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Moringa oleifera supplemented cereal granules: physical quality, nutritional composition, and antioxidant activity

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Abstract

Moringa oleifera leaf powder has been incorporated into functional food products to enhance the nutritional value and to address stunting problems. This study aimed to evaluate the physical quality, nutritional content and antioxidant activity of cereal granules supplemented with *M. oleifera* leaf powder (5% MOLP) produced at different drying temperatures of 50°C (F1), 55°C (F2), 60°C (F3), and 65°C (F4). The evaluation of physical quality included hedonic organoleptic test, specific gravity, pH, and moisture content. Concurrently, the nutritional composition involved proximate analysis (water, ash, crude fiber, carbohydrate, fat, and protein content), total flavonoid, total phenolics, total alkaloids and fatty acids compositions. Antioxidant activity was assessed by using the DPPH method. Physical characteristics identified F1 as the best formula based on the highest average score value of the hedonic organoleptic test, specific gravity, and moisture content. Further proximate analysis revealed that F1 contained 83.08% carbohydrate, 10.32% protein, 3.26% fat, 0.98% crude fiber, 2.67% water, and 0.67% ash contents. Meanwhile, the total alkaloid, total phenolic, and total flavonoid contents were found to be 334.08 µg/g, 4.05%, and 2.60%, respectively. Methyl laurate, methyl myristate, and methyl palmitoleate were found to be abundant with concentrations of 37.40%, 16.87% and 14.72%, respectively. The antioxidant activity exhibited moderate efficacy, with an IC_{50} value of 150.51 µg/mL. These results indicated that F1 possessed potential for advancement as a functional food ingredient to mitigate stunting.

1. Introduction

Data on the development of stunting rates in Indonesia, released by the Indonesian Ministry of Health in January 2023, shows that from 2021 to 2022, Indonesia experienced a 2.8% decrease in stunting rates. Meanwhile, in Palu City, Central Sulawesi, the number of stunted children reached 1,221 out of a total of 22,400 toddlers (Suaib and Huda, 2023). Researchers have revealed that the direct causes of stunting are inadequate nutritional intake and the presence of diseases, especially infectious diseases (Daracantika *et al.*, 2021). Nutritional deficiencies can weaken the body's immune system, making it more susceptible to infections. Currently, many studies have explored the use of *Moringa oleifera* leaves to address nutritional problems. In Africa and Asia, *M. oleifera* leaves are used as a nutritional supplement for pregnant women and growing children. *Moringa oleifera* leaf powder is known to contain carbohydrates (38.2%), protein (27.1%), fiber (19.2%), fat (2.3%), and water (7.5%) (Zainal *et al.*, 2019). Additionally, one of the most notable components of the *M. oleifera* plant is its antioxidants. Antioxidants are essential for the body to maintain a healthy immune system and to prevent diseases (Hasanuddin *et al.*, 2022). This activity is related to its high total flavonoid content, which is 9.6 mg per 100 mg (Sulastri *et al.*, 2018).

Bioactive compounds in plants have long been used for medicinal purposes due to their therapeutic effects. A new focus is on how to preserve these bioactive compounds, which have physiological benefits for living organisms (Kussmann *et al.*, 2023). Therefore, developing efficient and environmentally friendly methods for retaining such compounds presents a challenge for the food industry. Concurrently, there is a growing trend towards the development of foods enriched with bioactive components to address

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nutritional deficiencies. This trend has spurred researchers to explore new ways in which bioactive compounds impact human health. However, emerging technologies sometimes come with significant disadvantages concerning food safety, efficiency, energy consumption, and operational costs. For instance, advanced oxidation processes like cold plasma and ultrasound may negatively affect bioactive lipids rather than preserve their activity. Furthermore, the ability of nanoemulsions or cold plasma to penetrate cell membranes has uncertain implications for the biological matrix (Galanakis, 2013). On the other hand, processing food at low temperatures allows for the gentle treatment of essential vitamins, preserving both nutrients and flavors. Additionally, interactions between different bioactive compounds in food present another consideration when applying emerging technologies. For instance, in low-processed foods, the interaction of antioxidants with macromolecules may be more favorable compared to high-processed foods, where antioxidants might bind to dietary fiber instead of proteins (Galanakis, 2021).

Moringa oleifera leaves can be processed into powder form, which can then be added to various food products or used in pharmaceuticals. One of our previous studies demonstrated that incorporating MOLP with soybeans (*Glycine max* (L.) Merill) into chocolate biscuits enhances their nutritional content and health benefits (Zainal *et al*., 2019). Therefore, creating snack foods that appeal to children and incorporate *M. oleifera* leaf powder, rich in antioxidants, can serve as an additional source of nutrition for children suffering from stunting. Developing a cereal granule formula using MOLP can be achieved through the wet granulation method, which improves the physical characteristics of the powder, such as flow properties, prevents component segregation, and ensures content uniformity. A study on the development of *M. oleifera* leaf cereal nutraceuticals using arrowroot flour (*Maranta arundinaceae* L.) as a binder utilized the wet granulation method because of *M. oleifera*'s stability and resistance to water (Rani *et al*., 2019). Therefore, this study aims to investigate the effect of drying temperature using low temperature (50-65°C) on processing *M. oleifera* leaves into functional foods in the form of MOLP-supplemented cereal granules, which contain metabolites, possess antioxidant properties and are appealing to children and toddlers for stunting prevention, particularly in Central Sulawesi.

2. Materials and methods

2.1 Materials

Moringa oleifera was collected from South Tinggede Village, Marawola District, Sigi Regency, Central Sulawesi Province, Indonesia (0.9408°S, 119.8728°E). Sorghum flour, xanthan gum, sucrose, vegetable creamer, sodium benzoate, eggs, vanilla flavorings, milk, and distilled water were purchased from the local market in Palu, Indonesia. DPPH, ethanol and other chemical substances were purchased from Sigma Aldrich.

2.2 Sample preparation

The harvested *M. oleifera* leaves were cleaned and washed with running water. After that, the leaves are shredded and then dried by aerating at room temperature until completely dry. After drying, the leaves were ground into powder using a blender and then sieved using an 80 mesh number sieve. The powdered leaves were then tested for organoleptic properties such as color, aroma and taste. Plant identification was carried out at the Plant Biosystematics Laboratory, Department of Biology, Faculty of Science, Tadulako University.

2.3 Phytochemical screening of Moringa oleifera leaf powder

Phytochemical screening was performed by several tests.

2.3.1 Alkaloid test

MOLP powder (0.5 g) was mixed with 1 mL of 2 N hydrochloric acid and 9 mL distilled water. The mixture was heated in a water bath for 2 mins, cooled to room temperature, and filtered. To 0.5 mL of the filtrate, 2 drops of Dragendorff's reagent were added. The formation of an orange-brown precipitate indicated alkaloid presence.

2.3.2 Terpenoid/steroid test

MOLP powder (0.5 g) was macerated in 10 mL *n*-hexane for 1 hr and filtered. The filtrate was evaporated to dryness, and the residue was treated with 10 drops of glacial acetic acid followed by 1 drop of concentrated sulfuric acid. The transition from a transient purple or red coloration to blue-green confirmed the presence of terpenoids or steroids.

2.3.3 Flavonoid test

MOLP powder (1 g) was boiled in 10 mL distilled water for 5 mins and hot-filtered. To 5 mL of filtrate, 0.1 g magnesium powder, 1 mL HCl, and 2 mL amyl alcohol were added. The mixture was vigorously shaken and allowed to separate. A yellow-red or orange-red coloration in the organic (amyl alcohol) layer indicated the flavonoids content.

2.3.4 Saponin test

MOLP powder (0.5 g) was mixed with 10 mL hot

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water in a test tube, cooled, and shaken vigorously for 15 mins. The formation of persistent foam stable for at least 10 mins confirmed saponin.

2.3.5 Tannin test

MOLP powder (1 g) was boiled in 10 mL distilled water, cooled, and filtered. The filtrate was diluted with distilled water, and 2 mL of the solution was treated with 1–2 drops of 5% $FeCl_3$. A blue or blackish-green color change indicates tannins (Sabdoningrum *et al*., 2021).

2.4 Formulation of cereal granule of Moringa oleifera leaf powder

The formula consisted of 5% MOLP, 20% sorghum flour, 1% xanthan gum, 20% sucrose, 15% vegetable creamer, 0.1% sodium benzoate, 2% vanilla, 13% eggs, and the remainder milk. A total of 100 g of the formula was prepared by mixing and stirring the ingredients: MOLP, sorghum flour, xanthan gum, sucrose, vegetable creamer, sodium benzoate, and vanilla in a container. Eggs and milk were gradually added to the mixture until a moist cereal granule mass formed. The cereal granule mass was then sieved using a 10-mesh number sieve and dried in an oven at 50°C (F1), 55°C (F2), 60°C (F3), and 65°C (F4) for 3 hrs until the moisture content of the granules was about 2-4%. The granules were then evaluated (Rani *et al*., 2021).

2.5 Physical and chemical evaluation of cereal granule of Moringa oleifera leaf powder

Physical evaluation of cereal granules of MOLP was performed by determining the hedonic organoleptic, specific gravity, pH, and moisture content. Meanwhile, the chemical evaluation was performed on total alkaloids, total phenolics, total flavonoids and fatty acid components by using a gas chromatography-mass spectrophotometer (GC-MS).

2.5.1 Hedonic test

The hedonic test was conducted with a total of ten untrained panelists to evaluate the color, taste, aroma, and texture of MOLP cereal granule product by asking them to express their preference on the 4-point hedonic scale (1 = very dislike, 2 = dislike, 3 = like, 4 = very like). Furthermore, this hedonic scale data was analyzed to facilitate statistical analysis (Dwiloka *et al*., 2022).

2.5.2 Specific gravity test

Specific gravity test was conducted using a clean and dry pycnometer. First, the empty weight of the pycnometer is recorded. Next, the pycnometer is filled with distilled water, and its weight is measured again. Finally, the pycnometer is filled with the MOLP cereal granule solution, and its weight is recorded for comparison (Iskandar *et al*., 2021).

2.5.3 pH test

The pH measurement was conducted using a pH meter. The MOLP cereal granule was dissolved in 20 mL of distilled water and homogenized using a mortar for 1 min. The homogenized sample was then transferred into a 10 mL glass beaker. Subsequently, the pH meter was immersed in the sample solution, and the pH value was recorded once the indicator stabilized on the screen (Dwiloka *et al*., 2022).

2.5.4 Moisture content

Measurement of the moisture content of MOLP cereal granules was carried out using a moisture content analyzer with a sample amount of 5 grams for each test (Rani *et al*, 2019).

2.5.5 Total alkaloids

MOLP cereal granules (50 mg) were added to 5 mL of 2N HCl, shaken, and then filtered. The solution was washed three times with 10 mL of chloroform in a separating funnel, and the chloroform phase was discarded. The solution was neutralized by adding 0.1 N NaOH, and then 5 mL of BCG solution and 5 mL of Phosphate Buffer were added. After that, the solution was extracted with 5 mL of chloroform and stirred with a magnetic stirrer at 500 rpm for 15 mins. The sample extraction with chloroform was repeated twice. The chloroform phase was collected and evaporated using nitrogen gas, then chloroform was added to bring the volume to 5 mL. The absorbance of the sample was read at a wavelength of 470 nm Meanwhile, A quinine calibration curve was constructed using standard solutions with concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 μg/mL. The experiment was performed in triplicates (Khairani *et al*., 2024).

2.5.6 Total flavonoids

MOLP cereal granules (50 mg) were mixed with 0.3 mL of 5% sodium nitrite ($NaNO_2$) and allowed to react for 5 mins. Subsequently, 0.6 mL of 10% aluminum nitrate ($Al(NO_3)_3$) was added, followed by another 5-min incubation. Next, 2 mL of 1 M sodium hydroxide (NaOH) was introduced, and the volume was adjusted to 10 mL with distilled water. The mixture was then diluted five-fold, and absorbance was measured at 510 nm. Meanwhile, A quercetin calibration curve was constructed using standard solutions with concentrations of 0.5, 1, 2, 5, 10, 25, 50, 75, and 100 μg/mL. The experiment was performed in triplicates (Zan *et al*., 2022).

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2.5.7 Total phenolic

MOLP cereal granules (50 mg) were added 0.5 mL of Folin-Ciocalteu phenol reagent along with 7.5 mL of distilled water (aquadest). Thoroughly mix the solution and allow it to stand for 10 mins. Then, add 1.5 mL of 20% sodium carbonate solution, mix again, and let it rest for another 10 mins. Finally, adjust the volume to 10 mL with distilled water, dilute the mixture by a factor of 20, and measure the absorbance at a wavelength of 760 nm. Meanwhile, A gallic acid calibration curve was constructed using standard solutions with concentrations of 0.5, 1, 2, 5, 10, 25, 50, 75, and 100 µg/mL. The experiment was performed in triplicates (Sulastri *et al*., 2018).

2.5.8 Fatty acid analysis

The MOLP cereal granules were converted into fatty acid methyl esters (FAME) for fatty acid analysis. To prepare the sample, 2 mL of 0.5 M NaOH was added and heated in a water bath at 100°C for 20 mins. After cooling, a solution of 14% BF_3 in methanol was added, and the mixture was reheated at 100°C for another 20 mins. Once cooled, the mixture was shaken until it reached 30°C, followed by the addition of 2.0 mL of saturated NaCl. The solution was vortexed for approximately 2 mins, then n-hexane was added, and the mixture was vortexed again for 2 mins. The solution was allowed to settle at room temperature, and the hexane layer containing the methyl esters was collected and transferred to a 10 mL volumetric flask, where it was diluted with n-hexane. Before injecting the n-hexane layer of methyl esters into the gas chromatography for fatty acid composition analysis, a first solution of FAME from external standard was diluted and added to the 10 mL flask containing a standard solution of 500 mL. Finally, 1 mL of the standard solution was injected into the gas chromatography, followed by the injection of 1 mL of the sample solution (Jamaluddin *et al*., 2016).

2.6 Proximate analysis of cereal granule of Moringa oleifera leaf powder

Proximate analysis of cereal granules was conducted according to the quality requirements of the Indonesian National Standard (SNI 01-4270-1996) regarding cereal (National Standardization Agency, 1996). Parameters analyzed include protein content, fat, crude fiber, carbohydrate, ash content, and water content. The nutrient content was determined following the procedures outlined in SNI 01-2891-1992 (National Standardization Agency, 1992).

2.6.1 Protein

MOLP cereal granule (1 g) was accurately measured and placed into a 100 mL Kjeldahl flask, where 5 g of a selenium catalyst mixture and 25 mL of concentrated H_2SO_4 were added. The mixture was heated using an electric heater or open flame until boiling, resulting in a clear greenish solution. The solution was then allowed to cool, diluted with distilled water, and transferred to a 100 mL volumetric flask, ensuring the final volume reached the calibration mark. For blank preparation, 25 mL of concentrated H_2SO_4 was mixed with 150 mL of distilled water and 3 drops of phenolphthalein (PP) indicator. Subsequently, 5 mL of the sample solution was pipetted into the distillation apparatus, followed by the addition of 5 mL of 30% NaOH and several drops of PP indicator. Distillation was performed for 10 mins, with the distillate collected in a container containing 10 mL of 2% boric acid solution mixed with an indicator. The condenser outlet was rinsed with distilled water to ensure accuracy. The same distillation procedure was applied to the blank sample. Finally, the collected distillate was titrated using a 0.01 N HCl solution to determine the analyte concentration. The protein was calculated using the equation 1:

$$Protein\ Content = \frac{(\text{volume of titration sample} - \text{titration blank}) \times \text{N HCl} \times 0.014 \times \text{conversion factor (6.25)} \times \text{dilution factor}}{\text{Sample weight}} \times 100\% \quad (1)$$

2.6.2 Fat

The fat content of MOLP cereal granules was determined using the Soxhlet extraction method. The sample was weighed about 5 g and placed into a paper sleeve lined with cotton. The paper sleeve containing the sample was sealed with cotton and dried in an oven at a temperature not exceeding 80°C for approximately 1 hr. The dried sample was then transferred into a Soxhlet apparatus equipped with a fat flask containing boiling stones that had been pre-dried and weighed. The extraction was carried out using hexane or another fat solvent for approximately 6 hrs. Following extraction, the hexane was distilled, and the fat extract was dried in an oven at 105°C. The extract was then cooled, weighed, and subjected to repeated drying cycles until a constant weight was achieved. The fat content was calculated using the equation 2:

$$\text{Fat Content} = \frac{\text{Weight of sohxlet flask and fat after extraction} - \text{weight of flask before extraction}}{\text{Sample weight}} \times 100\% \quad (2)$$

2.6.3 Crude fiber

The MOLP cereal granules were carefully measured between 1-2 g. The fat was removed either by Soxhlet extraction or by stirring the sample in an organic solvent three times. The defatted sample was then dried and transferred into a 500 mL Erlenmeyer flask. A total of 50 mL of 1.25% H_2SO_4 solution was added, and the mixture was boiled for 30 mins using a vertical condenser. Subsequently, 50 mL of 3.25% NaOH solution was added, and the mixture was boiled again for another 30

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mins. While still hot, the mixture was filtered using a Buchner funnel containing Whatman filter paper that had been pre-dried and weighed. The residue retained on the filter paper was successively washed with hot 1.25% H_2SO_4, hot water, and 96% ethanol. The filter paper and its contents were then carefully removed and placed in a pre-weighed drying box. The sample was dried at 105°C, cooled, and weighed repeatedly until a constant weight was achieved. The crude fiber content was then calculated using Equation 3:

$$\text{Crude fiber} = \frac{\text{Residue weight on filter paper}}{\text{Sample weight}} \times 100\% \quad (3)$$

2.6.4 Carbohydrate

The analysis of carbohydrate content was conducted using the "by difference" method, which involves subtracting the sum of the moisture, ash, protein, and fat content from 100%. The carbohydrate was calculated using equation 4:

Carbohydrate = 100% - (% water content + % ash content + % protein content + % fat content (4)

2.6.5 Ash content

The MOLP cereal granules were weighed into a porcelain or platinum crucible of known weight. Then, the dried sample was charred over a burner flame before being ashed in an electric furnace at a maximum temperature of 550°C until complete ashing was achieved. During ashing, the furnace door was occasionally opened slightly to allow oxygen to enter. After ashing, the crucible was cooled in a desiccator and weighed repeatedly until a constant weight was obtained. The ash content was calculated using Equation 5:

$$\text{Ash content} = \frac{\text{Total weight of ashes and crucible} - \text{empty crucible}}{\text{Sample weight}} \times 100\% \quad (5)$$

2.6.6 Water content

The water content of MOLP cereal granules was determined using the oven method. First, an empty crucible was dried in the oven for at least 2 hrs. After that, it was moved into a desiccator for 30 mins until reached room temperature, and then the empty crucible was weighed. Furthermore, the sample was precisely measured in a crucible of known weight. The sample was then dried in an oven at 105°C for 3 hrs. After drying, it was cooled in a desiccator and weighed. The drying process was repeated until a constant weight was achieved. The water content was then calculated using Equation 6:

$$\textit{Water content} = \frac{\text{Total weight of dry sample and crucible} - \text{empty crucible}}{\text{Total weight of sample and dry crucible}} \times 100\% \quad (6)$$

2.7 Antioxidant activity

The antioxidant activity of cereal granules of MOLP against the DPPH radical was determined in our previous study. About 10 mg extract was dissolved in 10 mL ethanol p.a (1,000 µg/mL) and then diluted to reach the concentration series of 7.8125, 15.625, 31.25, 62.5, and 125 µg/mL. About 0.1 mM DPPH was added to each sample solution with a series of concentrations of 100, 200, 400, 800, and 1000 µg/mL) (1:1). After 30 mins incubation, the absorbance of each mixture was measured at 515 nm on spectrophotometry UV-Vis. The blank solution (ethanol and DPPH) and ascorbic acid with the series concentration of 2, 4, 6, 8, and 10 µg/mL as positive control were also prepared and measured at the same wavelength. The experiment was carried out in triplicates. The percentage of inhibition was calculated and further analyzed to determine the IC_{50} (Sulastri *et al*., 2018).

2.8 Statistical analysis

Statistical analysis tests were conducted using the Kruskal-Wallis method. If there is a significant difference in the effect of treatment from the results of the analysis, then continue with the Mann-Whitney test. Calculations were performed using SPSS 17.0 (SPSS. Inc, USA).

3. Results

The resulting *M. oleifera* leaf powder (MOLP) is dark green in color, has a plant-like aroma, and has a bitter taste (Figure 1). Phytochemical screening results indicated the presence of alkaloids, steroids, flavonoids, saponins, and tannins.

Figure 1. *Moringa oleifera* leaf powder.

3.1 Physical evaluation of cereal granule of Moringa oleifera leaf powder

Organoleptic evaluation of F1-F4 (Figure 2) showed the color dark green. Based on the data presented in Table 1, no significant differences ($P > 0.05$) were observed among all formulas in the hedonic test for color, aroma, taste, and texture. Although the color attribute did not differ significantly ($P > 0.05$) between the formulas, there was a tendency for higher scores with increasing MOLP concentration. Similarly, the aroma attribute showed no significant differences ($P > 0.05$) among the formulas. Overall, other sensory attributes, such as taste and texture, exhibited similar trends, likely because the ingredients influencing these attributes remained unchanged in concentration. Despite these findings, all attributes were generally well-received by the panelists. However, the physical evaluation showed that F1 has a higher value of specific gravity and moisture content suggesting further analysis (Table 2).

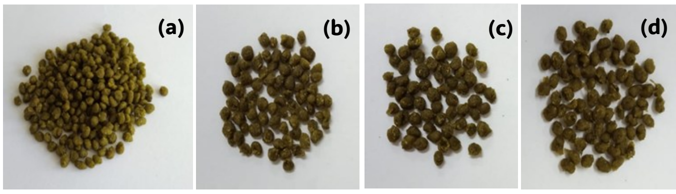

Figure 2. Product of cereal granule of MOLP 5% with variation of temperature (a) Formula 1 at 50°C, (b) Formula 2 at 55°C, (c) Formula 3 at 60°C, (d) Formula 4 at 65°C.

Table 1. Hedonic organoleptic test scores.

Parameters	F1	F2	F3	F4
Color	3.6±0.51[a]	3.5±0.52[a]	3.4±0.51[a]	3.3±0.48[a]
Aroma	3.7±0.48[a]	3.6±0.51[a]	3.5±0.52[a]	3.4±0.51[a]
Taste	3.5±0.52[a]	3.4±0.51[a]	3.4±0.51[a]	3.3±0.67[a]
Texture	3.5±0.52[a]	3.2±0.63[a]	3.2±0.63[a]	3.2±0.63[a]

Values are presented as mean±SD (n = 3). Values with different superscripts in the same row are statistically significant difference (P > 0.05) between formula at the Mann -Whitney test level.

Table 2. Analysis of specific gravity, pH and moisture content.

Sample	Specific gravity (g/mL)	pH	Moisture content
F1	1.00±0.02[a]	6.29±0.14[a]	3.16±0.28[a]
F2	0.94±0.03[a]	6.56±0.43[a]	2.50±0.50[a]
F3	0.96±0.08[a]	6.53±0.04[a]	2.16±0.28[a]
F4	0.84±0.07[a]	6.37±0.58[a]	3.16±0.28[a]

Values are presented as mean±SD (n = 3). Values with different superscripts in the same row are statistically significant difference (P > 0.05) between formula at the Mann -Whitney test level.

3.2 Proximate analysis of cereal granule of Moringa oleifera leaf powder

The proximate analysis test of MOLP cereal granules was carried out on F1. F1 was chosen based on the highest average score value of hedonic organoleptic and physical evaluation tests. The proximate analysis showed that F1 met the requirements for nutritional contents except for fat and crude fiber (Table 3).

3.3 Phytochemical and fatty acids analysis of cereal granule of Moringa oleifera leaf powder

The phytochemical analysis showed that F1 contained alkaloids with a value of 334.08 µg/g quinine equivalent, and its total phenolic value was higher than the total flavonoid (Table 4). Meanwhile, the fatty acid components were successfully identified 17 saturated fatty acids and 20 unsaturated fatty acids (Figure 3).

3.4 Antioxidant analysis of cereal granule of Moringa oleifera leaf powder

The antioxidant activity of F1 showed that the 50% inhibition of DPPH radical appears in concentrations above 100 µg/mL, possessing the IC_{50} value of 150.51 µg/mL that was categorized as moderate activity (Table 5).

4. Discussion

Antioxidants are compounds capable of slowing down the oxidation process initiated by free radicals (Ratnayani *et al*., 2012). An increase in free radicals within the body can lead to oxidative stress, which damages cell structures, fatty tissues, proteins, the immune system, and DNA. This condition can contribute to several degenerative diseases, including cancer, diabetes, inflammation, and cardiovascular problems. Therefore, meeting antioxidant requirements through the

Table 3. Proximate analysis of cereal granule of *Moringa oleifera* leaf powder (F1).

Parameters	Concentrations			Mean value±SD (n = 3)	SNI-01-4270-1996 (%)
	I (%)	II (%)	III (%)		
Carbohydrate	83.343	82.858	83.048	83.083±0.244	Min 60.0
Protein	10.18	10.33	10.46	10.323±0.140	Min 5
Fat	2.977	3.312	3.492	3.260±0.261	Min 7.0
Crude fiber	0.99	0.97	0.96	0.975±0.126	Max 0.7
Water	2.5	3	2.5	2.667±0.288	Max 3.0
Ash	1	0.5	0.5	0.667±0.288	Max 4

Table 4. Phytochemical analysis of cereal granule of Moringa oleifera leaf powder (F1).

Formula	Total alkaloids (µg/g QE)	Total phenolics (%w/w GAE)	Total flavonoids (%w/w QE)
F1	334.08	4.05	2.60
	334.09	4.05	2.60
	334.07	4.05	2.60
Mean value±SD (n=3)	334.08±0.0099	4.05±0.000049	2.60±0.0000055

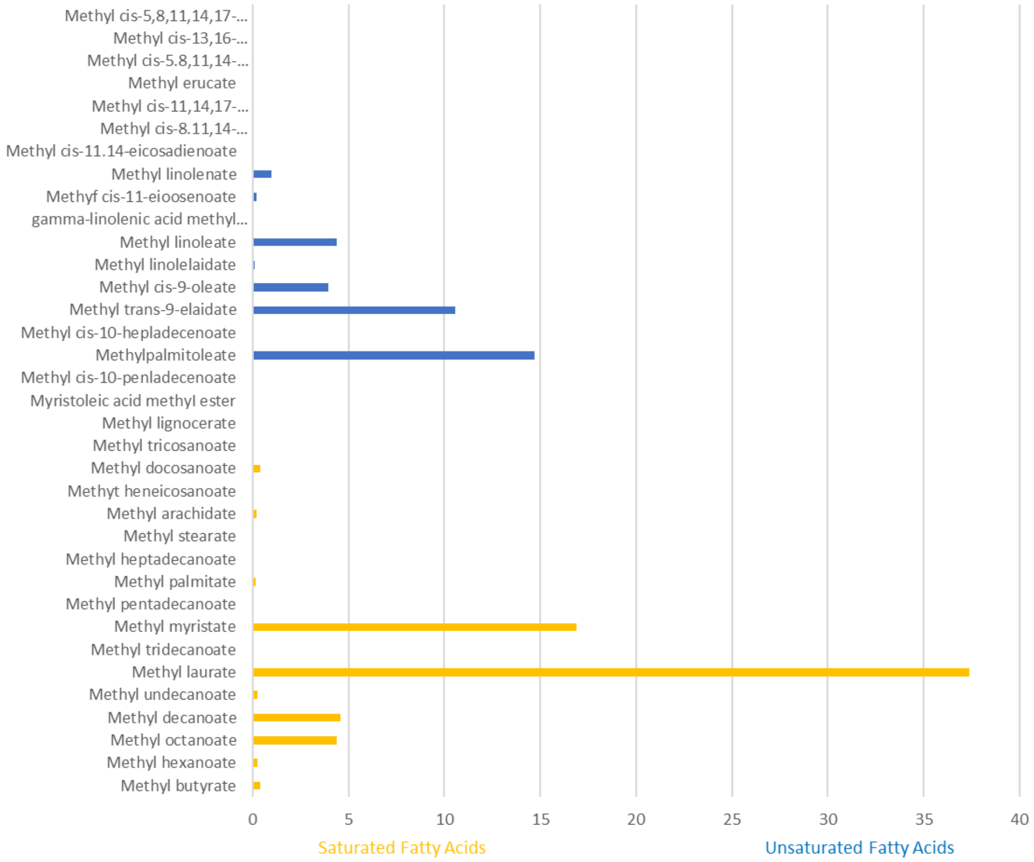

Figure 3. Fatty acids analysis of cereal granule of *Moringa oleifera* leaf powder (F1).

Table 5. Antioxidant analysis of cereal granule of *Moringa oleifera* leaf powder (F1).

Sample	Conc. (µg/mL)	Replication I	Replication II	Replication III	Mean value±SD (n=3)	Inhibition (%)	IC_{50} (µg/mL)
Formula1	1000	0.553	0.552	0.549	0.551±0.002	60.5915	150.510
	800	0.561	0.581	0.569	0.570±0.010	56.0352	
	400	0.572	0.569	0.589	0.576±0.010	54.5164	
	200	0.577	0.588	0.591	0.585±0.007	52.4382	
	100	0.595	0.618	0.610	0.607±0.011	47.0823	
Vitamin C	10	0.539	0.538	0.539	0.538±0.000	63.6290	7.456
	8	0.567	0.567	0.566	0.566±0.000	53.5750	
	6	0.629	0.629	0.629	0.629±0.000	41.9660	
	4	0.655	0.654	0.654	0.654±0.000	30.9220	
	2	0.721	0.722	0.723	0.722±0.001	19.6640	

consumption of antioxidant-rich foods is crucial (Tukiran *et al*., 2020). *Moringa oleifera* is known to contain high levels of polyphenols and flavonoids, which contribute to its strong antioxidant activity. Additionally, *M. oleifera* leaves are rich in minerals, essential amino acids, vitamin C, vitamin E, and other secondary metabolites that act as antioxidants (Asisi *et al*., 2021). Phytochemical screening tests have shown that the powder of *M. oleifera leaves* is positive for alkaloids, steroids, flavonoids, saponins, and tannins, indicating their potential as functional foods with antioxidant properties.

In evaluating food products, organoleptic testing is essential to measure the level of acceptance of a product. A questionnaire is used as a tool, comprising a list of questions to be answered by the respondents being evaluated. In this case, the organoleptic test utilized was the hedonic test to assess the MOLP-supplemented cereal granules (Govender and Siwela, 2020). The hedonic organoleptic test is a crucial method in food product evaluation. The purpose is to gauge consumer acceptance or preference for a product. This organoleptic assessment encompasses various aspects such as color, aroma, taste, and texture. In a hedonic test, panelists are asked to respond based on a scale of preference, ranging from "really like," "like," "neutral," "dislike," to "really dislike." This information is valuable for researchers to determine whether their products are well-received by the panelists (consumers). Formula F1 received the highest score for color, aroma, taste and texture, while

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F4 received the lowest score (Table 1). Statistical analysis using the Kruskal-Wallis method showed that there is no significant difference among the formulas. All formulas produced a dark green color, with the highest color acceptance of 3.6 (F1) achieved with the addition of 5% MOLP at a drying temperature of 50°C. The similarity in taste may be attributed to the consistent use of ingredients in the cereal granule formula that exhibit a slightly bitter taste.

Further physical characteristics were specific gravity, pH and moisture content (Table 2). It was found also that all formulas are in accordance with the reference. The specific gravity test results for each formula revealed the following average values: F1 had a specific gravity of 1.00, F2 measured 0.94, F3 recorded 0.96, and F4 showed 0.84. A Kruskal-Wallis analysis indicated no significant differences ($P > 0.05$) in the specific gravity of the cereal granules produced. Notably, F1 met the standard requirements outlined by Citra Rani et al. (2021), which state that the acceptable range for specific gravity is between 1.00 and 1.25 g/mL. The pH is in the range of 5.0-6.5 which indicates that cereal preparation with low temperature maintains the chemical stability of phenolic and flavonoid compounds found in *M. oleifera* leaves, which are known for their benefits in protecting against chronic diseases associated with oxidative stress mechanisms. The moisture content in granules is also allowable (<5%). Adequate moisture content is necessary for particles to bond via liquid bridges and prevent component separation (Sun, 2008; Rani *et al*., 2021).

Regarding the proximate analysis (Table 3), Formula F1 meets the Indonesian National Standard (SNI) requirements, except for fat and crude fiber contents. This discrepancy is due to the naturally low-fat content of *M. oleifera* leaves, which contain 1.7% fat when fresh and 2.3% after powdering (Gopalakrishnan *et al*., 2016). Meanwhile, the increase in crude fiber is attributed to the addition of sorghum flour, known for its higher fiber and mineral content compared to wheat flour (which contains approximately 2.74% fiber) (Meilinda and Batubara, 2021). Another study indicates that sorghum flour typically contains between 2.76% and 3.96% crude fiber, with higher proportions leading to the increased fiber content in the resulting cereal granules (Mohapatra *et al*., 2019). The phytochemical analysis found that formula F1 still has high total alkaloids, total phenolics and total flavonoids (Table 4). Meanwhile, the analysis of fatty acids found that there are 17 saturated fatty acids and 20 unsaturated fatty acids. Among them, methyl laurate, methyl myristate and methyl palmitoleate were the highest concentrations of 37.40%, 16.87% and 14.72%, respectively (Figure 3). Our previous study of *M. oleifera* metabolite compounds found that alkaloid and aromatic fatty acids may be responsible for the prevention of stunted growth in children by interaction with albumin protein receptor (ALB) (Arwansyah *et al*., 2023). In addition, several fatty acids were also found to significantly improve children's growth in clinical tests (Adjepong *et al*., 2018). The antioxidant properties of Formula F1 showed moderate activity with IC_{50} of 150.51 µg/mL (Table 5). This antioxidant activity was better than other MOLP products such as bread and biscuits that are processed at high temperatures (Ferreira *et al*., 2023). This result indicates the importance of using low temperature to produce the granule cereal of 5% MOLP (F1) that has moderate antioxidant activity and meets the physical and nutritional composition. Further in vivo and clinical studies are required to validate the results to address the stunted growth problems in children.

5. Conclusion

In conclusion, Formula F1 is the best product based on the physical characteristics of cereal granules with a specific gravity of 1.00 g/mL, pH test of 6.29 and moisture content of 3.16%. Formula F1 have also complete nutritional content although there are two nutrients that still have different levels from the provisions of the Indonesian National Standard (SNI) where fat content is lower than 7.0%, (3.260 %) and crude fiber content greater than 0.7% (0.975 %). The high total alkaloids, total phenolics, total flavonoids, and fatty acid components, as well as its moderate antioxidant activity, support the application of Formula 1 for treating stunting in children.

Conflict of interest

The authors declare no conflict of interest.

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