Antioxidant Activity and Quality of Frozen Yoghurt with *Moringa oleifera* Lamk. Leaves Extract

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ABSTRACT

Moringa oleifera Lamk. (Moringa) is a plant widely distributed across various regions in Indonesia. The leaves of *Moringa oleifera* Lamk. contain higher nutritional value in protein and minerals compared to the green vegetables that are commonly consumed. This plant can be used to improve the quality of frozen yoghurt, a functional food that is currently popular due to the existence of Lactic Acid Bacteria (LAB), which is good for digestion. Therefore, this research aimed to improve the nutritional quality of frozen yoghurt using *Moringa oleifera* Lamk. Leaves Extract (MOLE), which is high in antioxidants and protein. However, MOLE does not reduce the viability of LAB. The viability of LAB decreases when phytochemical content with the ability as an antibacterial is added. The soluble fiber content of MOLE is able to improve the texture of frozen yoghurt. In this research, four different frozen yoghurt samples were produced with the addition of MOLE in various ratios (0%, 10%, 20%, and 30%). The analysis shows that frozen yoghurt with 30% MOLE produces the highest antioxidant activity and viability of probiotics. The content of product with 30% MOLE includes 2.85±0.13% fat, 10.07±0.44% protein, 1.10±0.01% ash, 26.4±0.35% total solid (TS), 21.67±0.58% total soluble solid (TSS), 78.66±1.31% overrun, 807±5.77 (s) melting rate, 0.86±0.02% titratable acidity, and 5.4±0.02 pH. It also contains 8.58±0.52 log CFU/mL LAB, *Salmonella* negative/25 g, 102.26±0.13 mg GAE/100 mL of total phenolic, and 87.78±0.64% of antioxidant activity.

Keywords: Antioxidant capacity; frozen yoghurt; *Moringa oleifera* Lamk. leaves; viability probiotic

INTRODUCTION

Frozen yoghurt is characterized by the physical appearance of ice cream with a distinctive taste. The production stage consists of mixing yoghurt, thickener, and stabilizer (Hui *et al.*, 2004). Currently, frozen yoghurt has become a popular product due to the presence of probiotics and the lower fat contents compared to ice cream (Mahrous and Salam, 2016). However, consuming this product increases the consumption of sugar because of the addition of toppings with high sugar content (An and Jiang, 2017), and further, it has lower nutrition of vitamins and minerals compared to ordinary yoghurt. Due to the lack of nutrition, the improvement of frozen yoghurt can be made with the addition of *Moringa oleifera* Lamk. (Moringa) Leaves Extract (MOLE).

Moringa oleifera Lamk. leaves are rich in nutrients. Further, it increases spermatogenesis (Widiastini *et al.*, 2022), prevents degenerative diseases (Islam *et al.*, 2021), and has a significant therapeutic effect on human health (Patil *et al.*, 2022). These leaves contain antioxidant compounds carotenoids of 34.000 mg/kg ($_{db}$) (Nobossé *et al.*, 2018) and beta-carotene of 234.03 mg/kg ($_{db}$) (Owusu *et al.*, 2011). *Moringa oleifera* Lamk. leaves also contain flavonoid and total phenolic compounds, with greater beta-carotene compared to carrots 62.28 mg/kg (Imsic *et al.*, 2010). In comparison, *Moringa oleifera* Lamk. leaves have 415 μg/mL of flavonoid and 60 μg/mL of total phenolic content (TPC),

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while green spinach contains 155 μg/mL and 5 μg/mL, respectively (Raghavendra *et al*., 2013). The chlorophyll content of moringa leaves is higher than the chlorophyll content of spinach leaves. Fresh and powdered moringa leaves have a chlorophyll content of 80 and 1268 mg/100g, respectively (Kashyap *et al*., 2022). Spinach leaves have a chlorophyll content of approximately 60 mg/100g (Leite *et al*., 2018). Moringa leaves also have a high protein content, calcium (Angelina *et al*., 2021), dietary fiber (Milla *et al*., 2021), iron (Peñalver *et al*., 2022), and ash (Nisha *et al*., 2012), but low fat (Milla *et al*., 2021). Therefore, this research aims to improve the nutritional quality of frozen yoghurt through the addition of MOLE.

Antioxidant content of *Moringa oleifera* Lamk. leaves can be reduced due to the susceptibility to high temperatures (Kanika *et al*., 2015). According to Nazarni *et al*. (2016), the fermentation process with lactic acid bacteria (LAB) in frozen yoghurt can increase the content of flavonoids and polyphenols. The addition of phenolic compounds as antioxidants tends to decrease the LAB (Jaffar *et al*., 2024). The LAB in frozen yoghurt products is tolerant to acidic conditions and capable of improving the absorption of iron in the body (Siebenthal *et al*., 2023). Despite the numerous advantages, there is a paucity of scientific information regarding the potential of MOLE in frozen yoghurt to enhance LAB viability and antioxidant activity. Therefore, this research aims to investigate the potential of making frozen yoghurt with high LAB and antioxidant activity.

METHODS

Materials

The experiment was carried out in the food research and production laboratory of the Faculty of Biotechnology of Universitas Atma Jaya Yogyakarta, Yogyakarta, Indonesia. *Moringa oleifera* Lamk. leaves were collected from Martani.co Garden located in Kalasan, Yogyakarta, Indonesia. Pure isolates of *Lactobacillus acidophilus* FNCC 0051, *Lactobacillus bulgaricus* FNCC 0041, and *Streptococcus thermophilus* FNCC 0040 in agar preparations were obtained from Microbiology Laboratory of Food Nutrition of Universitas Gadjah Mada, Yogyakarta, Indonesia. "Lactona" Skim milk, "Dancow" full cream milk, "Gulaku" sugar, and CMC were used in this research.

The materials for analysis are 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) powder, gallic acid powder, Na_2CO_3, Folin-ciocalteu reagent (purchased from Sigma-Aldrich St. Louis, MO, USA), ascorbic acid powder Sigma-Aldrich (Steinheim, Germany), diethyl ether (purchased from Merck Stockholm, Sweden), hexane (purchased from J.T. Baker USA), hydrocloric acid 37%, 96% of ethanol Merck (Damstadt, Germany), methanol PA (purchased from Sigma-Aldrich Damstadt, Germany). Buffer peptone water, Lactose Broth (LB), Selenite Cystine Broth (SCB), Salmonella Shigella Agar (SSA), Triple Sugar Iron Agar (TSIA), Man Rogosa Sharpe (MRS) Agar, and Man Rogosa Sharpe (MRS) Broth were purchased from Oxoid (Basingstoke, England).

Preparation and Extraction of *Moringa oleifera* Lamk. Leaves

Moringa oleifera Lamk. leaves was collected from Martani.co Garden located in Kalasan, Yogyakarta, Indonesia. The criteria of the leaves are dark green color and undamaged condition. The leaves were harvested 1 day before treatment. Subsequently, *Moringa oleifera* Lamk. leaves were blanched for 1 minute at 70°C and reduced in size using a blender. This was followed by the addition of water solvent with a ratio of leaves to water of 1:10 in the extraction process for 3 minutes at room temperature. *Moringa oleifera* Lamk. leaves were filtered to get an aqueous extract, which was stored in a bottle covered with a lid.

The Making of Yoghurt Starter and Plain Yoghurt

Yoghurt starters and plain yoghurt were made using the method suggested by Trisnaningtyas *et al*. (2013) with a slight modification. Pure cultures of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* bacteria were taken with an ose. Each of the pure cultures was put into 10 mL of sterile MRS broth and incubated at 37°C for 24 h. The bacterial cultures were prepared to make a starter. The ratio of LAB on MRS Broth is 1: 1: 1, and the amount of each LAB is 9% of the 200 mL water. Then, LAB was inoculated into 100 mL sterile skim milk and incubated at 37°C for 24 h to form a yoghurt starter. Subsequently, the yoghurt starter in the amount of 9% of 200 mL water, 15% skim milk, and 1% corn starch were incubated at 37°C for 24 h to make plain yoghurt which would be added to the frozen yoghurt mixture.

The Making of Frozen Yoghurt with MOLE Variations

MOLE frozen yoghurt was made according to the methods suggested by Hui *et al*. (2004) with a slight modification. A 2 g full cream milk, 14 g skim milk, 4 g sugar, and 0.35 g CMC were mixed with boiled water and heated at 40 – 50°C. Then, 0%, 10%, 20%, and 30% of MOLE were added. After that, it was pasteurized at 70°C for 10 minutes. The mixture was cooled to 43°C, added with 50 g plain yoghurt, placed in a sterile closed container, and incubated at 4°C for 4 h. The incubated mixture was put into an ice cream maker (ICM) for the dynamic freezing process and stored in a freezer for 24 hours for the hardening process.

Determination of Total Phenolic Content (TPC)

TPC of frozen yoghurt and MOLE was quantified using Folin-Ciocalteu methods of Barku *et al*. (2013) with a slight modification. A 1 mL of frozen yoghurt and MOLE was diluted in 9 mL distilled water and centrifuged at 14.000 rpm for 15 minutes. A 1 mL supernatant was mixed with 5 mL Folin-Ciocalteu reagent, incubated for 3 min, and added with 4 mL of 7.5% Na_2CO_3 solution and incubated in the dark at 25°C for 1 h. The mixture absorbance was measured by UV-Vis Spectrophotometer at 765 nm. TPC measurement was done in triplicate and measured in mg GAE/g.

Antioxidant Activity Assay

The antioxidant activity of frozen yoghurt and MOLE was determined using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), as described by Rauf *et al*. (2010). Antioxidant activity was measured on the basis of the ability of the antioxidant to scavenge DPPH cation radicals. The ability of antioxidants to donate hydrogen atoms or electrons changed the purple color of the DPPH solution into yellow pale. DPPH powder was diluted in methanol. A 1 mL frozen yoghurt and MOLE were diluted in 9 mL distilled water and centrifuged at 14.000 rpm for 15 minutes. A 1 mL supernatant was mixed with 3 mL DPPH solution and vortex vigorously. The reaction tubes were incubated in a dark room for 30 minutes at a room temperature. The discoloration of DPPH was measured at 517 nm using a UV-Vis Spectrophotometer. The assessment of antioxidant activity was repeated three times. The antioxidant value was calculated by dividing the absorbance difference of the blank from the test sample by the absorbance of the blank and then multiplied by 100%.

Chemical Analysis

Chemical analysis of MOLE and frozen yoghurt were conducted using (AOAC, 1995) for ash and fat content. Protein analysis was conducted using AOAC (1990). The acidity was measured using a pH meter (AOAC, 1975), titratable acidity was measured using the method according to Sia (2014), total solid (TS) was measured using a *thermogravimeter* according to Askar and Sugiarto (2003), and total soluble solid (TSS) was measured using a refractometer (Magwasa and Opara 2015).

Physicochemical Analysis

Overrun was measured by dividing the difference between the initial volume from the expanded volume by the initial volume of the frozen yoghurt and then multiplied by 100% (Marshall and Arbuckle 2000). The melting rate was determined according to the methodology proposed by Clark *et al*. (2009), where 10 g frozen yoghurt was placed in a closed petri dish at room temperature (25°C). The melting rate was recorded using a stopwatch and measured in seconds.

Microbiological Analysis

LAB count was performed using the pour plate method (Robertson 1993) with a slight modification. The frozen yoghurt samples were diluted in sterile 2% buffer peptone water and incubated in Man Rogosa Sharpe (MRS) Agar combined with 0.5% $CaCO_3$ solution. The plates were incubated at 37°C for 48 h, and the number of colonies was calculated using the Aerobic Plate Count (APC) method (CFU/mL). *Salmonella* was enumerated using the streak plate method (Hocking *et al*. 1997). A 25 g frozen yoghurt sample was added to lactose broth and incubated at 37°C for 24 h. A 1 mL sample was inoculated in Selenite Cystine Broth and incubated at 37°C for 48 h. The sample was inoculated in *Salmonella Shigella* Agar (SSA) using the streak plate method and incubated at 37°C for 24 h. *Salmonella* colony appeared transparent with black centers.

Hedonic Test

Hedonic testing included aroma, taste, color, and texture properties. 30 untrained panelists (15 men and 15 women) completed the 4-point hedonic scaling questionnaire. Score 4: like very much, 3: like, 2: like slightly, 1: dislike (Tjokrokusumo *et al*., 2019).

Data Analysis

All samples were made and analyzed in triplicate. The data was analyzed using a one-way Analysis of Variance (ANOVA) for a completely randomized design. If a significant difference was found, the Duncan Multiple Range Test (DMRT) with a confidence level of 95% was conducted using the SPSS program ver. 17 (George and Mallery, 2018).

RESULTS AND DISCUSSION

Proximate and Antioxidant Value of MOLE

The proximate and antioxidant values of MOLE are shown in Table 1. This research finds that MOLE contains 2.58% fat, 9.63% protein, 2.55% ash, 9.19 mg GAE/100 g TPC, and an antioxidant activity of 77.82%. It shows that MOLE is a healthy foodstuff, and it is

Table 1. Nutritional properties of MOLE

Parameter	Value
Fat (%)	2.58±0.56
Protein (%)	9.63±0.88
Ash (%)	2.55±0.19
Moisture (%)	75.33±0.79
Total phenolic content (mg GAE/100 g)	9.19±0.06
Antioxidant Activity (%)	77.82±0.20

Values are presented as mean ± standard deviation (n= 3).

suitable as a supplementary material in frozen yoghurt production.

Chemical Value of Frozen Yoghurt With MOLE

The chemical values of frozen yoghurt with MOLE variations are shown in Table 2. Overall significant differences of protein, fat, and ash contents are found between control (0%) and frozen yoghurt with MOLE variation (10%, 20%, 30%). Control (0%) has the lowest value of protein, fat, and ash content compared to the samples with MOLE variations. The values of TS, TSS, titratable acidity, and pH are 24.24 – 26.40%, 20.00 – 21.67%, 0.81– 0.86%, and 5.48 – 5.40, respectively. The higher addition of MOLE in frozen yoghurt increases the values of TS, TSS, and titratable acidity but decreases the value of pH.

The fat content of frozen yoghurt ranges from 2.10 – 2.85%. The control has 2.10% fat. According to Tamime and Robinson (2000), frozen yoghurt has a fat content between 2% to 6%. The frozen yoghurt control meets the requirements. The results of this study are higher than pure skim yoghurt (1 %) because this research uses a little bit of full cream milk as an ingredient. Nevertheless, this result is lower than full cream yoghurt by 4 % (D'Andrea *et al*., 2023). The fat content of frozen yoghurt lubricates and increases the richness of flavor (Goff and Hartel 2013). However, an ingredient with a high-fat content is not recommended because it produces high calories. Therefore, low-fat frozen yoghurt was made for this study. The addition of MOLE with variations increases the fat content because MOLE has 2.58% fat content. Dried Moringa leaves contain fat approximately 5-7 % (Cuellar-Nuñez et al., 2017). The fat content of MOLE is a suitable type of fat in the body because it is composed mainly of unsaturated fatty acids, specifically oleic and palmitic acids (Lee *et al*., 2017).

The protein content of frozen yoghurt ranges from 5.57 – 10.07%. The control sample has a protein content of 5.57% stemming from casein in milk. The protein content of yogurt is approximately 4.5% (D'Andrea *et al*., 2023), lower than that of MOLE. MOLE contains 9.63% protein. The dominant amino acids in Moringa leaves are glutamic acid, leucine, and aspartic acid (Cattan *et al*., 2022). The addition of MOLE increases the protein content. Frozen yoghurt with the 30% treatment has the highest protein content. The protein of frozen yoghurt acts as an emulsifier and water-binder agent (Goff and Hartel 2013). In the making of frozen yoghurt, an aeration process infiltrates the air in a frozen yoghurt mixture. The process brings about a 50% expansion of the volume. Protein is an active surface component that stabilizes air and water expansion (Fox and McSweeney 2003).

Ash content is an essential parameter in determining the nutritious quality of food products. Frozen yoghurt control has a 1.01% ash content. The ash content of the control is contributed by the milk as the main ingredient. MOLE has 2.55% ash content. The dominant macromineral content in Moringa leaves is calcium, which is 907.7 mg/100g; the dominant micromineral content in Moringa leaves is iron, which is 3.7 mg/100g (Cattan *et al.,* 2022). The addition of MOLE increases the ash content of frozen yoghurt. According to Lawalata *et al*. (2004), ingredients containing high ash increase the ash content of the product.

The pH and titratable acidity value are important factors in determining the quality of frozen yoghurt

Table 2. Chemical value of frozen yoghurt with MOLE

MOLE (%)	Fat (%)	Protein (%)	Ash (%)	pH	Titratable acidity (%)	TS (%)	TSS (%)
0	2.10±0.32[a]	5.57±0.10[a]	1.01±0.05[a]	5.48±0.19[a]	0.81±0.00[a]	24.24±0.09[a]	20.00±0.00[a]
10	2.24±0.19[a]	8.35±0.22[b]	1.03±0.02[a]	5.42±0.03[a]	0.83±0.01[b]	24.90±0.05[b]	20.67±0.58[ab]
20	2.53±0.16[b]	9.72±0.31[c]	1.04±0.01[a]	5.42±0.00[a]	0.84±0.01[b]	25.91±0.08[c]	21.00±0.00[bc]
30	2.85±0.13[c]	10.07±0.44[d]	1.10±0.01[b]	5.40±0.02[a]	0.86±0.02[c]	26.40±0.35[d]	21.67±0.58[c]

Numbers followed by the same letter in the same column show no significant difference according to DMRT at the 95% confidence level (n= 3).

products. Frozen yoghurt is a product of ice cream and yoghurt fusion. The distinctive characteristic of this product is the sour taste of yoghurt and the sweetness of the ice cream. The sour taste comes from the formation of lactic acid due to fermentation carried out by LAB (Abedi and Hashemi, 2020). The acidity level of frozen yoghurt is detected by titratable acidity, which affects pH value.

The titratable acidity of frozen yoghurt ranges from 0.81– 0.86%, and it meets the SNI standard of yoghurt 2981:2009 - 0.5 – 2.0%. The titratable acidity comes from acids formed by LAB of frozen yoghurt. LAB uses lactose to produce lactic acid. The higher amount of lactic acid leads to a lower pH value and increases the acidity in the product (Maitimu *et al.*, 2013). There is no standard for the pH value of frozen yoghurt. The standard is only based on the level of consumer preference. According to Chandan *et al.* (2017), consumers in the United States preferred frozen yoghurt with a pH of 6, made from a 90% ice cream mixture and 10% plain yoghurt. Frozen yoghurt made from 100% yoghurt with the addition of stabilizers and sweeteners has a pH value of 4.5 or below is less preferred. The frozen yoghurt in this research is made by adding 50 g plain yoghurt to 100 g frozen yoghurt mixture and it has a pH value ranging from 5.48 to 5.40, which is preferred by consumers.

TS and TSS are important aspects of the quality of frozen yoghurt. TS plays an important role in forming a soft and firm structure and reducing the freezing sensation in the mouth. In addition, the high TS indicates the high content of nutrients in frozen yoghurt (Goff and Hartel 2013). In this research, TS and TSS range from 24.24 – 26.40% and 20.00– 21.67%, respectively.

The TS of frozen yoghurt comes from stabilizers and other milk nutrients such as protein and fat. According to Arbuckle (1986), TS standard of frozen yoghurt ranges from 20 – 31%. TS of frozen yoghurt comes from sugar, lactic acid, minerals, and water-soluble proteins. In addition, pigments and phenolic compounds in MOLE contribute to TSS (Magwaza and Opara, 2015). Moringa leaves contains soluble fiber ranging from 1.86-2.29% (Cuellar-Nuñez *et al.*, 2017). Previous research has also stated that soluble fiber in dried moringa leaves contains 9.54-11.6% and has a viscosity 29-157 cP (Fidyasari *et al.*, 2024)

Table 3. Physicochemical value of frozen yoghurt with MOLE

MOLE (%)	Overrun (%)	Melting rate (s)
0	71.43±0.00[a]	600±0.00[a]
10	73.33±1.65[a]	657±5.77[b]
20	77.90±1.31[b]	770±6.93[c]
30	78.66±1.31[b]	807±5.77[d]

Numbers followed by the same letter in the same column show no significant difference according to DMRT at the 95% confidence level (n= 3).

Physicochemical Value of Frozen Yoghurt With MOLE

The physicochemical analysis of frozen yoghurt with MOLE variation shows the presence of 71.43 – 78.66% overrun and 600 – 807s of melting rate. The higher addition of MOLE in frozen yoghurt increases the values of overrun and melting rate. Physicochemical test results of frozen yoghurt can be seen in Table 3.

The structural properties of frozen yoghurt are the most important aspects in determining product acceptance. The sensory parameters of the structure of frozen yoghurt include a sturdy shape, resistance to melting, and texture. Generally, a good frozen yoghurt has a soft texture with undetectable ice crystals (Vafiadis 1997).

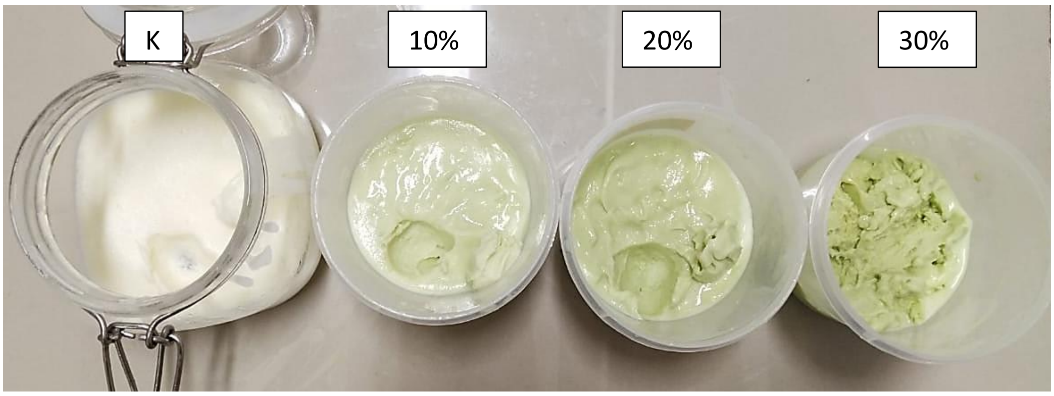

Figure 1. Frozen yoghurt with MOLE

Overrun is the percentage of ice cream mixture expansion. Ice cream and related products, such as frozen yoghurt, are aerated in the making process. The air from the aeration process gives a light and soft texture and affects the physical properties of hardness and melting speed of frozen yoghurt (Figure 1). The overrun value of frozen yoghurt ranges from 71.43 – 78.66%.

According to Goff and Hartel (2013), the overrun of frozen yoghurt with MOLE variations comes within the premium category because the expansion rates range from 60 – 90%. It is better than the standard category, which has an overrun rate of 100 – 120%. The component responsible for the overrun of the product is milk protein, such as casein and whey. Milk protein is an active surface agent, with the ability to bind air during the foam-forming aeration (Westerbeek 1996). Protein and soluble fiber of *Moringa oleifera* Lamk. Leaves may also contribute to the overrun (Cuellar-Nuñez *et al*., 2017).

The value of overrun of frozen yoghurt increases with the addition of MOLE, which contains TS stemming from protein and fat. However, the overrun value is insignificant because the TS values are approximately the same for all treatments. According to Kurultay *et al*. (2010), the good overrun of frozen yoghurt is two to three times the TS value.

The melting rate of frozen yoghurt with MOLE variations ranges from 600 – 807 seconds. According to Masykuri *et al*. (2012), the melting time of frozen yoghurt with MOLE variations comes within a good category ranging from 600 to 900 seconds. Melting time is related to TS. The higher the TS value, the longer the melting time is.

TPC and Antioxidant Activity of Frozen Yoghurt with MOLE

TPC of frozen yogurt ranges from 62.26 – 102.26 mg GAE/100 mL. The control sample (0%) has a total phenolic value of 62.26 mg GAE/100 mL (Table 4).

Table 4. TPC and antioxidant activity of frozen yoghurt with MOLE

MOLE (%)	TPC (mg GAE/100 g)	Antioxidant activity (%)
0	62.26[a]±1.11[a]	65.96[a]±0.86[a]
10	78.86[b]±0.99[b]	81.61[b]±0.25[b]
20	82.69[b]±4.75[b]	82.61[b]±0.37[b]
30	102.26[c]±0.13[c]	87.78[c]±0.64[c]

Numbers followed by the same letter in the same column show no significant difference according to DMRT at the 95% confidence level (n= 3).

Generally, frozen yoghurt is made from milk containing phenolic compounds such as phenol, cresol, thymol, and carvacrol (O'Connell and Fox 2001). Therefore, a frozen yoghurt product without the addition of MOLE has a total phenolic content.

Frozen yoghurt with the addition of 30% MOLE has the highest TPC of 102.26 mg GAE/100 mL. Dried moringa leaves contain a TPC of around 11.8 g GAE/100g (Cattan *et al*., 2022). Adding approximately 10% MOLE to food products increases the nutritional value and acceptable sensory characteristics (Kashyap *et al*., 2022). This extract also has the potential for phenolic and antioxidant supplementary sources (Peñalver *et al*., 2022). MOLE contains a TPC of 9.19 mg GAE/100 mL. Phenolic compounds are secondary metabolite products of *Moringa Oleifera* Lamk. Leaves. The main phenolic components of Moringa leaves are kaempferol and quercetin (Bajpai *et al*., 2005). Another study states that chlorogenic acid and ρ-coumaric acid are the dominant phenol components in moringa leaves (Cuellar-Nuñez *et al*., 2017). Phenolic compounds have the ability to capture free radicals because of the presence of hydroxyl groups that can donate hydrogen or electrons to free radicals. Therefore, total phenolic concentration can be used as a basis for the quick determination of antioxidant activity (Soobrattee *et al*., 2005).

Antioxidant activity in foods is detected from its ability to inhibit DPPH by donating electrons or hydrogen atoms. Antioxidant scavenges radicals to produce a stable compound (Kedare and Singh 2011). As shown in Table 4, the antioxidant activity of frozen yoghurt with MOLE has the inhibitory percentages of DPPH ranging from 65.96 – 87.78%. The control (0%) has the lowest value of 65.96% due to phenolic compounds of 62.26 mg GAE/100 mL. The antioxidant activity of control is attributed to the presence of hydroxyl groups, which are capable of donating hydrogen or electron atoms to stabilize free radicals (Soobrattee *et al*., 2005). Furthermore, the control has an antioxidant activity because components in milk as the main ingredient of frozen yoghurt contain lactoferrin, vitamin C, beta-carotene, and vitamin E (Lindmark-Månsson and Åkesson 2000).

The addition of MOLE can increase antioxidant activity. The highest percentage of DPPH inhibition is found in the treatment with the 30% MOLE addition. MOLE has 77.82 % antioxidant activity (Table 1). Dried Moringa leaves have approximately 80 % antioxidant activity (Cuellar-Nuñez *et al*., 2017). In addition to total phenolic content, the components of MOLE that affect the increase of antioxidant activity are chlorophyll, beta-carotene, xanthophyll, and ascorbic acid (Gopalakrishnan *et al*., 2016).

Table 5. Microbiological quality of frozen yoghurt with MOLE

MOLE (%)	LAB viability (log CFU/mL)	Salmonella (/25 g)
0	7.84[a]±0,62[a]	Negative
10	8.23[a]±0,51[a]	Negative
20	8.46[a]±0,50[a]	Negative
30	8.58[a]±0,52[a]	Negative

Numbers followed by the same letter in the same column show no significant difference according to DMRT at the 95% confidence level (n= 3).

The chlorophyll structure acting as a free radical scavenger is the porphyrin ring. However, the magnesium ions in the porphyrin ring decrease antioxidant activity because nitrogen-free electrons are bound to magnesium atoms. Under acidic conditions, the magnesium atoms undergo dechelation. This process transforms chlorophyll structure into phaeophytin with a free nitrogen atom (Hsu *et al*., 2013). The free electrons of nitrogen atoms in pheophytin form may react with the DPPH radical and turn it into a stable compound (Rothemund, 1956). Beta-carotene and xanthophyll in MOLE belong to the flavonoid group, which has antioxidant activity and prevents free radical damage through two mechanisms: hydrogen ions donation and reaction as a direct free radical scavenger (Arora *et al*., 1998).

Microbiological Quality of Frozen Yoghurt with MOLE

Microbiological quality of frozen yoghurt is observed in terms of LAB viability and absence of *Salmonella* contamination. According to Charalampopoulos *et al*. (2002), the good viability of LAB is a prerequisite for the functionality of fermented products such as frozen yoghurt. Viability is a measurement of the ability of LAB to form colonies and multiply. *Salmonella* bacteria are pathogenic gram-negative bacteria causing *salmonellosis.* The microbiological quality is presented in Table 5.

The viability of LAB in frozen yoghurt with MOLE variations ranges from log 7.84 – 8.58 CFU/mL. Total value of LAB of the product meets SNI 2981: 2009 yoghurt with a minimum threshold of log 7 CFU/mL. Total LAB tends to increase with the addition of MOLE, but statistically, it is not significantly different. This occurred due to the presence of other nutrients, such as protein and fat, that could support LAB viability. The addition of MOLE also increased TS in frozen yoghurt. According to Chairunnisa (2009), a higher value of TS corresponded to a greater total LAB because the nutrients needed were sufficient for growth and development.

Salmonella contamination is not found in all samples of frozen yoghurt products, both control, and treatments with MOLE variations. This means that frozen yoghurt products are safe to consume. The pasteurization process of frozen yoghurt eliminates *Salmonella* bacteria. According to D'Aoust (1989), *Salmonella* is not able to survive at 63°C. MOLE contains phenolic compounds capable of inhibiting the growth of pathogenic bacteria such as *Salmonella* in frozen yoghurt products. According to Torres-Castillo *et al*. (2013), the secondary metabolites of *Moringa oleifera* Lamk. leaves, namely phenolic compounds, inhibit the growth of pathogenic bacteria in the genus *Shigella, Pseudomonas,* and *Salmonella.*

Hedonic Properties of Frozen Yoghurt with MOLE

The color properties of the product affect the level of panelists' preference. As shown in Table 6, panelists preferred the frozen yoghurt product with the addition of 30% MOLE. The more MOLE is added, the greener the color. The green color of frozen yoghurt comes from the chlorophyll of *Moringa oleifera* Lamk. leaves. Chlorophyll is the main pigment found in leaves. There are two types of chlorophyll, namely chlorophyll-a, which emits a green-blue color, and chlorophyll-b, which emits a green-yellow color (Leite *et al*., 2018).

Taste and aroma properties can influence acceptance as well as interest in consuming a product (Chodur *et al*., 2018). Based on the level of panelists`preference for the taste and aroma, frozen

Table 6. Hedonic properties of frozen yoghurt with MOLE

MOLE (%)	Properties				Average	Ranking
	Color	Taste	Aroma	Texture		
0	1.53	1.80	1.97	1.77	1.77	4
10	2.00	2.30	2.33	2.26	2.26	3
20	3.07	3.07	3.17	3.13	**3.13**	**1**
30	3.47	2.90	2.70	2.97	2.97	2

yoghurt with a 20 % addition of MOLE is the most liked product. However, the highest addition of MOLE at 30% was not liked by the panelists due to the dominant taste and aroma of *Moringa oleifera* Lamk. leaves.

The texture properties of the product with the addition of 20% MOLE were the most liked by panelists. Based on categorization, the order of frozen yoghurt products from most liked to disliked was a product with 20% MOLE, followed by 30%, 10%, and control. Frozen yoghurt with the addition of 20% MOLE was superior in terms of taste, aroma, and texture, while 30% MOLE was the highest regarding color.

CONCLUSIONS

In conclusion, this research shows that MOLE with a certain concentration can be used for the making of frozen yoghurts. The addition of MOLE improves the antioxidant activity, chemical value, physicochemical, microbiological, and sensory properties of frozen yoghurt. The results show that frozen yoghurt with 30% MOLE is the best product.

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