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Coliform Assessment of Commercial Buko Juice with Milk in Sogod, Southern Leyte, Philippines

Ramel E. Claros* & Pet Anthony L. Pascual**

**Department of Food Science & Technology, Southern Leyte State University, Sogod, Southern Leyte, Philippines*

***Director of Center for Organic & Natural Food Research (CONFoR), Southern Leyte State University, Sogod, Southern Leyte, Philipines*

ABSTRACT

Buko juice is rich in minerals, amino acids, vitamins and antioxidants. It is added with milk resulting to its more delicious and nutritious drink. However, it is processed and placed in a bottle without any heat application. Two (2) different sources of buko juice with milk were collected and analysed for pH, TSS, aerobic plate count and coliform detection. The pH results from source A was 6.3 and from source B was 6.2. The oBrix were 10.8 and 11.0 for source A & B, respectively. Further, the aerobic plate count (APC) were 16,000 CFU/mL (source A) and 22,000 CFU/mL (source B). The coliform results confirmed the presence of coliform bacteria.

KEYWORDS: *Cocci, rod, TCW, APC and CFU/mL*

INTRODUCTION

Coconuts are economic plants in many tropical countries like Philippines, and often called ‘‘trees of life’’ because a large amount of coconut fruit and its products are consumed and utilized by humans (Prades et al 2012). It is the principal permanent crop in Region 8 in terms of the number of trees/vines/hills. About 25.5 million coconut trees were planted in 234.9 thousand farms. Most of these trees (16.8 and 16.1 million) were grown in Northern Samar and Leyte, respectively. Southern Leyte contributes 2.3 million trees (NSO, 2002).

Tender coconut water (TCW) is derived from young coconut fruit having a soft & white endosperm. It is colorless and has natural sweet taste with approximation of 6 ^{o}Brix. TCW is often called buko juice in the Philippines.

According to DebMandal and Mandal (2011), Singh (2009), Athukorale (2008), Fife (n.d), and Yong et al (2009) that TCW is rich in minerals, amino acids, vitamins and antioxidants. Thus, it is sold in the Philippine market as fresh buko juice either in the form of whole fruit or bottled buko juice. Majority sold this juice in bottled product for less volume and weight during transportation.

In the concept of natural and fresh buko juice, it is bottled without any heat application. Thus, it is bottled and placed with more ice inside the bucket for its preservation. However, the human intervention during preparation may contribute microbial contamination that could compromise health safety to the consumers.

Hence, this study was conducted to documents the prevalence of microorganisms that could harm health safety. Specifically this study aims the following: (a) to determine some chemical properties and aerobic plate count and (b) to conduct coliform analysis of the commercial buko juice with milk in Sogod, Southern Leyte, Philippines.

MATERIALS AND METHODS

Sample Collection. Two samples of buko juice with milk from two different sources (A & B) in Sogod, Southern Leyte were collected and placed in the ice bucket to prior of bringing into the laboratory. It was observed during collection that these samples were properly stored in the retail outlet using ice bucket with full of ice.

Total Soluble Solids (TSS). TSS was determined using refractometer in degree Brix unit. A drop of each sample was placed on the prism before reading was taken.

pH. The pH meter was calibrated using two buffer solutions of known pH of 4 and 7 before it was used on the samples. Approximately, 50 mL of sample was placed in a beaker and the pH meter was submerged to take the acidity reading.

Aerobic Plate Count (APC). APC was determined using plate count agar. A 25 mL sample was dissolved in sterile peptone water serving as 10^{-1}. One mL from 10^{-1} was transferred to 9 mL peptone water serving as 10^{-2} until 10^{-3} dilutions were prepared. Each dilution was plated into sterile petri plates and incubated for 24 hrs.

Coliform Analysis. The following tests from US-FDA (2001) procedures such as presumptive, confirmed and completed tests were conducted to detect the presence coliform bacteria in commercial buko juice. Further, gram staining was conducted to verify the shape and color.

Statistical Treatment of Data. Data from instrumental analyses will be subjected to paired comparison or T-test.

RESULTS AND DISCUSSION

TSS and pH. Table 1 shows that the pH of the two sources of buko juice were 6.3 and 6.2 for source A & B, respectively. It is identical on the study conducted by Kannangara et al (2018) that coconut water at 7-month old has pH of 5.39 and 9-month old with of pH= 6.34. In the review of Yong et al (2009) that young green coconut water has pH of 5.6. Further, Prades et al (2012) made summary on pH and TSS value of different varieties of immature coconut water with mean value of 5.16 for pH & 5.8 for TSS. Hence, buko juice is considered low acid food due to its pH value greater than 4.5. According to Tucker and Featherstone (2011) that this food is more susceptible to microbial spoilage as its pH > 4.5.

On the other hand, the freshly obtained buko juice from 7 & 9 months old maturity were 5 & 6.16 ^{o}Brix, respectively (Kannangara et al 2018). However, the commercial buko juice has TSS value of 10.8 ^{o}Brix from source A and 11 ^{o}Brix from source B. It implies that the commercial buko juice has added sugars even with the presence of its natural sugars.

Table 1. Average TTS and pH values of the 2 buko juice sources in 3 replications.

Buko Juice	pH	TSS (^{o}Brix)
A	6.3[a]	10.8[a]
B	6.2[a]	11.0[a]

[a]- same subscript indicates no significant difference

Microbiological tests are mainly used to make the critical decisions regarding food safety and shelf-life (FSAI, 2017). The (FDA-Phil, 2013) sets microbiological criteria for non-alcoholic beverages for Aerobic Plate Count (10-100 CFU/mL) and Gulf Standards (2000) in United Arab Emirates sets for fruit juice and drink for APC is 100-300CFU/mL. Table 2 implies that the commercial buko juice with milk in Sogod, Southern Leyte is not safe for human consumption.

Table 2. Estimated aerobic plate count (APC) per mL of commercial buko juice with milk.

Buko Juice	10^{-1}	10^{-2}	10^{-3}	CFU/mL
A	TNTC	160	14	16,000
B	TNTC	211	31	22,000

TNTC - too numerous to count
EAPC - estimated aerobic plate count

Table 3. Lauryl Tryptose Broth at 24 hours incubation of buko juice with milk from source A.

Dilution	TT_1	TT_2	TT_3	TT_4	TT_5
1:10 (10^{-1})	(-)	(-)	(-)	(-)	(-)
1:100 (10^{-2})	(-)	(-)	(-)	(-)	(-)
1:1000 (10^{-3})	(-)	(-)	(-)	(-)	(-)

TT =test tube
(-) =no bubble inside durham tube

Table 3 shows negative results of buko juice from source A in lauryl tryptose broth after 24 hours incubation. However, after 48 hours incubation, it shows positive presumptive test except one test tube (TT_5) at 10^{-3} dilution (Table 4).

Table 4. Lauryl Tryptose Broth at 48 hours incubation of buko juice with milk from source A.

Dilution	TT_1	TT_2	TT_3	TT_4	TT_5
1:10 (10^{-1})	+	+	+	+	+
1:100 (10^{-2})	+	+	+	+	+
1:1000 (10^{-3})	+	+	+	+	(-)

TT =test tube
+ =bubble inside durham tube

On the other hand, source B after 24 hours incubation in lauryl broth, shows positive results in all test tubes in 10^{-1} dilution and TT_3 in 10^{-2} and 10^{-3} dilutions as shown in Table 5. Then, the rest tubes were negative results. However, these negative results changed into positive after 48 hours incubation except TT_1 from 10^{-3} dilution and TT_5 from dilution 10^{-2} & 10^{-3} (Table 6).

Table 5. Lauryl Tryptose Broth at 24 hours incubation of buko juice with milk from source B.

Dilution	TT_1	TT_2	TT_3	TT_4	TT_5
1:10 (10^{-1})	+	+	+	+	+
1:100 (10^{-2})	(-)	(-)	+	(-)	(-)
1:1000 (10^{-3})	(-)	(-)	+	(-)	(-)

TT =test tube
(-) =no bubble inside durham tube
+ =bubble inside durham tube

Table 6. Lauryl Tryptose Broth at 48 hours incubation of buko juice with milk from source B.

Dilution	TT_1	TT_2	TT_3	TT_4	TT_5
1:10 (10^{-1})	+	+	+	+	+
1:100 (10^{-2})	+	+	+	+	(-)
1:1000 (10^{-3})	(-)	+	+	+	(-)

TT =test tube
(-) =no bubble inside durham tube
+ =bubble inside durham tube

Table 7. Brilliant Green Bile Broth at 48 hours incubation of buko juice with milk from source A.

Dilution	TT_1	TT_2	TT_3	TT_4	TT_5
1:10 (10^{-1})	+	+	+	+	+
1:100 (10^{-2})	+	+	+	+	+
1:1000 (10^{-3})	+	+	+	+	No tube

TT =test tube
+ =bubble inside durham tube
No tube =due to negative result from lauryl broth

On confirmed test, source A of buko juice showed positive results in all test tubes using brilliant green bile broth (Table 7). Further, buko juice from source B showed positive confirmed test except TT_2 from 10^{-3} dilution (Table 8). It implies that buko juice from source A and B were contaminated with coliform bacteria. Since FDA-Phil (2013) sets microbiological criteria for non-alcoholic beverages for coliform at 1CFU/mL; therefore, these products were unsafe for human consumption. Even the Gulf Standards (2000) in UAE sets for fruit juice and drink for coliform (0-10 CFU/mL).

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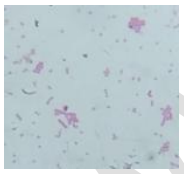
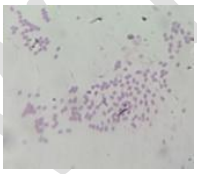
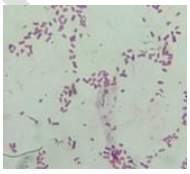
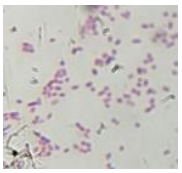
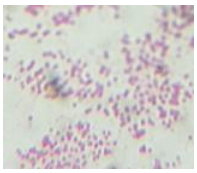
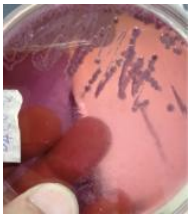
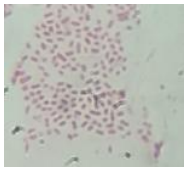
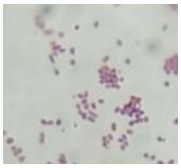
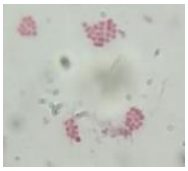
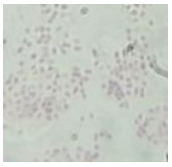
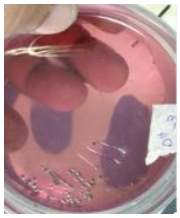
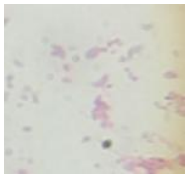
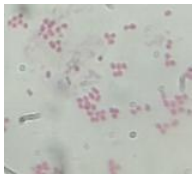
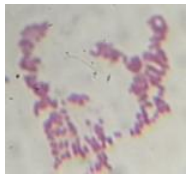
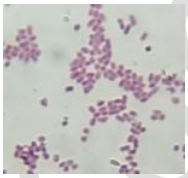
Table 8. Brilliant Green Bile Broth at 48 hours incubation of buko juice with milk from source B.

Dilution	TT_1	TT_2	TT_3	TT_4	TT_5
1:10 (10^{-1})	+	+	+	+	+
1:100 (10^{-2})	+	+	+	+	No tube
1:1000 (10^{-3})	No tube	(-)	+	+	No tube

TT =test tube
+ =bubble inside durham tube
(-) =no bubble inside durham tube
No tube =due to negative result from lauryl broth

Table 9. EMB agar at 24 hours incubation and its shape of buko juice with milk from source A.

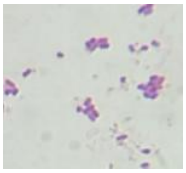
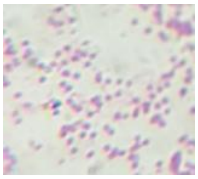
Dilution	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
1:10					
	White colony w/green metallic sheen	White colony	White colony w/ dark center & w/ green metallic sheen	White colony w/ dark center	White colony w/ dark center
					
	Rod	Cocci	Short rod	Short rod	Cocci
1:100					
	White colony	White colony	White colony	White colony w/ dark center	White colony w/ dark center
					
	Short rod	Cocci	Cocci	Cocci	Cocci

1:1000					No plate due to negative result from brilliant green bile broth
	White colony	White colony	Green metallic sheen colony	Green metallic sheen colony	
					No slant due to negative result from brilliant green bile broth
	Rod	Cocci	Short rod	Cocci	

According to Leininger et al (2001) that EMB contains dyes that are toxic to gram-positive bacteria. It is a differential culture media intended for gram-negative bacteria. Hence, these bacteria are gram-negative. Table 9 shows green metallic sheen in plate 1 and plate 3 with rod-shape bacteria from 10^{-1} dilution and plate 3 and 4 from 10^{-3} dilution. It implies presence of coliform bacteria from source A of buko juice.

However, plate 4 at 10^{-3} dilution had cocci shape bacteria. According to Gotschlich (2009) that gram-negative cocci are almost isolated from nasopharyngeal cultures of human beings as pathogenic bacteria. It was a consequence of non-application of heat during preparation of buko juice with milk as finished product.

Table 10. EMB agar at 24 hours incubation of buko juice with milk from source B.

Dilution	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
1:10					
	White colony w/ dark center	White colony w/ dark center	White colony w/ dark center	Green metallic sheen colony	White colony w/ dark center
					
	Short rod	Short rod	Cocci	Cocci	Cocci

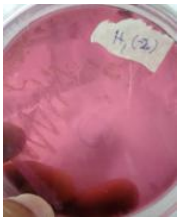
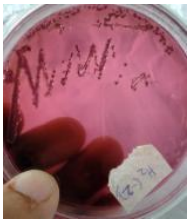
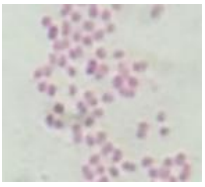
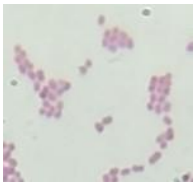
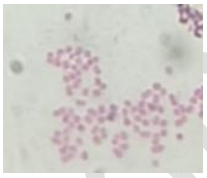
1:100	 White colony	 White colony w/ dark center	 White colony w/ dark center	 White colony w/ dark center	No plate due to negative result from brilliant green bile broth
	 Cocci	 Short rod	 Cocci	 Cocci	No slant due to negative result from brilliant green bile broth
1:1000	No plate due to negative result from brilliant green bile broth	 White colony	 Green metallic sheen colony	 White colony	No plate due to negative result from brilliant green bile broth
	No slant due to negative result from brilliant green bile broth	 Cocci	 Cocci	 Short rod	No slant due to negative result from brilliant green bile broth

Table 10 shows green metallic sheen at plate 4 (10^{-1}dilution) with cocci shape bacteria. The same green metallic sheen on plate 3 (10^{-3} dilution) with cocci bacteria.

CONCLUSION

The two (2) different sources of buko juice with milk in Sogod, Southern Leyte had pH reading of 6.3 from source A and pH=6.2 from source B. The ^{o}Brix were 10.8 and 11.0 for source A & B, respectively. Further, the aerobic plate count (APC) were 16,000 CFU/mL (source A) and 22,000 CFU/mL (source B). The coliform analysis revealed the presence of coliform bacteria. Hence, these products are unsafe for food consumption.

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LITERATURE CITED

i. **Athukorale**, DP (2008). Tender Coconut Water- its Health Benefits In: Experts' Findings on the Health Benefits of Coconut Water. Cocoinfo International. Vol 19 (2): 63-64 pp.

ii. **DebMandal M and S Mandal** (2011). Coconut (*Cocos nucifera L*): In health promotion and disease prevention. Asian Pacific Journal of Tropical Medicine. Science Direct, Elsevier. 241-247 pp.

iii. **FDA-Philippines** (2013). Revised Guidelines for the Assessment of microbiological Quality of Processed Foods.

iv. **Fife, B** (n.d). Coconut Water for Health and Healing. Healthy Ways Newsletter In: Experts' Findings on the Health Benefits of Coconut Water. Cocoinfo International. Vol 19 (2): 63-64 pp.

v. **FSAI** (2017). Validation of Product Shelf Life. Guidance Note.

vi. **Gotschlich EC** (2009). Gram-Negative Cocci, Pathogenic. The Rockefeller University, New York, USA. Elsevier Inc.

vii. **GULF Standards** (2000). Microbiological Criteria for Foodstuff –Part I. United Arab Emirates.

viii. **Kannangara AC, Chandrajith VGG and KKDS Ranaweera** (2018). Comparative analysis of coconut water in four different maturity stages. Journal of Pharmacognosy and Phytochemistry. 7(3): 1814-1817 pp.

ix. **Leininger DJ, Roberson JR and F Elvinger** (2001). Use of eosin methylene blue agar to differentiate Escherichia coli from other gram-negative. Journal of Vet Diagn Invest 13:273–275 pp.

x. **NSO** (2002). Census of Agriculture: Eastern Visayas. Vol 2: 17pp.

xi. **Prades A, Dornier M, Diop N and JP Pain** (2012). Coconut water uses, composition and properties: A Review. Journal of Fruits. Vol. 67: 87–107 pp. EDP Sciences and available at http://www.fruits-journal.org

xii. **Tucker, G and S. Featherstone** (2011). Essentials of Thermal Processing. Blackwell Publishing Ltd. John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK.

xiii. **US-FDA** (2001). Bacteriological Analytical Manual. Accessed at www. fda.gov.

xiv. **Singh R** (2009). Coconut Water: A Rehydrating Drink after Exercise. Cocoinfo International. Vol 16 (1): 19-20pp.

xv. **Yong JWH, Ge L, Ng YF and SN Tan** (2009). A Review: The Chemical Composition and Biological Properties of Coconut (Cocos nucifera L.) Water. Molecules. Vol 14: 5144-5164 pp.