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EFFECT OF PASTEURIZATION, BOILING, AND STERILIZATION ON BUFFALO MILK I. ACIDITY, PH, LACTOSE, REDUCING CAPACITY AND LIBERATION OF -SH GROUPS

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Sterilization of fresh milk significantly increases its acidity and decreases its pH while the some variations due to boiling are insignificant. Pasteurization insignificantly lowers the acidity and raises the pH of fresh milk. The loss of lactose due to pasteurization and boiling are insignificant, while the loss due to sterilization is significant. Pasteurization significantly decreases while sterilization significantly increases the reducing capacity of fresh milk. The decrease due to boiling is insignificant. The liberation of -SH groups is measurable only in boiled and sterilized samples.

The conventional heat treatments used in milk processing are pasteurization, boiling and sterilization. They differ in their effect on the physical and chemical properties of milk. Changes in the pH value, acidity, lactose and the liberation of -SH groups cause obvious changes in its physical and organolyptic properties. Moreover, the prolongation of the period of heating caused apparent changes in the reducing capacity of milk. The sulfur containing compounds are known to bear a direct relationship to the development of cooked flavor which is favoured by the local consumers. The recent tendency for the local production of sterilized milk for liquid consumption encourages studies along this line of research. With these views in mind, besides the meager work that has been reported on buffalo milk the object of this investigation was to determine and compare the changes in the acidity, pH value, lactose and reducing capacity in fresh pasteurized, boiled and sterilized milk. The detection of -SH groups due to heat treatments was also measured.

Experimental and Method of Analysis

Twenty nine morning fresh buffalo milk samples were collected from the Baragil collecting centre. The peroxidase test, Ling (1956) was applied to ensure no previous heat treatment. Each was divided into 4 equal parts. The first was pasteurized at 63°C for 30 min., the second was heated up to boiling and the third was sterilized according to the method of Patton (1952) for laboratory sterilization (20 min. at 120°C.). The fourth was untreated and used as control.

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Titratable acidities of fresh, pasteurized and boiled milk samples determined according to the Standard Methods while of sterilized milk samples was according to Higgin-bottom and Taylor (1960). The pH values were measured by using Beckman zeromatic pH meter with glass electrode (Ling 1956). Lactose was quantitatively determined by the colorimetric method of Barrnett and Abdel Tawab (1957) using standard curve. The lactose percent in milk samples was given by the following expression.

$$\text{Lactose percent} = \frac{\text{ug. lactose in 2 ml. extract}}{20}$$

The method of chapman and Mc Farlane as modified by Crowe, and Coulter (1948) was used for the determination of reducing capacity. The color intensity was read after 10 min. using the spectrophotometer at 660 mu wavelength with reagent blank set at 100 percent transmission. Standard curve was obtained for calculating the concentration of reducing capacity in fresh and heat treated samples. The procedure of Josephson and Doan (1949) for the qualitative nitroprusside test was used for detection of free sulphydryl groups in fresh and heat treated samples.

Analysis of varience was carried out and the significant differences between the mean values were calculated by Duncan's new multiple range test according to Steel and Torrie (1960). Ninty five and 99 percent levels of confidence were chosen for all tests of significance. Statistical analysis concerning the pH values were carried out on the antilog values of pH, taking in consideration that $pH = -\text{Log}\ (H^+)$.

Results and Discussion

The mean values of pH, acidity, lactose, and reducing capacity of fresh milk samples as affected by heat treatments are shown in table (1) and figures 1, 2, 3, and 4. The significance between mean values of hydrogen ion concentration, acidity, lactose, and reducing capacity in fresh and heat treated samples are presented in table (2).

TABLE 1.—THE MEAN VALUES OF pH, ACIDITY, AND LACTOSE OF FRESH, PASTEURIZED BOILED, AND STERILIZED MILK SAMPLES

Treatment	pH	acidity percent	Lactose percent	Reducing capacity g/litre
Fresh	6.576	0.162	4.7555	0.3490
Pasteurized	6.385	0.157	4.7328	0.2315
Boiled	6.532	0.164	4.7045	0.3380
Sterilized	5.993	0.279	4.6453	1.1743

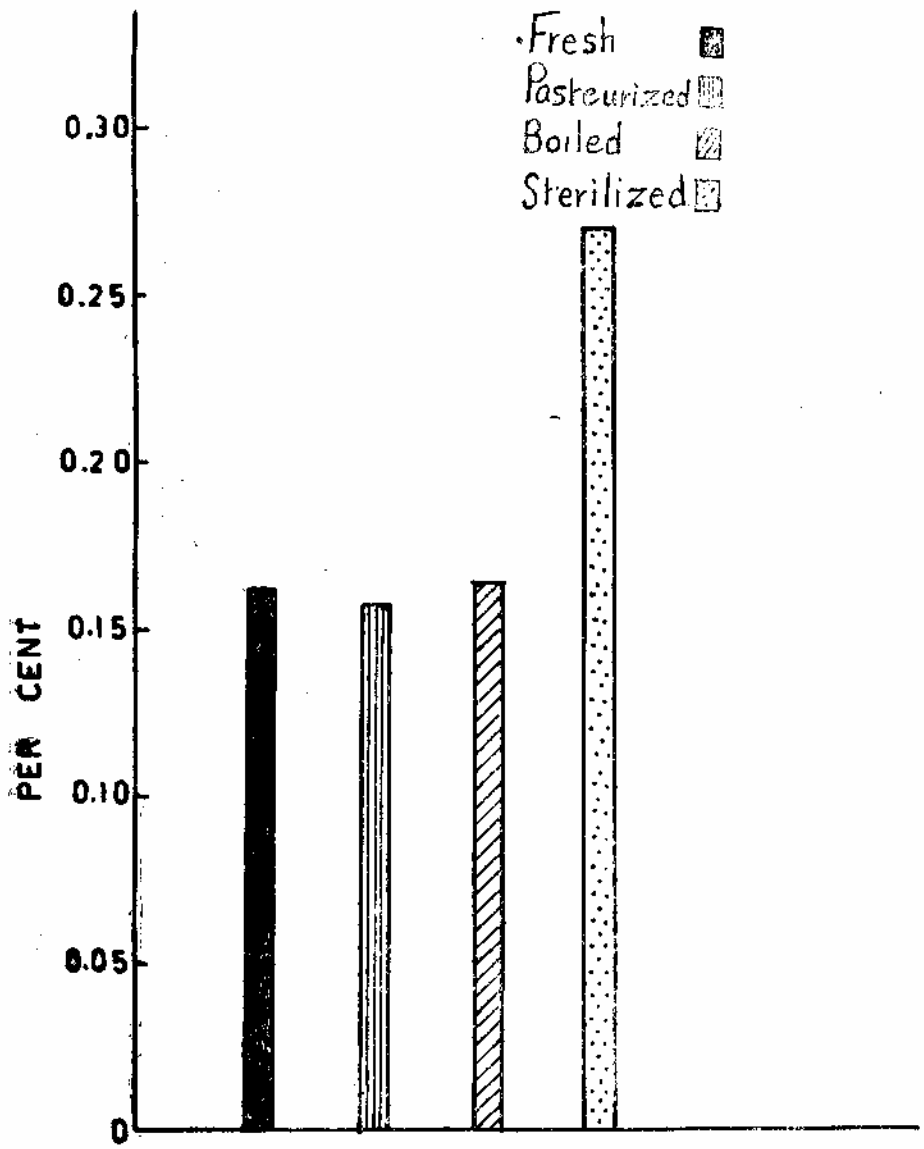

FIG. 1.—The average values for acidity per cent in fresh, pasteurized, boiled and sterilized samples.

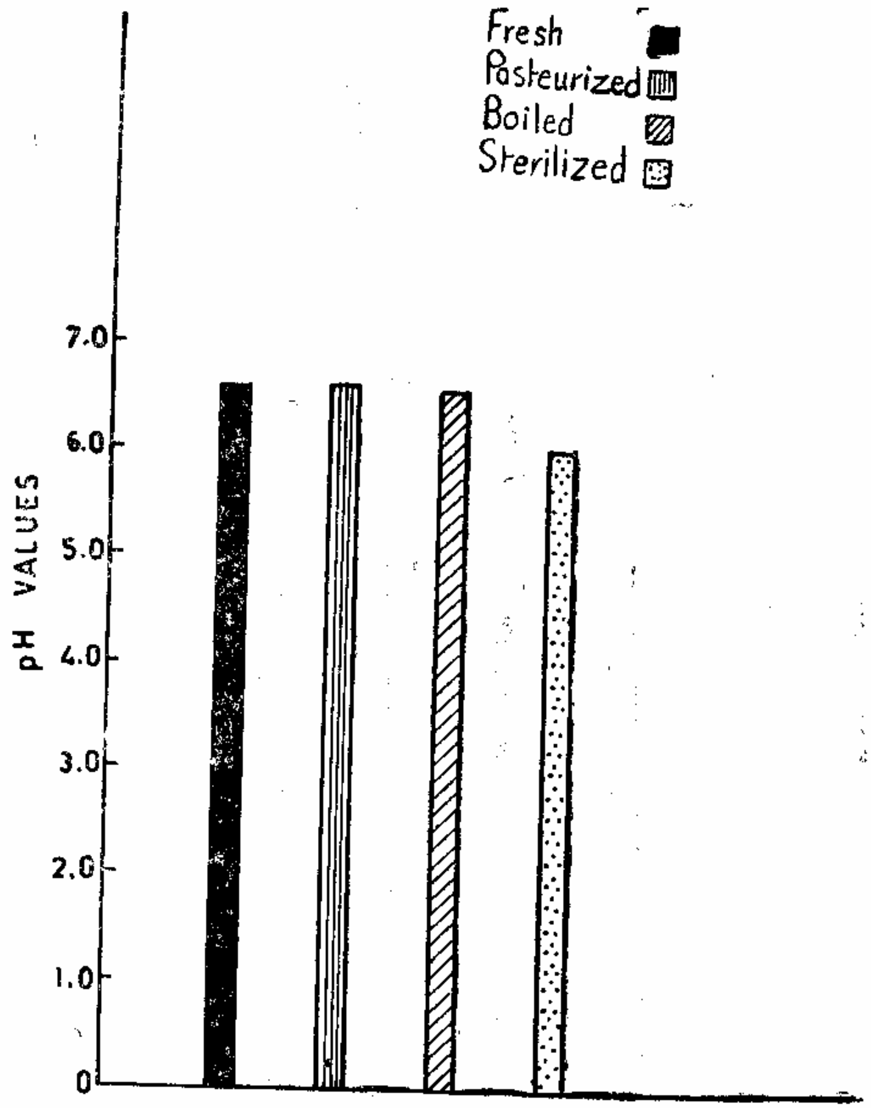

FIG. 2.—The mean values for pH in fresh, pasteurized, boiled and sterilized milk samples.

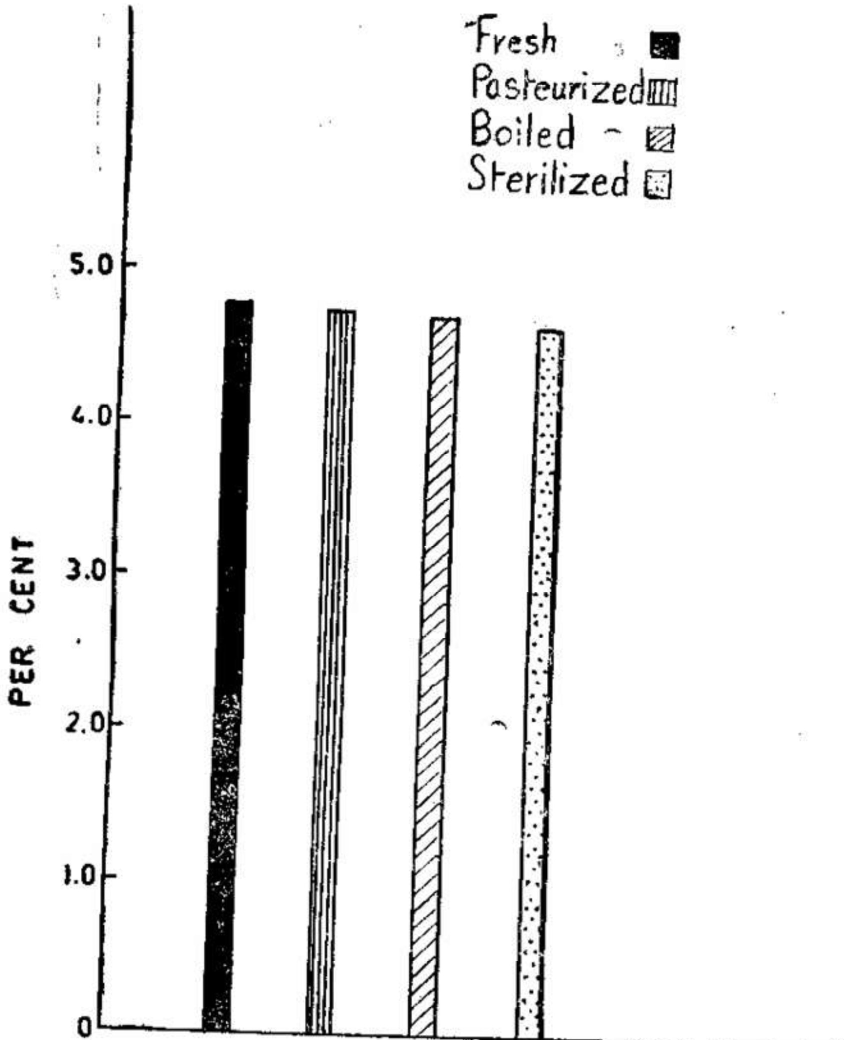

Fig.(3). The mean values for lactose per cent in fresh, pasteurized, boiled and sterilized samples.

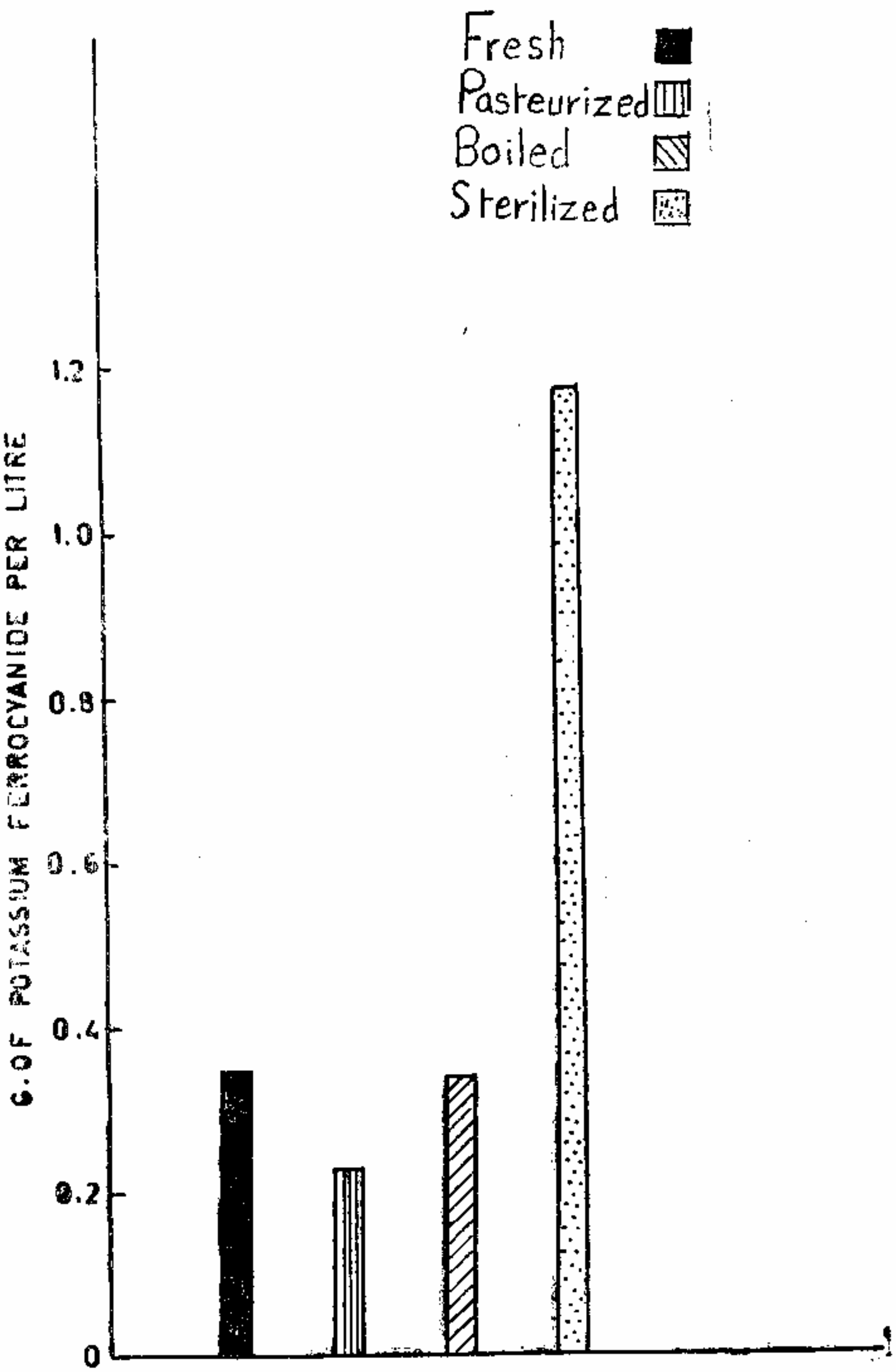

FIG. 4—The mean values of reducing capacity in fresh, pasteurized, boiled and sterilized samples

TABLE 2.—THE SIGNIFICANCE BETWEEN MEAN VALUES OF HYDROGEN-ION CONCENTRATION, ACIDITY, AND LACTOSE CONTENT, IN FRESH AND HEAT-TREATED SAMPLES.

Difference between	Hydrogen-ion concentration		Acidity		Lactose		Reducing capacity	
	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01
Fresh and sterilized	+	+	+	+	+	+	+	+
Fresh and boiled	—	—	—	—	—	—	—	—
Fresh and pasteurized	—	—	—	—	—	—	+	—
Pasteurized and sterilized	+	+	+	+	+	+	+	+
Pasteurized and boiled	—	—	—	—	—	—	+	—
boiled and sterilized	+	+	+	+	+	+	+	+

\+ = The difference is significant.

— = The difference is insignificant.

It was clear from the results that pasteurization and boiling caused a slight insignificant changes in acidity and pH. They were mostly due to the removal of CO_2 during heating of fresh milk, Hofi *et al* (1966). The significant increase of acidity or decrease in pH of sterilized milk samples were attributed to formation of organic acids such as lactic, formic acetic, propionic acid.... etc., from the heat degradation of lactose or the interaction between amino acids and lactose or lactose fragments as reported by several investigators (Grimbleby 1954, Jenness and Patton 1959, Higginbottom and Taylor 1960). Also, Kometaini (1931), attributed the high acid value to the hydrolysis of casein in which calcium was split off from calcium caseinate. Grimbleby (1954), added that the increase in acidity was due to the loss of basic amino groups of proteins by combining with lactose. Lactose content decreased gradually by raising the temperature of fresh milk namely pasteurization, boiling or sterilization respectively. This conclusion was arrived at Grimbleby (1954), and others (Andross 1940, Gould 1945, Larsen 1951, Patton and Flipse 1957). The percentage loss due to pasteurization was slight and amounted only to 0.48, boiling almost doubled the percentage of loss to 1.07 and sterilization redoubled the loss further to 2.32 percent. Similar findings were reported by Shuval (1956). Neilsen *et al* 1963) using isotope techniques attributed this loss to the combining reaction between lactose and milk proteins.

Statistical analysis of results showed that the decrease in reducing capacity due to pasteurization was significant while insignificant due to boiling. Sterilization, however, caused a significant increase in this phenomenon.

The fresh and pasteurized samples gave negative nitroprusside test while boiled and sterilized samples were positive.

It might be concluded accordingly, that the three heat treatments caused a significant effect of the reducing capacity of fresh milk. Relatively excessive heat treatment such as boiling or sterilization liberated -SH groups as reported by Hammett and Chapman (1938). The insignificant difference in reducing capacity between fresh and boiled milk did not present the true mechanism involved because of the difference in the compounds causing the reducing capacity of fresh or boiled milk as reported by Gould (1940). In accordance with the present results regarding the increase in reducing capacity as a result of sterilization, Mori (1963), Boyd, and Gould (1957) and crowe and Coulter (1948) found the same trend. Moreover, Jenness and Patton (1959), reported that the large increase in the reducing capacity of sterilized milk was considered as a result of the changes related to the browning phenomenon which involved -SH compounds as well as ascorbic acid, and other substances associated with the browning reaction.

Agreeing with the present results as for the formation of -SH groups in heated milk were the results of Lyster (1964), and Wahba (1965). Blankengle and Humbert (1963) explained that increasing the processing temperatures of milk resulted first in serum protein denaturation followed by-SH groups formation and finally the appearance of cooked flavor. Serum milk proteins were reported by many authors as the source of heat labile sulfides of milk (Harland *et al* 1949, Hutton 1953, Jenness 1954). In addition, Towenly and Gould (1943), found that other substances associated with the fat globules were also responsible for -SH liberation. Jenness and Patton (1959) fully explained the activation of -SH groups in milk and concluded that the amino acid cysteine of B-lactoglobulin was the principal site of these groups in milk serum proteins.

From the above discussion and results, the reducing capacity of milk and the formation of heat labile sulfides could be used as reliable tset to detect the previous heat treatments of fresh milk. These tests also could be considered as indices for the detection of added evaporated condensed or powder milk to pasteurized market milk. Choi *et al* (1953) detected the reconsituted dried skim milk in pasteurized milk by an increase in the ferricyanide reducing properties of the acid precipitated milk proteins.

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تأثير البسترة والغلى والتعقيم على اللبن الجاموسى
١ ـ الحموضة و pH واللاكتوز ـ القدرة الاختزالية وانطلاق مجموعات السلفاهيدريل

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الملخص

يزيد تعقيم اللبن الطازج حموضته وينقص رقم الـ pH جوهريا بينما كانت نفس التغييرات نتيجة الغليــان غير جوهرية ـ تخفض البســترة الحموضية وترفع الـ pH فى اللبن الطازج بنســبة غير جوهرية ـ كان النقص فى اللاكتوز نتيجة البسترة أو الغليان غير ذى قيمة بينما كان ذو قيمة نتيجة التعقيم .

تقلل البســترة فى حين يرفع التعقيم المقدرة الاختزالية للبن الطازج بدرجة ملحوظة .

كان النقص الناتج نتيجة الغليان فى المقدرة الاختزالية نقصا جوهريا ـ كذلك كان خروج مجموعات السلفاهيدريل واضحا فى العينات المغلية المعقمة فقط .