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Antibacterial Effects of Aromatic Materials Produced in Indonesia on the Preservation of Skimmed and Whole Milk in Storage

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Abstract: Antibacterial effects of aromatic materials, produced in Indonesia, on the preservation of stored milk were investigated. Organoleptic evaluation, bacterial growth, protease activities, lipase activities, protein degradation and acidities of milk with addition of 10% aromatic materials were assessed by panelists, total counts, azocasein method, modified dole extraction, formol and base-acid titrations, respectively. At 5 days, after the expiry date, 19 of the 28 aromatic milk were better than control, and 10 of these were better than the others, while the aromatic whole milk were shown to be better than the skimmed milk. These 10 were the ones with the additions of honey, cinnamon, ginger, turmeric, zingiber, wild ginger, nutmeg, pepper, garlic and galangale. Bacterial growth, protease activities, lipase activities, protein degradation and acidities of 10 aromatic whole milk were in the range: 2.4×10^2 - 7.4×10^5 cfu/ml; 0.18 - 0.32 U/ml; 0.22 - 0.26 μequiv.ml^{-1}h^{-1}; 2.50 - 2.58% - 0.27 - 0.32%; while that of skimmed milk were 7.3×10^4 - 8.2×10^5 cfu/ml; 0.33 - 0.43 U/ml; 0.15 - 0.22 μequiv.ml^{-1}h^{-1}; 2.68 - 2.95% - 0.31 - 0.38%; respectively.

Keywords: Whole milk, skimmed milk, antibacterial and aromatic materials, protease, lipase, preservation

INTRODUCTION

The qualities of pasteurized milk within refrigerated storage were affected by temperatures and types. The temperatures used to keep the quality of the milk in storage were between 4 - 7°C, up to their expiry date (Chandler *et al.*, 1990). At higher than storage temperatures, decrease in quality may have resulted. Furthermore, the different types of milk in storage may have resulted in the different qualities and shelf lives (Deeth *et al.*, 2002). It can be seen that there were differences in term of qualities and shelf lives between the skimmed milk and whole milk. The qualities and shelf lives of skimmed milk were lower than that of whole milk in refrigerated storage at 4 - 7°C (Janzen *et al.*, 1982), and the bacterial growth and protease activities activities of skimmed milk in storage were higher than that of whole milk (Janzen *et al.*, 1982; Deeth *et al.*, 2002).

The problem in the pasteurization process in plants which affected the qualities of the pasteurized milk in storage were that the psychrotrophic bacteria were killed by the pasteurization process, however, after the pasteurization process, particularly if hygiene in the processing plant is of a poor standard, these psychotrophic bacteria can re-contaminate milk (Craven and Macauley, 1992). The growth of these bacteria, especially *Pseudomonas* spp. occurred during the time of milk storage on or after the expiry date, and the longer the time of storage, the higher the growth and enzyme activities of the psychrotrophic bacteria in the milk, which may result in spoilage of the milk in storage (Bucky *et al.*, 1986; Chandler *et al.*, 1990; Deeth *et al.*, 2002). The spoilage of pasteurized milk in refrigerated storage may result in the microbial and chemical changes, and the volatile compounds of the milk (Reinheimer *et al.*, 1993). The biochemical changes of milk during spoilage may be a result of the activities of extracellular enzymes especially protease which degrades protein (Janzen *et al.*, 1982); and lipase which degrades lipid (Bucky *et al.*, 1986).

In Indonesia, the spoilage of commercially pasteurized milk packaged in cartons and produced from factories located mainly in Java and then distributed throughout the local area and outside Java, was due to the activities of

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psychrotrophic bacteria. These bacterial activities may have an affect on the shelf life of the milk, with the average shelf life of the milk being around 7 days (Khusniati *et al.*, 2006). However, Heo (1989) examined commercial milk samples stored at 7.2°C and it was found that after 10 days of storage, 91% of the whole milk was acceptable, and after 14 days, 82% were still acceptable.

In commercially pasteurized milk, distributed both in and out of the local area from the factories in Indonesia, the spoilage within refrigerated storage may be reduced by the addition of aromatic materials. It is well known that there are a lot of commercial aromatic materials produced in Indonesia, such as: honey, cinnamon and citronella (sweet); ginger, radish, turmeric, galingale, zingiber and wild ginger (roots); nutmeg, cardamon, cumin and pepper (seeds); garlic, clove, javanoni and galingale (bubs); green tea (dried leaf); laurellike, bamboo leaf, banana leaf, guava leaf, avocado leaf, betel vine, celery, garlic leaf, aloe vera and pandan (fresh leafs). The antibacterial and aromatic compounds of these 28 aromatic materials are shown in Table 1. To extend the shelf life of the commercially skimmed and whole milk produced in Indonesia, these aromatic materials may be used as preservatives.

The effectiveness of the antibacterial and aromatic compounds of the aromatic materials as preservatives in inhibiting spoilage bacteria and/ or pathogenic bacteria may differ from each other. This difference may be due to the different types of antibacterial and aromatic compounds between each of them, as shown in Table 1. The aromatic materials which contain different antibacterial and volatile compounds may have an affect on the preservation of the milk, and it is thought that these different materials may have resulted in the different rates of suppression of the growth and enzymatic activities of psychrotrophic bacteria. The differences in suppression of the bacteria may result in the differences in increased quality and shelf lives (from start of pasteurization process to expiry date), of pasteurized milk. Furthermore, the mechanisms of the antibacterial and aromatic compounds in the suppression of the bacterial growth and enzymatic activities of psychrotrophic bacteria on the preservation of the milk in storage haven't been reported to date. However, it has been reported that aromatic materials (sweets, roots, seeds, bulbs, dried leafs and fresh leafs) which contain antibacterial and aromatic compounds, as shown in Table 1, inhibited spoilage bacteria and/ or pathogenic bacteria.

This paper reports the antibacterial effects of aromatic materials produced in Indonesia on the preservation of skimmed and whole milk in storage.

MATERIALS AND METHODS

Milk Samples

The same lots of commercially pasteurized milk (the same factory and times of the expiry dates of commercially pasteurized milk) processed at 85ºC for 30 minutes, packaged in 1 and 2 litre glass bottles were used. The milk samples kept in glass bottles were put in an ice box to maintain quality during transportation to the laboratory and the samples were then stored at 4°C until used.

At 5 days before the expiry date, 100 ml aliquot of each milk sample was transferred aseptically into 200 ml sterile bottles and the samples were organoleptically assessed. The samples at 5 days after the expiry date were then analyzed for total aerobic count, protease activities, lipase activities, protein degradation and acidities. Each treatment was performed in triplicate. Five days period was chosen because there were significant differences in growth and enzymatic activities of *Pseudomonas* spp. in stored milk at 5 days intervals (Deeth *et al.*, 2002).

Preparation of Aromatic Materials

Aromatic materials produced in Indonesia were collected from various supermarkets and traditional markets in the city of Bogor. These materials contained both antibacterial and aromatic compounds. The 28 materials used in these treatments were honey, cinnamon and citronella (sweets); ginger, radish, turmeric, galingale, zingiber and wild ginger (roots); nutmeg, cardamom, cumin and pepper (seeds); garlic, clove, javanoni and galingale (bubs); green tea (dried leaf); laurellike, bamboo leaf, banana leaf, guava leaf, avocado leaf, betel vine, celery, garlic leaf, aloe vera and pandan (fresh leafs). The sweet material of honey was prepared in liquid form, while cinnamon and citronella were prepared from blended fresh materials. The other materials of roots, seeds, bulb and fresh leafs were also prepared by blending, while the dried leaf of green tea was prepared by blending the dry materials.

Ten grams of each of the materials prepared both in liquid and blended fresh and dry materials were put in opened petri dishes ∅ 3 cm in the middle of 100 ml of milk and placed in the closed petri dishes ∅ 15 cm for the detection of antibacterial effects of the aromatic materials on

Table 1: Antibacterial and aromatic compounds of aromatic materials produced in Indonesia

No.	Antibacterial and aromatic materials	Antibacterial and aromatic compounds	References
1.	honey	”inhibine”, hydrogen peroxide, flavonoid	Lusby *et al.*, 2005
2.	cinnamon	cinnamic acid, cinnamon oil, ethanol, methylene chloride, eugenol, benzyl benzoate	Tabak *et al.*, 1999
3.	citronella	citronella oil, kingisidic acid	Deans and Ritchie, 1987
4.	ginger	“gingerin”, gingerols, a-zingiberene, shorgaols, ginger oil	Chrubasik *et al.*, 2005
5.	radish	some glucosinolates, hexenyl acetat	Tirranen *et al.*, 2001
6.	turmeric	turmeric oil	Lantz *et al.*, 2005
7.	galingale	aromatic galingale	Anonymous, 2006
8.	zingiber	zingiber oil, acyclic oxygenated monoterpenes	Deans and Ritchie, 1987
9.	wild ginger	wild ginger oil	Deans and Ritchie, 1987
10.	nutmeg	diphenylpropanoids, ethyl acetate	Sherry *et al.*, 1982
11.	cardamon	aromatic cardamon	Mummenhoff and Hurka, 1991
12.	cumin	glycosides of 2-C-methyl-D-erythritol, cumin oil	Deans and Ritchie, 1987
13.	pepper	adipic acid, piperine, oleorecine	Chitwood *et al.,* 2003
14.	garlic	‘allicin’, free phenolics, p-coumaric, ferulic, p-hydroxybenzoic, garlic oil and vanillic acid and specific garlic aromatic	Tirranen *et al.*, 2001
15.	clove	clove oil, isobiflorin, biflorin, eugenol, phenolic compound, ferulic acid	Deans and Ritchie, 1987
16.	javanoni	anthraquinon and aromatic javanoni	Anonymous, 2001a
17.	galangale	galangale oil (cineol, a-pinene, eugenol, camphor, methyl cinnamate and sesquiterpenes)	Deans and Ritchie, 1987
18.	green tea	polyphenolic, phenolic acid, catechins, caffeine, flavonoid, epicatechin and ascorbic acid	An *et al.*, 2004
19.	laurellike (Indonesian bay-leaf)	aromatic laurellike	Anonymous, 2001b
20.	bamboo leaf	cinnamic derivatives	Tachibana *et al.*, 1992
21.	banana leaf	starch phosphorylase, polycyclic aromatic hydrocarbon, non volatile organic acid	Palmer and Wyman, 1965
22.	avocado leaf	(R)-2-hydroxy-4-oxohenicosan-1-yl acetat, aldehydes, ketones, alcohols, terpenoides, estragole, and 2-hexenal	Carman and Duffield, 1995
23.	celery	apiole, 3-butylphthalide, sedanenolide, monoterpene, hydrocarbon, phthalides, limonene	MacLeod and Ames, 1989
24.	garlic leaf	garlic leaf lectin	Tirranen *et al.*, 2001
25.	aloe vera	glucomannans, uronic acid, glycoproteins, anthraquinones, saccharrides, and phenolic compounds	Ni *et al.*, 2004
26.	pandan	pandan oil, monoterpene hydrocarbon and sesquiterpene hydrocarbon	Deans and Ritchie, 1987
27.	betel vine	betel vine oil, eugenol, 1,3 benzodioxol (5)-2-propenyl, anethole	Deans and Ritchie, 1987

preservation of the milk in storage. The control (s) used were sterilized water, placed in opened petri dishes located in the middle of the milk which was then placed in the closed petri dishes.

Aromatic Materials Assessments

One sample from each of the 28 different aromatic materials (10 gram) was placed in the middle of 100 ml aliquots from the batch of pasteurized milk. The milk samples with the addition of aromatic materials, together with their controls, were incubated at 4°C for up to 5 days after their expiry dates. At 5 days before the expiry date, samples of the controls of the 28 samples with additional aromatic materials were assessed organoleptically; and at 5 days after the expiry date, all samples were assessed visually and analyzed for total aerobic counts, acidities, protein degradation and then used for the production of cell-free supernatants to be assessed for protease activities and lipase activities. The results presented for bacterial counts, acidities, protein degradation, protease activities and lipase activities are mean values for the three replicates.

Sensory Assessments

Sensory assessments were randomly conducted for evaluation by 18 panelists (Indonesians). The assessments were based on the evaluation of taste, colour, flavour and performance. The tastes were noted as acidic, sweet, flat, milky or others; the colours were noted as white, green, red or others; the flavours were noted as flat, acidic, sweet, milky or others; and the performances were noted as homogeneous, non-homogeneous or others.

Total Aerobic Bacterial Counts

Ten-fold serial dilutions of the milk samples were made and spread plate counts performed according to Australian Standard AS 1766.1.4 using Nutrient Agar. The plates were incubated for 2 - 3 days at 30°C.

The Acidities of Aromatic Milks

The acidities of the pasteurized milk containing antibacterial and aromatic materials at various times of storage were measured using the base-acid titration method (Cunniff, 1999). The acidities of 28 samples of skimmed and whole milk, containing aromatic materials, were measured by the titration method at the time of storage 5 days after the expiry dates. Ten ml of milk containing aromatic materials were poured into 100 ml Erlenmeyer flasks and several drops of phenolphthalein added. The solutions were titrated and the acidity was based on [volume of NaOH used x concentration of NaOH standardized x 90.000] per volume of milk titrated.

Preparation of Cell Free Extracts

Bacterial cells were removed from the incubated milk samples (with and without aromatic materials by centrifugation at 24,000 x g for 10 minutes at 4°C). The milk samples used to prepare the free extracts for detecting protease activities and lipase activities were 100 ml for each. The resulting supernatants were collected and stored at -20°C in sterile bottles until assayed for enzyme activities.

Protease Activity Assay

Proteolytic activity was assayed by the azocasein method, using sulphanilamide-azocasein (Sigma Chemical Co., USA) as the substrate, according to the method of Christen and Marshall (1984), with some modifications. The reaction mixture contained 2 ml of azocasein (10 g/l, dissolved by heating 0.1 M Tris-HCl buffer pH 7.4 (sterile) containing 2 mM $CaCl_2$ at 63°C for 30 min) and 0.5 ml enzyme solution (cell free supernatant), and was incubated for 1 hour at 37°C. One unit of proteolytic activity was defined as the volume of enzyme solution (ml) required to produce an absorbance increase at 345 nm of 0.01 A.U. in 1 hour under the assay conditions.

Lipase Activity Assay

The lipase activity assay was carried out based on the assay procedure of a modified Dole extraction procedure (Deeth *et al.*, 1975). Cell-free culture supernatants (crude enzyme, 0.5 ml), 0.25 ml buffer (2 M diethanolamine-HCl, pH 8.5), 3 ml UHT cream, and 1 ml sterile water (in a stoppered test tube) were incubated at 40°C for 2 hours in a shaking (100 rpm) water-bath, and lipase activities were measured by titration. The activities of the cell-free supernatants are expressed as μequiv.$ml^{-1}h^{-1}$.

Protein Degradation

Protein degradation was detected using the method of formol titration (Lakin, 1978) with some modifications. Ten ml of milk was added to 20 ml sterilized water, 0.4 ml saturated di-potassium oxalate monohydrate ($K_2C_2O_4.H_2O$), and 1 ml phenophthalein 1%. These solutions were then kept for 2 minutes and titrated. Protein degradation was calculated as a percentage (%) of milk protein = 1.83 x the amounts of NaOH 0.1 M for titration.

Statistical Analysis

All treatments were statistically analyzed by ANOVA with Factorial Complete Randomized Design (Snedecor and Cochran, 1989) using General Linear Model with three replications.

RESULTS

Organoleptic evaluation (tastes, flavours, colours and performances) of the skimmed and whole milk containing aromatic materials during cold storage are shown in Table 2. The organoleptic evaluation of these aromatic skimmed and whole milk with scores in the range 2.65 - > 2.65 were more acceptable (very good colour, flavour and performance and very nice in taste); 2.50 - < 2.65 were acceptable (good in colour, flavour and performance and nice in taste); 2.00 - < 2.50 were quite acceptable (quite good in colour, flavour and performance and quite nice in taste); and 1.00 - < 2.00 were unacceptable (bad in taste, colour, flavour and performance).

The organoleptic performances of all the milk with the addition of 10% aromatic materials were classed generally as acceptable by the panelists, although some of them were unacceptable. Twenty six of the 28 aromatic skimmed and whole milk were classed as more acceptable, acceptable and quite acceptable; while the other two were classed as unacceptable.

From the 26 selected aromatic whole milk, the organoleptic evaluation of these showed that 10 were more acceptable, 14 were acceptable, and 2 were quite acceptable. The 10 that were aromatic whole milk containing honey, cinnamon, citronella, ginger, galingale, nutmeg, galangale, green tea, aloe vera and pandan; and the 14 that were aromatic whole milk with turmeric, zingiber, wild ginger, cumin, pepper, clove, javanoni, laurellike, bamboo leaf, banana leaf, guava leaf, avocado leaf, celery and garlic leaf were acceptable;

Table 2: Organoleptic evaluation of skimmed and whole milk with the addition of aromatic materials (taste, color, flavor, performance)

The antibacterial and aromatic materials	Whole milk	Skimmed milk
Milk	more acceptable	more acceptable
Honey	more acceptable	more acceptable
Cinnamon	more acceptable	acceptable
Citronella	more acceptable	more acceptable
Ginger	more acceptable	more acceptable
Radish	unacceptable	unacceptable
Turmeric	acceptable	acceptable
Galingale	more acceptable	acceptable
Zingiber	acceptable	quite acceptable
Wild ginger	acceptable	acceptable
Nutmeg	more acceptable	acceptable
Cardamon	unacceptable	unacceptable
Cumin	acceptable	acceptable
Pepper	acceptable	quite acceptable
Garlic	quite acceptable	quite acceptable
Clove	acceptable	quite acceptable
Javanoni	acceptable	acceptable
Galangale	more acceptable	acceptable
Green tea	more acceptable	acceptable
Laurellike	acceptable	acceptable
Bamboo leaf	acceptable	acceptable
Banana leaf	acceptable	acceptable
Guava leaf	acceptable	acceptable
Avocado leaf	acceptable	quite acceptable
Betelvine	quite acceptable	acceptable
Celery	acceptable	quite acceptable
Garlic leaf	acceptable	quite acceptable
Aloe vera	more acceptable	more acceptable
Pandan	more acceptable	acceptable

Notes: more acceptable (> 2.65), acceptable (≥ 2.50);
quite acceptable (≥ 2.00); unacceptable (< 2.00)

Table 3: Antibacterial effect of aromatic materials produced in Indonesia on preservation of whole milk at storage up to 5 days after expiry date

No.	Aromatic materials	Total bacterial counts (cfu/ml)	Protease activities (U/ml)	Lipase activities (μequiv. ml^{-1} h^{-1})	Protein degradation (%)	Acidities (%)
1.	Milk(control)	8.6 x 10^5 c	0.51 c	0.35 a	2.98 c	0.42 bc
2.	Honey	7.5 x 10^4 f	0.23 h	0.25 lmno	2.55 j	0.31 rstu
3.	Cinnamon	7.4 x 10^4 f	0.22 h	0.25 klmno	2.55 j	0.29 tuvw
4.	Citronella	7.5 x 10^5 e	0.37 f	0.28 fghi	2.78 g	0.34 lmnop
5.	Ginger	2.5 x 10^2 g	0.19 I	0.23 opqrs	2.51 k	0.28 vw
6.	Radish	8.3 x 10^5 d	0.43 e	0.34 ab	2.84 f	0.40 cdef
7.	Turmeric	7.3 x 10^4f	0.22h	0.24 mnop	2.54j	0.29uvw
8.	Galingale	7.5 x 10^5 e	0.37 f	0.27 hijkl	2.78 g	0.33 opqrs
9.	Zingiber	7.5 x 10^4 f	0.23 h	0.25 klmno	2.55 j	0.31 rstu
10.	Wild ginger	7.4 x 10^5 e	0.32 g	0.26 ijklm	2.58 m	0.31 qrstu
11.	Nutmeg	7.4 x 10^4 f	0.22 h	0.25 klmno	2.55 j	0.30 stuv
12.	Cardamon	8.3 x 10^5 d	0.43 e	0.33 abc	2.84 f	0.39 cdefg
13.	Cumin	7.5 x 10^5 e	0.37 f	0.27 hijk	2.78 g	0.33 opqrs
14.	Pepper	7.3 x 10^4 f	0.22 h	0.24 mnop	2.54 j	0.29 uvw
15.	Garlic	2.4 x 10^2 g	0.18 I	0.22 pqrst	2.50 k	0.27 w
16.	Clove	7.5 x 10^5 e	0.37 f	0.26 ijklm	2.78 g	0.32 pqrst
17.	Javanoni	7.5 x 10^5 e	0.37 f	0.27 hijkl	2.78 g	0.33 opqrs
18.	Galangale	7.4 x 10^5 e	0.32 g	0.26 jklmn	2.58 i	0.32 pqrst
19.	Green tea	7.5 x 10^5 e	0.37 f	0.28 ghij	2.78 g	0.33 pqrst
20.	Laurellike	8.2 x 10^5 d	0.42 e	0.30 def	2.83 f	0.35 jklmno
21.	Bamboo leaf	8.2 x 10^5 d	0.42 e	0.30 cde	2.83 f	0.35 jklmno
22.	Banana leaf	8.2 x 10^5 d	0.42 e	0.30 def	2.83 f	0.35 klmno
23.	Guava leaf	8.2 x 10^5 d	0.42 e	0.31 cde	2.83 f	0.35 jklmno
24.	Avocado leaf	7.5 x 10^5 e	0.37 f	0.29 efgh	2.79 g	0.34 mnop
25.	Betelvine	8.3 x 10^5 d	0.43 e	0.32 bcd	2.84 f	0.36 Hijklm
26.	Celery	8.3 x 10^5 d	0.43 e	0.32 bcd	2.84 f	0.37 ghijkl
27.	Garlic leaf	7.5 x 10^5 e	0.37 f	0.29 efgh	2.79 g	0.34 lmnop
28.	Aloe vera	8.2 x 10^5 d	0.43 e	0.32 bcd	2.84 f	0.38 fghij
29.	Pandan	7.5 x 10^5 e	0.37 f	0.30 defg	2.79 g	0.34 lmnop

Note: Different letters in the same column represent significant differences ($P < 0.05$)

while 2 were aromatic whole milk with garlic and betelvine.

From the 26 selected aromatic skim milk, the organoleptic evaluation of these showed that 4 were more acceptable, 15 were acceptable, while 2 were unacceptable. The 4 that were aromatic skimmed milk containing honey, citronella, ginger and aloe vera; and the 15 that were aromatic skimmed milk had cinnamon, turmeric, galingale, wild ginger, nutmeg, cumin, javanoni, galangale, green tea, laurellike, bamboo leaf, banana leaf, guava leaf, betelvine and pandan; and the 7 that were aromatic skimmed milk had zingiber, pepper, garlic, clove, avocado leaf, celery and garlic leaf.

So, the 26 selected aromatic skimmed and whole milk were classed as good based on organoleptic assessments. However, the good milk were then analysed for total bacterial counts, enzymatic activities, protein degradation and acidities, to know more about the quality and shelf life of aromatic milk. Antibacterial effects of aromatic materials produced in Indonesia on the preservation of whole milk in storage are shown in Table 3, while that of skimmed are shown in Table 4.

On the preservation of skimmed and whole milk in refrigerated storage using aromatic materials, the total bacterial counts, proteolysis, protein degradation, lipolysis and acidities of these aromatic skimmed and whole milk were lower than that of the control (without aromatic materials). This was because production of psychotrophic bacteria in the aromatic milk was lower than for the control. The decrease in production of psychrotrophic bacteria in the aromatic milk may have resulted in the decrease of the total bacterial counts, proteolysis, protein degradation, lipolysis and acidities in the milk.

The total bacterial counts, protease activities, protein degradation and acidities of 19 out of the

Table 4: Antibacterial effect of aromatic materials produced in Indonesia on preservation of skimmed milk at storage up to 5 days after expiry date

No.	Aromatic materials	Total bacterial counts (cfu/ml)	Protease activities (U/ml)	Lipase activities (µequiv. ml^{-1} h^{-1})	Protein degradation (%)	Acidities (%)
1.	Milk	9.8×10^6 a	0.62 a	0.27 hijkl	3.18 a	0.45 a
2.	Honey	7.4×10^5 e	0.38f	0.19uv	2.87 e	0.34 mnopq
3.	Cinnamon	7.4×10^5 e	0.38 f	0.19 uv	2.85 ef	0.33 opqrs
4.	Citronella	8.3×10^5 d	0.48d	0.21 rstu	2.98 c	0.38 efghi
5.	Ginger	7.4×10^4 f	0.33 g	0.16 w	2.68 h	0.31 rstu
6.	Radish	7.9×10^6 b	0.54 b	0.26 jklmn	3.04 b	0.43b
7.	Turmeric	7.4×10^5 e	0.38 f	0.17vw	2.85 ef	0.33 opqrs
8.	Galingale	8.3×10^5 d	0.48 d	0.20 tu	2.98c	0.38 efghi
9.	Zingiber	7.4×10^5 e	0.38 f	0.22 pqrst	2.87 e	0.34 mnop
10.	Wild ginger	8.2×10^5 d	0.43 e	0.20 stu	2.95 d	0.36 ijklmn
11.	Nutmeg	7.4×10^5 e	0.38 f	0.19 uv	2.87 e	0.33 nopqr
12.	Cardamon	7.9×10^6 b	0.54b	0.26 jklmn	3.04 b	0.41bcd
13.	Cumin	8.3×10^5 d	0.48d	0.20 tu	2.98c	0.38 efghi
14.	Pepper	7.4×10^5 e	0.38 f	0.17 vw	2.85 ef	0.33 opqrs
15.	Garlic	7.3×10^4 f	0.33 g	0.15 w	2.68 h	0.31 qrstu
16.	Clove	8.3×10^5 d	0.48d	0.19 uv	2.98 c	0.38 fghij
17.	Javanoni	8.3×10^5d	0.48d	0.21 rstu	2.98c	0.38 fghijk
18.	Galangale	8.2×10^5 d	0.43 e	0.19 uv	2.95 d	0.38 efghi
19.	Green tea	8.3×10^5 d	0.48 d	0.21 qrstu	2.98 c	0.38 fghijk
20.	Laurellike	7.8×10^6 b	0.53 bc	0.23 nopq	3.03 b	0.39 cdefgh
21.	Bamboo leaf	7.8×10^6 b	0.53 bc	0.23 nopq	3.03 b	0.40 cdef
22.	Banana leaf	7.8×10^6 b	0.53 bc	0.24 mnop	3.03 b	0.39 cdefg
23.	Guavaleaf	7.8×10^6 b	0.53 bc	0.24 mnop	3.03 b	0.39 cdefgh
24.	Avocado leaf	8.3×10^5 d	0.48 d	0.22 opqr	2.98 c	0.39 defghi
25.	Betelvine	7.9×10^6 b	0.54 b	0.23 opqrs	3.04 b	0.40 cdef
26.	Celery	7.9×10^6 b	0.54 b	0.23 opqrs	3.04 b	0.40 cdef
27.	Garlic leaf	8.3×10^5 d	0.47 d	0.22 opqr	2.98 c	0.39 fghijk
28.	Aloe vera	7.9×10^6 b	0.54 b	0.25 klmno	3.04 b	0.41 bcde
29.	Pandan	8.3×10^5 d	0.47 d	0.23 nopq	2.98 c	0.38 cdefgh

Note: Different letters in the same column represent significant differences ($P < 0.05$)

26 selected aromatic skimmed and whole milk, 10 of the 19 aromatic skimmed and whole milk, and 10 of the 19 aromatic whole milk were lower than that of control; the others, and the skimmed milk, respectively, at time of storage 5 days after the expiry date ($P < 0.05$). Furthermore, the lipase activities of the 19 aromatic skimmed and whole milk were lower than the control; and the activities of the 10 aromatic skimmed and whole milk weren't different to the others, while the activities of the 10 aromatic whole milk were higher than that of the skimmed, at the same time of storage ($P < 0.05$). The 19 that were aromatic skimmed and whole milk contained honey, cinnamon, ginger, turmeric, zingiber, wild ginger, nutmeg, pepper, garlic, galangale, citronella, galingale, cumin, clove, javanoni, green tea, avocado leaf, garlic leaf and pandan; while the 10 milk that were aromatic skimmed and whole milk with honey, cinnamon, ginger, turmeric, zingiber, wild ginger, nutmeg, pepper, garlic and galangale.

So, based on organoleptic assessments, total bacterial counts, protease activities, protein degradation and acidities, the qualities of the 19 aromatic skimmed and whole milk, the 10 aromatic skimmed and whole milk, and the 10 aromatic whole milk were better than that of control, the others, and the skimmed milk, respectively, at time of storage 5 days after the expiry dates.

DISCUSSION

The organoleptic performances of 19 of the 26 aromatic skimmed and whole milk, 10 of the 19 aromatic skimmed and whole milk, and 10 of the 19 aromatic whole milk, were better than the control, the others, and the skimmed milk, respectively, at storage up to 5 days before the expiry date. This may be attributable to the fact that the 19 aromatic skimmed and whole milk were more fresher than the control, the nutritional and aromatic compounds of the 10 aromatic skimmed and whole milk were better than that of the others, and the lipid contents of the 10 aromatic whole milk were higher than that of the skimmed milk, respectively, at 5 days before the expiry date.

It has been reported that at 5 days before the expiry date (the designated expiry date), the taste, colour, flavour and performance of the pasteurized milk were fresh because at that time there was no growth of psychotrophic bacteria (Khusniati *et al.*, 2006). Furthermore, the different nutritional and aromatic compounds of the aromatic milk may have resulted in the different organoleptic performance of the milk. Moreover, the differeing lipid content between skimmed and whole milk may have resulted in the different organoleptic performance between the two milk. The lipid contents of whole milk were higher than that of the skimmed milk (Chandler *et al.*, 1990), and the shelf life of the whole milk was higher than that of the skimmed milk (Deeth *et al.*, 2002).

The bacterial growth, protease activities, protein degradation and acidities of 19 of the 26 aromatic skimmed and whole milk, 10 of the 19 aromatic skimmed and whole milk, and 10 of the 19 aromatic whole milk were lower than that of control, the others, and the skimmed milk, respectively, at storage of 5 days after the expiry date ($P < 0.05$). Furthermore, lipase activities of the 19 aromatic skimmed and whole milk were higher than the control, and the activities of the 10 aromatic skimmed and whole milk showed no difference to the others, while the activities of the 10 aromatic whole milk were lower than that of the skimmed milk ($P < 0.05$). These results may be due to the antibacterial and aromatic compounds in the 19 aromatic skimmed and whole milk can suppress bacterial growth and protease activities of psychrotrophic bacteria, especially *Pseudomonas* spp. Furthermore, the antibacterial and aromatic compounds in the 10 aromatic skimmed and whole milk and the 10 aromatic whole milk were more effective than in the others and the skimmed milk, respectively, for 5 days of storage after the expiry date.

The suppression of bacterial growth and protease activities of the bacteria in those three may be due to a reduction in protein degradation and acidities of the milk, at the same times of storage. Moreover, the different characteristics of lipase activities in those three milk may be because of the activities of the antibacterial and aromatic compounds in inhibiting lipase activities of psychrotrophic bacteria in the 19 aromatic skimmed and whole milk were lower than the controls, and the activities of the compounds in the 10 aromatic skimmed and whole milk weren't different to that of the others, while the activities in the 10 aromatic whole milk were higher than that in the'skimmed milk, respectively.

Several reports showed that the bacterial growth, protease activities and lipase activities of psychotrophic bacteria, especially *Pseudomonas* spp. in refrigerated milk increased at various storage times (Janzen *et al.*, 1982; Bucky *et al.*, 1986; Chandler *et al.*, 1990; Deeth *et al.*, 2002). The protease activities of the bacteria may have resulted in the protein degradation of the pasteurised milk in refrigerated storage, and this degradation may have resulted in the change of the acidities of the milk (Reinheimer *et al.*, 1993). The bacterial growth and protease activities of the psychrotrophic bacteria in the whole milk in refrigerated storage were lower than that within the skimmed milk (Chandler *et al.*, 1990). The lipase activities of the bacteria in whole milk in storage were higher than that in the skimmed milk (Janzen *et al.*, 1982; Bucky *et al.*, 1986: Deeth *et al.*, 2002). Furthermore, the antibacterial and aromatic compounds of aromatic materials inhibited spoilage and/or pathogenic bacteria and the different compounds of the different materials may have resulted in the different effects in inhibiting the bacteria. However, some compounds of the different materials may have resulted in the same effects in inhibiting the bacteria (Deans and Ritchie, 1987; Tirranen *et al.*, 2001; Lusby *et al.*, 2005).

Moreover, the compounds inhibiting the bacteria in whole milk were more effective than that in skimmed milk, because the lipid contents of whole milk may be used for protection in attacking the bacteria. It has been shown that the lipid contents in whole milk were higher than that in skimmed milk (Chandler *et al.*, 1990).

CONCLUSION

The antibacterial effects of 19 of the 28 aromatic materials produced in Indonesia, on the preservation of milk in storage were investigated. At 5 days after the expiry date, based on organoleptic performances, bacterial growth, protease activities, protein degradation and acidities, the qualities of 19 of the 28 aromatic milk were better than that of the control, and 10 of these had an addition of 10% aromatic materials (honey, cinnamon, ginger, turmeric, zingiber, wild ginger, nutmeg, pepper, garlic and galangale), and these were all found to be better than the others, while the aromatic whole milk were better than the skimmed milk.

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