Microbial contamination on dangke fresh white cheese from traditional home industry in Enrekang Regency

Muhammad Askari Zakariah¹, Ratmawati Malaka², Amran Laga³, Ambo Ako², Failal Ulf Mauliah⁴, and Muhammad Zakariah¹

¹Islamic Institute (IAI) of Al Mawaddah Warrahmah Kolaka, Jalan Pondok Pesantren No.10 Kolaka 93511, Indonesia.
²Department of Animal Product Technology, Faculty of Animal Science, Hasanuddin University, Jalan Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia.
³Department of Animal Product Technology, Faculty of Animal Science, Hasanuddin University, Jalan Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia.
⁴Department of Agriculture Technology, Faculty of Agriculture, Hasanuddin University, Jalan Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia.
⁴Faculty of Medicine, Hasanuddin University, Jalan Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia.

E-mail: askari@iaialmawar.ac.id

Abstract. Dangke is local cheese from Enrekang regency, South Sulawesi, Indonesia. Dangke was made from buffalo or cow milk as a raw material and processed with help of milk clotting protease enzyme from papaya leaves and fruit. The mixture was beaten and then the milk protein clots, the clot is kept in mold of a coconut shell. Dangke has been well known by South Sulawesi community but information is very limited regarding to its characteristics with standardized quality. The presence of pathogenic bacteria in food potentially cause health problem to consumers. This study was aimed to chemical quality and microbiology evaluation a product of dangke in Enrekang regency. The experiment was conducted in a one simple t-test on chemical characteristics, while microbiological characteristics were identified using exploratory descriptive. Ash content on this study 1.83%, it was lowest significant different than previous study. Microbiology evaluation was tested, the result showed that nothing a Staphylococcus aureus, nothing a Bacillus cereus, negative result for a Salmonella. Most probable number Eschericia coli and Coliform is >1100 MPN/100g respectively. Dangke white cheese have microbial contamination on most probable number coliform and E. coli, it could be potentially causes health problem for consumers if it was eaten raw.

1. Introduction

Indonesia have many traditional food which made from milk. One of product is Dangke, dangke is local cheese from Enrekang regency, South Sulawesi. Dangke was made from buffalo or cow milk as a raw material and processed with help of milk clotting protease enzyme from papaya leaves and fruit. The mixture was beaten and then the milk protein clots, the clot is kept in mold of a coconut shell. Dangke is natural cheese from Enrekang, South Sulawesi, Indonesia produced through agglutination buffalo milk, cow, goat or sheep using the sap of papaya [1]. The name dangke is derived from “dank u wel”, which is Dutch means “thank you very much”, according to local stories, natives presented...
this product to the Dutch on their first visit to South Sulawesi and subsequently said “dank je”, a shortened form of “dank u wel”.

Some cheese varieties have similarities with Dangke, like Paneer from India, Akawieh, Baladi, Karichee from Lebanon, shosim from Nepal, Kesong puti from Philippines, Mashanza from Congo, Altiplano from Bolivia [2]. The distribution has been reported to reach out of the province, including to other countries such as Brunei Darussalam and Malaysia. Nationally, dangke is being already distributed to Java and Sumatra islands, consistent with the increase in the number of tourism activities [3]. Therefore, preservation have main role to maintain the quality of dangke could be arrived to consumers. Dangke was produced by traditional processing that cause of many bacterial contaminated, it was affected to long storage duration time relatively ± 3 days with addition of salt at room temperature. Study of evaluation of quality control of dangke is important to identify a factor destroyed for extending the shelf life of dangke.

This research consists of mainly two sections. First, our current knowledge of the chemical conten of dangke was compared with our research recently and other literatures. Second, we describe the current status of microbial invention on dangke fresh soft cheese from traditional home industry Enrekang regency. A small number of studies on the contamination of pathogenic bacteria in dangke In Enrekang Regency have been carried; therefore, the availability of scientific information on the topic is limited. This study discovers the contamination level of *E. coli*, *Salmonella* spp, *Bacillus cereus*, and *Staphylococcus aureus* in dangke. This study will help the researcher to uncover critical control point of level microbial contamination in National Standard (SNI) for Dangke as traditional cheese. Thus, a new finding could be applied in the food technology.

2. Material And method

Dangke was get from tradition home industry Producers in Enrekang Regency, research was done on April 2018, while the dangke chemical quality was evaluated in Feed Chemical Laboratory, Animal Nutrition Program, Universitas Hasanuddin, then Microbiology analysis in Health Laboratory of Makassar (Balai Besar Laboratorium Kesehatan Makassar). The analysis of protein, water, ash, and carbohydrate contents of dangke samples were conducted according to AOAC [4], then fat analysis was conducted zakariah [5].

Total calculation of *Staphylococcus aureus* was done by method dilution / Platting Method through series dilution. Traditional cheese samples (sequence) of each trader different taken in the market and weighed in laboratory as much as 10 grams later samples put into a bottle has sterile water as much as 90 mL and homogenized to obtain rank dilution 10-1 then 1 mL suspension on 10-1 dilution ago put in a tube that has been contains 9 mL of sterile water to obtain 10-2 dilution rank. Sample is planted by taking 1 mL suspension on dilution 10-2 and placed on a cup sterile petri which is then added with the media Mannitol Salt Agar (MSA), homogenized and incubated at temperature 37 oC for 24 hours. Calculation *Staphylococcus aureus* is performed with count the number of colonies of each cup 30-300 CFU / g [6].

Solid-shaped samples and large chopped and homogenized using the tools has been sterilized. A total of 10 g each sample is inoculated into in 90 mL Mac Conkey sterile broth and homogenized using vortex. Then 0.5 mL of the suspension taken and inoculated into tube containing 4.5 mL Mac Conkey broth sterile to obtain sample suspension which has been subjected to dilution 100 x. The tube is taken again 0.5 mL suspension and inserted into the tube next, so on up obtained a 10-8 dilution suspension. Next, the sterile Durham tube put in each tube, and incubated for 48 hours at 37ºC [7]. After 48 hours, all the tubes will be checked the results, and recorded for calculated the estimated number of bacteria Coliform in accordance with MPN table series 3 tube FDA-BAM [8]. Suspension from the tube show positive results on the presumptive test will be inoculated to in *Escherichia coli* (EC) sterile broth. Then the Durham tube is inserted into in each tube, and incubated for 24 hours at a temperature 37ºC. The tube containing a murky suspension is interpreted as a positive tube of *Escherichia coli*. Suspension from the tube show positive results on confirmed test will be inoculated to Eosin Methylen Blue (EMB) Media, and incubated for 18-24 hours at temperature 37ºC [7]. Single
Isolate which is metallic black on The EMB agar surface is a the hallmark of the colony of Escherichia coli [9].

Test method used in this research is qualitative test taken from the Indonesian National Standard Method which refers to Bacteriological Analytical Manual, Food and Drug Administration, AOAC International [10]. Each testing process is always accompanied by a control positive and negative.

The experiment on chemical characteristics of dangke was conducted one sample t-test. One sample t-test is a statistical procedure used to determine whether a sample of observation could have been generated by a process with specific mean. Significance was assessed at $p \leq 0.05$. While microbiological characteristics were identified using exploratory descriptive which is research explore temporary information or unknown cases or only little is known about data collection to provided description or affirmation of a concept or symptom. The aim is to describe bacterial contamination. Tested microbial quality by Most Probable Number (MPN) method, with formula:

$$\text{Most probable number} = \text{MPN on table} \times \frac{1}{\text{dilution middle stage}}$$

After obtaining the results data for the sample, then the data will then be compared to the standard values set by the Head of the Agency of Drugs and Food, Republic Indonesia (BPOM RI) Number HK.00.06.1.52.4011 in 2009 concerning the maximum limit of contamination microbes in food and beverages, if the data are analyzed exceed the maximum threshold, it can be concluded that processed drink is not suitable for consumption.

3. Result and discussion

The data showed that organic matter highest than the previously study Table 1. showed that water content in this research higher than another result. High water content in cheese causing soft cheese. On the other hand the more high in protein content in cheese, then the more the amount of fat can be tied and maintained in cheese, so the resulting cheese becomes high in content the fat [11]. Water content of dangke was normally as type of fresh cheese. Typical fresh cheese have water, fat were 54 and 34.8 % respectively [12]. Water content, protein density, fat forming have affected on structure characteristic of dangke. Microstructure features has a difference in protein density, fat distribution and presence of water in the dangke [13]. The hardness and texture were related to various chemical component within microstructure level that manifestation in the protein network by aggregation and interaction between caseins, fat and another component [14].

Water content were calculated from dry matter of dangke cheese, the major content of dry matter of dangke cheese is fat and protein. Water content have correlation with milk component. Correlation coefficient between the major milk component and adjusted fresh soft cheese yield were highly positively correlated with cheese yield adjusted ($r=0.71$ and 0.73 respectively) [15]. As mentioned before, fat and protein in milk are the main component that affects the cheese yield [16].

Protein content on this study is low content, it might be heating temperature. Higher heating temperature decreased protein and fat content. Malaka [17] showed that increasing temperature could be decrease protein and fat content, percentage of protein dangke on 75°C, 80°C, 90°C, 95°C, and 100% were 17.16%, 16.62%, 16.28%, 15.16%, 14.33%, 12.99% respectively. Turkish Standard 591 have total solid in the cheese not to exceed 60 g/100 g, fat in solid should be minimum 45 g/100 g. Composition of cheese would be different because different condition in milk and fresh cheese, furthermore protein will be increased by using more of a coagulant, lower pH at curd drainage, probably by low scalding temperature, and more intensive milk pasteurization [2].

Microbial evaluation of dangke was got from traditional home industry Enrekang regency that nothing a Staphylococcus aureus, nothing a Bacillus cereus, nothing a Salmonella. Contamination Staphylococcus aureus of milk and dairy product may occur direct from infected food production animals or may result from poor hygiene during production process or at the retail and storage stage, investigation on the presence of toxin and antimicrobial resistant S. aureus in Turkish cheese 6% total samples, S. aureus the mean was 4.31 log cfu/g [23]. Contamination Staphylococcus aureus in
traditional white cheese from 3 different Basrah city local market is al basrah, al asrah, al jumhurya were 53.33%, 50%, 13.33% respectively [24]. Difference between the result may be based on the difference in the cheese production technique, and whether the milk used was raw or pasteurized. It could be also related to unclean condition where the cheese is produced and personnel involved in production.

Table 1. Chemical characteristics of dangke

| Fraction                          | Fresh Dangke | Dangke with Papaya 0.3% and heat 80[18] | Dangke with Papaya 0.3% and heat 80[19] | Sample were get from dangke producer in Enrekang [20] | Dangke was added Lactococcus lactis FNCC 00866[21] | Dangke was added Lactobacillus plantarum[21] | Dangke by Ripening time 6 day and added L. lactis[22] |
|----------------------------------|--------------|----------------------------------------|----------------------------------------|----------------------------------------------------|--------------------------------------------------|--------------------------------------------------|---------------------------------------------------|
| Water*                           | 58.28        | 58.75                                  | 55.00                                  | 45.65                                              | 60.65                                             | 44.93                                             |                                                   |
| Ash*                             | 1.83         | 2.31                                   | 2.10                                   | 1.94                                               | 1.94                                              |                                                   |                                                   |
| Crude protein*                   | 15.57        | 16.86                                  | 17.94                                  | 23.80                                              | 19.23                                             | 14.80                                             | 24.54                                             |
| Extract ether*                   | 20.71        | 15.19                                  | 2429                                   | 14.80                                              | 32.82                                             | 17.31                                             | 8.03                                              |
| Carbohydrate*                    | 3.60         | 5.88                                   | 0.53                                   | 4.68                                               | 19.36                                             |                                                   |                                                   |

*Means within the same row with different superscripts differed significantly (p<0.05)

Most probable number E. coli and Coliform is >1100 MPN/100g respectively on recent study. The presence of pathogenic bacteria in food potentially causes health problem to consumers, such as infectious and food poisoning, contamination level of E. coli in dangke, workers hand, mold and packaging material were 73%, 40%, 63%, and 40% respectively [20]. The workers and equipment of dangke processing might become risk factors for dangke to be contaminated by E. coli. Coliform can multiply very rapidly in the curd during the first few hours, when the pH and temperature are favorable. However, the problem is rare in modern dairies, provided that efficient pasteurization and good manufacturing practices. Furthermore, the activities of the starter culture is crucial for the control coliform by reducing the pH and the amount of lactose in the curd. De Reu [25] found higher levels of coliform in soft cheeses when compared semi-hard and hard, because higher pH, shorter ripening periods, higher water activity and lower salt concentration.
Table 2. Microbial Evaluation of dangke

| Parameters          | Recently study on April 2018 | Number on other literatures |
|---------------------|------------------------------|-----------------------------|
| Staphylococcus aureus | 0 Colony/g                   | 5.03 Log cfu[^26]           |
|                     |                              | 3.64 Log cfu[^27]           |
|                     |                              | 4.37 Log cfu[^28]           |
| Bacillus cereus     | 0 Colony/g                   |                             |
| E. coli             | Most probable number         | > 1100 MPN/100 g            |
|                     |                              | 6.16 Log cfu[^21]           |
|                     |                              | 3.4 Log cfu[^29]            |
|                     |                              | Negative[^30]               |
| Coliform            | Most probable number         | > 1100 MPN/100 g            |
|                     |                              | 6.03 Log cfu[^26]           |
|                     |                              | 3.64 Log cfu[^27]           |
|                     |                              | Negative[^30]               |
| Salmonella          | Negative /25 g               | Negative[^30]               |
|                     |                              | Positive 7%, 2/30 sample[^20]|

European commission regulation No. 2073[^31] determined that maximum threshold for coliform contamination was 3 log cfu/g. National Standard of Indonesia on microbial contamination limit and limit maximum residues in foodstuffs of animal origin, on this case raw material cheese is milk pasteurization, have criteria Coliform, E. coli, S. aureus, Clostridium, Salmonella, Listeria were lower 0.1 x 10^3, 0 unit, 1x 10^5, 0 unit, and negative respectively[^32]. Then, Indonesian standard for cheese (all varieties) 10/g MPN E. coli, negative/25 g salmonella, 1 x10^2 cfu/g S. aureus, Listeria monocytogenes negative/25 g[^33]. Most probable number of E. coli above Indonesian Standard of cheese and could be food poisoning. However, never found a patient case on hospital because microbial contamination on dangke. Served dangke on fok by fryed could be reduce decomposition and pathogenic bacteria.

4. Conclusion

Dangke white cheese have microbial contamination on most probable number coliform and E. coli, and Salmonella spp test showed negative results in all samples, then Staphylococcus aureus and Bacillus cereus are 0 Colony/g in dangke. Microbial contamination on Dangke potentially causes health problem for consumers if it was eaten raw.

Reference

[1] Malaka R, Hatta W and Baco S 2017 Evaluation of using edible coating and ripening on Dangke a traditional cheese of Indonesia Food Research 1 51-56
[2] Fox PF, McSweeney PLH, Cogan TM and Guinee TP 2004 Cheese Chemistry, Physic and Microbiology (London: Elsevier Academic Press)
[3] Al Baari AN, Legowo AM, Arum SK, Hayakawa S 2018 Extending shelf life of Indonesian soft milk cheese (dangke) by Lactoperoxidase system and Lysozyme. International Journal of Food Science. 1-7
[4] AOAC 2005 Official Methods of Analysis (Washington: Benjamin Franklin Station)
[5] Zakariah MA 2016 Teknologi dan Fabrikasi Pakan (Makassar: Pusaka Al Maida)
[6] Rahayu NPN, Kawuri R, and Suriani NL 2014 Availability test of Staphylococcus aureus in the traditional sausage in traditional market in Denpasar, Bali (Jurnal Simbiosis) 2 147-157
[7] Feng P, Weagant SD, Grant MA and Burkhardt W 2002 BAM: Enumeration of Escherichia coli and Coliform bacteria. (U. S. Food and Drug Administrtion)
[8] Blodgett R 2010 BAM Appendix 2: (Most Probably Number from Serial Dilations) (U. S. Food and Drug Administration)
[9] Juwita U, Haryani Y, dan Jose C 2014 Jumlah bakteri coliform dan detreksi Escherichia coli pada daging ayam di Pekanbaru. JOM FMIPA. 1 48-55.

[10] AOAC 2001 Bacteriological Analytical Manual. Division of Microbiology (USA: Food and Drug Administration AOAC International)

[11] Sunarya H, Legowo AM, and Sambodho P 2016 The concentration of water, fat, and texture of mozzarella cheese made of buffalo and cow’s milk and it is combination. Animal Agriculture Journal 5 17-22.

[12] Malin EL, and Tunick MH 1995 Chemistry of structure-function relationship in cheese (New York: Springer Science)

[13] Sabil S, Malaka R, and Maruddin F 2017 Chemical and Microstructure characteristics of dangke at various temperature ripened IJCRGG 10. 611-615.

[14] Malaka R, Hatta W, and Baco S 2017 Properties and microstructure of dangke fresh cheese made with passion fruits juice as coagulant IJAIR 5 601-606.

[15] Mohammed AHM, Perez CFA, Burgos AJA, Lazardo MBB, Sanchez FJS, Vera JCK 2016 Evaluation of milk composition and fresh soft cheese from an intensive silvopastoral system in the tropics. Dairy Sci. & Technol. 96 159-172.

[16] Hattem HE, Taleb AT, Manal AN, Hanaa SS 2012 Effect of pasteurization and season on milk composition and ripening of Ras cheese. J Brew Distill. 315–22

[17] Malaka R, Baco S, Prahesti KI 2015 Characteristic and mechanism of gelatination of dangke curd by physico-chemical properties and microstructure JITP 4(2) 56-62

[18] Mukhilash AN 2017 Physical, microbial, and chemical qualities of dangke produced by different temperatures and papain concentration. Med. Pet. 40(1)63-70.

[19] Malaka R, Baco S, Prahesti KI 2015 Characteristic and mechanism of gelatination of dangke curd by physico-chemical properties and microstructure JITP 4(2) 56-62.

[20] Hatta W, Sudarwanto M, Sudirman I, and Malaka R 2013 Prevelance and sources of contamination of Escherichia coli and Salmonella spp. In cow milk dangke, Indonesia fresh soft cheese Global Veterinaria 11 352-356.

[21] Arini N, Sudarwanto M, Sudirman I, and Indrawati A 2011 Utilization of Lactobacillus plantarum supernatant as an inhibitor of Escherichia coli growth in cow’s milk dangke. Jurnal Veteriner 17(3) 365-373.

[22] Malaka R, Hatta W, and Baco S 2017 Properties and microstructure of dangke fresh cheese made with passion fruits juice as coagulant IJAIR 5 601-606.

[23] Can HY, and Celik TH 2012 Detection of enterotoxigenic and antimicrobial resistant S. aureus in Turkish cheeses. Food Control 24 100-103.

[24] Jaber NN 2011 Isolation and biotyping of Staphylococcus aureus from white cheese in basrah local market. Bas. J. Vet. Res. 10(2) 55-66

[25] De Reu K, Debeuckelaere W, Boitteldoorn N, De Block J, and Herman L 2002 Hygienic parameters, toxins and pathogen occurrence in raw milk cheese. J. Food Safety 22 183-196.

[26] Oner Z, Karahan AG, Aloglu H 2006 Changes in the microbiological and chemical characteristics of an artisanal Turkish white cheese during ripening LWT 39: 494-454.

[27] Temelli S, Anar S, Sen C, and Akyuva P 2006 Determination of microbiological and chemical characteristics of an artisanal Turkish white cheese during ripening LWT 39: 494-454.

[28] Heikal GI, Khater DF, and Al Wakeel SA 2014 Bacteriological hazard of white cheese processed in some small primitive plants (dairy shops) in tanta city. Benha Veterinary Medical Journal. 26. 185-194.

[29] Arum RH,Satiawihardja B, Kusumaningrum HD 2014 Antibacterial activity of dried papaya latex toward Staphylococcus aureus in dangke. J. Teknol. Dan Industri Pangan 25 65-71

[30] Hafsan 2013 Microbial contamination of Buffalo milk dangke from curio Enrekang of South Sulawesi. Proceeding of Buffalo International Conference 2013 (Makassar: Hasanuddin University) Nopember 2013 p. 344-346
[31] Indonesia National Standard 2000 SNI 01-6366-2000 on microbial contamination limit
European Commission. (2005). Commission Regulation (EC) No 2073/2005 (OJ L338, p1, 22/12/2005 ) of 15 November 2005 on microbiological criteria for foodstuffs. https://www.fsai.ie/legislation/food_legislation/hygiene_of_foodstuffs/microbiological_criteria.html.

[32] Indonesia National Standard 2009 SNI 7388:2009 Maximum limit of microbial contamination in food. (Jakarta: National Standardization Agency)

[33] Indonesia National Standard 2000 SNI 01-6366-2000 Microbial contamination limit maximum residues in foodstuff of animal origin. (Jakarta: National Standardization Agency)