Supplementation of matoa (*Pometia pinnata*) leaf extract and alginate suppressed the growth of *Staphylococcus aureus* and *Escherichia coli* in pasteurized milk

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**Abstract.** The usage of natural additives in pasteurized milk processing allows enhancing the functionality of the product. In this matter, matoa (*Pometia enhancing*) and alginate become a precious ingredient that exerts antimicrobial properties against *Staphylococcus aureus* and *Escherichia coli*. This present work aimed to investigate the proper level of matoa leaf extract and alginate showing the best inhibitory effects on the growth of *Escherichia coli* and *Staphylococcus aureus*. Completely randomized design with factorial was arranged, consisting of 2 factors: matoa leaf extracts (0%, 0.1%, 0.15% dan 0.20%) and alginate (0%, 0.1%, 0.2%, dan 0.3%). The experiment was performed by triplicate. As a result, the treatments showed a wide range of inhibition towards *S. aureus* and *E. coli* from 2.68 to 10.48 mm and from 3.15 to 12.34 mm, respectively. In this case, matoa leaf extract was believed capable of suppressing the growth of both bacteria, and the inhibition seemed to increase as more extract was added. Meanwhile, alginate showed the antibacterial effect on *E. coli*, but not *S. aureus*. The higher level of alginate also produced more suppressing effects on the growth of *E. coli*. However, no interaction effect was found between matoa leaf extract and alginate in reducing the population of *S. aureus* and *E. coli*. In short, the best inhibition was achieved in the use of the matoa leaf extract of 0.20% and alginate of 0.2-0.3%.

1. Introduction
Currently, milk-derived products have been massively developed particularly related to their functionality. Thus, numerous researches focused on the manufacture of milk products with antibacterial effects and high antioxidative properties, including fermented dangke [1,2], the fermented beverage of dangke whey [3], kefir [4,5], and pasteurized milk supplemented with binahong [6]. Besides, pasteurized milk is also innovated with the addition of flavoring agents, aroma, and color [6]. The antibacterial ingredient is also added, including matoa (*Pometia pinnata*). The plant belongs to family of Sapindaceae widely spread in tropical areas such as Indonesia. It has been utilized by people in Asia (Malaysia and Indonesia) for traditional medicinal plants [7]. Matoa leaf contains flavonoid, saponin, tannin, alkaloid and kumarin, in which they act as antimicrobial agents. Previous studies [8,9] showed that antibacterial activity of matoa leaf could deactivate the growth of pathogens such as *Staphylococcus aureus* and *Escherichia coli*. 

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Practically, matoa leaf extract is acid; therefore, when added to milk, the stabilizer is required to stabilize the dispersion. Alginate may show a satisfying performance in such stabilization, while also enables to serve as an antibacterial agent. This current work was to understand the storability of pasteurized milk enriched with antibacterial agents, matoa leaf extract and alginate, towards pathogens *E. coli* and *S. aureus*.

2. Materials and methods
Pasteurized milk was made from full cream milk, added with matoa leaf extract and alginate. Cells of *E. coli* and *S. aureus* were cultured in media of tryptone soy broth and agar nutrients (Merck). Other chemicals, including alcohol 70%, NaCl, distilled water, ethanol 70% and NaOH (Sigma Aldrich), were used.

Experimental design was arranged according to completely randomized design with 2 factors: matoa leaf extract (0%, 0.1%, 0.15%, 0.20%) and alginate (0%, 0.1%, 0.2%, 0.3%). The experiment was carried out by triplicate.

2.1. Research and procedure
An amount of Matoa leaves were washed, drained, and air-dried at room temperature. Subsequently, they were pulverized using an electric blender to produce the powder. The powder was weighed and added with distilled water as the solvent, and left for 2×24 h (maceration). The fresh extract was freeze-dried to collect dried extract [10].

Pasteurized milk was prepared from reconstitution milk, using full cream milk at a concentration of 10% (w/v). The milk was treated with matoa leaf extract and alginate at particular concentrations, then pasteurized using HTST (±72°C for 15 sec.) [11].

2.2. Parameters and statistical analysis
The efficacy of treatments was evaluated according to the inhibitory zone. For statistical evaluation, data were tested using analysis of variance (ANOVA) in SPSS 16. The significant difference between means was verified using the Duncan test.

3. Results and discussion
3.1. Inhibition of *Staphylococcus aureus*
The inhibitory activity represented the ability of treatment given in retarding the growth of *S. aureus*, indicated by the formation of a clear zone around the disk (table 1).

| Levels of matoa extract | Clear zone (mm) | Averages |
|-------------------------|-----------------|----------|
|                         | Levels of alginate | 0% | 0.1% | 0.2% | 0.3% | Averages |
| 0%                      | 3.46 ± 0.78      | 4.05 ± 0.17 | 3.06 ± 0.34 | 4.07 ± 0.42 | 3.66 ± 0.60* |
| 0.1%                    | 7.23 ± 0.16      | 7.50 ± 1.23 | 7.89 ± 0.17 | 6.06 ± 0.73 | 7.17 ± 0.94b |
| 0.15%                   | 7.32 ± 1.44      | 8.39 ± 0.73 | 7.95 ± 1.07 | 7.28 ± 0.53 | 7.73 ± 0.98c |
| 0.20%                   | 9.13 ± 1.16      | 9.84 ± 0.34 | 10.06 ± 0.77 | 10.00 ± 0.48 | 9.76 ± 0.75d |
| **Averages**            | 6.78 ± 2.32      | 7.45 ± 2.31 | 7.24 ± 2.74 | 6.85 ± 2.29 |

Note: Different superscripts following means in the same column indicate a significant difference (p<0.05)

The results indicated that the presence of matoa leaf extract in pasteurized milk reduced the growth of *S. aureus*. However, alginate and interaction of both factors did not alter *S. aureus*. As shown in the Duncan test, inhibition of *S. aureus* increased as more matoa leaf extract was added. This inhibition could be associated with antibacterial compounds in the extracts as well as the natural antimicrobial properties of milk. Former studies [9,12-14] reported that matoa leaves contained a variety of functional
compounds, acting as antimicrobial agents and antioxidants. Besides, a study [15] suggested that antibacterial properties of milk closely related to the activity of lactoperoxidase and lactoferrin. These components retard bacteria through the devastating permeability of the cell walls [16]. In this work, clear zone can indicate the inhibitory activity, ranging from 2.72 to 10.48 mm, classified as weak-moderate. Furthermore, as reported by some studies [6,17], clear zone can be classified into four levels according to diameter (in mm) as follows: weak (≤5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (20 mm).

3.2. Inhibition of Escherichia coli

E. coli belongs to Enterobacteriaceae, which is a member of pathogenic, Gram-negative, motile, and non-spore forming bacteria. This bacteria is often used as a bioindicator for food contaminants since it frequently occurs in food products such as pasteurized milk. To deal with this, natural additive as an antibacterial and non-toxic ingredient is supplemented in order to ameliorate the functionality of the product. The antibacterial performance of matoa leaf extract and alginate in pasteurized milk towards E. coli is presented in table 2.

| Levels of matoa extract | Levels of alginate | Averages |
|-------------------------|--------------------|---------|
| 0%                      | 0%                 | 4.21 ± 0.84ab |
| 0.1%                    | 0.1%               | 7.29 ± 1.27b |
| 0.15%                   | 0.2%               | 8.57 ± 0.57c |
| 0.20%                   | 0.3%               | 9.85 ± 1.13d |
| Averages                | 7.01 ± 2.62A       | 8.06 ± 2.61C |

Note: Different superscripts following means in the same column indicate a significant difference (p<0.05)

Experimental data suggested that the use of matoa leaf extract and alginate in pasteurized milk enabled to reduce the growth of E. coli; but such effect in the interaction of both factors. Duncan test exhibited that alginate added a dose of 0.3% showed the highest inhibition against E. coli, as presented in table 2. In short, it is revealed that the concentration of matoa positively correlated with the diameter of a clear zone, suggesting that it can more reduce the growth of E. coli as a higher concentration of matoa leaf extract is used.

Suppression of E. coli seemed to be higher in more addition of matoa leaf extract and alginate. In the absence of matoa leaf extract and alginate, pasteurized milk also showed inhibitory activity against bacteria. This may relate to enzymatic actions by lactoperoxidase and lactoferrin [15]. Additionally, Duncan test also showed that matoa leaf extract could exert a very significant effect on the parameter observed; this possibly associates with the content of antibacterial agents in the extract [18].

Furthermore, alginate also provides a significant effect on the formation of clear zone. Alginate is extracted from seaweeds, and it is reported to show antibacterial activity, fungicidal, anticancer, and antiseptic. This is augmented by another study [19], finding that alginites derived from either red or brown seaweeds are rich in bioactive compounds capable of devastating bacterial cells. Besides, alginate is widely applied as an external medicine since it possesses antiseptic properties [20]. A report [21] also found that alginate might reduce the growth of pathogens responsible for Staphylococcus epidermidis, Pseudomonas, E. coli and other diseases caused by pathogenic bacteria.

4. Conclusion

This study concluded that supplementation of matoa leaf extract and alginate in pasteurized milk was evidenced able to exert inhibitory effects on Staphylococcus aureus and Escherichia coli.
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