



# Total Microbiological and Chemical Quality of Dry Fermented Mare's Milk Sumbawa Using Gum Arabic (*Acacia senegal*) as Encapsulating Agent

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## Authors' contributions

This work was carried out in collaboration among all authors. Authors LKR, KUAA and AHU did the conceptualization. Authors AHU, YS, Kasri, PA and RF did the formal analysis. Authors AHU, YS, Kasri, PA and RF searched for resources. Author AHU wrote original draft. Author AHU wrote, reviewed and edited the manuscript. Authors LER and KUAA supervised the study. All authors read and approved the final manuscript.

## Article Information

DOI: 10.9734/AFSJ/2024/v23i3703

## Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/114346>

**Original Research Article**

**Received: 13/01/2024**  
**Accepted: 17/03/2024**  
**Published: 20/03/2024**

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## ABSTRACT

Dry fermented mare's milk Sumbawa by applying gum arabic (*Acacia senegal*) as an encapsulant agent and can improve the functionality of protein peptides to avoid their damage during process of making milk powder and can be fully absorbed by the body. This study aimed to improve the level and quality of dry fermented mare's milk Sumbawa by applying gum arabic (*Acacia senegal*) as an encapsulant agent by a vacuum foam drying oven method. The method used in this research was a laboratory experimental using a completely randomized design consisting of 5 treatments and 4 replications, which is T1 (20% with encapsulation gum arabic), T2 (24% with encapsulation gum arabic), T3 (28% with encapsulation gum arabic), and T4 (32% with encapsulation gum arabic). The difference in the concentration ratio of encapsulation gum arabic in dry fermented mare's milk Sumbawa gave a very significant difference ( $P < 0.01$ ) in total plate count (T4 :  $6.09 \times 10^5$  CFU/ml), potential of hydrogen (T4 : 4.60), total titration lactic acid (T4 : 1.27%), and total protein (3.72%). This analysis has shown that encapsulation can be achieved by adding gum arabic to the preparation of dry fermented mare's milk Sumbawa.

*Keywords: Dry Fermented Mare's milk; gum arabic; encapsulation; vacuum foam drying oven.*

## 1. INTRODUCTION

Milk is a nice source of protein for human nutrition and the one of livestock product that has many benefits. Milk also contains many bioactive molecules, which protect against microbial infections or antimicrobials and contribute to immune maturation or antioxidants [1]. Mare's milk is a one type of livestock milk production that has many benefits in the health sector. While mare's milk does contain some antibacterial and antioxidant properties, these benefits are not as well-established as those of other foods or supplements [2]. Mare's milk has a high content of lactic acid bacteria, so it has the privilege that it can fermentation a milk into lactic acids because contains the highest lactose and produce antimicrobial and antioxidant compounds such as bacteriosin, lactic, organic acid. (acetate and hydrogen peroxide) [3,4].

The important aspects related to functional foods are the composition and processing of raw materials, as well as the survival and productivity of probiotics used. Several parameters can control the safety aspects, sensory properties, organoleptic characteristics, and stability of fermented dairy products, including fermented mare's milk [5,6]. However, so far people can only consume mare's milk in a fresh condition, so it is necessary to develop its production, one of which is the development of dry milk processing production by encapsulating its nutrients, in an effort to increase the level of consumption of nutrients from mare's milk in a practical way [7].

Fermented mare's milk containing probiotics can be used in the management, prevention, and treatment of several diseases, such as diseases related to the digestive and immune systems, so it is necessary to carry out an encapsulation process [8]. Encapsulation is a process of trapping enclosing a substance, such as a drug, nutrient, flavor, inside a protective coating or matrix made from various materials, such as polymers, lipids, carbohydrates and protein from environmental factors such as moisture, heat, and oxygen, which can degrade its stability and potency [9].

Encapsulation is more effective in the implementation of vacuum foam drying, where the method has stagnant and regular heat conditions in the radiation process, so it is necessary to carry out protections process for the nutritional content of dry fermented mare's milk [10,11]. Vacuum foam drying oven is generally a very ideal an in terms of drying sample materials that are sensitive to heat or oxygen (such as enzymes and microorganisms), because its advantages eliminate high water content especially in dairy products by minimizing the possibility of oxydation reactions [12]. The vacuum drying process without encapsulation will result in dry fermented horse milk breaking down easily. it aims to protect the damage to the fresh product and facilitate the transport process . One form of protection in the drying process is to utilise gum arabic as an encapsulant agent, because it is one of the products with heat stability in protecting other substances well [13].

In this study, gum Arabic is a polymer that is utilized as fillers, stabilizers, thickeners, and encapsulants in confectionery beverages [14]. These samples can be made better by protecting biopeptides from mare's milk and preventing infectious diseases caused by pathogenic microorganisms [15]. The potential of gum arabic as an encapsulant for Sumbawa dried horse milk, provides an advantage in the development of processed food products, where the properties of horse milk remain protected from heat damage caused by the heating process and contribute antioxidants and antibacterials inhibitor indirectly to the final product [16]. The development of dry fermented mare's milk Sumbawa products with gum arabic encapsulant is unprecedented. Therefore, in this case study, the potential applying gum arabic as an encapsulant in the preparation of dry fermented mere's milk Sumbawa to increase its functional in microbiology and chemistry activities was concerned.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The materials used in this research were mare's milk Sumbawa, polysorbate/tween 80 food grade, petrifilm AC 3M™, buffered peptone water, aquadest, alcohol 70%, buffer for calibration 6.8 and 4.0, aquadest, NaOH 0.1 N, PP (*phenolphthalein*) indicator.

### 2.2 Methods

The method used in this research completely randomized design with 5 treatments and 4 replications with 20 trial samples. The experimental design used in this study was a complete randomized design with a vacuum drying oven drying method using gum arabic as a dressing material for peptides in mare's milk. The experiment and continued with anova analysis was designed. The research design can be seen as follows T0 (without non encapsulation gum arabic), T1 (20% with encapsulation gum arabic), T2 (24% with encapsulation gum arabic), T3 (28% with encapsulation gum arabic), and T4 (32% with encapsulation gum arabic).

### 2.3 Preparation of Dry Fermented Mere's Milk Sumbawa Production with Vacuum Foam Drying Oven

Mere's milk was obtained from Sumbawa, Nusa Tenggara Barat and preparation for analysis was

150 ml/samples with preparation of 150 ml milk requirement analysis per sample by homogenizing with gum arabic mixer as 5 treatments and 4 replicates with percentages of 20%, 24%, 28% and 32% plus 1% tween 80 as a frothing agent during the heating process with the vacuum foam drying oven method for 420 minutes and a temperature of 70°C-100°C [12].

### 2.4 Raw Materials Analysis

Sample analysis was carried out with the initial stages of pasteurization of fresh sumbawa horse milk and weighing of gum arabic which was already in powder form. Analysis on both raw materials concerned protein content, total titratable acid and pH measurement [17]. Total plate count and *Escherichia coli* contained in the sample by counting the total colonies that grow on petrifilm AC 3M™, petrifilm *E. coli/coliform* 3M™ growth culture media. Taking with a micropipette as much as 1000 μ of samples that have been diluted as much as 9 ml of BPW dilution media, then put into a petrifilm AC 3M™, petrifilm *E. coli/coliform* 3M™ and gently homogenized. The samples were then incubated in an incubator at 37°C for 24 hours. After 24 hours, the number of bacterial colonies was counted with a colony counting tool [18].

### 2.5 Raw Materials Analysis

The physico-chemical analysis carried out in this study were protein content, total acid, and pH. The protein content of dry fermented mere's milk Sumbawa with Kjeldhal method. Protein content in dry fermented mere's milk Sumbawa calculated by Eq. 1 [17]

$$\% \text{ Protein} = \frac{(\text{VA} - \text{VB})\text{HCL} \times \text{N HCL} \times 14,007 \times 100\%}{\text{W sampel} \times 1000}$$

Then, pH analysis dry fermented mere's milk Sumbawa using a pH meter with reference to the AOAC (2005) [17]. The total lactic acid of dry fermented mere's milk Sumbawa was carried out by calculating about lactic acid contain in a samples using the acid-base titration analysis method, i.e. a sample of 10 ml of each fermented horse milk that has been diluted with distilled water is put into a 250ml erlenmeyer and marked to differentiate. Each added 2 drops of 1% phenoltptalin (PP) indicator. Perform the titration process of the sample using 0.1 N NaOH

solution until pink [19]. Recorded the final result of 0.1N NaOH solution needed to titrate the sample. The total lactic acid content in dry fermented mare's milk Sumbawa calculated by Eq. 2 :

$$\% \text{ Lactic Acid Content} = \frac{\text{ml NaOH} \times N \text{ NaOH} \times 90 \times 100\%}{W \text{ sampel} \times 1000}$$

### 3. RESULTS AND DISCUSSION

#### 3.1 Gum Arabic and Raw Mare's Milk Fermentation Sumbawa Analysis

The results of the analysis of the value of fermented mare's milk Sumbawa with in total plate count ( $3.09 \times 10^5$  CFU/ml), potential of hydrogen (3.90), total titration lactic acid (1.66%), total protein (2,02%) and total *Eschericia coli* >3 MPN.

It was previously reported that mare's milk Sumbawa contains a high nutritional value, so there are several standardizations that are determined including chemical criteria, including: protein content, pH, and total lactic acid titrated, then microbiological criteria (total plate count), including: lactic acid bacteria and antimicrobial pathogens that are specified with the aim as a reference to maintain safety and quality control from time to time [8].

However, gum arabic can be used as a protein binder in the manufacture of foods and supplements. For example, a study using gum arabic as protein binders in milk powder extracts in the protection of milk nutrients found [15]. The results of the analysis of the value of fermented milk protein content of 1.99%, total acid of 0.14%, pH value of 5.95, total plate count  $0.12 \times 10^1$  CFU/ml.

Reviewed from the parameters of Table 1 shows that the content contained in sumbawa horse milk has good quality in terms of protein content, tal plate count, pH value and total lactic acid titrated has a low value due to the high content of microbacteria in mare's milk and it becomes acidic ambience [20]. The content of *Acacia senegal*, has physical qualities that are denser and smoother in powder form and has less charge, less hydrolyzed by enzymes, surface active, more stable in solution, richer in minerals, easy to bind proteins, bind probiotics to prevent damage and high polyphenol content and less rich in protein and biopeptide [21].

#### 3.2 Chemical Analysis of Dry Fermented Mare's Milk Sumbawa

The results of the analysis that has been carried out physicochemical analysis content in dry fermented mare's milk Sumbawa had a statistical value that was significantly very different ( $p < 0.01$ ) from the addition of gum arabic as an encapsulant. The results of statistical analysis can be presented in Table 2.

The results obtained through study show that the use of gum arabic encapsulation can be a protect bioactive contained in Sumbawa mare's milk. Where the content of stabilizers can help reduce the presence of precipitation in the product and can maintain its stability. Precipitation in the product and can maintain stability. Stabilizers also have properties as emulsifiers characterized by the presence of polar (*hydrophilic*) and non-polar (*hydrophobic*) groups, when mixed into liquid food ingredients, the polar groups will bind to water and maintain the consistency, nutritional content and shape of the product [22]. The results of the protein content test observations on dry sumbawa horse fermented milk with stabilizers have an average value which can be seen in Table 2. Table 2 can show that the average value of protein content of dry fermented mare's milk ranges from 2.02% -3.72% with high protein value in the treatment of 32% addition of gum arabic. Raw mare's milk has a protein content of about 6.5 grams / 100 ml of pure milk, and Indonesian national standards SNI No. 01-2981-2009 showed that the protein content of horse milk is 1.89% and for fermented processed milk it is at least 2.7% [23].

The results of previous observations show that protein is a good compound in improving health and enhancing the immune system, where protein is a substance that is easily damaged in the event of heat stress and oxidation [24]. The results of previous research analysis stated that the physicochemical activity of gum arabic was reported in the original article after biological experiments using the total content of gum arabic, which was found to contain mostly macropolysaccharide content (more than 80% w/w) and had little protein (1%-3.5% w/w) [21]. Thus the functionality of gum arabic as a protector can be maximized. In addition, increasing the amount of gum arabic as an igniter will also have an impact on increasing the protein in the sample.

**Table 1. Analysis results of raw material fermented mare's milk Sumbawa and gum arabic encapsulant**

Parameters	Gum arabic	Fermented mare's milk Sumbawa
Protein contain (%)	1.99 ± 0.22	2.02 ± 0.07
Total acid titration (%)	0.14 ± 0.06	1.66 ± 0.07
pH value	5.95 ± 0.06	3.43 ± 0.10
<b>Total Plate Count cfu/ml</b>	<b>0.12x10<sup>1</sup> ± 0.34</b>	<b>6.70 x 10<sup>4</sup> ± 0.22</b>

All values are mean ± SD. Values with different letters are significantly different ( $P < 0.05$ ) according to Duncan's multiple range test and the best value is taken

**Table 2. Analysis results of chemical analysis from fermented mare's milk Sumbawa with gum arabic encapsulant**

Treatments*	Protein content (%)	pH Value	Lactic Acidity Value (%)
T0 : 0%	2.02 ± 0.07a	3.43 ± 0.10a	1.66 ± 0.07bc
T1 : 20%	2.74 ± 0.22b	4.08 ± 0.10b	1.45 ± 0.06b
T2 : 24%	2.98 ± 0.16b	4.15 ± 0.13b	1.41 ± 0.04b
T3 : 28%	3.44 ± 0.26bc	4.35 ± 0.13b	1.37 ± 0.04ab
T4 : 32%	3.72 ± 0.27c	4.60 ± 0.14b	1.27 ± 0.05a

All values are mean ± SD. Values with different letters are very significantly different ( $P < 0.01$ ) according to Duncan's multiple range test

The results of the pH and total acid lactic content test observations on dry sumbawa horse fermented milk with stabilizers have an average value which is given in Table 2. Table 2 can show that the average value of pH content of dry fermented mare's milk sumbawa ranges from 3.43 - 4.60 and the average value of total acid lactic content 1.66%-1.27%. From the analysis results in Table 2 that the pH value and total acid have a inverse continuous effect where the lower the pH value, the higher the total acid value in the sample. In acidic samples, the relationship between acid content and pH value is inverse proportional. This is due to the fact that chemicals that form acids, namely carbon dioxide (CO<sub>2</sub>) and hydrogen ions (H<sup>+</sup>). When acid levels increase, the number of hydrogen ions (H<sup>+</sup>) also increases, so the pH decreases [25]. Conversely, if the acid level decreases, the amount of hydrogen ions (H<sup>+</sup>) also decreases, so the pH increases [10]. Previous analytical results show that the amount of acid produced during the fermentation from LAB contained in fermented milk can cause a decrease pH value and increase total acid value [8]. The activity of homofermentative bacteria in sumbawa horse milk naturally produces more than 85% lactic acid as a metabolite product, so that the fermentation of milk lactose will be converted into lactic acid and acidic conditions will be produced in horse milk [26].

In addition, the amount of total titratable acid also affects the stability of proteins in mare's milk. Proteins in mare's milk depend on pH for stability, and a higher amount of total titratable acid can result in a more neutral pH, which minimizes the risk of protein denaturation in horse milk [20]. In addition, the drying process of making Sumbawa mare's milk powder will have an influence in reducing the total acid value of horse milk. This is due to the total acid content found in Sumbawa mare's milk, which is between 0.5%-1.5% [27]. Sumbawa mare's milk is a type of fermented milk produced from mare's milk using *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* bacteria. Both types of bacteria are included in LAB (*Bacteria acidolactici*) which has a homofermentative fermentation pattern, which is the end result in the form of lactic acid only. The total amount of titratable acid in Sumbawa mare's milk ranges from 0.3%-1.9% [28]. Fermented milk has a pH value of at least 3.0 and the allowable value of fermented milk for the total acid contained in the product is 0.5-2.0% [23]. The results showed that the best treatment is the treatment that has been given the effect of adding 32% gum arabic which has the highest protein content value of 3.72%, pH value of 4.60 and total acid of 1.27% which is inversely proportional. Where the protein value, pH value and total acid of dry fermented mare's milk products can be said to be in accordance with the standards that have been imposed.

### 3.4 Total Plate Count of Dry Fermented Mare's Milk Sumbawa

The results of the analysis that has been carried out total plate count analysis content in dry fermented mare's milk Sumbawa had a statistical value that was significantly very different ( $p < 0.01$ ) from the addition of gum arabic as an encapsulant. The results of statistical analysis can be presented in Table 3.

Total Microorganisms is the number of microorganisms with certain types of microorganisms contained in a product, both pathogenic and non-pathogenic. Total Plate Count (TPC) is a population of all microorganisms contained in food products without indicating certain types of microorganisms, so it can be used as a general description of microorganisms in a food product without indicating certain types of [29]. The maximum number of live microbes desired for specific fermented milk products is 106 cfu/ml or 6 log cfu/ml and the number of live microbes that should be present in probiotic products is 106-108 cfu/ml [30,31].

The average value of total microorganisms (total plate count) of Sumbawa horse milk powder has the value of the analysed treatments ranging from 6.7 - 6.9 x 104 cfu/ml, the results of bacterial analysis derived from the total plate count derived from the average value of the highest total lactic acid from treatment T4 where with the total percentage value of tertirasi acid of 1.27% with the addition of gum arabic as a microencapsulant as much as 32% of the volume of mare's milk used in the manufacture of powder. While the lowest average value was obtained from T0 parameter with no addition of gum arabic as much as 0%. The increase in the mean value of total microorganisms in the content of sumbawa horse milk powder is caused by many factors that affect the growth of microorganisms including the amount of nutrients obtained by microorganisms to grow and multiply, the content of water and oxygen that supports the life of aerobic bacteria that require water and oxygen to grow [32,33]. The process of adding gum arabic has reduced the total plate count content because there are some bacteria that are resistant to heat so that they maintain their life due to the help of gum arabic protection as a microencapsulant agent, besides that gum arabic also functions as an additional temporary

nutrient substitute for bioactive horse milk in the process of making milk powder [34]. Viability or resistance to microbacteria in fermented milk or milk powder that has been processed is a very important consideration in the development of probiotic products, where the benefits of probiotics become a reference for the successful development of a milk processing product, especially Sumbawa horse milk powder [35].

**Table 3. Analysis results of total plate count fermented mare's milk Sumbawa and gum Arabic encapsulant**

Parameters	Total Plate Count x104 cfu/ml
T0 : 0%	6.70 ± 0.10bc
T1 : 20%	6.51 ± 0.12b
T2 : 24%	6.44 ± 0.09b
T3 : 28%	6.23 ± 0.11ab
T4 : 32%	6.09 ± 0.11a

All values are mean ± SD. Values with different letters are very significantly different ( $P < 0.01$ ) according to Duncan's multiple range test

## 4. CONCLUSION

The application of gum arabic (*Acacia senegal*) affects the microencapsulated content of sumbawa horse milk powder by protecting the probiotic content as well as the existing chemical activity content, emulsion activity, and inhibition of pathogenic bacteria that are not heat resistant. The results obtained in this study showed that T4 was the best treatment, where the content was neither too high nor low so that it was at the threshold of the established standards that can be consumed by the general public. Thus, the application of gum arabic microencapsulation has the potential to protect the peptides and chemical content of dry fermented mare's milk Sumbawa.

## ACKNOWLEDGEMENTS

The Authors thanks to Department of Animal Product Technology, Faculty of Animal Science, Animal Product Technology Laboratory and LPPM Brawijaya University for support this research.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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