are otherwise indigestible, yielding short-chain fatty acids (SCFAs) that can either serve as substrates for lipid and glucose metabolism or be oxidized by colonocytes, thereby contributing to the host's energy harvest (Fluitman et al., 2017) .

Malnutrition is generally caused by an imbalance in the nutrient intake (Ratnayani, Sunardi, et al., 2024). Children who experience malnutrition often experience infectious diseases caused by a weak immune system and an imbalance in the gut microbiota composition. This causes malabsorption, which affects the nutrients that can be absorbed by the body (Rahayu et al., 2016). For example, an imbalance in the microbiota can affect the absorption of vitamin B, with vitamin B1 being vital for SCFA production by the gut microbiota, especially in the butyrate synthesis pathway. *Faecalibacterium*, the predominant rumen cocci, depends on vitamin B1 as a coenzyme to transform pyruvate into acetyl coenzyme, which is crucial for the production of butyrate (Wan et al., 2022). Therefore, enhancing the composition of gut microbiota is essential for improving nutrient absorption.

Dietary fiber is a polysaccharide that cannot be digested by digestive enzymes. Some polysaccharides can also be degraded by the bacteria. Fermentable fiber is a substrate for the microbial population in the large intestine, which stimulates the growth of certain organisms and promotes the production of various metabolites including short-chain fatty acids (SCFA). Certain types of fibers, such as fructans, can be classified as "prebiotics" if they are selectively consumed by host microorganisms and offer health benefits (Kashtanova et al., 2016). Similarly, for children, fiber is still required to maintain digestive health, with the amount adjusted according to age. In cases related to gastrointestinal tolerance, fiber intake can be gradually increased until the daily requirement is met (Hojsak et al., 2022).

One of the activities carried out by the government in an effort to address nutritional issues in children is the Supplementary Feeding Program (PMT). In the implementation of the PMT, the use of local food ingredients is recommended (Ministry of Health of the Republic of Indonesia, 2024). Tempes and dates are local foods in Indonesia that have the potential to be developed into food for the PMT program. Several tempeh-based products that can be used in the PMT activities include nuggets (Susianto et al., 2023) and cookies (Sogen et al., 2022).

Tempeh is an Indonesian food that is widely known to have nutritional content and health benefits (Ahnan-Winarno et al., 2021). In addition to serving as a protein source, the fiber content in tempeh provides many benefits, especially for digestive health and growth of beneficial bacteria. After the age of 6 months, when complementary foods rich in protein and fiber are introduced, there is an increase in the diversity of infant gut microbiota, signaling a shift toward a more complex microbiota (Bäckhed et al., 2015). There is substantial evidence suggesting that the gut microbiota composition in children adjusts according to their diet, which is likely to influence both the metabolic function of the microbiota and the child's nutritional status (Iddrisu et al., 2021). Previous studies have shown that tempeh increases IgA production (Soka et al., 2015). Moreover, 16-day supplementation with tempeh has been shown to boost the population of *Akkermansia muciniphila* (Stephanie et al., 2019). Tempeh is increasingly being made into flour that can be combined to make various foods (Dianingtyas et al., 2018; Yuspitasari et al., 2023).

In addition to tempeh, dates are one of the foodstuffs that have good nutritional content and are known throughout the world. There are many types of dates, and one of the most widely consumed is the Tunisian date. Research on dates was conducted in the seed sections. Based on the research results, the total dietary fiber content in Tunisian date seeds is 73,5%. This is greater than the fiber content found in Tunisian dates, which is just 18,4% (Wahini, 2016). However, date seeds are not widely used. Previous studies have shown that date seeds contain soluble dietary fiber (Kiesler et al., 2024; Fu et al., 2022).

Date seed flour has been utilized as a food product, one of which is sourdough bread (Ameur et al., 2022). Research on date seed flour has focused on its potential as an antioxidant and for protein digestibility (Muñoz-Tebar et al., 2024). The potential of date seed flour as a source of prebiotics was determined by examining its Short Chain Fatty Acid (SCFA) (Noorbakhsh & Khorasgani, 2023) content and increasing the production of lactic acid bacteria (Al-Thubiani & Khan, 2017). Thus, date seed flour has the potential to improve the composition of gut microbiota.

To date, research on the potential of tempeh flour and date seed flour has been conducted separately. There has been no research combining tempeh and date seed flours. Previous studies have demonstrated the potential of tempeh and date seed flours, particularly in terms of digestive health. Based on this, this study aimed to combine tempeh flour and date seed flour into a mixed complementary food to explore its potential in improving microbiota composition. Before creating the mixed complementary food, preliminary research was conducted to determine the nutritional profile of both tempeh and date seed flour. The results of this characterization can be used to determine the formula for mixed complementary foods. Furthermore, this mixed complementary food formula will be tested in malnourished mice to assess improvements in microbiota composition. In addition, the selected formula can be developed into various food products that can be used in the Supplementary Feeding Program (PMT) for malnourished children.

Methods

Design, Place, and Time of Research

This study used an experimental design to analyze tempeh and date seed flour. Flour production and analysis were carried out at the Food Technology and Process Research Center, BRIN, Yogyakarta, and the SIG laboratory. The study was conducted between August and October 2024.

Material

The main material used in this study was the Doga variety of soybean obtained from Balai Benih Gunungkidul. The Doga variety was chosen because it is widely used in tempeh production in the Gunungkidul region. The dates used to make date seed flour were Tunisian and purchased from an online marketplace. Tunisian dates were used because they are among the types of dates that are widely consumed and relatively affordable. Sodium bisulfite ($NaHSO_3$) was used as the other material for soaking in date seed flour.

From a food safety perspective, tempeh is commonly consumed daily. Regarding date seeds, a review concluded that, to date, no cytotoxic effects have been reported from consuming date seed coffee (Wahini, 2016). Therefore, it can be stated that both these ingredients are safe for use.

Procedure

Process of making tempeh flour

The production of tempeh flour was optimized in Rumah Tempe Gunungkidul. Tempeh was then processed into flour following the method described previously Herawati et al., (2024). Fresh tempeh was thinly sliced, soaked, and boiled to improve its texture. After boiling, the tempeh was drained and baked in a convection oven at 60°C for 8 h to ensure thorough dehydration, without compromising its nutritional properties. The choice of 60°C was based on its ability to dry tempeh efficiently while preserving its protein content and preventing nutrient loss. Once dried, the tempeh was blended and sieved through an 80-mesh sieve to obtain fine flour. The milling process, including blending and sieving, was optimized to ensure a high yield of flour with a consistent texture and quality, which is crucial for its application in food products.

Process of making date seed flour

The production of date seed flour was to Wahini (2016). Fresh date seeds were washed and soaked in sodium bisulfite ($NaHSO_3$) solution for 24 h to preserve the color and stop the browning reaction. The seeds were rinsed and boiled at 90ºC. In this study, variations in boiling time were carried out for 10 min (A1) and 20 min (A2). Boiling was performed to soften the texture of the seed. In previous research, the boiling time used was 10 minutes (Wahini, 2016). Owing to the hardness of date seeds, this study made a modification by increasing the boiling time to 20 min for comparison. The date seeds were then drained and dried in an oven at 55ºC °C for 24 h. The dried date seeds were then ground using a cross-beater mill and sieved using a 60-mesh sieve.

Parameter Analysis

Physical Characteristics

The physical characteristics include color and microstructure. The color of tempeh flour and date seeds was analyzed using a Hunter Laboratory Calorimeter (model SN 7877, Ultrascan, Hunter Associates Laboratories, Inc., Virginia). The microstructures of the two flours were described using the method proposed by Relucenti *et al.* (2020), using a scanning electron microscope (S-4800, Hitachi Ltd., Tokyo, Japan). The samples were immersed in acetone twice for 10 min each and air-dried in a fume hood. Once dried, they were carefully mounted onto aluminum stubs using carbon tape to ensure stability. A thin layer of gold, approximately 10 nm thick, was then sputter-coated onto the samples for 15 min to enhance the conductivity. SEM imaging was conducted at a voltage of 3 kV with an S-4800 microscope (Hitachi Ltd., Tokyo, Japan).

Proximate (nutrients) analysis

The proximate (nutrients) analyzed included water content, ash content, macronutrients (protein, fat, and carbohydrates), micronutrients (minerals), and antioxidant activity. The measurements were conducted in triplicate. Proximate analysis was carried out based on AOAC International (2019) standards to determine the content of water, fat, ash, protein, and carbohydrates(AOAC International, 2019). The methods used were as follows: gravimetric method (No. 925.10) for water content, Soxhlet extraction (No. 920.39) for fat, gravimetric method (No. 923.03) for ash, the Kjeldahl method (No. 955.04) for protein, and carbohydrate by difference method for carbohydrates (AOAC International, 2019).

The mineral content analysis was performed using an XRF spectrometer (XRF PANalytical Epsilon 4). The pellet-shaped flour samples were then analyzed using an XRF Spectrometer. The pellet sample was placed in the XRF chamber and measured at 8 kV and 12 KV with an intensity of 0.32-0.34 mA. The results of the analysis were in the form of a printout of the composition and mineral content of the flour samples. Detection limits for most elements are 2-20 μg/cm^2 (Rajapaksha et al., 2017).

Functional groups and chemical bonds

Functional groups and chemical bonds in date seed flour and tempeh flour were measured using FTIR Spectroscopy, according to Nandiyanto et al. (2019). FTIR spectrometer (Vertex 70, Germany) operating in ATR (Platinum accessory) mode between 4000 and 400 cm-1 (Nandiyanto et al., 2019).

Statistical Analysis

Microsoft Excel and Statistical Package for the Social Sciences (SPSS) version 20 were used to analyze the data. Tempeh and date seed flours were descriptively analyzed. To examine differences in characteristics between tempeh flour, date seed flour A1, and date seed flour A2, one-way ANOVA was performed. If significant differences were found ($p<0,05$), a post-hoc Duncan's test was applied to determine where differences occurred. Prior to performing ANOVA, the data were tested for normality using the Shapiro-Wilk test to ensure appropriate statistical analysis.

Result and Discussion

Physical characteristics

The physical characteristics of tempeh and date seed flours are presented in Table 1.

Table 1. Physical characteristics of tempeh flour and date seed flour

Parameters	Tempeh Flour	Date Seed Flour (A1)	Date Seed Flour (A2)
Colour:			
*L**	86,28	59,43	57,52
*a**	1,88	11,82	11,49
*b**	17,03	16,42	15,96
Picture of flour			

Data are presented as means; A1:10 min boiling treatment; A2:20 min boiling treatment.

The color is represented by the letters *L, a*, and b**, where *L* indicates a scale from black to white, *a** ranges from red to green, and *b** varies from yellow to blue. The *L* value denotes the brightness level. Tempeh flour had a high level of brightness, as indicated by its *L* value of 86,28. This value is still below the brightness level of wheat flour (approximately 100), which is 94,04 (Agba et al., 2024). In addition, A1 date seed flour had a higher brightness value than A2 date seed flour. This aligns with a study Azzollini et al., (2018) that reported that boiling, blanching, and drying in an oven at a temperature of 50°C can maintain color more effectively. The *a** values of A1 and A2 flours range from 11,49 to 11,82, while tempeh flour had *a** value of 1,88. The *a** value indicates red color. The *b** values of A1 and A2 were lower than those of tempeh flour. Lee et al., (2023) reported that the blanching treatment resulted in lower *b** values compared to the samples. In general, color influences consumer acceptance, with higher *L* values contributing to greater food acceptability. Cetinkaya (2022) stated that consumer perceptions and views play an important role in the food industry because color is considered an aesthetic parameter. Additionally, the relationship between color and consumer preference indicates that consumers tend to favor lighter-colored products, associating them with freshness and quality. Studies have shown that color differences, especially in food items like steamed brownies or cookies, significantly affect consumer perceptions, as they are often drawn to visually appealing products that match their expectations of taste and quality (Isaskar et al., 2021).

Microstructure and Morphology

The surface morphology and microstructure of tempeh flour (TK) and date seed flour are depicted in Figure 1. Scanning electron microscopy (SEM) was used to analyze the surface structure of both flours (Relucenti et al., 2020). Additionally, the effect of pretreatment, specifically boiling, on the surface structure of the date seed flour was assessed.

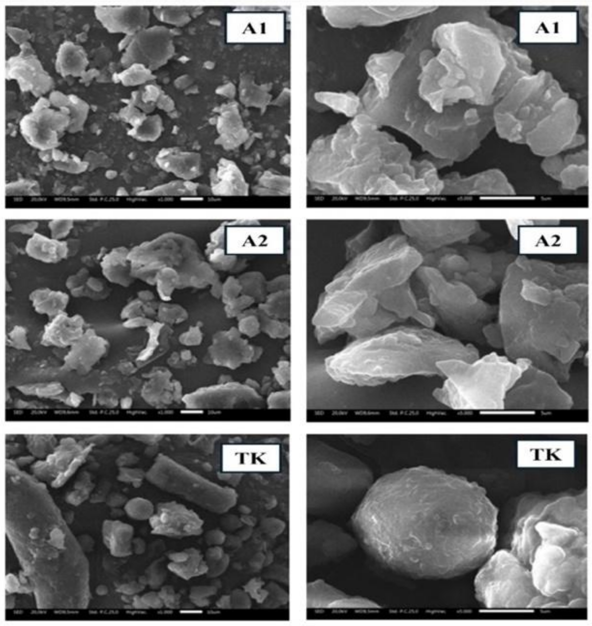

Figure 1. Microstructure of date seed flour processed by soaking for 10 minutes (A1); A2: date seed flour processed by soaking for 20 minutes (A2); soybean tempeh flour (TK) (magnification 1000x and 5000x)

The microstructures of the A1 and A2 flours have irregular aggregate fragments, and the surface is clearly visible owing to the drying process that leaves aggregates and porous structures (Monisha & Loganathan, 2022). The dense surface structure and grain shape became more porous because of the extended boiling process. The microstructure of A2 flour exhibits increased aggregation and a more pronounced porous texture compared to A1 flour. This outcome is attributed to the initial soaking and blanching treatments, which promote particle expansion owing to the absorption capacity during the process (Locali-Pereira et al., 2022).

Proximate Analysis (Nutrient Content)

The findings of the proximate (nutrient) analysis are presented in Tables 2 and 3. Table 2 shows the water, ash, protein, fat, and carbohydrate contents of tempeh and date seed flour. As shown in Table 2, the water content of date seed flour was higher than that of tempeh flour.

Table 2. Proximate analysis of tempeh flour and date seed flour

Parameters (%)	Tempe Flour	Date Seed Flour (A1)	Date Seed Flour (A2)	p-value
Water content	4,68[a]	8,46[b]	8,30[c]	< 0,01
Ash content	2,34[a]	0,88[b]	1,06[c]	< 0,01
Protein	48,11[a]	4,40[b]	4,49[b]	< 0,01
Fat	16,01[a]	11,35[b]	8,78[c]	< 0,01
Carbohydrate	28,86[a]	74,91[b]	77,37[c]	< 0,01
Dietary Fiber	37,64[a]	82,13[b]	77,59[c]	< 0,01
Soluble Dietary Fiber	0,93[a]	6,63[b]	6,01[c]	< 0,01
Insoluble Dietary Fiber	36,71[a]	75,49[b]	71,58[c]	< 0,01

Data are presented as means; A1:10 min boiling treatment; A2:20 min boiling treatment. Analysis using ANOVA test. Different letters in the same row indicate significant differences.

As shown in Table 2, the water content of tempeh flour was 4,68% lower than that of A1 date seed flour (8,46%) and A2 (8,30%). The high water content of date seed flour indicates a higher potential for susceptibility to quality degradation and the need for additional storage or processing methods, whereas tempeh flour with lower water content can have a longer shelf life. Compared with the maximum allowable moisture content in wheat flour, which is 14,5% (SNI 3751-2018), tempeh flour has a much lower water content. This lower moisture content in tempeh flour may contribute to its longer shelf life, as lower water levels help inhibit microbial growth and reduce the risk of spoilage (Winarno, 2004). In addition, lower moisture content may affect the processing behavior of tempeh flour, potentially reducing the need for drying or rehydration in certain applications, thus improving its stability during storage and transport.

The ash content in tempeh flour was recorded at 2,34% higher than that in A1 date seed flour (0,88%) and A2 (1,06%). The higher ash content of tempeh flour indicates a higher mineral content than that of date seed flour, which may provide additional benefits as a source of micronutrients (Teoh et al., 2024a).

The protein content of tempeh flour was 48,11% higher than those of A1 date seed flour (4,40%) and A2 (4,49%). The fat content of tempeh flour was 16,01% higher than that of A1 date seed flour (11,35%) and A2 (8,78%). Based on the carbohydrate content, A1 date seed flour (74,91%) and A2 (77,37%) had higher carbohydrate content than tempeh flour (28,86%). In addition, the dietary fiber content in A1 (82,13%) and A2 (77,59%) date seed flours was higher than that in the tempeh flour (37,64%).

The data showed that tempeh flour is a better source of protein and fat than date seed flour, making it ideal for meeting the high protein needs of the diet. In contrast, date seed flour contains abundant carbohydrates, making it an excellent energy source. Date seeds are known to have a high carbohydrate content, thus supporting their use as a good source of energy (Kiesler et al., 2024).

Date seed flour has a higher dietary fiber content than tempeh flour, both soluble and insoluble. Dietary fiber is a good food source for the digestive tract microbiota. Fibers can improve the balance of the digestive tract microbiota (Bailén et al., 2020; Tian et al., 2021). Based on its bioavailability, tempeh is commonly consumed and has good digestibility with potential benefits for the microbiota (Teoh et al., 2024b). In contrast to tempeh, the bioavailability of date seeds has not been established. However, biologically, dates have been widely reviewed and shown to be rich in phytochemicals and dietary fiber, which have been associated with various beneficial biological activities (Fernández-López et al., 2022).

In addition to proximate analysis, mineral content analysis was also carried out, and information regarding the mineral content of tempeh flour and date seed flour is presented in Table 3. The data show the macro- and micro-mineral contents of tempeh and date seed flours subjected to different boiling treatments. Tempeh flour has a relatively high calcium (0,694 mg/100 g, and phosphorus (0,714 mg/100 g compared to date seed flour. Extending the boiling time during the preparation of date seed flour causes an increase in the content of several minerals, such as calcium from 0,231 mg/100 g to 0,304 mg/100 g and phosphorus from 0,121 mg/100 g to 97,050 mg/100 g.

Based on micro minerals, tempeh flour has an iron content of 18,660 mg/100 g, while date seed flour has a much higher iron content of 29,34 mg/100 g (A1) and 54,050 mg/100 g (A2). Statistically, there was a difference in the mineral content between date seed flours A1 and A2. Iron is a vital mineral responsible for hemoglobin production and the transport of oxygen throughout the body; therefore, the consumption of tempeh flour has the potential to help prevent anemia (Teoh et al., 2024a). The iron content of A2 date seed flour was much higher than that of A1. These data indicate that boiling can increase the iron content of date seed flours.

Table 3. Minerals content of tempeh flour and date seed flour

Parameters	Tempe Flour	Date Seed Flour (A1)	Date Seed Flour (A2)	p-value
Macro mineral (mg/100 g)				
Magnesium	t.t	19,97	t.t	-
Potassium	t.t	0,490[a]	0,489[a]	0,288
Calcium	0,694[a]	0,231[b]	0,304[c]	< 0,01
Phosphor	0,714[a]	0,121[b]	97,050[c]	< 0,01
Chloride	t.t	0,133[a]	0,124[b]	< 0,01
Micro mineral (mg/100 g)				
Iron	18,660[a]	29,34[b]	54,050[c]	< 0,01
Zinc	14,970[a]	11,54[b]	24,940[c]	< 0,01
Copper	10,800[a]	6,210[b]	14,560[c]	< 0,01
Iodine	0,210[a]	0,340[b]	0,290[c]	< 0,01
Manganese	4,410[a]	2,800[b]	2,340[c]	< 0,01
Molybdenum	0,770[a]	0,190[b]	0,310[c]	< 0,01
Selenium	0,270	t.t	t.t	-

Data are presented as means; A1:10 min boiling treatment; A2:20 min boiling treatment. Analysis using ANOVA test. Different letters in the same row indicate significant differences.

The data in Table 3 show that tempeh flour has a higher mineral content than A1 date seed flour. These minerals are zinc (14,970 mg/100 g), copper (10,8 mg/100 g), manganese (4,410 mg/100 g), molybdenum (0,770 mg/100 g) and selenium (0,270 mg/100 g). As with macrominerals, the number of microminerals (iron, zinc, copper, and molybdenum) increases with increasing boiling time.

Increasing the boiling time can increase the mineral content of date seed flours through several mechanisms. Longer boiling times can soften or break down the cellulose matrix so that the previously trapped minerals can be released. In addition, heating can reduce the amount of antinutrient compounds, such as phytate and oxalate, which are known to bind essential minerals, such as calcium, iron, and zinc, making them more easily absorbed and available. The boiling process can also break down protein or carbohydrate bonds that bind minerals and cause these minerals to dissolve in boiling water, thereby increasing the mineral concentration in foods that have been boiled for longer (Motta et al., 2022). Thus, longer boiling times can increase the availability of minerals, such as calcium, phosphorus, iron, copper, zinc, and molybdenum, in date seed flour.

In addition to increasing the mineral content, increasing the boiling time can also reduce the levels of several minerals in the date seed flour. Several minerals, such as potassium, chloride, iodine, and manganese, decrease in levels with increasing boiling time. Increasing the boiling time can reduce the mineral levels in food ingredients, owing to several factors. These factors include the solubility of minerals in water, so that longer boiling times can cause these minerals to dissolve in boiling water and disappear from the food ingredients(Motta et al., 2022).

Antioxidant Activity

Antioxidant activities are presented in Table 4. This table shows that date seed flour has much higher antioxidant activity than tempeh flour. The date seed flour with A1 treatment had an antioxidant activity of 5059 mg TEAC/100 g sample, whereas A2 treatment showed an increase in antioxidant activity to 5504 mg TEAC/100 g. In contrast, tempeh flour had a much lower antioxidant activity (1904 mg TEAC/100 g sample). Statistically, there was a difference in the antioxidant activity between tempeh flour and date seed flour (A1 and A2).

This difference suggests that date seeds have greater potential as a source of antioxidants, especially after additional boiling, which may increase antioxidant capacity. The high antioxidant activity of date seeds may be due to the abundance of phenolic compounds recognized for their capacity to neutralize free radicals and safeguard the body against oxidative damage. The longer boiling treatment (A2) likely broke down the phenolic bonds, thereby increasing its antioxidant capacity (Alkhoori et al., 2022).

In this study, tempeh flour had lower antioxidant activity. However, tempeh contains isoflavones and other bioactive compounds that play a role in fighting free radicals (Hong et al., 2022). Isoflavones in tempeh are known to have protective effects against numerous chronic conditions linked to oxidative stress, such as cardiovascular diseases and type 2 diabetes(Rizzo, 2020).

Compared to popular fruits, cranberries exhibited the highest antioxidant activity at 6738 mg TEAC/100 g, while raspberries (5317 mg TEAC/100 g) and blackberries (2593 mg TEAC/100 g) also showed significant antioxidant levels. Blueberries (1917 mg TEAC/100 g) and strawberries (1569 mg TEAC/100 g) had relatively lower TEAC values (Frangu et al., 2020). Overall, the antioxidant activity of date seed flours, particularly A1 and A2, was comparable to or even surpassed that of many fruits, highlighting their potential as rich sources of antioxidants.

Table 4. Antioxidant activity of tempeh flour and date seed flour

Sample	Antioxidant Activity (mg TEAC/100g sample)	p-value
Tempeh flour	1904[a]	< 0,01
Date seed flour (A1)	5059[b]	
Date seed flour (A2)	5504[c]	

Data are presented as means; A1:10 min boiling treatment; A2:20 min boiling treatment. TEAC: Trolox equivalent antioxidant capacity. Analysis using ANOVA test. Different letters in the same column indicate significant differences.

Functional Groups and Chemical Bonds

FTIR analysis was conducted to determine the functional groups of the activated carbon with a wavenumber range of 450–4500 cm-1. The FTIR spectra revealed that some flour samples exhibited peak shifts, as determined by FTIR functional group analysis. These differences in peak values were mainly attributed to the formation of chemical bonds between the functional groups in date seed flour that underwent boiling treatment (Nandiyanto et al., 2019) (see Table 5).

Table 5. Functional groups of tempeh flour and date seed flour

Wavenumber (/cm)	Wavenumber Reference (/cm)	Peak Shape	Component type	Label
3303,73	3,350-3,450	Wide	OH carbohydrate protein and polyphenol	A1
3010,08	3010-3020	Sharp	CH alkene	TK
2853,49 – 2923,60	2850 – 2950	Sharp	CH dan CH_2 aliphatic	A1, A2, TK
2111,39 – 2111,81	2100 – 2500	Sharp	C=C and C≡C conjugated	A1, A2, TK
1608,49 – 1626,87	1550 – 1650	Sharp	N–H amina primer	A1, A2, TK
1440,36 – 1450,57	1400 – 1460	Wide	C=O inorganic carbonate	A1, A2, TK
1371,72 -1397,11	1450 – 1370	Wide	CH alphatic bending	A1, A2, TK
1242,59 – 1312,55	1240 – 1340	Sharp	C-N amide III bond	A1, A2, TK
1147,15 – 1157,37	1120 – 1160	Wide	C-O-C polysaccharide	A1, A2, TK
1029,98 – 1044,14	1000 – 1050	Sharp	Eter	A1, A2, TK
869,16 – 870,09	850 – 890	Sharp	Aromatic	A1, A2
764,26 – 764,61	730 – 780	Wide	Mono-substituted aromatics	A1, A2

A1:10 min boiling treatment; A2:20 min boiling treatment. TK=tempeh flour

The IR spectrum of date seed flour with boiling treatment (A1 and A2) was not significantly different, except for the peak spectrum at 3303,07 cm-1, for the -OH components of carbohydrates, proteins, and polyphenols (Table 5). The peaks of the IR spectrum were seen at Wavelengths 3303, 3010, 2111, 1608, 1440, 1371, 1242, 1147, 1029, 869, and 764 cm-1. These results indicate that boiling treatment did not change the IR spectrum of date palm seed flour. However, a lower peak spectrum was observed for A2 than that for A1.

The band in the wavenumber region of 2900 - 3600 cm-1 is a stretching vibration in the O-H functional group. The band at 2923 cm-1 shows the stretching vibration of the C-H group.

The C-N functional group was detected at a wavenumber of approximately 1200 cm-1. The band at 1147 cm-1 shows the C-O stretching vibration in the phenol and alcohol groups, and at wave number 764 cm-1, there is a C-H group produced by the bending vibrations of the aromatic ring. These results are consistent with a study conducted by Desmagrini et al., (2021) who characterized date seeds using FTIR and reported that the functional groups owned by the date seed carbon electrode were identified as C-C, C≡C (alkyne), C-H (alkane), and (O-H) at wave numbers 981,81, 2866,34 and 3065,0 cm-1.

Conclusion

Based on the research results, tempeh and date seed flours have different characteristics, but complement each other in terms of nutritional content. Tempeh flour has advantages in terms of protein content and fat, whereas date seed flour has a higher carbohydrate content, especially dietary fiber, several minerals, and antioxidant activity. Therefore, in future research, the formulation of tempeh flour and date seed flour is expected to become a mixed food ingredient with complete nutritional content. Furthermore, the formula for mixed food made from tempeh flour and date seed flour can be developed into various products that can be used in the Supplementary Feeding Program (PMT) local food to improve the nutritional status of undernourished children.

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