

The Effect of Olea afrikana and Cassia didymobotrya and Aging Period, on the Level of Heavy Metal Residues in Fermented Milk A Case of Kabianga Division

Wangila Tsikhungu Phanice[1] Tsimbiri Pamella[2] Peter Nyongesa[3]
1.University of Kabianga, School of Science and Technology, P.O box 2030, Kericho, Kenya
2.Pamella Tsimbiri, school of Health Sciences, Egerton University, P.O box 536, Njoro, Kenya
3.Peter Nyongesa, Ministry of Labour, Occupational Health and Safety, P.O box 12242, Nakuru, Kenya
tsikhungu@yahoo.com

Abstract
The harvesting of indigenous herbs and plants materials for use in form of ash as food additives by different communities dates back to 6000 BC. The present study gives findings on the aging period and use of ash from two types of herbs, *Cassia didymobotrya (senetwet)* and *OleaAfrikana (emitiot)* on the levels of sodium and heavy metals lead, chromium, iron and cadmium in fermented milk. Samples were purposely collected from selected households within Kabianga division,of Kericho County. The samples were prepared and analysed as per the AOAC (1996) methods. Lead, chromium, iron and cadmium were determined using an atomic absorption spectrometer (AAS) while a flame emission photometer was used in the determination of sodium. From a survey carried, 52.6% of the sampled respondents were male and 47.1% female of which 95% take fermented milk and 90.4% of the respondents take fresh milk, with 19.5% of this taking fresh milk with herbs. Of those who take fermented milk, 28.4% take fermented milk without herbs. The average pH of the milk during processing decreased from 6.67 to 4.14 from 0 day to 6th day for *Cassia didymobotrya* (senetwet) ash and 6.61 to 4.37 for *Olea afrikana* (emitiot) herb. Fermented milk with *Cassia didymobotrya* (senetwet)ash recorded a higher pH of 4.65 than 3.96 of *Olea afrikana* (emitiot). Showing that the later is generally better for acidity reduction. Cd, Cr, Pb, Fe and Zn were detected in all milk samples at the initial stage of 0 days, in the order of Pb>Zn>Cr>Fe>Cd (3.75, 1.25, 0.30, 0.168 and 0.03 ppm respectively). Addition of 5 g of course *Cassia didymobotrya* (senetwet) ash decreased the concentration of Pb, Fe and Zn while Cd remained constant. On the other hand, addition of 5 g of course *Olea afrikana* (emitiot) ash decreased the concentration of Fe and Zn while Cd was unaffected and Pb and Cr being undetected. *Olea afrikana* (emitiot) herb is better for removal of lead which is toxic to human body. However, for the retention of Fe and Zn, *Olea afrikana* (emitiot) herb is preferable. Comparatively, grinding ash increases the surface area for both *Olea afrikana* (emitiot) and *Cassia didymobotrya* (senetwet) which further reduces the concentration of the metals Cd, Cr, Fe, Zn, and Pb in fermented milk. Neither *Olea afrikana* (emitiot) nor *Cassia didymobotrya* (senetwet) retained Pb and Cr during the fermentation process. In addition, *Olea afrikana* (emitiot) retained upto 100 % zinc compared to *Cassia didymobotrya* (senetwet) that retained only 60 % of zinc. For reduction of toxicity from Pb, Cr and Cd, *Olea afrikana* (emitiot) herb is preferred.
Keywords: Indigenous herbs, *Cassia didymobotrya, Olea afrikana,* Lead, chromium, iron and cadmium

1. Introduction

Traditional fermented milk, *Mursik* is a basic product of the Kalenjin community of south rift Kenya. It is made from curdled dairy products with plant material additives in form of ash and cooked in specially made gourds. The herbs have been judged suitable for the purpose of imparting the preservative and aromatic effect to milk. Some of the plant herbs used by Kalenjins in Kericho County include *sertwet* (*Acacia meansii*), *simotwet (Ficus thoningii) , suriat (Rhus natalensis), muokiot (Lippia kituiensis*, *OleaAfrikana (emitiot)* with a majority preferring to use *Cassia didymobotrya (senetwet)*, mostly citing its ease of availability. Due to the daily consumption of this herb-based fermented milk, any contamination even in low level could create health risks to the consumers.

It seems to prepare quality mursik requires knowledge of properly preparing the gourd, herb slection and ashing and a master of the processing technique. Preparation of fermented milk entails preparation of a gourd from its raw form to a dried well cleaned one, ashing with a selected herb, cooling after ashing, filling the gourd with cooled boiled milk either to fullness or by portions depending on the availability of the milk, corking and setting it in a cool dry place for fermentation. Because of the many causes of unusual milk flavor, including the effect of plain gourd walls (which give a bittersweet, rotten like tang) and the plainness of white ripened milk, the role of ash additive is paramount. While these communities seem to master the processes involved in fermentation of milk, little is known about the toxicity it. Numerous factors affect the outcome of the fermented milk product, including the chemical composition of the milk, additives, processing of the product and individual breed, animal, and stage of lactation, age, and health status of animal, feeds, milking utensils and equipment and environmental conditions. These factors tend to affect the ultimate flavor, texture and consistency of the final product as well as its composition. High hygiene standards must be observed to avoid potential food poisoning that could result from exposing the milk to harmful bacteria. Contamination of milk may be due to additives, equipment used, wash water, air contamination, packing materials and personnel. Food-borne illnesses come in due to food contamination with microorganisms and/or their toxin. However, health hazard from contamination of foods with other toxins like heavy metals could also create acute poisoning as well as long-term health problems. In addition to being a nutritious food, milk can provide a favorable environment for the growth of microorganisms like yeast, moulds and a broad spectrum of bacteria, particularly at temperatures above 16^{o}C. Adverse health effects of heavy metals contamination in food has been reported (Wu Leung and Butrum, 1972; Conti et al, 2000; Saracoglu et al., 2004; Das, 1990). Cadmium, mercury and chromium have been reported as contaminants, following consumption of contaminated foods. International Agency for Research and Cancer classified lead and cadmium as human carcinogen (IARC, 1993; Steenland and Boffetta, 2000). The effect of the aging period and herb additives are factors investigated in this study.

2. Methodology

2.1 Study area

The study was carried out between October 2011 and May 2012 and between the month of February and April, 2013 in Kabianga division, Kericho County. Target group was mainly females between the ages of 25years to 65 years. Snow balling and purposive sampling methods with simple randomization design were used in the sampling of the respondents as well as sample collection.

2.2 Sample Collection and Preparation

Gourds were prepared by thoroughly washing with tap water and rinsed with distilled water. They were filled with distilled water and left overnight. The water was emptied and further rinsing using distilled water done. This process was repeated for seven days. The gourds were then sun dried for two days. Nine gourds of 2 litre volumes were used, one for milk without any treatment as a control, while 8 gourds had 5 and 10 g of fine and course ashes of *Cassia didymobotrya* (senetwet) and *Olea afrikana* (emitiot) respectively. All the gourds were filled with boiled, cooled milk to the brim and corked. The gourds were kept in a cool dry place for fermentation process.

The *Cassia didymobotrya* (senetwet) and *Olea afrikana* (emitiot) herbs approximately one year old were deliberately sampled in the areas near the University of Kabianga. The *Olea afrikana* (emitiot) and *Cassia didymobotrya* (senetwet) were purposely collected from Kabianga village between 5th to 17th February. Several stems were cut using a stainless steel machete and carried in polythene papers to the laboratory for further preparation and analysis. The herbs were washed and rinsed with distilled water. The herbs were then dried for two weeks under a shade. The stems were burnt to obtain pieces of charcoal which were ground using a pestle and mortar into ash. The course ash was divided into two equal portions. The first portion was further ground and sieved using sethi standard test sieve of 150 microns to obtain fine ash. The two types of ash were labelled as coarse (C.A) and fine ash (F.A) and kept in plastic bottles for use. About 3 liters of fresh milk samples was collected from Ayrshire cows from selected homesteads in Kabianga division, Kabianga village, into plastic bottles (500 ml each) and transported at 4 ^{o}C to the laboratory. Each of the samples was filtered using a sieve followed by use of Whatman No. 40 filter papers to remove any solid particles. The samples were boiled in aluminium utensils using a hot plate model SB 162. The boiled samples were cooled to room temperature (25˚C) before being fermented in prepared gourds.

3. Sample analysis

Analytical grade nitric acid (Analytical Rasayan, Nitric acid (1.42) AR, %purity 69.0-71.0, specific gravity 1.42 g/cm^3) was used in digesting the samples. Distilled water was used in rinsing apparatus and diluting digested samples. Dried plant materials were digested using the wet digestion method with 1:1 HCl: HNO_3 for 45 minutes at 150˚C, to obtain a clear solution, filtered using Whatman No. 40 filter into respective 50ml volumetric flasks and made up to the mark using distilled water. Standard solutions of metals (Cd, Cr, Fe and Pb) were used to obtain calibration curves. Milk samples were analyzed for PH using a pH meter model HI 2211/ORP before every sample digestion. Samples were analysed as per the AOAC (1996) methods for the presence of selected heavy metals. The milk samples were analyzed after 0, 2, 4 and 6 days respectively after shaking to obtain a uniform mixture. Milk samples were analyzed for the concentration of heavy metals Cr, Cd, Fe, Zn and Pb using a Thermo Jarrell Ash S11 atomic absorption spectrophotometer while a flame emission photometer model was used in the determination of sodium.

4. Results and Discussion

Fermented milk in South Rift region is prepared using various types of herbs. In Kericho region, 95% of the respondents interviewed take fermented milk treated with *Cassia didymobotrya (senetwet).* Some of the findings indicated that procedures used were well articulated by knowledgeable old women of over 60 years in Kericho. The communities value their product and have a willingness to improve on the methodologies for value addition. In addition, it was noted that pasteurization of milk, adsorption of particulates; flavoring, coloring are some of the relevant procedures practiced by these people.

4.1 pH

The PH of the nine samples from each gourd were measured at a temperature of 18.8˚C before digestion and found to be as table 1 below. The fine ash samples were black while the course ash samples were greyish. These colours persisted from the first day to the last day

From the results, the pH of milk during the fermentation process is independent of surface area of ash additive, amount and herb used. However, the pH value decreases as the increase in number of days of fermentation. However the amount of ash used insignificantly affects the pH of the milk. The milk tends to be more acidic implying its dependence on aging period. It was observed that addition of ash additive to all samples either in course or fine form reduced the acidity of the milk as compared with the control sample. Fine ash is better in reduction of acidity than course ash. Course *Cassia didymobotrya* (senetwet) ash recorded a higher acidic value than *Olea afrikana* (emitiot) ash, implying that *Olea afrikana* (emitiot) is generally better for acidity reduction than *Cassia didymobotrya* (senetwet) with a pH value of 4.65 while *Cassia didymobotrya* (senetwet) was 3.96 when 10 g of each of its fine ash used. On average the pH values from 0 day to 6th day decreased from 6.67 to 4.14 for *Cassia didymobotrya* (senetwet) ash and 6.61 to 4.37 for *Olea afrikana* (emitiot) herb.

4.2 Heavy metals

The conditions for analysis of heavy metals were Cadmium (Cd) Lamp current:3.0 , Wavelength:228.8 , Bandpass:1.0 , Flame description: Air acetylene oxidizing, Fuel lean, blue; Lead (Pb) Lamp current: 5.0, wavelength: 217.0, band pass: 1.0, Flame description: Air acetylene , Fuel lean, Blue; Chromium (Cr) Lamp current: 6.0, Wavelength: 357.9, band pass: 0.5, Flame description: Acetylene, Flame rich red cone 20mm high; Iron (Fe) Lamp current: 8.0, Wavelength: 248.3, Band pass: 0.3, Flame description: Air acetylene, oxidizing, Fuel lean, Blue; Zinc (Zn) Lamp current:3.0,Wavelength:213.9 , Band pass:1.0, Flame description: Air acetylene, oxidizing, Fuel lean, Blue.

At 0 days, all metals Cd, Cr, Pb, Fe and Zn were detected, with lead being highest in the order Pb>Zn>Cr>Fe>Cd with 3.75, 1.25, 0.30, 0.168 and 0.03 ppm respectively. At 0 days, only cadmium had its concentration remaining constant while all other metals either decreased as for the case of Pb, Fe and Zn or were not detected as for Cr, on addition of 5 g of course *Cassia didymobotrya* (senetwet) ash. On the other hand, when 5 g of course *Olea afrikana* (emitiot) ash was added, only Cd remained the same, Fe and Zn decreased and Pb and Cr were undetected. With 5 g of ash additive during fermentation, appreciable amounts of heavy metals Pb and Cr can be removed from the end product while also removing Fe and Zn which are required by the human body. *Olea afrikana* (emitiot) herb is better for removal of lead which is toxic to human body. Therefore should be the most preferred for fermentation. However, for the retention of Fe and Zn, *Olea afrikana* (emitiot) herb is preferable.

At 0 days, only Pb had its concentration remaining constant, Cd increased from 0.03 ppm to 0.085 ppm while all other Fe, Cr, and Zn metals were undetected, on addition of 10 g of course *Cassia didymobotrya* (senetwet) ash. On the other hand, when 10 g of course *Olea afrikana* (emitiot) ash was added, only Pb remained the same, Cd increased from 0.03 ppm to 0.195 ppm while Fe, Cr and Zn were undetected. With 10 g of ash additive during fermentation, appreciable amounts of heavy metals Fe, Zn and Cr are removed from the end product while retaining Pb and increasing levels of Cd. This implies that less ash additive should be recommended for fermentation process to reduce cases of toxicity that comes from Pb and Cd.

Comparatively, increase in surface area by grinding ash to fine powder for both OleaAfrikana (emitiot) and *Cassia didymobotrya* (senetwet) tends to reduce the concentration of the metals Cd, Cr, Fe, Zn, and Pb in fermented milk. For 0 days, neither *Olea afrikana* (emitiot) nor *Cassia didymobotrya* (senetwet) retained Pb and Cr. Increase in surface area with 5 g of *Olea afrikana* (emitiot) removes more heavy metals from milk as shown in the above table where Cd, Pb and Cr were completely undetected when fin *Olea afrikana* (emitiot) of 5 g was used. Equally fine powder had more zinc retained compared to all cases of ash additive in the study. In addition, *Cassia didymobotrya* (senetwet) also had considerable retention of zinc when 5 g was used, of about 60 % while *Olea afrikana* (emitiot) retained upto 100 % zinc when 5 g was used.

Comparatively, *Olea afrikana* (emitiot) ashes tend to remove Cd, Pb, Zn and Cr completely since by the 6 day none of these were detected. Only 48.4 % of Fe was present in the fermented milk by the 6th day. Samples with *Cassia didymobotrya* (senetwet) herb lost Cd, Cr and Fe while 4. 8 % Zn was retained. Lead was constant after 6 days implying no loss. For reduction of toxicity from Pb, Cr and Cd, *Olea afrikana* (emitiot) herb is preferred.

The concentration of heavy metals in wash water, fresh milk and fermented milk samples were compared to establish whether the heavy metals in fermented samples was as a result of the preparation aof gourds and fresh milk samples. Results indicate that Cu metal increases in concentration when fresh milk has been put in gourds washed with the water samples, from 0.12 ppm to 0.133 ppm. On the other hand high Pb levels in fresh milk seem to be independent of the Pb levels in wash water. Cr is quite high in fermented milk despite low amounts in wash water and fresh milk, implying that the herbs may be imparting it in the milk during preparation. Hg levels decreases with the process, from wash water to fresh milk to the fermented sample, 0.0046 ppm 0.0014 to 0 ppm, meaning the fermentation process decreases its presence in fermented milk. Cd also decreases in concentration on processing, however the effect wash water has on its concentration in fermented milk is minimal, figure 1 below.

5. Conclusion

Olea afrikana (emitiot) herb is most suitable for reduction of acidity and toxic heavy metals such as Cd, Cr and Pb in traditionally fermented milk. *Olea afrikana* (emitiot) herb is better for removal of lead which is toxic to human body whilst retaining Fe and Zn,. Less ash additive with increased surface area should be recommended in fermentation process of milk to reduce cases of toxicity that comes from Pb and Cd. Equally fine powder had more zinc retained compared to all cases of ash additive in the study. In addition, *Cassia didymobotrya* (senetwet) also had considerable retention of zinc when 5 g was used, of about 60 % while *Olea afrikana* (emitiot) retained up to 100 % zinc when 5 g was used. The results of the study indicated that *Olea afrikana* (emitiot) herb did not retain heavy metals. Therefore being recommended especially for the removal of the toxic heavy metals (Pb, Cr) while retaining essential metals such as Zn and reduction of acidity in the fermented milk. Small amounts of the ashes should be used in the preparation of mursik as the large amounts contribute to high retention of the heavy metals. Equally fermentation should not exceed 4 days as longer period increases acidity that affects the retention of iron in the milk. Although there were records of presence of harmful trace elements like Cd and Pd in mursik as indicated by the study, the concentrations were low and therefore this result suggests that mursik is safe as important minerals like Fe and Zn are also retained. From the results of this study mursik is safe to be produced in large scale especially using the *Olea afrikana* (emitiot) ash since it shows the least concentration of the harmful heavy metals. With this it can be introduced to the public both nationally and internationally and people who desire the food can be able to enjoy it without any fear.

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Figure1: Presence of heavy metals in water and milk samples

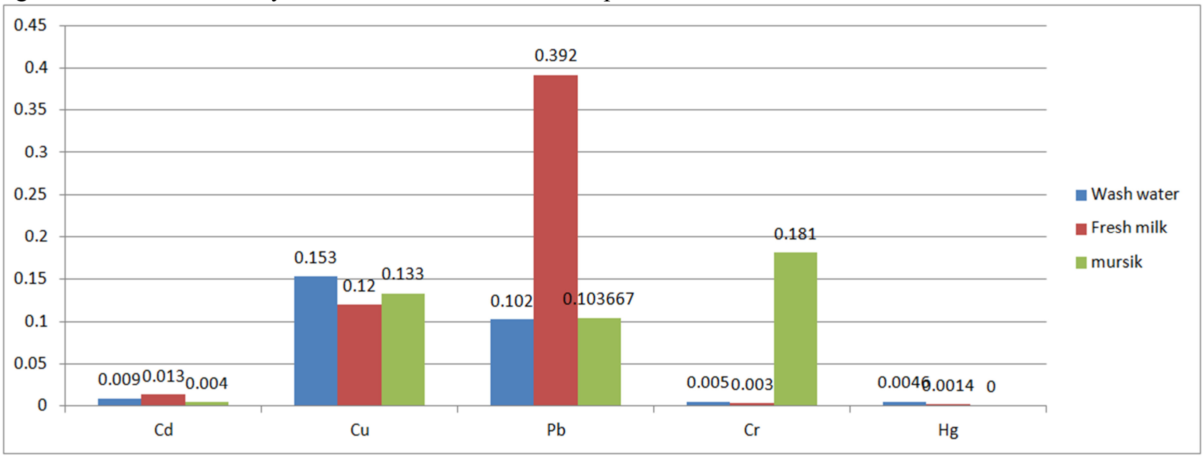

Table 1: pH of various forms of milk samples using *Olea afrikana* (emitiot) and *Cassia didymobotrya* (senetwet) herbs.

No of days	pH values								
	Control	5g C.S	5g C.E	5gF.S	5gF.E	10gC.S	10gC.E	10gF.S	10gF.E
Day 0	6.48	6.59	6.57	6.73	6.45	6.72	6.70	6.64	6.72
Day 2	6.54	4.68	4.81	5.07	4.93	4.51	4.50	4.58	4.86
Day 4	4.37	4.43	4.46	4.72	4.58	4.39	4.33	4.24	4.57
Day 6	4.26	4.24	4.22	4.34	4.39	4.04	4.24	3.96	4.65

Table 2: concentration of heavy metals with aging period and varying amounts of *Olea afrikana* (emitiot) and *Cassia didymobotrya* (senetwet) herbs.

Analyte	days	Control	5g C.S	5g C.E	5gF.S	5gF.E	10gC.S	10gC.E	10gF.S	10gF.E
Cd	Day 0	0.030	0.030	0.030	0.055	ND	0.085	0.195	0.055	ND
	Day 2	0.085	ND	ND	ND	ND	ND	ND	ND	ND
	Day 4	0.055	ND	0.085	ND	ND	ND	ND	0.140	ND
	Day 6	0.030	ND	ND	ND	0.085	0.085	ND	ND	0.170
Pb	Day 0	3.75	0.500	ND	ND	ND	3.75	3.75	ND	ND
	Day 2	2.50	2.50	ND	2.50	ND	ND	3.75	ND	ND
	Day 4	2.50	2.50	ND	ND	ND	0.500	0.500	0.500	ND
	Day 6	3.75	3.75	ND	ND	ND	ND	ND	ND	ND
Cr	Day 0	0.031	ND	ND	ND	ND	ND	ND	ND	ND
	Day 2	0.031	ND	ND	ND	ND	ND	ND	ND	ND
	Day 4	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Day 6	ND	ND	ND	ND	ND	0.019	0.019	ND	0.019
Fe	Day 0	0.168	0.015	0.015	ND	ND	ND	ND	0.015	0.065
	Day 2	1.5	ND	0.015	ND	1.5	0.015	ND	1.44	0.015
	Day 4	0.065	ND	ND	ND	ND	ND	ND	ND	ND
	Day 6	0.03	ND	0.015	0.005	0.5	ND	0.015	ND	0.005
Zn	Day 0	1.250	0.250	ND	0.750	1.254	ND	ND	0.150	0.350
	Day 2	1.150	0.300	ND	ND	ND	ND	ND	ND	ND
	Day 4	0.350	0.150	0.150	ND	ND	ND	ND	ND	ND
	Day 6	0.540	0.150	ND	0.005	ND	ND	ND	ND	0.150