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EFFECT OF PASTEURIZATION, BOILING, AND STERILIZATION ON BUFFALO MILK

II.—Determination of Hydroxymethyl Furfural (HMF), Detection of Maltol and Changing in Color

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Unheated fresh milk contains free and potential HMF. The time and temperature of pasteurization; 63°C. for 30 min. are not sufficient to increase their amounts, while boiling significantly increases the potential HMF. Sterilization at 120°C. for 20 min. carries the browning reaction to completion with the production of large amount of free and potential HMF and the browning discoloration of milk. The temperature and time of pasteurization, boiling or sterilization are not enough to cause the formation of maltol. Sterilization is the only heat treatment which gives measurable change in color of milk while no effect can be observed after pasteurization or boiling.

The nature and mechanism of reactions during heating of milk are diverged and complicated. However, formation of HMF is taken as a criterion for the first step in heat degradation of lactose. Further-more, Maltol is considered as an intermediate compound formed during heating of milk (Patton 1950). Whether the temperature and duration of pasteurization or boiling cause the formation of HMF or maltol are not definitely known.

Therefore both free and potential HMF and maltol were determined in fresh, pasteurized, boiled and sterilized milk samples. The changes in color of fresh milk due to heat treatments were also estimated.

Experimental and Method of Analysis

Morning buffalo milk samples were collected and tested for previous heat treatment by the peroxidase test (Ling 1956). Each sample was divided into 4 parts. The first was pasteurized at 63°C. for 30 min., the second was heated up to boiling, the third was sterilized at 120°C. for 20 min., and the fourth was left unheated and used as a control.

The free HMF was determined spectrophotometrically by the thiobarbituric acid (TBA) method. The potential HMF was determined by digesting the sample with 0.03 N oxalic acid and then reacting with 0.05M TBA

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(Keeney and Bassett 1959). Maltol was detected chromatographically (Potter and Patton 1956) using the three following resolution systems :

a) isoamyl alcohol, conc. NH_4OH and water (6 : 3 : 1 v/v).

b) isoamyl alcohol and 5N formic acid ; (1 : 1 v/v).

c) ethyl acetate, pyridine and water ; (5 : 2 : 7, v/v).

Equal parts of 0.1 N Ag NO_3 and 0.1 N NH_3OH or 0.5 N aqueous solution of ferric chloride were used as spraying reagents for the chromatograms.

The measurements of the color of milk samples followed the procedure of Higginbottom and Taylor (1960), using a tintometer in a white cabinet.

Results and Discussion

Potential HMF measures the amount of 1-amino-1-deoxy-2-ketoses ; ADK (Hodge 1953) the precursor of HMF which will be formed later. Accordingly, the digestion of samples with oxalic acid will selectively convert the ADK to HMF.

Table (1) showed that the average values of tree HMF in fresh, Pasteurized, and boiled samples were 0.2209, 0.1951, and 0.3571 uM per litre of milk; and 0.9401, 1.5912, and 3.4750 uM per litre of milk for potential HMF respectively. On the other hand, sterilized samples had an average of 10.4490 uM per litre of free HMF and 22.8090 uM per litre of milk as potential HMF. Results indicated also that out of 22 untreated samples, 5 (about 22.7%) contained free HMF, and 14 (64%) potential HMF. The conclusion therefore, was that HMF or its precursor were found in raw milk due to reasons ought to be studied. These samples when pasteurized, their potential HMF contents highly increased while the increase in free HMF was slight. No free HMF was found in raw milk samples which were originally devoid of it, when boiled. However, the potential HMF increased noticably on boiling. On sterilization, all the samples contained high levels of free HMF and the increase in the potential HMF was great.

TABLE 1.—THE MINIMUM, MEAN AND MAXIMUM VALUES OF FREE AND POTENTIAL HMF IN FRESH AND HEAT TREATED SAMPLES.

Treatment	Minimum		Mean		Maximum	
	Free	Potent.	Free	Potent.	Free	Potent.
Fresh	0.0	0.29	0.9411	1.2968	1.2968	4.8125
Pasteurized	0.0	0.0	0.1951	1.5912	1.0530	5.6875
Boiled	0.0	0.0	0.3571	3.4750	3.8880	10.1725
Sterilized	5.2650	12.1195	10.4490	22.8090	17.4150	36.9625

The results in table 1, also indicated that boiling of milk would not free HMF and if it was present it would be in traces. On the other hand, boiling would significantly increase the amount of potential HMF formed. These compounds were progressively intensified by sterilization at 120°C. for 20 min. Agreeing with these results, Patton (1952) found that the range of 100° to 120° was critical in the browning reaction of skim milk. Patton (1950)[b] succeeded in obtaining HMF from condensed milk heated for 2.5 hours at 127°C.

Therefore, the reaction between lactose and milk proteins was most probable initiated during boiling of fresh milk to at least the stage of HMF formation, while sterilization carried the reactions to completion with the formation of melanoids. The discussion of browning discoloration of milk samples in the present investigation and of Patton's (1952) ascertained these conclusions boiling or heating for 1 hr. at 100°C. produced no appreciable discoloration whereas the browning was readily evident in sterilized samples.

The formation of maltol in the browning reaction was explained by Jenness and Patton (1959). All the chromatograms were void of spots indicating the absence of maltol in pasteurized, boiled, or sterilized milk. Accordingly, the temperature and its duration in the three heat treatments were not sufficient to form maltol. Potter and Patton (1956) reported that milk receiving limited heat treatments such as 65.6°C. for 20 min. did not contain any maltol. It seemed that maltol would be formed by more intensive heat treatment than in the present study for prolonged time namely 120° or 127°C. for 2½ hrs. as reported by Potter and Patton (1956), and Patton (1950).

Fresh, Pasteurized, and boiled samples showed no measurable color units as determined tintometrically. On the other hand, the sterilized samples gave a measurable readings in the yellow and red units with average values of 1.208 and 1.016 respectively while the mean of total units were 2.224 in the tintometer and produced brownish discoloration. The findings of Higginbattom and Taylor (1960), on autoclaved bottled milk using the same method of the estimation agreed with the present results. Moreover, the brownish discoloration was observed after heating of milk to temperature near to sterilization or higher for short or long periods by other investigators (Burton 1955), Burton and Rowland (1954), Nelson (1948). The browning of milk was attributed mainly to the interaction between milk proteins and carbohydrate contents of milk in Maillard type of reaction to yield polymers and co-polymers which would end to the formation of melanoids. This combining reaction, however was preceded by splitting of dioxyacetone which would react readily by amino acids or proteins to give the Schiff's base as explained by Krauss (1955). The latter compound by hydrolysis gives HMF which is one of the intermediates the browning phenomenon of heated milk as reported by Hodge (1953).

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تأثير البسترة والغلى والتعقيم على اللبن الجاموسى
٢ ــ تقدير هيدروكسى ميثيل فورفورا الـ HMF والكشف عن المالتول والتغير فى اللون

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الملخص

يحتوى اللبن الطازج الغير معامل حراريا على HMF حرومتكون ــ كان الوقت ودرجة الحرارة المستعملة فى البسترة البطيئة غير كاف لرفع كمياتهما بينما ادى الغلى الى رفع نسبة الـ HMF المتكون جوهريا أما التعقيم فى درجة ١٢٠ درجة مئوية لمدة ٢٠ دقيقة يؤدى الى نهاية تفاعل اللون البنى وينتج كميات كبيرة من HMF الحر والمتكون مع وجود اللون البنى فى اللبن كذلك كان الوقت ودرجة الحرارة المستعملان سواء فى البسترة أو الغلى أو التعقيم غير كافيين لتكوين مركب المالتول . كان التعقيم هو المعاملة الحرارية الوحيدة التى أعطت تغيرا ملحوظا فى لون اللبن فى حين لم يلاحظ اى تأثير على اللون نتيجة البسترة أو الغلى .