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Antibacterial study of cocoa skin liquid smoke in raw milk

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Abstract. This study aims to investigate the antibacterial effectiveness of adding liquid smoke from cocoa skin as antibacterial properties in raw milk and to determine the proper concentration of liquid smoke from cocoa skin as antibacterial properties in raw milk. The design used in this study was a completely randomized design (CRD) with 5 levels of treatment and 3 replications. The observations were analyzed using ANOVA with the F test and the Duncan`s New Multiple Range Test (DNMRT) continued to test at the 5% level. Observations were made on *Staphylococcus Aureus*, *Coliform*, and reduction time by using the Total Plate Count (TPC) method. The results showed that the concentration of liquid smoke from cocoa skin affected *Staphylococcus Aureus*, *coliform*, and the reduction time in raw milk by using the total plate count method. Based on the test results, it was found that the best antibacterial liquid smoke from cocoa skin in raw milk was the addition of 2% liquid smoke from cocoa skins with a reduction time of 642 minutes (10.7 hours).

1. Introduction

Indonesia is an agricultural country with large and diverse agribusiness potential. The agricultural sector has an important and strategic role in the socio-economic activities of the community. One of the agricultural sub-sectors in the livestock sub-sector. Animal husbandry has a potential opportunity to be developed and has a very important role in the growth of Indonesia's agricultural economy. The livestock subsector produces various kinds of commodities, one of which is raw milk, which is a potential commodity from dairy cows. Milk is one of the most important foodstuffs for the nutritional needs of the community. Milk plays an important role in health, intelligence, and growth, especially for children [1].

Along with population growth, and increased income followed by public awareness of the importance of a healthy lifestyle, the demand for fresh and processed raw milk will increase. This makes milk an economic commodity that has strategic value. The demand for milk is growing rapidly, this can be seen from the level of milk consumption of Indonesians in 2019, which is still around 16.23 kg/capita/year. When the national demand for milk (2019) reaching 4.3 million tons, domestic fresh milk production (SSDN) is only able to meet 22% of national needs [2].

Fresh milk is a liquid that comes from healthy and clean cows udder, which is obtained by milking it properly, whose natural content is not reduced or not added by anything and has not received any treatment except cooling [3].

Milk is a food material with a complete nutritional content consisting of protein, carbohydrates, fats, and minerals so that it is one of the important foodstuffs in meeting the nutritional needs of the community. The content of milk in the form of protein is a good medium for the growth of microorganisms so that milk is a food material that is very susceptible to contamination by microorganisms. Contamination of pathogenic microorganisms into the milk can cause milk and its processed products to become a source of disease transmission (foodborne diseases). Contamination of non-pathogenic microorganisms into the milk can cause milk and its products to spoil quickly, smell, become rancid and decrease the quality of the milk. Contamination of microorganisms in milk can make milk a dangerous drink and a food/beverage ingredient that is easily damaged [4]. Microorganisms found in milk include bacteria, yeast, and fungi. With the presence of these microorganisms, fresh raw milk will be easily damaged. Only an interval of four hours after milking fresh milk will gradually be damaged or rot.

Milk is an excellent medium for bacterial growth. Bacterial populations can double every 30 minutes at 25 ° C, where the pH ranges from 6.0 to 6.5 [5]. According to Jorgensen, et.al. [6], microorganisms in milk will naturally be found, but the number of these microorganisms will increase in the presence of contamination from hands and milking clothes, milking tools, cages, milk collection equipment (buckets, rags, filters) and certain diseases in animals, these raw milk is collected from the dairy farms in Lubuk Minturun, the milking process follows the health standardization by using a hygienic milking tool in the morning around 6-8 AM.

Besides, the number of microorganisms can increase up to 100-fold or more when stored at 250C degree for a long time [7]. The results of research by Balia et.al. [8] from smallholder dairy farms in Lembang, West Java, showed that the total number of bacteria in fresh and pasteurized milk exceeds the maximum limit of SNI microbial contamination in the year 2000 (the total bacterial contamination requirement is in the minimum of 1 x 10^6 CFU/ml).

According to SNI No. 3144.1: 2011 concerning the quality requirements for fresh milk, fresh milk that is good for consumption must meet the requirements in terms of nutritional content and also food safety. There are conditions for contamination, maximum microbial content, antibiotic residues, and maximum hazardous metal contaminants that have been determined. To obtain good fresh milk, all efforts must be made to reduce the number of bacteria present in the milk by taking into account several factors that affect the quality of the milk, such as sanitation and cage hygiene, handler health and hygiene, animal health, and hygiene, clean milking equipment and maintenance fresh milk purity.

Efforts to overcome food damage due to bacteria, in general, are using chemical preservatives such as formalin and borax [9]. Preservatives such as formaldehyde and borax are very harmful to humans when consumed. Currently, many researchers suggest alternatives to preservatives made from natural ingredients such as liquid smoke. Liquid smoke is a result of condensation from the combustion steam directly or indirectly. Liquid smoke is obtained from the pyrolysis of softwood and hardwood, one of which is empty palm fruit bunches, coconut shells, and wood [10].

Liquid smoke can be used as a preservative because it has antibacterial and antioxidant properties. This antibacterial property is related to the content of compounds in liquid smoke, namely phenolic, carbonyl compounds, and carboxylic acids [11]. In research by Ginayati, et.al. [12], regarding the use of liquid smoke from the pyrolysis of palm oil shells as a natural preservative tofu shows that the liquid smoke from palm oil shells is capable of preserving tofu food products.

Another liquid smoke that can be used as an antibacterial agent is liquid smoke from cocoa skins waste. Cocoa pod skin contains a lot of phenolic compounds, such as resinol, tannins, quercetin, cinnamic acid, pyrogallol, and epicatechin-3-gallate [13]. According to Mashuni, et.al. [14] the chemical components of cocoa skins are ammonia, hexane, alcohol, ketones, acetic and phenolic acids. Phenolic compounds, flavonoids, tannins, and terpenoids in the skin of cocoa pods are known to have antimicrobial activity. Phenolic active compounds have an antifungal activity which can damage the membrane of fungal cells resulting in changes in cell permeability which can result in inhibition of cell growth or death of fungal cells [15].

According to research [16] regarding the antimicrobial test of liquid smoke from cocoa skin against *Escherichia coli* and *Staphylococcus aureus* bacteria, it was found that liquid smoke of cocoa skin at 10% moisture content was able to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria at the highest level compared to the liquid smoke moisture content of cocoa skins 15%, 20%, and 25%. The process of preserving food by smoking traditionally can be replaced with liquid smoke. Apart from being considered more practical and efficient, the process of preserving food with liquid smoke is safer than smoking.

Based on the description above, the liquid smoke of cocoa skins has antibacterial properties that can inhibit microbial growth. Therefore, the objectives of this study are: (1) To determine the effect of the addition of liquid smoke from cocoa skins on microbial contamination in raw milk, (2) To determine the proper concentration of liquid smoke in inhibiting microbial contamination in raw milk.

2. Materials and Methods

The research has been carried out at the Agricultural Product Technology Laboratory of Ekasakti University and the Microbiology Laboratory of the Padang Industry Standardization Research Institute (BARISTAND). The research was conducted from May to June 2020.

The materials used are the raw milk obtained from the healthy and unstressful crossbred Friesian Holstein (FH) cows from one of the cattle breeders in the Koto Luar Limau Manis area and the liquid smoke of cocoa skins that have been purified by the distillation method to produce grade I liquid smoke [12].

There are several materials used for microbiological analysis: (1) The reduction time test is methylene blue (2) The Total Plate Count (TPC) test is Plate Count Agar (PCA) and 0.1% Buffered Peptone Water (BPW), (3) Test *Staphylococcus aureus* is Baird-Parker Agar (BPA), Egg yolk tellurite emulsion, Brain Heart Infusion Broth (BHIB), TSA, rabbit plasma coagulase (coagulase rabbit plasma) with EDTA 0.1% and BPW 0.1%. (4) The coliform test is Buffered Peptone Water (BPW) 0.1%, Lactose Broth (LB), and Brilliant Green Lactose Bile Broth (BGLBB).

The tools used for microbiological analysis consist of (1) the reduction time test is a reduction tube, a water bath, a volumetric pipette, and an incubator; (2) A test tube, petri dish, volumetric pipette, media bottle, counter colony, incubator, autoclave, and water bath are used for the Total Plate Count (TPC) test; (3) A petri dish, test tube, measuring pipette, media bottle, bunsen burner, incubator, autoclave, and water bath are used for the Staphylococcus aureus test; (4) A petri dish, test tube, measuring pipette, inoculation needle, media bottle, incubator, autoclave, and bunsen burner are used for *the Coliform* test.

The tools used for microbiological analysis consist of (1) A reduction tube, a water bath, a volumetric pipette, and an incubator are used for the reduction time test (2) The Total Plate Count (TPC) test is a test tube, petri dish, volumetric pipette, media bottle, counter colony, incubator, autoclave, water bath (3) The Staphylococcus aureus test is a petri dish, test tube, measuring pipette, media bottle, bunsen burner, incubator, autoclave, water bath (4) Coliform test is a petri dish, test tube, measuring pipette, inoculation needle, media bottle, incubator, autoclave, bunsen burner.

The design used in this study was a completely randomized design (CRD) with 5 levels of treatment and 3 replications. Observation data were analyzed using analysis of variance (ANOVA) with the F test and advanced test of Duncan's New Multiple Range Test (DMNRT) at $\alpha = 0.05$.The treatment in this study was the addition of different concentrations of liquid smoke modified from Nahak, et.al. [17] namely the concentrations of: A = 0%; B = 0.5%; C = 1%; D = 1.5%; and E = 2%. Furthermore, the variance fingerprint test is carried out, if there is a significant difference, it will be continued with the 5% DMRT test. The formulations of the main ingredients and additives in this study are presented in table 1.

Table 1. Formulation of cocoa skins liquid smoke in 250 ml of raw milk

Material	Unit	Treatment				
		A	B	C	D	E
Raw milk	ml	250	250	250	250	250
Cocoa skins liquid smoke	%	0%	0.5	1	1.5	2

Source: [17] modified

2.1. Research Implementation

2.1.1 Provision of Raw Materials

The raw materials in this study were liquid smoke of cocoa skins that had been purified using activated charcoal and distillation methods to produce liquid smoke of grade I cocoa skins and raw milk obtained from one of the cattle breeders in the Koto Luar Limau Manis area. The collection of raw milk is carried out in the morning at 06.00 WIB as much as 1,250 ml (for one replication) and stored in a cooler box with added ice cubes.

2.1.2 Procedure for making liquid smoke of grade I cocoa skins [12].

The process of making liquid smoke for grade I cocoa skins includes (1) Liquid smoke of grade III cocoa skins, (2) Purification of liquid smoke from cocoa skins using activated charcoal, (3) Producing grade II liquid smoke, (3) Purification of liquid skin smoke. Grade II cocoa uses the distillation method, (4) Produces grade I liquid smoke.

2.1.3 Procedure for sample preparation of raw milk

a. Raw milk is obtained from one of the cattle farms in the Koto Luar Limau Manis area
b. The milking of cows by breeders is done in the morning at 06.00 WIB
c. Take 1,250 ml of raw milk (one replication)
d. Storage of raw milk in a cooler box
e. Raw milk

2.1.4 The procedure for adding grade I cocoa skins liquid smoke to raw milk (Nahak et al. 2014)

a. Sample in the form of fresh raw milk for each treatment is provided as much as 250 ml
b. Each sample was added with liquid smoke grade I cocoa skins with a concentration according to treatment

2.2. Observation Variable

Observations on raw milk with the addition of liquid smoke from grade I cocoa skin were carried out at 0 hours as control and at 6 hours covering Total Plate Count (TPC) Test (SNI 2897, 2008), Staphylolococcusaureus Test (SNI 2897, 2008), Coliform Test (SNI 2897,2008) and Reduction Time Test (SNI 3141, 1998).

3. Results and Discussion

3.1. Total Plate Count (TPC)

The results of the analysis of diversity showed that the addition of liquid smoke from cocoa skin to raw milk had a very significant effect ($p < 0.01$) on the total number of bacteria (TPC) produced. Based on the DNMRT further test at $\alpha = 1\%$, it turns out that each treatment shows a very significant difference to the total number of bacteria (TPC) in raw milk. The average total bacterial count (TPC) in raw milk which was transformed to log x is presented in Table 2.

Table 2. Average total bacteria in raw milk which was transformed to log x

Addition of Liquid smoke (%)	TPC (CFU/ml) at 0 hour	Log x	TPC (CFU/ml) at 6 hour	Log x	
A = 0,0%	$2{,}0x10^2$	2,30	$1{,}32x10^6$	6,12	a
B = 0,5%	$2{,}0x10^2$	2,30	$5{,}8x10^5$	5,76	b
C = 1,0%	$1{,}9x10^2$	2,27	$3{,}8x10^5$	5,57	c
D = 1,5%	$1{,}9x10^2$	2,27	$2{,}2x10^5$	5,34	d
E = 2,0%	$1{,}9x10^2$	2,27	$9{,}2x10^4$	4,96	e
NB				0,98 %	

Figures in the same row followed by different lowercase letters show significantly different results in the DNMRT follow-up test at α = 1%

The total number of bacteria (TPC) in raw milk with the addition of liquid smoke from cocoa skins at 0 hours ranged from 1.9 x102 - 2.0x102 cfu/ml or transformed into Log x, namely 2.27 - 2.30 cfu/ml. While the total number of bacteria (TPC) at the 6th hour ranged from 9.2x104-1.32x106 cfu/ml or transformed into Log x, namely 4.96 - 6.12 cfu/ml [11]. The higher the concentration of liquid smoke added causes a decrease in the total number of bacteria (TPC) in raw milk (Figure 3). The highest total bacterial count (TPC) at the 6th hour was found in treatment A (without the addition of liquid smoke from cocoa skin or 0%) which was 1.32x106cfu/ml, while the lowest total bacterial count (TPC) was found in treatment E (addition of 2% liquid smoke from cocoa skins) that was 9.2 x 104 cfu/ml.

The results showed that the addition of liquid smoke from cocoa skin to raw milk had a very significant effect on reducing the number of bacteria contained in raw milk. The greater the concentration of liquid smoke added, the greater the value of the decrease in the total number of bacteria (TPC), and vice versa. This can be seen in the results of the research on the total number of bacteria (TPC) at 6 hours, namely, in treatment A the total number of bacteria (TPC) was 1.32x106 cfu/ml and decreased until treatment E was 9.2x104 cfu/ml.

This is due to the antibacterial and antioxidant substances contained in the liquid smoke of the cocoa skins. Phenolic compounds, flavonoids, tannins, and terpenoids in the skin of cocoa pods are known to have antimicrobial activity. Phenolic active compounds have an antifungal activity which can damage the membrane of fungal cells resulting in changes in cell permeability which can result in inhibition of cell growth or death of fungal cells [15].

This is following the results of research [16] regarding the antimicrobial test of liquid smoke from cocoa skin against *Escherichia coli* and *Staphylococcus aureus* bacteria which obtained results that liquid smoke of cocoa skin at 10% water content was able to inhibit the growth of E.coli and Staphylococcus aureus bacteria. at the highest level compared to liquid smoke, the moisture content of cocoa skins was 15%, 20%, and 25%.

3.2. Staphylococcus Aureus

Milk is a livestock product that has high nutritional value. However, the low hygiene in farms causes these livestock products to be contaminated by bacteria. One of the bacteria-contaminated by milk is *Staphylococcus aureus*. Contamination of *Staphylococcus aureus* in milk does not cause physical changes in milk, so consumers often do not realize its existence.

Based on research on the use of liquid smoke from cocoa skins as prevention of microbial contamination in raw milk, the results show that there is no contamination of *Staphylococcus aureus* bacteria, either at 0 or 6 hours. equipment and environment of the pen, the condition of the cow, and the temperature and duration of storage of raw milk after milking The research shows that the results of testing for *Staphylococcus aureus* bacteria are following the Indonesian National Standard (SNI 3141.1: 2011) which stipulates the limit of Staphylococcus aureus bacteria contamination in fresh raw milk is a minimum of 1 x 102 cfu/ml. The average *Staphylococcus aureus* bacteria in raw milk is presented in Table 3.

Table 3. Average total of Staphylococcus *aureus* bacteria in raw milk

Addition of Liquid smoke (%)	*Staphylococcus aureus* at 0 hour (CFU/ml)	*Staphylococcus aureus at* 6 hour (CFU/ml)
A = 0,0%	0	0
B = 0,5%	0	0
C = 1,0%	0	0
D = 1,5%	0	0
E = 2,0%	0	0

Staphylococcus aureus also plays a role in food safety because it can cause mastitis in dairy cows and has the potential to contaminate raw milk products [18]. *Staphylococcus aureus* is a Gram-positive facultative anaerobic bacteria, spherical, produces enterotoxins, found in air, dust, waste, water, milk, food or tableware and on environmental surfaces [19]. In humans or animals, it is usually found on the skin and nose. Humans and animals are the main reservoirs with a rate of 20-30% of the total population as carriers of staphylococcus.

3.3. Coliform

Based on the *coliform* bacteria test at 0 o'clock, all treatments showed that there were no *coliform* bacteria in raw milk with the addition of liquid smoke from cocoa skins while in the 6th-hour test treatment A (without the addition of cocoa skins liquid smoke) showed a result of 4 APM/ml. The total number of *coliform* bacteria produced at the 6th hour has met the quality requirements for fresh milk set by the Indonesian National Standard [3], namely a maximum of 2x101 colonies/ml. The average *Coliform* bacteria in raw milk is presented in Table 4.

Table 4. Average total *Coliform* bacteria in raw milk

Addition of Liquid smoke (%)	*Coliform* at 0 hour (APM/ml)	*Coliform* at 6 hour (APM/ml)
A = 0,0%	<3	4
B = 0,5%	<3	<3
C = 1,0%	<3	<3
D = 1,5%	<3	<3
E = 2,0%	<3	<3

Based on the results of this study, it can be seen that the contamination of raw milk with the addition of liquid smoke of cocoa skin by *coliform* bacteria can come from equipment, unclean storage rooms, dust, air, and storage time of milk at room temperature during the study.

The results showed that there was no difference between 0 and 6 hours, and the development of *coliform* bacteria in raw milk was inhibited by the addition of liquid smoke from cocoa skins. This can be seen in the results that only in the treatment without the addition of liquid smoke from cocoa skins showed an increase in the total number of *coliform* bacteria, while in all treatments with the addition of liquid smoke from cocoa skins, there were no *coliform* bacteria.

This is due to the antibacterial and antioxidant substances contained in the liquid smoke of the cocoa skins. Phenolic compounds, flavonoids, tannins, and terpenoids in the skin of cocoa pods are known to have antimicrobial activity. Phenolic active compounds have an anti-fungal activity that can damage fungal cell membranes resulting in changes in cell permeability which can result in inhibition of cell growth or death of fungal cells [15]. The presence of *Coliform* bacteria in fresh raw milk is undesirable because it can cause health problems in humans, especially in the digestive tract, such as stomach cramps and diarrhea [20].

3.4. Time of reduction

The results of the analysis of diversity showed that the addition of liquid smoke from cocoa skin to raw milk had a very significant effect ($p < 0.01$) on the resulting reduction time (Appendix 12). Based on the DNMRT further test at $\alpha = 1\%$, it turns out that each treatment shows a very significant difference in the reduction time in raw milk with the addition of liquid smoke from cocoa skin. The average reduction time values for raw milk are presented in Table 5.

Table 5. Average reduction time values in raw milk

Addition of Liquid smoke (%)	Reduction time in minutes (hour)	
A = 0,0%	287 (4,78)	a
B = 0,5%	458 (7,63)	b
C = 1,0%	503 (8,38)	c
D = 1,5%	567 (9,45)	d
E = 2,0%	642 (10,7)	e
NB	2,64 %	

NB : Figures in the same row followed by different lowercase letters show significantly different results in the DNMRT follow-up test at $\alpha = 1\%$

Reduction time in raw milk with the addition of liquid smoke from cocoa skins ranged from 287/4.78 minutes/hour - 642/10.7 minutes/hour. The higher the concentration of liquid smoke added causes an increase in the value of reduction time in raw milk (Figure 4). The best reduction time was found in treatment E (addition of 2% liquid smoke from cocoa skins), with a reduction time of 642 minutes (10.7 hours). Meanwhile, the lowest reduction time was found in treatment A (without the addition of liquid smoke or 0% cocoa skin) with a reduction time of 287 minutes (4.78 hours).

The results showed that the addition of liquid smoke from cocoa skins to raw milk had a very significant effect on the reduction time value of raw milk. The higher the concentration of liquid smoke added causes an increase in the value of the reduction time in raw milk, and vice versa. This is due to the activity of the reductase enzyme produced by bacteria in reducing methylene blue. The more the number of bacteria in the milk, the more enzymes will be produced, and the faster the blue to white color changes. This is following the opinion of Fardiaz (1989), that the more bacteria in the milk, the faster the blue color changes to white.

4. Conclusion

Based on the results and discussion above, the following conclusions are, the addition of liquid smoke from cocoa skin to microbial contamination in raw milk affects the bacterial count in Total Plate Count (TPC), coliform, and reduction time. The addition liquid smoke at raw milk has the different effect on temperature quality (number of microbes), the addition of liquid smoke is able to suppress microbial development. All treatments met the quality requirements following the Indonesian National Standard

(SNI 3141.1: 2011) regarding fresh raw milk, except for the treatment without the addition of liquid smoke from cocoa skins or treatment A. The best combination of raw milk and liquid smoke from cocoa skin based on the results of microbiological tests is treatment E, namely the addition of liquid smoke with a concentration of 2%

References

[1] Farid, M., dan Sukesi, H. 2011. Pengembangan Susu Segar Dalam Negeri Untuk Pemenuhan Kebutuhan Susu Nasional. Buletin Ilmiah Litbang Perdagangan, 5(2), 196–221.
[2] Badan Pusat Statistik. 2019. Tingkat Konsumsi Susu Masyarakat Indonesia 2019
[3] BandanStandarisasiNasional. 2011. Susu Segar SNI 3141
[4] Harpini, B. (2008). Upaya menyongsong industri pengolahan dan pemasaran susu pada peternakan rakyat dalam Prosiding Prospek Industri Sapi Perah Menuju Perdagangan Bebas 2020. Pusat Penelitian dan Pengembangan Peternakan bekerjasama dengan Sekolah Tinggi Ilmu Ekonomi Keuangan dan Perbankan Indonesia". Jakarta, Hal. 13
[5] Morandi, S., Brasca, M., Alfieri, P., Lodi, R., and Tamburini, A. (2005). *Influence of pH and temperature on the growth of Enterococcus faecium and Enterococcus faecalis*. *EDP Sciences*, *85*(3), 181–192. https://doi.org/10.1051/lait
[6] Jorgensen HJ, T Mork, HR Hogasen, and LM Lorvik. (2005). *Enterotoxigenic Staphylococcus aureus in bulk milk in Norway*. J. Appl. Microbiol. 99, 158-166.
[7] Chye FY, Abdullah A and Ayob MK. (2004).*Bacteriological quality and safety of raw milk in Malaysia*. *Food Microbiol* 21 (5): 353-541
[8] Balia RL, E Harliadan D Suryanto. 2008. Jumlah Bakteri Total dan Koliform pada Susu Segar Peternakan Sapi Perah Rakyat dan Susu Pasteurisasi Tanpa Kemasan di Pedagang Kaki Lima. Dalam: Prosiding 'Prospek Industri Sapi Perah Menuju Perdagangan Bebas 2020'. Pusat Penelitian dan Pengembangan Peternakan bekerjasama dengan Sekolah Tinggi Ilmu Ekonomi Keuangan dan Perbankan Indonesia. Jakarta.
[9] Tirtodiharjo, K. (2011). Strategi mengatasi bacteria yang resisten terhadap antibiotika. Universitas Gadjah Mada, Yogyakarta.
[10] Lestari, Y. I., Idiawati, N., dan Harlia. 2015. Aktivitas Antibakteri Asap Cair Tandan Kosong Sawit Grade 2 yang sebelumnya di Adsorbsi Zeolit Teraktivasi. *Jkk*, *4*(4), 45–52.
[11] Panagan, A.T., Syarif, Nirwan.2009. Uji Daya Hambat Asap Cair Hasil Pirolisis Kayu Pelawan (*Tristaniaabavata*) terhadap Bakteri *Escherichia coli*. *Jurnal* Penelitian sains 9 (12): 30-32
[12] Ginayati, L., Faisal, M., dan Suhendrayatna. 2015. Pemanfaatan Asap Cair Dari Pirolisis Cangkang Kelapa Sawit Sebagai Pengawet Alami Tahu. *Jurnal* Teknik Kimia USU, 4(3), 7–11. https://doi.org/10.32734/jtk.v4i3.1474
[13] Fapohunda and Afolayan. 2012.*Fermentation of Cocoa Beans and Antimicrobial Potentials of the pod Husk Phytochemicals, Journal of Physiology and Pharmocology Advances*, 2 (3), 158-164
[14] Mashuni, M., Kadidae, L. O., Jahiding, M., Dermawan, M. A., dan Hamid, F. H. (2019). Pemanfaatan Kulit Buah Kakao sebagai Antibakteri *Escherichia coli* dan *Staphylococcus aureus*. *Jurnal* Penelitian Biologi, *6*(2), 1017–1026. https://doi.org/10.33772/biowallacea.v6i2.9009
[15] Rachmawaty, Mu'nisa, A., Hasri, Halifah P., Hartati, and Zulkifli, M. (2018).*Active Compounds Extraction of Cocoa Pod Husk (Thebroma Cacao l.) and Potential as Fungicides.Journal of Physics* 10(28): 1-8
[16] Budaraga, I. K., and Putra, D. P. (2019). *Liquid Smoke Antimicrobial Test of Cocoa Fruit Peel Against Eschericia Coli and Staphylococcus Aureus Bacteria.IOP Conference Series: Earth and Environmental Science*, *365*(1), 1–10. https://doi.org/10.1088/1755-1315/365/1/012049

[17] Nahak, R. J., Khotimah, S., dan Turnip, M. (2014). Aspek Mikrobiologis Susu Sapi Murni dengan Penambahan Sari Rimpang Bangle (*Zingiber cassumunar Roxb*). *Jurnal* Protobiont, *3*(3), 69–74.
[18] Savitri M, Yasni S, Bintang M, Ranti AS. 2011. Antioxidation activity and inhibitor of the enzyme a-glucosidase functional drink red betel (Piper crocatum) and cinnamon (Cinnamomum burmannii). Proceedings of the National Seminar of the Indonesian Food Technology Expert Association (hlm. 282-286), 15-17 September.
[19] Wertheim, E. G. 2008. http://web.cba.neu. edu/wertheim/intro/history 12 September 2020
[20] Muchtadi, Tien R dkk, 2010.Ilmu Pengetahuan Bahan Pangan. CV ALFABETA : Bandung.