

Analysis of Microbial Contamination and Identification of *Escherichia coli* on Unbranded Soymilk which is Circulated in Jaya Baru Sub-District, Banda Aceh

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Abstract: Soy milk is a liquid extraction of soy beans produced by home industry that needed to increase concern about hygiene and sanitary to prevent bacterial contamination. This study aimed to determine the presence of bacterial contaminants and detect *Escherichia coli* on unbranded soymilk from 9 locations in Jaya Baru Sub-District, Banda Aceh. Research were conducted at AKAFARMA Laboratory and Laboratory in Faculty of Mathematics and Natural Sciences, Syiah Kuala University from June to July 2020. The research method consisted of Total Plate Count (TPC) and IMViC. The results showed that were found 8 out of 9 samples were negative *Escherichia coli*. Meanwhile only one sample from Geuceu Meunara (GM) showed that positive *Escherichia coli*. In addition, the unbranded soy milk from Jaya Baru Sub-District were not eligible to National Standard (SNI No.06.8-7388-2009).

Keywords: soy milk, microbial contamination, *Escherichia coli*, total plate count, IMViC test

1. Introduction

Soy milk is produced by plant-based ingredients which have the highest nutritional value¹. Besides the highest nutritional value, soy milk is also a good medium for the microbial growth, both are beneficial and harmful microorganism. *Escherichia coli* is one of the harmful microorganisms, which is when the large numbers of *Escherichia coli* transmitted to humans with the low-immune system can cause acute diarrhea. *Escherichia coli* is an organism that lives in human guts and also as indicators bacteria for fecal contamination, the sanitary quality of water, food safety, as well as milk hygiene^{2,3}.

The contamination of microorganisms on soy milk due to some factor such as, the usage of dirty equipment, dirt remaining on soy milk processing equipment, filthy raw materials, dust, etc^{4,5,6}. The presence of microorganism contaminants can cause damage to the quality of the soy milk⁷. However, soy milk which has been contaminated by bacteria is not suitable for drinking.

Banda Aceh is one of the city that produced various of homemade drinks such as soy milk. The study takes place in Jaya Baru Sub-District because there are so many home industry produced an unbranded soy milk with low cost and good taste. That is why many people like to drink unbranded soy milk.

According to Ref.⁸, the locally made soy milk in Lembang contained $3,70 \times 10^6$ CFU/ml of bacteria and unpackaged pasteurized soy milk in street vendors contains bacteria of $3,45 \times 10^6$ CFU/ml. It was assumed that CFU value higher than the maximum limit of microbial contamination as permitted by SNI No. 06.8-7388-2009 about the maximum limit of microbial in soybean extract products of 5×10^4 colonies / ml^{8,9}.

Based on the previous study, we need to conduct research about analysis of microbial contamination and identification of *Escherichia coli* on unbranded soymilk which is circulated in Jaya Baru Sub-District, Banda Aceh. So that would be determined the presence of bacterial contaminants on unbranded soy milk.

2. Material and Method

Material

The material which were used in this study are soy milk was obtained from 9 locations in Jaya Baru Sub-District, Plate Count Agar (PCA), alcohol, aquadest, gauze, cotton, Kovac reagents, Nutrient Agar (NA) media, Simmons Citrate (SC) media, SIM media (Sulfur, Indole), Alpha reagents-naphtol, methyl red (MR) reagent, potassium hydroxide, and ethanol 70%.

Analysis Total Plate Count

Total microbial analysis was carried out by taking 1 ml of each dilution sample and put it in a sterile petri dish. In this study, the dilution was made from 10^{-1} to 10^{-5} . All dilution sample must be carried out in Duplo. Pour cooled Mueller Hinton Agar (MHA) media into sterile petri dishes as much as 12 ml. Petri dishes are carefully rotated until the suspension is evenly distributed. Incubation was carried out at 37°C for 24-48 hours. And then, calculation and recording of colony growth is carried out in colony forming unit per ml (cfu/ml) or per gram (cfu/g).

Identification of *Escherichia coli*

Indole test

The separate colony was taken by a sterile loop then inoculated into SIM media and was incubated for 24-48 hours at 37°C. After incubation, add 10-12 drops of Kovac's reagent. A positive indole test is indicated by the formation

of a red color in the reagent layer on top of the culture¹⁰.

Methyl red test

The separate colony was taken by a sterile loop then inoculated into MR-VP media and was incubated for 24-48 hours at 37°C. Add 3-4 drops of methyl red indicator. A positive methyl red (MR) test is indicated by the culture medium turns red. In other words there is acid formation in cultures¹⁰.

Vogesproskauer (VP) test

The separate colony was taken by a sterile loop then inoculated into MR-VP media and was incubated for 24-48 hours at 37°C. One ml of the suspension was pipette into the tube, then add 0.6 ml of naphthol solution and 0.2 ml of potassium hydroxide solution and shake well. Let stand for 2-4 hours. A positive vogesproskauer test is indicated by the culture medium turns pink until dark red¹¹.

Simmons citrate (SC) test

The separate colony was taken by a sterile loop then inoculated into simmons citrate media and was incubated for 24-48 hours at 37°C. A positives simmons citrate test is indicated by the culture medium turns blue¹².

3. Result

Total Plate Count (TPC)

The result of total plate count bacteria on unbranded so milk which is circulated in Jaya Baru Sub-District is presented in Table1.

Table 1: The total plate count analysis on unbranded soy milk from Jaya Baru Sub-District, Banda Aceh

Code of sample	Dilution	The number of colonies (col/ml)		TPC (CFU/ml)
		PD1	PD2	
LT	10 ⁻¹	TNTC	TNTC	23x10 ⁶
	10 ⁻²	TNTC	TNTC	
	10 ⁻³	TNTC	TNTC	
	10 ⁻⁴	TMTC	TNTC	
	10 ⁻⁵	236	230	
E	10 ⁻¹	TNTC	TNTC	49x10 ⁶
	10 ⁻²	TNTC	TNTC	
	10 ⁻³	TNTC	TNTC	
	10 ⁻⁴	TNTC	TNTC	
	10 ⁻⁵	50	47	
LD	10 ⁻¹	TNTC	TNTC	86x10 ⁶
	10 ⁻²	TNTC	TNTC	
	10 ⁻³	TNTC	TNTC	
	10 ⁻⁴	TNTC	TNTC	
	10 ⁻⁵	98	73	
B	10 ⁻¹	TNTC	TNTC	2,8x10 ⁶
	10 ⁻²	TNTC	TNTC	
	10 ⁻³	TNTC	TNTC	
	10 ⁻⁴	67	200	
	10 ⁻⁵	147	201	
LB	10 ⁻¹	TNTC	TNTC	26x10 ⁶
	10 ⁻²	TNTC	TNTC	
	10 ⁻³	TNTC	TNTC	
	10 ⁻⁴	TNTC	TNTC	
	10 ⁻⁵	271	241	
	10 ⁻¹	TNTC	TNTC	
	10 ⁻²	TNTC	TNTC	

PBC	10 ⁻³	TNTC	TNTC	50x10 ⁵
	10 ⁻⁴	32	31	
	10 ⁻⁵	42	TNTC	
GM	10 ⁻¹	TNTC	TNTC	44x10 ⁵
	10 ⁻²	TNTC	TNTC	
	10 ⁻³	TNTC	TNTC	
	10 ⁻⁴	TNTC	277	
	10 ⁻⁵	43	207	
L	10 ⁻¹	TNTC	TNTC	45x10 ⁵
	10 ⁻²	TNTC	TNTC	
	10 ⁻³	TNTC	TNTC	
	10 ⁻⁴	289	298	
	10 ⁻⁵	209	201	
UP	10 ⁻¹	TNTC	TNTC	32x10 ⁵
	10 ⁻²	TNTC	TNTC	
	10 ⁻³	TNTC	TNTC	
	10 ⁻⁴	141	224	
	10 ⁻⁵	153	206	
Blank	1		0	
	2		0	

*LT : LamteumenTimur, E: Emperom,
 LD: LampohDaya, B: Bitai,
 LB: Lamteumen Barat,
 PBC: PungeBlang Cut,
 GM: GeuceuMeunara,
 L: Lamjame, UP: UleePata
 *PD : Petri Dishes
 *TNTC : Too Numerous To Count

As Table 1 shows, mostly of samples is considered Too Numerous To Count (TNTC), especially for the dilution 10⁻¹ up to 10⁻⁴. Meanwhile, some samples have a statistically significant number of colonies at dilution 10⁻⁴. i.e B (67/200), PBC (32/31), GM (277), L (289/298), and UP (141/224). In addition, the number of colonies can be calculated for the whole sample at dilution 10⁻⁵. In line to Ref. [13], a plate was chosen should have between 30 and 300 colonies because this range is considered statistically significant. According to Ref. [9] the maximum limit of microbial contamination as permitted by SNI No. 06.8-7388-2009 about the maximum limit of microbial in soybean extract products of 5x10⁴ colonies/ml. Accordingly to unbranded soy milk from Jaya Baru Sub-District were not eligible to SNI standard, No. 06.8-7388-2009.

In other hand, there are several factor that affects the presence of bacteria on unbranded soy milk, such as poor sanitary conditions, unhygienic, soy milk production by conventional method will always increase the presence of pathogen contaminants, etc^{14,15}. The source of pathogenic contamination occurred due to raw materials, food materials, additives, packaging materials, equipment, environmental, as well as worker. Contamination of microorganisms in soy milk has a potential risk for human health (Opportunistic infection) that can cause diarrhea¹⁶.

IMViC Test of Escherichia coli

The IMViC Test of *Escherichia colion* unbranded soy milk from 9 locations in Jaya Baru Sub-District, Banda Aceh is presented in Table 2.

Table2: IMViC Test Results of *Escherichia coli* on soy milk

Code of sample	Indole test	MR test	VP test	SC test
LT	-	-	-	-
E	+	-	-	-
LD	-	+	-	+
B	-	-	-	-
LB	-	-	-	-
PBC	-	-	-	+
GM	-	+	-	-
L	-	+	-	+
UP	-	+	-	+

*+ : positive

*- : negative

Indole Test

Based on Table 2, the results of indole test showed that were found 1 samples was positive *Escherichia coli*. Whereby the sample was purchased from Emperum (E). Meanwhile, other samples were negative *Escherichia coli*. According to¹⁷, a positive indole test is indicated by the formation of a pink to red color. The red coloured ring is formed due to the indole produced by bacteria reacts with *para-dimethyl amino benzaldehyde*, which is contained in Kovac's Reagent.

MR Test

The result of methyl red (MR) test showed that were found 4 samples were positive *Escherichia coli*. Where, we collect the sample from different location *i.e.* Lampoh Daya (LD), Geuceu Meunara (GM), Lamjame (L), and UleePata (UP). Whereas, other samples showed negative results. Ref.¹⁷ reported that the positive methyl red (MR) test is indicated by a change in the colour of methyl red from yellow to red.

VP Test

The result of vogesproskauer (VP) test showed that the whole sample were negative *Escherichia coli*. The positive vogesproskauer (VP) test is indicated by no color change in the culture medium. In line to Ref¹⁷, a positive vogesproskauer (VP) test is indicated by the culture medium turns pink until dark red. It was happening due to the presence of acetone on *Escherichia coli*.

Simmons Citrate Test

The result of simmons citrate (SC) test showed that were found 4 samples were positive *Escherichia coli*. Where, we purchased the sample from different location *i.e.* Lampoh Daya (LD), PungeBlang Cut (PBC), Lamjame (L), and Ulee Pata (UP). The positive simmons citrate (SC) test is indicated by the culture medium turns blue. Meanwhile, other samples were negative *Escherichia coli*. According to Ref.¹⁸ a positive simmons citrate (SC) test is indicated by a change in the medium colour from green to blue.

Generally, based on 9 samples of unbranded soy milk was obtained from 9 locations in Jaya Baru Sub-District, Banda Aceh showed that were found 8 out of 9 samples were negative *Escherichia coli*. Conversely, only one sample from Geuceu Meunara (GM) showed that positive *Escherichia coli*. It can be assumed by the biotyping of *Escherichia coli* those are indole test (negative/positive), methyl red test (positive), vogesproskauer test (negative), and simmons

citrate test (negative). This results were not eligible to SNI standard, No.06.8-7388-2009 about coliform bacteria are normally not present in natural mineral water sources, include in soy milk drinks. Commonly was detected 0 most probable number per 100 millilitres⁹.

4. Conclusion

In conclusion, unbranded soy milk from Jaya Baru Sub-District were not eligible to National Standard (SNI No.06.8-7388-2009). The results of the identification of the *Escherichia coli* showed that there are eight samples were negative *Escherichia coli*. Conversely, there is one sample was positive *Escherichia coli*.

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