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Combining thermization and evaporative cooling toward milk freshness preservation at the smallholder farm level

Edison J. Sempiira[1] | Dana J. Mugisa[1] | Joseph Galiwango[2] | William S. Kisaalita[1]

[1]College of Engineering, University of Georgia, Driftmier Engineering Center, Athens, Georgia
[2]Nsangi Trading Center, Smallholder Fortunes, Kampala, Uganda

Correspondence
William S. Kisaalita, College of Engineering, University of Georgia, Driftmier Engineering Center, Athens, GA 30602.
Email: williamk@engr.uga.edu

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Abstract

Thermization and low-cost evaporative cooling have been seamlessly combined (evakuuling process) to preserve milk freshness for next day market. For proper regulation, a more exact profile of thermized milk in the hands of smallholders is needed. The purpose of this paper was to establish the milk quality-profile of "evakuuled" milk. Thermization was done using temperatures between 55 and 70°C. Evakuuled milk quality-profile (total viable and psychotrophic microbial counts, fat, protein, and pH) was compared to chilled and fresh milk. A consumer sensory test was also performed to evaluate consumer preference. The results showed that the quality-profile of milk thermized at 65°C, followed by evaporative cooling (evakuuled) was indistinguishable from chilled and fresh milk. A consumer preference test scored evakuuled, fresh and chilled milks on average equal in terms of taste, appearance, aroma, and general acceptability.

Practical applications: The finding of evakuuled milk having the same quality attributes as fresh or chilled milk and meeting consumer preference in terms of aroma, taste appearance and general acceptability has potential practical significance. These results provide evidence in support of inclusion of evakuuled milk in the East African raw milk standard.

1 | INTRODUCTION

The majority of smallholder dairy farmers in sub-Saharan Africa do not have access to grid electricity to keep their milk fresh. Current commercialized alternative solutions for on-farm milk freshness preservation developed for rural smallholder dairy farmers include Solar ISAAC (Erickson, 2009), Promethean rapid milk chiller (Kulkarni & Ganesh, 2013), Sun Danzer solar refrigerator, Cool Churn (Ndyabawe & Kisaalita, 2014), and the Hohenhein solar cooling system (Salvatierra Rojas, Torres Toledo, Mrabet, & Müller, 2018). These solutions have been found to be costly and, in some cases, limited in capacity in the context of sub-Saharan smallholder farmers (Sempiira, Mugisa, Galiwango, Katimbo, & Kisaalita, 2020). These available solutions have therefore not been widely adopted by smallholders. This explains why even with these solutions on the market, smallholder farmers still lose a substantial amount of milk due to spoilage, because they cannot preserve their milk freshness at the farm. For example, In Uganda, these milk losses have been approximated to 27% at the farm level, translating to $23 million per year (Kisaalita, Katimbo, Sempiira, & Mugisa, 2018). A system that combines two micro-biostatic steps of thermization and evaporative cooling as a solution has been developed (Kisaalita et al., 2018). Thermization works by reducing the population of spoilage microorganisms (Chouliara, Georgogianni, Kanellopoulou, & Kontominas, 2010; Walstra, Geurts, Noomen, Jellema, & Boekel, 2004) and evaporative cooling minimizes microbial growth, increasing the shelf life of raw milk (Wayua, Okoth, & Wangoh, 2012).

Thermization refers to the mild heat treatment at temperatures in the range of 57–68°C with holding times of 15–30 s. Thermization is used in commercial dairy processing to prolong the quality of raw milk for an extra 24–72 hr after receipt at processing plants (Walstra et al., 2004). This process results in the reduction of bacteria (psychotrophs) in milk (Chouliara et al., 2010; Walstra et al., 2004). Evaporative cooling occurs naturally when warm dry air is blown through a wetted porous medium, lowering the air temperature and increasing the relative humidity. Evaporative coolers will only provide a maximum drop in temperature of about 13°C below room temperature on a hot dry day (Manuwa & Odey, 2012; Ndukwu, Manuwa, Olukunle, & Oluwalana, 2013). In Uganda, the temperature on a hot dry day can be as high as 32 and on average 29°C. Smallholder farmers in Uganda live in rural places (off-grid electricity) and milk twice a day, both in the morning and evening. The morning milk usually gets to the market or collection centers; the challenge is with the preservation of the evening milk- amidst lack of affordable means to preserve on the farm. Application of evaporative cooling for milk preservation has been demonstrated with camel milk (Wayua et al., 2012). While evaporative cooling will not reduce bacteria count, storage of milk below room temperature will reduce the rate of bacteria growth compared to when stored at room temperature (Wayua et al., 2012). Although evaporative cooling provided promising results, milk preserved with evaporative cooling alone may not achieve the desired attributes for milk to enter the cold chain.

Milk that comes immediately from the cow contains a few bacteria but can be easily contaminated through handling. Psychotrophic bacteria (pseudomonas species) represent a substantial percentage of bacteria (65–70%) in raw milk (Ledenbach & Marshall, 2009). Studies have correlated the presence of psychotrophic bacteria in pasteurized milk to the levels of lipids and protein (Lu et al., 2013); therefore, psychotrophic bacteria are used in the determination of the shelf life of milk. Psychotrophs produce lipase and protease enzymes, which break down the lipids and proteins and reduce their concentration levels in the milk. The by-products of these hydrolysis reactions increase the milk acidity and directly correlate to milk spoilage (Lu et al., 2013). Most forms of bacteria can thrive at the pH of 6. At low pH levels, lactic acid bacteria can grow rapidly and produce lactic acid, which further lower the milk pH resulting in spoilage (Lu et al., 2013).

The combination of thermization and evaporative cooling have been packaged in a kit branded as "EvaKuula". With the EvaKuula, thermization can be performed in a wooden drum in the hands of the smallholder farmer without sophisticated controls. At the time of writing, EvaKuula has been deployed in Uganda, and according to Kisaalita et al., 2018, all evakuuled milk tested with resazurin assay was of good quality and successfully entered the cold chain. However, the resulting fat/protein content and microbiological quality parameters of milk were not determined. Also, the evakuuled milk consumer preference among the target consumer needs documentation.

The Food and Drug Administration (FDA) of the United States does not recognize thermized milk in the Grade-A Pasteurization Milk ordinance (PMO) as a grade of milk because milk in the United States is not allowed to be sold in the raw state, which is similar is to the case in European Union countries. However, thermization is practiced in commercial processing for extended raw milk storage and cheese processing (Walstra et al., 2004). The FDA and European Union have not defined the quality standards for thermized milk. Similarly, the East African standard (EAS) does not define thermized milk as a grade of milk even when the regulators allow retailing of milk in the raw state. In order to be properly regulated, a more exact profile of thermized milk in the hands of smallholders is necessary. Therefore, the purpose of this study was to characterize "evakuuled" milk with respect to bio-chemical properties and microbial counts in the context of the East African dairy regulatory space. We use the term "evakuuling" or "evakuuled" to refer to milk whose freshness has been preserved by thermization followed by evaporative cooling.

2 | METHODOLOGY

2.1 | Thermization temperature

Milk sample preparation followed a schematic shown in the Figure 1. Thermization of milk was done following the procedure by Kisaalita et al. (2018). The 20-L milk can used was equipped with a stainless-steel thermal couple inserted through the top cover to monitor the temperature of the milk during thermization. A data logger (model OMEGA HH309) was connected to the thermocouple to monitor temperature during the process. Thermization temperatures in the recommended range of 57–68°C were used. The specific temperature values used were; 55°C (two degrees below range), lower quartile temperature value (59°C, average temperature value (63°C), upper quartile temperature value (65°C), and 70°C (2°C above the range). The 55 and 70°C temperature values outside the range were added to better understand outcomes if a farmer under- or over-thermized the milk. Once the required temperature was attained, milk was immediately removed from the thermization drum and taken to an evaporative cooler until the next day. Milk was removed from the evaporative cooler after approximately 12 hr (between 6:00 and 8:00 a.m.) the following morning. A sample (100 ml) was immediately collected (Figure 1)

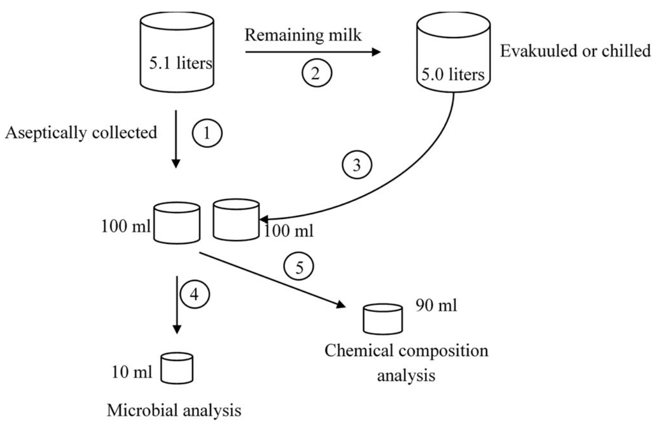

FIGURE 1 Sample preparation for both microbial and fat and protein composition analysis. 5.1 L of fresh milk were collected immediately after milking. One-hundred milliliters were aseptically taken off and subdivided into 10 ml and 90 ml for both microbial and fat and protein composition analysis respectively. The remaining milk 5.0 L were preserved by EvaKuula or chilling depending on the experiment and subjected to the same process. The 5 L was used because it is the minimum design volume for the EvaKuula Unit

aseptically, and analyzed for microbial, fat, and protein composition. Microbial analyses included determination of psychotrophic bacteria counts (PBC) and total viable counts (TVC). The counts were measured as colony forming units per ml (CFU/ml). Results of microbial and fat and protein were analyzed following ANOVA and post-hoc procedures using JMP software to compare quality of evakuuled to that of chilled, and fresh milk. Chilled milk was prepared by storage in a solar energy-powered refrigerator that was set at a temperature of 4 ± 1°C.

2.2 | Microbiological analysis

Milk samples (10 ml) were aseptically pipetted into 100 ml sterile dilution bottle and further diluted. Serial dilutions of 10^0, 10^{-1}, 10^{-2}, and 10^{-3} were prepared using sterile phosphate buffer. Plating and enumeration of the total viable counts and psychotropic bacteria in the sample was performed following the procedures that were established by Wehr and Frank (2004). For PBC, the plates were incubated in a solar refrigerator set at 7 ± 2°C for 10 days and for TVC, the plates were incubated at 32° ± 1°C for 48 hr in an isotherm forced conventional laboratory incubator model OIFA-32L. Colonies were counted using a colony counter model KUNHEWUHUA X-1253.

2.3 | Fat and protein analysis

Milk fat and protein were analyzed using a Milktech Ultrasound milk analyzer model MAP250. Additional parameters reported by the analysis included: lactose, solid-non-fat, solids, density, and freezing point depression. Milk pH was measured with a digital glass electrode pH meter (Just Utile, model COMINHKPR137640). The instrument was first calibrated using buffers of pH 6.86 and 4.01. The pH of the samples was measured by dipping the electrode of the pH meter into portions of milk samples in a beaker. Between samples, the electrode was rinsed with deionized water and wiped with tissue paper.

2.4 | Sensory evaluation

Consumer preference tests were administered in the School of Food Science Sensory Testing Laboratory at Makerere University, Kampala, Uganda. A total of 35 undergraduate students were recruited in the study. In order to prepare milk samples for testing, 5 L of milk samples were obtained in the evening within 5 min of milking. Milk was divided into two equal portions. One portion was evakuuled (thermized and stored in the evaporative cooler) and the other portion was kept in a refrigerator set at 5°C. A fresh milk sample (about 3 L) was also obtained in the morning.

The three samples (evakuuled, chilled, and fresh milk) were prepared in the morning by laboratory pasteurization at 63°C for 30 min followed by cooling to about 10°C. The three samples were then served in individual cups in quantities of 25 ml at chilled temperature of between 12 and 16°C. All milk handling was done with aluminum milk cans that were cleaned and dried under the sun, to imitate the process of milk handling within the cattle keeping communities. Mineral water at room temperature was provided for palate cleansing in between samples. Throughout the testing session, samples were randomly allocated to participants. An incentive (food) was given to the participants for their time at the end of the testing session. Participants scored the milk samples on a 9-point hedonic scale (9-like extremely to 1-dislike extremely) for four attributes: aroma, taste, appearance and general acceptability. A score above five was considered "liked." Participants were also asked to rank the samples based on order of preference as either 1, 2, or 3. Data was analyzed with Wilcoxon/Kruskal-Wallis Tests using JMP software at the 5% level of significance.

3 | RESULTS AND DISCUSSION

3.1 | Thermization temperature range

Table 1 presents results for PBC composition of fresh and evakuuled milk. Milk thermized at 55°C, 59°C, 63°C, 65°C, and 70°C, followed by evaporative cooling, had values of 140.0, 17.0, 3.2, 3.2, and 0.83 thousand CFU/ml, respectively. The Food and Drug Administration (FDA) and East African Community (EAC) standard do not define the quality standards for raw milk in terms of number of PBC. However, the European Union standard defines high quality (meeting grade 1 classification) raw milk to have a PBC counts lower than 5 thousand CFU/ml with 20 thousand CFU/ml being the acceptable maximum (Samaržija, Zamberlin, & Pogačić, 2012). Therefore, milk preserved by evaporative cooling after thermization at temperatures of 59°C, 63°C, 65°C, and 70°C is classified as high quality according to the EU regulatory standard.

Fresh milk samples were collected independently each day and thermization at each temperature was conducted using a different sample. Therefore, the initial CFU in the sample for each temperature is independent. This explains the difference in CFU for the fresh milk samples used for thermization at different temperatures. The thermization temperature of 70°C presented the lowest CFU/ml followed by 63°C, 65°C, and 59°C within the recommended range for

TABLE 1 Psychotrophic bacteria CFU for fresh and evakuuled milk

Temperature (°C)	Fresh (CFU/ml)[a]	Evakuuled (CFU/ml)[a]
55.0	880	140,000
59.0	6,200	17,000
63.0	7,500	3,200
65.0	420	3,200
70.0	29,000	830

[a]Geometric average of two samples.

thermization (57–68°C). The findings are similar to results reported by Samelis et al. (2009), who reported a maximum reduction in bacterial load at 67°C. Thermization temperatures of 63°C and 65°C presented similar counts after evaporative cooling, which is attributed to the initial high count for milk used at 63°C, compared to that at 65°C as shown in Table 1. As expected the temperature of 55°C presented the highest CFU/ml; it is outside the recommended range for thermization. At 55°C of thermization, a reasonable reduction in the PBC was not achieved and hence the high CFU/ml after evaporative cooling. From the results, the temperatures within the recommended thermization range presented PBC CFU/ml within EU standard recommended limits.

Table 2 shows the results of the fat and protein analysis. The fat content of the milk was reduced when the milk was subjected to the evakuuling process (thermization and evaporative cooling). The minimum change in milk fat on average was experienced at a temperature of 65°C whereas the maximum on average was observed at a temperature of 70°C. Statistical analysis of results indicated that there was no significant difference (p-value > .05) in the mean change in fat for temperatures of 55°C, 59°C, and 63°C. However, there was a significant difference between the change in fat at 55°C and 65°C (p-value < .05). There was also a significant difference between the change in fat at 70°C and at other temperature values (p-value < .05). For milk protein, a significant difference was observed between 55°C and other temperature values.

The change in fat and protein content can be attributed to both increase in CFU/ml in bacteria during storage and thermization temperature used. Heating of milk to temperatures of 70°C and higher affects the fat globular membrane (FGM) by denaturing the membrane proteins, leading to formation of a layer of denatured whey proteins around the fat globule (Fox, Uniacke-Lowe, O'Mahony, & Mcsweeney, 1998), which lowers the fat concentration. However, this effect is usually not an issue with milk heat processing because homogenization of milk after heat processing corrects the damage by creating an artificial layer that mainly constitutes Casein and whey proteins (Fox et al., 1998). Therefore, the EvaKuula process within the recommended range of temperature will deliver milk with desired fat and protein levels. The reduction in fat and protein after evakuuling is not significant because the milk pH that remained relatively constant for all temperature values as shown in Table 2. Thermized milk at 65°C provided the best results. As such, further fresh/chilled-evakuuled milk comparisons were performed with milk thermized at 65°C.

3.2 | Comparison of evakuuled to chilled and fresh milk

According to the East African raw cow milk standard, "EAS 67: 2000" (EAC, 2006), milk in graded based on total viable counts (TVC) as follows: grade I (less than 200 thousand CFU/ml), grade II (200–1,000, thousand CFU/ ml), and grade III (1,000–2,000 CFU/ml). Additionally, raw cow milk should contain: not less than 3.25% fat, not less than 8.5% non-fat solids, and added water—the freezing point depression should not be less than −0.525°C and not more than −0.550°C. Milk quality parameters such as fat, are affected by a number of factors, such as breed of the animal, climate and lactation period (Fox et al., 1998). Milk that used in this study came from a Frisian cow reared in tropical climate. Comparative evaluation of evakuuled, chilled, and fresh milk is shown in Table 3. On average the fresh milk had 4.43% fat, 8.90% SNF, −0.534°C freezing point, 0.0% added water and 1,800 cfu/ml of TVC. According to the EAS 67:2000, this fresh raw milk meets the grade I classification.

The fat, protein, and pH of evakuuled and chilled milk were practically identical to that of fresh milk (Table 3). There was a detection of added water in both evakuuled and chilled milk. This slight added water can be attributed to the evaporation and condensation of water vapor in the can during evakuuling and chilling, respectively; especially in the closed milk can. The authors expect that this is still part of the total percentage of water in the milk. The TVCs for Evakuuled milk were four times more than that in chilled milk but within the grade I milk specification limit of less than 200,000 counts/ml. This is as expected because of the expected growth inhibition of bacteria when milk is stored at 4°C and below. The results indicated that based on fat and TVC, milk from the EvaKuula process was similar to chilled

TABLE 2 Change in protein and fat for both fresh and evakuuled milk

		Temperature (°C)				
Parameters (mean)	**Milk type**	**55**	**59**	**63**	**65**	**70**
Fat (%)	Fresh	4.30 ± 0.15[bc]	4.41 ± 0.27[b]	4.43 ± 0.14[b]	4.28 ± 0.48[c]	4.80 ± 0.12[a]
	Evakuuled	3.95 ± 0.15[bc]	3.97 ± 0.28[b]	3.95 ± 0.09[b]	4.00 ± 0.52[c]	3.94 ± 0.10[a]
	Change[a]	0.35 ± 0.11[bc]	0.45 ± 0.06[b]	0.48 ± 0.08[b]	0.28 ± 0.05[c]	0.86 ± 0.08[a]
Protein (%)	Fresh	2.85 ± 0.17[b]	3.19 ± 0.20[a]	3.26 ± 0.16[a]	3.08 ± 0.06[a]	3.28 ± 0.13[a]
	Evakuuled	2.73 ± 0.05[b]	2.82 ± 0.08[a]	2.85 ± 0.06[a]	2.78 ± 0.02[a]	2.80 ± 0.06[a]
	Change[a]	0.12 ± 0.13[b]	0.37 ± 0.12[a]	0.41 ± 0.13[a]	0.3 ± 0.04[a]	0.48 ± 0.13[a]
pH	Fresh	6.72 ± 0.07[b]	6.66 ± 0.03[a]	6.61 ± 0.03[a]	6.61 ± 0.03[a]	6.62 ± 0.02[a]
	Evakuuled	6.86 ± 0.04[b]	6.70 ± 0.11[a]	6.76 ± 0.04[a]	6.77 ± 0.06[a]	6.75 ± 0.03[a]

Note: p-Value ≤ .0001 for fat, p-value = .0003 for protein. Values with the same letters in the same row are not significantly different.
[a]Change is the difference in parameter composition between fresh and Evakuuled milk.

TABLE 3 Milk quality parameters of fresh, evakuuled, and chilled milk

Parameters	Fresh	Evakuuled	Chilled
Fat (%)[a]	4.43 ± 0.31	4.25 ± 0.43	4.19 ± 0.39
Protein (%)[a]	3.26 ± 0.11	3.06 ± 0.06	3.07 ± 0.04
Solid non-fat (%)[a]	8.90 ± 0.030	8.34 ± 0.14	8.41 ± 0.11
Freezing point (°C)[a]	−0.534 ± 0.02	−0.516 ± 0.01	−0.518 ± 0.01
Added water[c] (%)[a]	0.0 ± 0.00	1.71 ± 0.57	1.33 ± 0.50
TVC[d] (cfu/ml)[b]	1800 (1,200, 2,600)	13,000 (6,800, 24,000)	3,500 (3,100, 4,000)

[a]Number of samples = 3.
[b]Number of samples = 2.
[c]Added water is a calculated value from the freezing point. Calculated as the percentage reduction from the freezing point base value of −0.525°C. The ultrasonic milk analyzer does not determine the added water directly.
[d]Calculated as geometric average of the cfu/ml for the two samples. The values in the brackets represent the cfu/ml for the samples tested. Samples of milk used for TVC were different from those used to measure for other quality parameters.

TABLE 4 Sensory evaluation results

	Mean score on quality attributes				
Milk type	Appearance	Aroma	Taste	Acceptability	Rank
EvaKuula	7.5 ± 1.5	6.3 ± 1.7	6.6 ± 1.7	6.9 ± 1.5	1 (10)[a]
Fresh	7.6 ± 1.1	6.5 ± 1.5	6.9 ± 1.7	6.9 ± 1.6	2 (13)[a]
Chilled	7.6 ± 1.1	6.8 ± 1.4	7.0 ± 1.5	7.2 ± 1.3	3 (10)[a]

[a]Number of participants who ranked the milk type as first preference one.

and fresh milk, and therefore can be graded as grade 1 based on the EAS.

Results of the sensory evaluation are shown in Table 4. A total of 33 participants (16 male and 17 female students) with an average age of 23 years (19–29) were recruited for the study. All the milk samples were scored above 6.5 on the hedonic scale, which means that on average all milk samples were liked by the subjects. Results from the Kruskal-Wallis statistical analysis test indicated that there was no significant difference within the samples in terms of all quality attributes (p value > .05). The chilled sample scored higher on average for appearance, aroma, and acceptability (Table 4). This is because most milk is kept cold in a refrigerator, and therefore, most of the subjects' palates were probably accustomed to chilled milk. In terms of ranking for general acceptability, on average the evakuuled ranked the best followed by fresh and chilled milk (Table 4). However, more participants (13) ranked fresh milk as first preference with evakuuled and chilled each getting 10 participants ranking them as first preference. The results of the ranking are consistent with the comments from the participants (Table 5)—participants reported that the milk samples were indistinguishable, and some reported added water to the evakuuled and chilled samples. However, some participants reported presence of solid particles in the evakuuled and chilled samples too, attributed to coagulation. This is a common trait to milk stored overnight. The participants reported evakuuled milk to be acceptable but in general, they equally like all the milk samples tested.

TABLE 5 Participants comments on milk type preference

404 (fresh milk) really has a great flavor and good mouth feel. 202 (evakuuled milk) has solids which may have biased my opinion
All the milk samples are good and pure
Appearances are not easy to tell apart
I prefer 404 (fresh milk) because it has a perfect taste, great aroma though 303 (chilled milk) and 202 (evakuuled milk) still works because people have different tastes
All the milk samples are of very good in quality, but I have issues with the aroma. The samples are lacking aroma
The milk looks watery. 303 (chilled milk) has solid particles. It has no aroma and tastes plain
Sample 202 (evakuuled milk) is super cool
Sample 202 (evakuuled milk) tastes better
Sample 404 (fresh milk) is the best because the aroma is not too much like that immediately from the cow
Product is so good, most like samples 202 (evakuuled milk) and 404 (fresh milk) are acceptable
202 (evakuuled milk) taste very dilute maybe needs less dilution the rest. 404 (fresh milk) tastes better
Sample 404 (fresh milk) and 303 (chilled milk) have the same taste and same aroma but different appearance
They are all slightly different but 303 (chilled milk) outstands them all
Sample 303 (chilled milk) is the best due to its aroma and after taste properties

Note: Code: 202—EvaKuula, 303—Chilled, and 404—freshly milked.

4 | CONCLUDING REMARKS

According to the East African raw cow milk standard, the EvaKuula process of thermization followed by evaporative cooling (evakuuling) yields milk that meets the highest (grade I) classification. Therefore, the thermization process can be seamlessly combined with evaporative cooling in the hands of the smallholder farmers who are typically low literacy. This is because any temperature within the recommended range delivers fat/protein and total viable counts that are within acceptable limits of the EAS standard for grade I milk quality. Evakuuled milk had the same quality attributes as fresh or chilled milk and satisfied consumer preference in terms of aroma, taste appearance and general acceptability. Therefore, the evakuuled milk was not distinguishable from milk preserved by the standard chilling methods. Therefore, the results support inclusion of evakuuled milk in the EAS standard as a form of raw milk.

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ORCID

William S. Kisaalita https://orcid.org/0000-0001-6999-8276

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