Canrea Journal: Food Technology, Nutritions, and Culinary, 2023; 6 (2): 100-118 OPEN ACCESS

e-ISSN : 2621-9468

Type of the Paper (Article)

Evaluation of the potential of matoa (*Pometia pinnata*) leaf extract as an antioxidant activity in pasteurized milk

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Article History
Received May 23, 2023
Accepted December 25, 2023
Published December 28, 2023

Keywords
Flavonoids,
Sensory Test,
Thiobarbituric Acid,
Viscosity.

Abstract

The research objective was to study the influence of the addition of matoa (*Pometia pinnata*) leaf extract (MLE) and various pasteurization methods (PM) on the antioxidant activity (AA) and physicochemical characteristics of pasteurized milk (PMi). The MLE was added at the level of 0%, 0.05%, 0.10%, 0.15%, and 0.20% into milk and pasteurized to low temperature for long-time pasteurization (LTLT) (63°C for 30 min) or a high-temperature short time (HTST) (72°C for 15 s). The HTST method increased AA and reduced the viscosity of pasteurized milk. On the other hand, the LTLT PMi showed lower thiobarbituric acid (TBA) values, improving whiteness, specific flavor, and taste among panelists. Each level of MLE affected AA, TBA values, and viscosity during pasteruization of milk under different conditions. Nevertheless, the relationship between MLE and PM did not milk. PMi exhibited an AA range from 14.05% to 57.79%, with an average AA of 34.36% for the LTLT method and 43.13 for the HTST method. In the LTLT method, the average TBA value for PMi was 0.08 mg MDA/kg, compared to 0.15 mg MDA/kg for the HTST method. Similarly, the average fat content in PMi was slightly higher for the LTLT method (0.98 %) than for the HTST (0.95%). PMi supplemented with MLE in both pasteurization methods exhibited viscosity levels ranging from 5 to 15 mPa.s. The preference test revealed that panelists generally favored the color white of the milk. Approximately 56% of panelists stated that the aroma and taste of the milk remained strong even with the addition of the MLE, with the most preferred sensory combination being the addition of 0.2% MLE with the HTST method of matoa leaf extract to pasteurized milk can be an innovation in the dairy industry to obtain healthful, functional pasteurized milk.

1. Introduction

Pasteurization, which involves heating milk below its boiling point, is the easiest milk processing method widely used by people and puny industries. Pasteurization was first used as a milk preservation technique in 1917 and serves to control milk-borne diseases (1). Pasteurization is expected to decrease the number of microbes found in milk (2). Pasteurized milk offers various innovations with specific taste, aroma, and color modifications to manufacture healthy drinks. Physicochemical properties must be considered because they are closely related to product quality in the dairy processing industry. One component that is easily denatured by heating is the beta-lactoglobulin protein, which affects the physical

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ISSN 2621-9468 online, DOI 10.20956/canrea.v6i2.1024

properties of milk and other dairy products as food and, consequently, the consumer's favorite level. This can be verified by organoleptic tests, which result in the panelist's response to the product, or the implementation of quality by the panelist assigned to assess the characteristics or quality of the food based on the actual subjective assessment (3).

The most common pasteurization methods used in the dairy industry are the low-temperature long time (63°C for 30 min), and the high-temperature short time (72°C for 15 s) HTST method (4). In addition, pasteurization methods can affect the protein content and quality of milk products. HTST is considered more effective than LTLT because it causes less damage to the nutritional content of the milk. The pasteurization conditions were intended to minimize nutrient loss in milk. The quality of fresh milk requires a minimum of 2.7% total protein (5). The process of heating milk from a high temperature to near its boiling point for a relatively long time causes some alterations in the nutritional quality of milk, including protein, fat, and vitamin contents, as well as color changes in milk products (6,7).

The addition of functional substances to milk products may help prepare healthy products as functional foods (8,9). Food products that can provide health benefits and at the same time function to help prevent or treat disease are called functional foods (10). Substances present in some functional foods are antioxidants, which can help prevent food spoilage, rancidity, or discoloration caused by oxidation (11–13).

Several communities use the matoa (*Pometia pinnata*) plant for its medicinal properties. Consuming matoa leaves boiled in water can relieve hypertension (11). It has been reported that mature leaf extracts (MLE) show anti-integration activity (4). These extracts could be beneficial as biological medicines made from plant material and are safer for consumers. Matoa leaves contain vitamins and antioxidant compounds that can defer, delay, and inhibit lipid oxidation (14). Flavonoid compounds have been proven to have antioxidant, antibacterial, and antifungal effects (15–19). Hence, the addition of matoa leaf extract is expected to increase the antioxidant activity of pasteurized milk.

The matoa plant, also called tava, is a tree with a height of up to 50 m. It can grow in various areas but is less well known because it is not cultivated. This tree naturally originates in Sri Lanka and the Andaman Islands. It has spread to South Asia, including China, Vietnam, the South Pacific, Samoa, Tonga, and Niau. This plant then spreads to Polynesia and Europe, namely Cook Island and French Polynesia (20). However, this tree is also found in Malaysia, Thailand, and Fiji (21).

The ethanolic extract of matoa leaves has antioxidant activity, and qualitative phytochemical screening has shown the content of flavonoids and tannins. Matoa leaf extract contains quercetin, procyanidin, and kaempferol (22). Phytochemical substances in mature leaves include alkaloids, flavonoids, saponins, tannins, and triterpenoids/steroids (23).

Therefore, adding matoa leaf extract to pasteurized milk can be an innovation in the dairy industry to obtain healthy, functional pasteurized milk. This study aimed to investigate the influence of different concentrations of matoa leaf extract and pasteurization methods and their interactions on the antioxidant activity, physicochemical quality, and sensory quality of pasteurized milk. The use of MLE in milk production is a new additive food in functional milk production.

2. Materials and Methods

The matoa leaves extract (MLE) was added at the level 0%, 0.05%, 0.10%, 0.15%, and 0.20% to milk and subjected to low-temperature long-time pasteurization (LTLT) (63°C for 30 min) or high-temperature short time (HTST) (72°C for 15 s).

2.1. Selection of Matoa Leaves

Matoa (*Pometia pinnata*) leaves were obtained from the Village of Romang Lompoa, Bontomarannu District, Gowa Regency, South Sulawesi, Indonesia. They were selected based on their light green color, and two to three leaves were taken from the youngest stems and shoots. The selection criteria also included a stiff and fresh texture and the absence of pest defects.

2.2. Preparation of MLE

Five kilograms of fresh matoa leaves were washed and then dried at room temperature for five days, washed, cleaned, dried, and aerated at room temperature. Dried matoa leaves were cut into small pieces and mashed using a blender. Fifty grams of matoa leaf powder was mixed with distilled water in a 1:10 ratio and then allowed to stand for 2x 24h (maceration). The matoa leaf powder was weighed to 50 g each with 500 mL of distilled water (ratio 1 10), placed in a closed measuring cup, placed in a refrigerator, and allowed to stand for 2 × 24 h. The suspension was stirred every four hours for 5 min during the maceration. The maceration process was carried out in a tightly closed container at room temperature. The moving process was performed every 4 h for 5 min during maceration. The maceration mix (250 mL) was freeze-dried, and the final powder extract (10.5 g) was used as powder extract according to the level of MLE for each treatment. The fine sediment was freeze-dried (Labist, 0.08m2 Stoppering Chamber Freeze Dryer -50°C). The dry extract was 375 mg/5000 mg*100% = 7.5%.

2.3. Preparation of Enriched Pasteurization Milk (PMi)

The 10% full cream powdered reconstituted milk (FCRM) is the raw material for making PMi. FCRM was added to 0% (without MLE) and 0.05%, 0.10%, 0.15%, and 0.20% (w/v) of MLE; each treatment consists of 5 samples. Later, milk samples were pasteurized using the LTLT (63°C for 30 min) and HTST (72°C for 15 s) methods (24). Reconstituted milk was prepared from commercial milk powder produced by Indomilk, with a nutritional composition of 6% protein, 6% fat, 10% lactose, 0.85% sodium, 2.3% potassium. The use of reconstituted milk ensured that each treatment unit was homogeneous. Fresh milk was avoided because its nutritional composition was different for each milking period. The average pasteurized milk in the industry is also made from reconstituted milk.

2.4. Antioxidant Activity (AA)

AA was determined using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method. Milk samples (0.1 g) were suspended in 20 mL of methanol in an Erlenmeyer flask and sterilized for 10(±1) min. Heating with sterilization will provide information on whether the food is rapidly oxidized. High heating speeds up oxidation. This method rapidly extracts malondialdehyde (MDA) from pasteurized milk samples (25). Afterward, the milk was centrifuged at 5000 rpm for 5 min. Then, 1.0 mL of filtrate was mixed with 0.5 mL DPPH reagent (4 x 10^{-4} M) and left for 20 min. Finally, the solution was diluted with methanol to obtain a total volume of 5 mL. Absorbance was measured immediately at 517 nm. Blanks were run using the same method but without samples. AA was expressed as the amount of DPPH

radicals (mmol) trapped by the sample (g). Calculations were performed based on absorbance readings of the samples. AA was calculated using the formula (25).

$$AA\ (\%) = 1-(A\_DPPH - A\_Sample)/A\_DPPH \times 100 \quad (1)$$

Where:
A_DPPH: Absorbance of DPPH
A_Sample: Absorbance of sample

2.5. Thiobarbituric Acid (TBA)

TBA numbers were determined following the 1960 Tarladgis method using a UV 1800 spectrophotometer (Shimadzu, Japan) (26). Ten grams of the PMi samples were weighed, diluted with distilled water (47.5 mL), and quantitatively transferred into a distillation flask. Afterwards, 2.5 (±0.1) mL of 4 M HCl was added until the pH reached 1.5. Boiling stones were added and the distillation flask was connected to a distillation system. Distillation was performed under high heating for 10 min until 50 mL of the distillate was obtained. The distillate was stirred and 5 mL was transferred to a closed test tube. The TBA reagent (5 mL of TBA reagent was added to the tube, which was then covered, mixed evenly, and heated for 35 min in boiling water. The samples were kept for seven days at room temperature. Blanks were prepared using 5 mL of distilled water and 5 mL of TBA reagent, following the same procedure as the samples. The test tubes were then cooled with water for 10 (±1) min. Their absorbances were measured (D) at a wavelength of 528 nm with a blank solution as a zero point and in 1 cm diameter cells. TBA values were expressed as mg of malonaldehyde (MDA) per kg of sample (TBA number = 7.8 x D).

According to Tarladgis et al. (26), the reagents used were TBA; 0.02 M 2-thiobarbituric acid in 90% glacial acetic acid dissolved by slight heating in a boiling water bath. The TEP standard was obtained from 1 x 10^{-3} M 1,1,3,3, -tetra-ethoxy propane in distilled water. This solution was stored for approximately a week in a refrigerator and diluted as needed. The HCl solution used in this method was obtained by mixing HCl and distilled water (1:2, approximately 4N HCl).

2.6. Fat Contents

A 10 g sample of PMi was mixed with 1.25 mL of concentrated NH_3 and heated in a 70°C water bath for approximately 15 min. 10 mL of 95% ethanol and 25 mL of diethyl ether were added to the milk-ammonia mixed solution. The mixture was then placed in a separation funnel and shaken for 1 min. Additionally, 25 mL of petroleum ether was added to the mixture and shaken for 1.5 min. The solution was left until two layers were formed. Finally, the top layer of the solution was placed in a previously weighed porcelain dish. It was then evaporated in a hot-water bath (27).

$$\text{Fat content} = (W_2-W_1)/ W_0 \times 100 \quad (2)$$

Where:
W_0 = weight of milk sample (g)
W_1 = weight of empty evaporating dish (g)
W_2 = weight of the evaporating dish and dry fat residue (g)

2.7. Viscosity (Vis)

Viscosity was measured using a Brookfield RVT viscometer (VM-BF-RV-01) (28). The PMi sample (250 mL) was warmed, poured into a 250 mL glass beaker, and measured using a viscometer with a spindle of a 3-unit gauge at a speed of 50 rpm.
The viscosity can be calculated with the formula:

$$\text{Viscosity} = \text{Readings X correction factor} \quad (3)$$

2.8. Sensory Evaluation (SE)

The SE panel consisted of 25 semi-trained individuals (29). The panelists for the sensory test were semi-trained panelists with an age range of 17-25 years, consisting of both women and men. The panelists had previously been accustomed to drinking milk and had not suffered from lactose intolerance (allergy to lactose). Sensory assessment based on the hedonic scale for color, flavor, taste, and preference was performed using a scale of 1-6 shown as follows Table 1.

Table 1. Sensory assessment based on the hedonic scale for color.

Scale	Type	Description
1	White	Bright white
2	Whitey	Milky white
3	Brownish white	White, but slowly turns to be seen brown color
4	Slightly brownish	The brown color is visible
5	Brown	The color looks brown
6	Very Brown	The color looks dark brown

Table 2. Sensory assessment based on the hedonic scale for flavor.

Scale	Type	Description
1	Milk odor	The smell of milk the typical smell of milk without there is another smell
2	Slightly milk odor	It slightly smells of milk; the smell of milk still feels dominant, but there are already other flavors besides the typical taste of milk
3	Somewhat milk odor	Slightly smells of milk; the smell of milk is still felt but the dominant scent is gone
4	Somewhat not milk odor	It doesn't smell like milk
5	Slight Milk odor	There is a slight smell of milk; the smell of milk is very little, and the scent is very dominant over the typical smell of milk
6	No milk odor	There is no milk taste. There is no longer a distinctive smell of milk

Table 3. The Taste testing method used a numerical scale from 1 to 6.

Scale	Type	Description
1	Not fresh taste	The taste is not fresh. That is, this dairy product does not taste fresh milk
2	Very slightly fresh taste	The taste is a little fresh. That is, this dairy product still has a little fresh milk taste
3	Slightly fresh taste	The taste is a little fresh. That is, this dairy product has a fresh milk taste
4	More slightly taste	The taste is fresher. That is, this milk product has a more pronounced fresh milk taste
5	Fresh taste	Fresh taste, namely milk products taste fresh milk, although there is still a little that is not new milk taste
6	Very fresh taste	The taste is fresh, that is, the milk product tastes like fresh milk, and there is absolutely no taste other than the taste of fresh milk

Table 4. The preference testing method used a numerical scale from 1 to 6.

Scale	Preference	Description
1	Really like	Like (very much like), i.e., panelists are ethnic or like the product
2	Like	Like. Panelists like this dairy product in general, like they like other similar dairy products
3	Quite like	A little like. The panelists feel a little like the product compared to the same kind of dairy product
4	Quite dislike	Slightly more to dislike. The panelists only like this product very little compared to the same kind of dairy product
5	Dislike	Dislikes. The panelists do not like the product but still want to taste during the panelist test
6	Really dislike	Don't like. The panelists don't like the smell, color, and taste

Preference tests are essential to people's acceptance of products.

2.9. Statistical Analysis

This study used three repetitions with a completely randomized design with a factorial pattern (5 × 2) and was processed using analysis of variance. Organoleptic test data were analyzed descriptively, and the data obtained from the panelist sensory test results were identified, tabulated, quantified, and subsequently described (30). Quantitative and continuous data were analyzed using IBM SPSS 23, with an accuracy of 0.05.

The AA TBA (31), fat content, and viscosity were analyzed using the CRD. The treatment that resulted in a real effect was further tested using Duncan's test (32). The mathematical model is as follows.

$$Y_{ijk} = \mu + \alpha_i + \beta_i + \alpha\beta_{ij} + €_{ijk} \quad (4)$$

i = 1,2,3,4,5 (Factor a)

j = 1,2 (Factor b)

k = 1,2,3 (repetition)

Note:

Y_{ijk} = The observation value of the k-antioxidant potential obtained by the combination of the i-th MLE level treatment and the j-PM

μ = average value of treatment

α_i = Effect of MLE level on antioxidant potential
β_j = Effect of pasteurization on jth antioxidant potential
$(\alpha\beta)_{ij}$ = Effect of the interaction between the level of the ith MLE and jPM
$€_{ijk}$ = Influence of errors that received the i-ML treatment and jth PM

3. Results and Discussion

3.1. Antioxidant Activity (AA)

The AA values of MLE-added PMi under different PM concentrations are listed in Table 1. The AA of PMi increased as the MLE level increased. Furthermore, the average AA of PMi using the LTLT method was 34.36%, lower than that HTST (43.13% AA).

Table 5. Antioxidant activities (%) of MLE-added PMi under different pasteurization methods.

Antioxidant activity (%)			
Matoa leaves extract	Pasteurization methods		Average
	LTLT (63°C, 30 min)	HTST (72°C, 15 sec)	
0%	14.05 ± 0.01^{a}	14.85 ± 2.68^{a}	14.45 ±1.75^{A}
0.05%	31.49 ±0.37^{b}	39.35 ± 1.07^{c}	35.42 ± 3.88^{B}
0.10%	32.42 ± 0.73^{b}	51.37 ± 2.13^{d}	41.89 ± 10.47^{C}
0.15%	38.19 ± 0.51^{c}	52.28 ± 2.13^{d}	45.24 ± 7.84^{D}
0.20%	55.66 ± 0.01^{e}	57.79 ± 0.96^{e}	56.72± 1.31^{E}
Average	34.36 ± 13.83^{A}	43.13 ± 15.98^{B}	

Note: ^{ABCDE}superscripts that follow the mean values in rows and columns that are the same show the real difference ($p<0.01$). ^{abcdef}superscripts that followed the mean values in the different treatment interactions indicate significant differences ($p <0.01$).

From Table 5, antioxidant activity can be known in pasteurized without MLE, which was an average of 14.45%. Adding 0.2% MLE antioxidants to pasteurized milk increased the antioxidant activity about 3-fold higher (on average 56.72%). This indicates that the addition of matoa leaves could become a functional milk drink with prospects for development. The antioxidant endurance of MLE in PMi can be determined by measuring the AA after the storage interval. In this study, the added MLE concentration limited the antioxidant levels.

In a review by Stobiecka et al. (33) that the addition of red ginseng extract to milk increased its antioxidant activity from 11.8 ± 0.00 g/mL (control without the addition of red ginseng extract) to 15.1 ± 0.5 g/mL (addition of 100 g/mL). Meanwhile, our results (Table 1) with the addition of 0.2% indicate that the moderate antioxidant activity is 56.72 ± 1.31%, where this value is equivalent to g/mL (weight/volume). Therefore, matoa extract has excellent antioxidant potential when added to pasteurized milk.

Different pasteurization methods showed a significant difference ($p<0.01$) between the LTLT and HTST pasteurization methods. Pasteurization of HTST yielded a higher AA value than LTLT. This was because LTLT pasteurization underwent prolonged heating for 30 min, which may cause damage to fat-soluble vitamins, such as antioxidants, especially Vitamin E (34). In the HTST pasteurization, the temperature was higher, but the heating time was short (approximately 15 s), and the heating was immediately stopped and quickly cooled. The fat, vitamins, and antioxidant substances were not damaged.

3.2. TBA Values

The TBA values of MLE-supplemented pasteurized milk and different pasteurization methods are presented in Table 6. The TBA values of PMi decreased with increasing MLE addition. The average TBA value of LTLT was 0.08 mg MDA/kg, which was lower than the HTST method (0.15 mg MDA/kg).

Table 6. TBA values (mg MDA/kg sample) of PMi samples added by MLE using different pasteurization methods.

	TBA (mg MDA/kg sample)		
	Pasterization Methods		
Matoa leaves extract	LTLT (63°C, 30 min)	HTST (72°C, 15 sec)	Average
0%	0.12 ± 0.00^{d}	0.19 ± 0.03^{f}	0.15 ± 0.04^{D}
0.05%	0.10 ±0.00^{cd}	0.19 ± 0.02^{f}	0.14 ± 0.04^{D}
0.10%	0.09 ± 0.00^{bc}	0.17 ± 0.00^{ef}	0.13 ± 0.04^{C}
0.15%	0.05 ± 0.00^{a}	0.16± 0.00^{e}	0.10 ± 0.06^{B}
0.20%	0.03 ± 0.00^{a}	0.07 ± 0.00^{b}	0.05 ± 0.02^{A}
Average	0.08±0.03^{A}	0.15 ± 0.04^{B}	

Note: ^{ABCDE}superscript follows the average value in the row and column and shows a very significant difference ($p < 0.01$). ^{abcdef}superscripts that follow the mean values for the treatment interactions differ significantly ($p < 0.01$).

Results demonstrated the addition of MLE 20% to PMi could decrease the TBA value about 4-fold compared with without MLE addition. This indicates that MLE prevents fat oxidation in dairy products. Using different PM shows that the HTST PM has a higher TBA value than the LTLT PM. Higher heating induces oxidation; therefore, fat is more decomposed into MDA in HTST than in LTLT. MDA is an oxidation product of unsaturated fatty acids (UFAs). MDA is a three-carbon dialdehyde with carbonyl groups at the C-1 and C-3 positions (35).

3.3. Fat Content

The levels of MLE-supplemented PMi fat with different PMs are shown in Table 7. There was no significant difference in fat content at each concentration of MLE added to the LTLT and the HTST Pasteurization methods. This indicated that adding matoa and pasteurization methods did not affect the fat content of pasteurized milk.

Table 7. Fat content (%) of MLE-added PMi using different pasteurization methods.

	Fat Content (%)		
Matoa leaves extract	Pasteurization Methods		Average
	LTLT (63°C, 30 min)	HTST (72°C,15 s)	
0%	1.03 ± 0.06	0.99 ± 002	1.01 ± 0.04^{B}
0.05%	1.05 ±0.00	1.02 ± 0.02	1.03 ± 0.02^{B}
0.10%	0.92 ± 0.00	0.92 ± 0.02	0.92 ± 0.01^{A}
0.15%	0.95 ± 0.00	0.94 ± 0.00	0.94 ± 0.00^{A}
0.20%	0.96 ± 0.02	0.91 ± 0.09	0.93 ± 0.06^{A}
Average^{ns}	0.98±0.05	0.95 ± 0.05	

Note: ^{ns}Non Significant, ^{ABCDE}superscripts that follow the mean values in the same column show significant differences ($p < 0.01$).

As shown in Table 7, the heating of HTST fat oxidized to MDA was greater than that of the LTLT method. As a result, although there was no statistically significant difference, the fat content of PMi with the HTST method was lower (0.95) than that with the LTLT method (0.98).

3.4. Viscosity

The viscosities of the MLE-added PMi using different PM are presented in Table 8. The average viscosity of the MLE-added PMi in the LTLT PM was 12 mPa.s, which is higher than that of the HTST method (7.2 mPa.s).

Table 8. Viscosity (mPa.s) of PMi supplemented with MLE using Different PM.

Matoa Leaves Extract	Viscosity (mPa.s)		
	Pasteurization Methods		Average
	LTLT (63°C,30 min)	HTST (72°C, 15 sec)	
0%	15.00± 0.00^{f}	9.45±0.47^{d}	12.22 ± 3.05^{D}
0.05%	15.00±0.00^{f}	7.92 ±1.25^{c}	11.46 ± 3.95^{C}
0.10%	12.50 ± 0.00^{e}	6.11 ± 0.48^{b}	9.30 ± 3.51^{B}
0.15%	12.50 ± 0.00^{e}	5.83 ± 0.83^{ab}	9.17 ± 3.68^{B}
0.20%	5.00 ±0.00^{a}	6.67 ±0.00^{b}	5.84 ± 0.91^{A}
Average	12.00 ± 3.80^{B}	7.20 ± 1.51^{A}	

Note: ^{ABCDE}superscript, which follows the average value in the row and column values, shows a very significant difference ($p < 0.01$). ^{abcdef}superscripts that followed the mean values of the treatment interactions differ significantly ($p < 0.01$).

As shown in Table 8, the PMi viscosity decreased with the addition of MLE, and the density was higher in the LTLT method than in the HTST method.

3.5. Organoleptic Test

The results of the organoleptic assessment of PMi color supplemented with MLE and different PM are shown in Figure 1a. Adding more MLE concentrations will affect the color of PMi; this organoleptic assessment used 25 panelists.

The organoleptic evaluation results of the PMi aroma supplemented with MLE using different PMs are shown in Figure 1b. Most panelists agreed that the higher the MLE level, the greater the decrease in milk aroma.

The results of the organoleptic assessment of the taste of PMi with the addition of MLE and different PM are shown in Figure 1c. The assessment of 25 panelists stated that the higher the use of MLE in PMi processing, the more the taste of PMi decreased. The organoleptic result test (Figure 1d) showed that the response to the preference test from 25 panelists was a higher level of MLE supplementation to PMi, caused by the lower panelists' likeness.

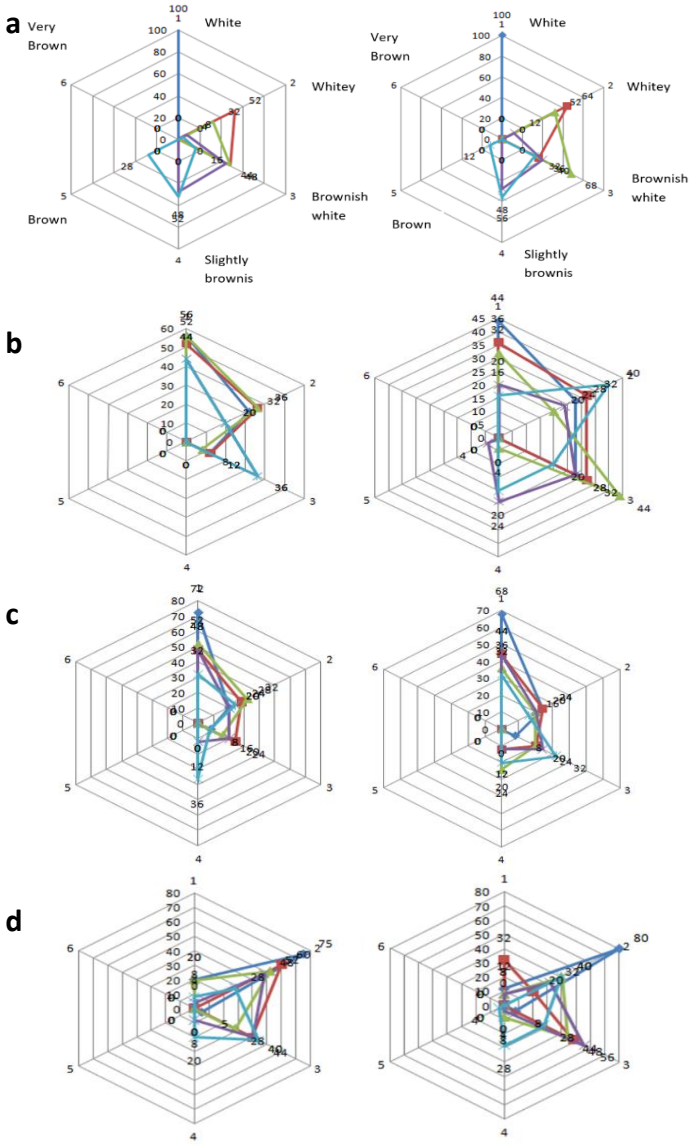

Figure 1. A spiderweb diagram.

(a) = PMi color by the addition with MLE (0, 0.05; 0.1; 0.15; and 0.2%) for LTLT (left) and HTST (right) pasteurization; (b) = PMi flavor by the addition of MLE (0, 0.05; 0.1; 0.15; and 0.2%) at LTLT (left) and HTST (right) of PMi (1= milk odor; 2= slightly milk odor; 3= somewhat milk odor; 4= somewhat no milk odor; 5= slight milk odor; 6= no milk odor); (c) = PMi taste addition by MLE (0, 0.05; 0.1; 0.15; and 0.2%) on LTLT (left) and HTST (right) PMi (1= not fresh taste; 2= very slightly fresh taste; 3= slightly fresh taste; 4= more slightly fresh taste; 5= fresh taste; 6 = very fresh taste); (d) = PMi hedonic test added with MLE (0, 0.05; 0.1; 0.15; and 0.2%) at LTLT (left) and HTST (right) PMi (1= really like; 2= like; 3= quite like; 4= quite dislike; 5= dislike; 6= really dislike).

3.6. Antioxidant Activity (AA)

The AA level (Table 5) ranged from 14.05% to 57.79%. The higher content of phenolic compounds in the MLE probably caused a higher AA. According to Khatun et al. (36), phenolic compounds can act as antioxidants by scavenging reactive oxygen species and binding to metals that catalyze oxidation reactions. Random analysis indicated that the various concentrations of MLE and PM in PMi manufacturing had a significant effect ($P<0.01$) on the AA of PMi.

The time difference between the LTLT and HTST methods caused a difference in the AA values. Although the HTST method used a lower temperature, the damage to milk nutrients was more significant than that in the LTLT method because the MLE-added PMi was exposed to heat for longer. Consequently, one of the causes of damage to antioxidant compounds is prolonged exposure to heat for a long time. This affected the amount and type of extracted phenolic components, which decreased the AA. Almost all thermal processes, including drying, pasteurization, or using microwaves, partially degrade phenolic substances, which reduces antioxidant capacity (37).

Duncan's test of MLE treatment showed that each treatment in PMi processing significantly increased the AA of PMi. MLE contains flavonoids in the form of phenolic compounds, which are highly active antioxidant components because of their ability to eliminate free radicals and peroxide radicals and effectively inhibit lipid oxidation (38). A higher TPC in natural materials accounts for a higher ability to prevent oxidation (39,40). Flavonoids in the form of polyphenols present in MLE have an additive effect on PMi. Flavonoids can remove free radicals by stabilizing reactive O_2 through reactions with radical reactive compounds. Certain flavonoids can directly trap superoxide anions, whereas other flavonoids capture O_2 radical derivatives that are very reactive, causing oxidation (31).

Duncan's test provides data analysis results that support the relationship between the MLE and PM treatments. Likewise, this treatment showed significant differences in each level of MLE and the LTLT and HTST pasteurization methods using PMi antioxidant activity. AA was higher in the HTST method than in the LTLT method because of the phenolic content in mature leaves, which contributed to increased antioxidant activity. Prolonged heating in the LTLT method affects the number and types of components of the extracted antioxidant compounds, thereby affecting the decrease in AA. Some active antioxidant ingredients, such as polyphenols, can be damaged by prolonged heating (41,42).

3.7. TBA Values

The decrease in the TBA value of PMi was proportional to the increase in AA in the PMi. A reduction in TBA values indicated that phenolic compounds in matoa leaves (*Pometia pinnata*) have good inhibitory properties against oxidative damage. Analysis of variance showed that the use of MLE and PM levels significantly ($P<0.01$) affected the TBA value.

Kapusta et al. (34) determined the relationship between lipid peroxidation and lactation period in Frishian Hollad (FH) dairy cows. Lipid peroxidation is a process in which free radicals attack the carbon double bonds of omega-3 and omega-6 fatty acids. The final product of this process is a reactive aldehyde, malondialdehyde (MDA). Lipid peroxidation causes adverse changes in the nutritional value of the milk. Therefore, the addition of antioxidant ingredients to milk is essential for increasing the value and durability of dairy products to become functional dairy products (34).

The Duncan's test proved that the treatment of MLE in the processing of PMi at concentrations of 0% and 0.05% showed no significant difference. However, TBA values of 0% and 0.05% MLE-added PMi showed significant differences of 0.10%, 0.15%, and 0.20% of MLE, respectively. Increasing MLE levels caused a decrease in fat oxidation and TBA values. This decrease was due to matoa leaves containing phenolic compounds and essential oils that could inhibit rancidity and oxidation reactions. Matoa leaves contain flavonoids as antioxidants that can donate protons to PMi, interact with free radicals, and reduce fat

oxidation. In addition to flavonoids, essential oils can act as antioxidants and prevent rancidity (43).

The heating process of PMi also affects the antioxidant level, which involves the duration and temperature. Damage to food fats can occur during processing, heating, and storage (44). This damage evokes terrible odors and tastes in fatty foods because of the oxidation of unsaturated fatty acids. High heat causes fat to be degraded; consequently, vitamin E, which is fat-soluble, also loses its function. Vitamin E is an essential milk fat-soluble antioxidants (34).

The results of Duncan's test (32) provided the relationship between MLE and PM treatments. TBA values decreased with the increasing levels of MLE and the use of the LTLT method compared to the HTST method. The difference in heating temperature induced this effect.

Gad and Sayd added two sources of phenol as antioxidants, rosemary and green tea, to reconstituted milk prepared for cheese production (45). This study concluded that the addition of rosemary and green tea increased antioxidant activity in the heating treatment and the presence of calcium chloride. The presence of antioxidants in dairy products is crucial to overcome the oxidation problem of unsaturated fatty acids found in milk (45).

3.8. Fat Content

PMi fat levels (Table 3) decreased with an increase in MLE during PM processing. The average levels of MLE-added PMi fat ranged from 0.91% to1.05%. Analysis of variance indicated that the addition of MLE had a significant effect ($P < 0.01$) on PMi fat content. However, PM and the relationship between the use of MLE levels and PM did not significantly affect PMi fat content.

Duncan's test results showed that supplementation of MLE in PMi at concentrations of 0% and 0.05% presented a non-significant difference. Significant differences existed in the concentrations of 0.10%, 0.15%, and 0.20% of MLE. The addition of MLE up to 0.10% could reduce PMi fat content. However, considering the data of LHLT and HTST separately, there were no significant differences in fat content with increasing MLE levels (46). Phytochemicals such as saponins, flavonoids, tannins, and other phenolic compounds from plants can affect the fat content of foods. Fat in food releases free radicals that oxidize fat to form ROO^{+}. The fat-free radicals multiplied after reacting with hydrogen ions (RH), increasing ROOH. These free radicals and fats are then broken down into ketones, aldehydes, alcohols/hydrocarbons, and epoxides, which produce rancid flavors in food. Natural chemicals such as phenolics, which are plant metabolites, in this term MLE, will prevent the oxidation process. Thus, it can control the rancidity and extend the product's shelf life. Various antioxidant compounds found in plants have long been used to reduce fat content (47).

The average fat content of PMi by the LTLT method was higher (0.98%) than that of HTST (0.95%). The lower ranges of PMi fat in the HTST method than those in the LTLT method are probably due to the higher temperature. High temperatures during food processing, such as frying, drying, steaming, and cooking, reduce the fat content in foods. Therefore, the measurement will present a low-fat content even though milk and its products are not considered (48).

3.9. Viscosity

The viscosity of PMi supplemented with MLE, LTLT, and HTST PM ranged from 5 to 15 mPa·s (Table 8). Analysis of variance demonstrated that the addition of MLE and PM, the relationship between MLE and PM, and viscosity significantly affected the treatment.

Based on Duncan's test, the supplementation with 0.10% and 0.15% MLE in PMi did not affect viscosity values significantly. However, there were significant differences between control (0% MLE) and 0.05%, 0.10%, 0.15%, and 0.20% MLE addition on the viscosity values of PMi. Increasing the concentration of MLE during PMi processing could reduce the viscosity. The decrease in viscosity was caused by matoa leaves containing acidic compounds, such as tannins. A higher concentration of MLE added to PMi led to a higher concentration of acidic tannins. The presence of organic acids leads to coagulation and clumping of milk proteins, thereby increasing the viscosity of PMi. Adding acid and heating melts casein micelles and forms lumps or milk clotting (49).

The difference in viscosity occurred because of the differences in heat pressure during the pasteurization process, which drove evaporation. The HTST process was carried out at 72°C for 15 s. This method involves a time duration that increases the viscosity of milk as it approaches the coagulation point of the milk protein (50).

Duncan's test results demonstrated a significant effect of the treatment and increased concentrations of MLE and PM on the viscosity of PMi. The Administration of 0.05% MLE could decrease the viscosity value, considering the acidic tannin compounds in matoa leaves. The organic acid content can agglomerate milk proteins, and heating also affects the viscosity of milk (51).

3.10. Organoleptic Test

3.10.1. Color

Most panelists generally assessed PMi color, which was ranked from white to brownish. The color of PMi was considered white in the treatment without PMi. The color changes began to appear at 0.05% - 0.20% MLE. The color is slightly white to brownish white and tends to be chosen by panelists. The spread of fat globules causes a white color in milk. The presence of β-carotene and riboflavin in animal feed makes fresh milk often results in a yellowish color. Because the MLE is brown, it causes the PMi to turn brown. Flavonoids, tannins, and saponins in mature plant leaves cause MLE to turn brown.

Flavonoids are the most abundant phenolic compounds in nature are flavonoids (52). Red, brown, purple, blue, and yellow dyes found in plants are generally the colors of flavonoid compounds. This is why the color of milk browns with an increase in the addition of MLE to PMi due to the presence of phenol compounds in matoa leaves.

The organoleptic test results shown in Figure 2 also explain pasteurization, which plays a role in the color change using LTLT and HTST PM. This is because the color of milk is affected by high-temperature heating. When milk is heated at very high temperatures, lactose reacts to form a brownish color change (53). Moreover, this browning reaction causes a color change owing to MLE degradation because of the temperature increase, which also affects the PMi color.

3.10.2. Aroma and Flavor

A change in the aroma of pasteurized milk occurred with the addition of MLE at a concentration of 0.05-0.20%. Triterpenoid compounds in mature leaves are typical and

fragrant volatile compounds that produce a characteristic flavor (not the smell of milk) and affect the PMi aroma. Triterpenoids are the main components of plants that contain specific odors. These compounds can be extracted and isolated by distillation to produce substances referred to as essential oils (52). Essential oils are oils from extraction, ingredients of volatile substances, and nonvolatile materials that induce a smell with specific properties (54).

Using LTLT tended to produce a more robust milk smell, approximately 56%) besides HTST (44%). The distinctive aroma of milk can be lost by high-temperature heating, which causes the evaporation of sulfides, especially hydrogen sulfide. The change in smell is also due to the MLE essential oil content, contributing to PMi. In contrast, heat induces protein denaturation. Whey protein beta-lactoglobulin was significantly degraded by increasing the heating temperature from the pre-heating temperature (60°C) to the UHT temperature (ultra-high temperature, 120°C) with a heating time of 5-300 seconds. This study's heating process above 74°C denatures sulfhydryl (-SH) and β-lactoglobulin (55,56). Thus, aggregation occurred because of disulfide bonds and hydrophobic interactions. The aggregation rate was higher when skim milk was heated to 85°C and 95°C. Protein aggregation mainly occurs in kaffa-casein, β-lactoglobulin, and other whey proteins (57).

3.10.3. Taste

When using MLE 0.05 - 0.20%, a change in PMi taste begins. Tannins in matoa leaves provide a sense of bitterness, which contributes to minimizing the characteristic milk taste. Tannins contain astringent substances in polyphenol groups that have a bitter taste (39).

The organoleptic test results in Figure 3 show that different PMs can affect the taste of the PMi. 72% of panelists agreed that LTLT PM gives a more pungent milky taste than HTST PM (assessed by 68% of panelists). Separate assessment according to the number of panelists judged. They were informed of the milk samples tested. It was concluded that LTLT PM provided a better milk taste than HTST PM. These results can be explained by the fact that an increase in temperature increases the solubility of tannins, which causes an increase in bitter taste.

3.10.4. Preference Test

Preference assessment generally depended on the panelists' likes and dislikes. Organoleptic tests measure consumer preference levels (20). A low percentage of 0-0.15 % MLE added to PMi was preferred while using 0.20% MLE convinced the panelists to dislike PMi. Every panelist received a different impression depending on their ability to sense the samples.

PMi milk added to MLE with a pasteurization system using the PM LTLT method is preferred by consumers based on the panelists' preference test data, with 20% of the panelists stating they like it very much. In comparison, 12% of panelists liked the HTST PM. This result was prompted by the higher temperature in HTST PM, which developed the cooked flavor and affected the panelist's response (29). Changes in taste generally influence a psychological panelist's reaction in the form of an impression or reply to the sample (36). This study is still limited and needs further research support for product endurance studies before the industry can adopt the downstream of the product.

4. Conclusions

The addition of matoa leaf extract to pasteurized milk can be an innovation in the dairy industry to obtain healthy, functional pasteurized milk. The addition of MLE to PMi has the potential to enhance the health benefits of pasteurized milk. In this research article, pasteurized milk (PMi) was added with matoa leaf extract (MLE) in various concentrations (0; 0.05; 0.1; 0.15; 0.20%) and pasteurized using two methods, namely LTLT and HTST. There was a significant difference between pasteurized PMi with LTLT and HTST in terms of antioxidant activity, TBA value, and viscosity but no significant difference in fat content. There was a significant difference in PMi between each MLE level with added antioxidant activity, but no significant difference between PMi without added MLE and the TBA value-added 0.05%. The fat content value differed between the 0.10% and 0.15% PMi. At the same time, the viscosity of PMi was not significantly different between the addition of 0.10% and 0.15% MLE. In the organoleptic test, PMi with varying levels of MLE produced PMi colors ranging from white to brownish in both LTLT and HTST. The aroma of PMi was assessed based on the smell of milk. The panelists agreed that PMi did not have a fresh taste and only had a slightly fresh taste.

The preference test conducted by the panelists indicated that they preferred white. About 56% of the panelists stated that the aroma and taste of milk remained strong even of MLE was added, with the most preferred sensory combination being the addition of 0.2% MLE using the HTST method. The results of this study can be applied to the dairy industry as functional pasteurized milk after further research is conducted on product durability and how long MLE functions as an antioxidant.

Acknowledgments

This work was funded by the Ministry of Research and Technology of Indonesia.

Author Contributions

Conceptualization, R.M. and F.M.; methodology, F.M.; software, M.A.V.V.; validation, A.J.S., R.M, F.M., and M.A.V.V; formal analysis R.M; investigation, F.M., M.A.V.V., A.J.S.; resources, A.J.S.; data curation, R.M.; writing—original draft preparation, F.M.; writing—review and editing, R.M.; visualization, M.A.V.V.; supervision, R.M.; project administration, R.M.; funding acquisition, M.A.V.V. and A.J.S. All authors have read and agreed to the published version of the manuscript.

Funding

This work was funded by the Ministry of Research and Technology, Indonesia.

Institutional Review Board Statement

This research did not use an animal laboratory.

Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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