The Effect of Boiling on Milk Microbial Contents and Quality

Ahmed M.M. Metwally[1], Nadia M.A.Dabiza[*2], Wagih I.El-Kholy[2] and Zeinab I.Sadek[2]

[1]Dairy Technol.Dept. Faculty of Agric. Cairo University, Giza, Egypt
[2]Dairy Science Department, National Research Center Dokki, Giza, Egypt
nadiadabiza@yahoo.com *

Abstract: Though milk boiling is a widespread heat treatment in number of countries, the process was not thoroughly studied. In this study, the effect of boiling buffaloes´ and cow's milk samples for different periods on their microbiological contents, keeping quality and bacterial ecology contents and chemical changes were determined. Lethality rate of 6.53, 6.77, 7.301 and 7.44 in buffaloes` and 6.76, 7.059, 7.012, 7.15 and 7.159 $\log_{10}$ cfu/ml in cows´ milk were obtained on boiling the samples for 0.5, 1,2 and 5min., respectively. Boiling milk for 0.5 and 1min decreased the bacterial count from 3.6×10^9 in cow's milk into 6.3×10^2 and 3.2×10^2 and from 7.8×10^9 in buffaloes 'milk into 2.26×10^3 and 1.3×10^3 cfu/ml, respectively. On cold storage, the microbial content of boiled milk, not only did not increase but also declined on the first week. Boiling destroyed bacterial vegetative cell leaving behind spores of the sporeformer which were dominated with *B.cereus* and *Micrococcus leteus.* Boiling affected milk quality far less than the effect occurred in UHT milk as determined by O.D- value measurements.
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1. Introduction:

In developed countries, only 2% of the milk produced is consumed in its raw form, most of the milk in underdeveloped countries is consumed raw. In number of African countries as well as in Egypt large percentage of milk is retailed directly to consumers by farmers and small-scale traders including hawkers. Most of the dairy farmers are small holders with 2 or 3 animals lacking the hygienic conditions of production and veterinarian services; therefore their milk is not of best quality. In many of these countries, there is no enough processing plants to coup up with the milk produced .Moreover, the industry processes mostly UHT milk, which is expensive for most consumers, a matter that encourages them to consume retailed raw milk.

There fore they have to rely on boiling to establish milk safety and a reasonable shelf life. Not only UHT milk is expensive but also the treatment produces number of undesirable changes and products such as maillard reaction products and lactulose (Seiquer, *et al.*2010). Boiling is a common practice in many countries and WHO (World Health Organization) recommended milk boiling for African countries and described the process in its training manual (Israel-Ballard and Chantry, 2010). Boiling milk is simple process and does not need temperature gauges or timing devices which limit the use of pasteurization.

However, period of boiling treatment, the effect of boiling milk on microbial contents, keeping quality and chemical changes were not thoroughly studied. Period of boiling varies between individuals, some turns off the heat as milk boils & foams and others boil milk for 5 or 10 min., however at such high temperature, period of heating has a decisive effect on milk quality. For example, furosine production in boiling milk was increased for different period of heating from 110.4 to 116.7 and 128.9 mg/100g proteins as the period increased from zero into 5 and 10min., respectively (Sun and Wang, 2009).

Therefore this research was carried out to determine the proper boiling period which results in the required safety through a proper shelf life with minimum quality deterioration. Moreover, chemical changes occurring in boiled milk and UHT-milk were compared using a UV- absorption method.

2. Materials and methods

Fresh buffaloes´ and cow's milk samples from the herd of the faculty of Agriculture, Cairo univ., *Bacillus cereus* NRRL, B.3711, from Northern Regional research Laboratory, Ill.USA and *Listeria monocytogenes* type 1 from Hungarian National Collection of Medical Bacteria, OKI, Budapest, Hungary.

Bacillus cereus and *Listeria monocytogenes* were activated in Brain- heart infusion broth (oxoid) at 37°C for 24hr. After sufficient growth, the cultures were diluted in saline to about 7×10^6 cfu/ml measured by standard plate count (SPC).

Thermal resistance of *Bacillus cereus and Listeria monocytogenes:*

The test tube method of Donnelly and Briggs (1987) was used to determine heat resistance of both microorganisms. One tenth of ml of 24 old culture (after the come up period) was inoculated into 10 ml of sterilized cow's milk in screw- capped tubes and were heated in a thermostatically oil bath. Milk was heated at 100ºC for 0.5, 1, 5 and 10 min, and then samples were cooled in ice bath. Aerobic plate counts using plate count agar (oxoid) at 37°C for 48h.were used to determine the survivals. Rates for thermal inactivation of each bacterium were determined graphically by plotting the $\log_{10}$ cfu/ml of surviving cell population versus heating time. A line was drawn through the date points and D-values were obtained from the slope of the best lit line (El-Shenawy *et al*, 1989).

Effect of milk different heat treatments on bacterial contents:

A half liter milk samples in glass beakers were heated at 80°C, 90ºC and 100ºC for 15 second in thermostatically controlled oil bath followed by rapid cooling in ice-bath to 5ºC. In another experiment buffaloes´ and cow's samples (0ne liter) were boiled for different periods and followed by rapid cooling. Samples were tested microbiologically for viable bacteria using aerobic plate count in nutrient agar (oxoid) (37°C for 48hr) after the standard methods for the examination of dairy products. Lethality was calculated as the difference between the log of colony counts of the untreated (No.) and treated samples (N_1) ($\log_{10}$ No- $\log_{10}$ N_1) (Roig-Saguès *et al*, 2009).

Chemically, the samples were tested using a UV- method for evaluation of heat treatment of milk (Sun and Wang, 2009).

Boiled milk keeping quality:

Cow's and buffaloes´ milk of one liter samples were boiled for different periods (1.0, 2.0., 3.0 and 5.0 min) and after cooling, the samples were kept in a household refrigerator (~ 7-8°C) for 10 days. Total bacterial contents of the samples were determined after 5, 7 and 10days of cold storage using plate count agar (oxoid) at
37ºC for 48h.

Bacterial ecology of boiled milk:

Buffaloes´ milk was boiled at 100ºC for 10 min and after cooling, milk was plated on nutrient agar at 37ºC for 48hr. Colonies present were visually examined and colonies representative of each distinct morphology were counted, isolated and identified. Identification was carried out by observing colony shape and color, microscopic examination, reaction to gram stain, catalase test, and production of acid from glucose, mannitol and zylose.

3. Results and Discussion:

The effect of heating milk at different temperatures on its microbial content was determined, Fig (1).Maximum lethality values of 5.793, 6.09 and 6. 60 $\log_{10}$ cfu/ml for buffaloes´ milk and 5.85, 6.18 and 6.959 $\log_{10}$ cfu/ml for cow's milk were obtained in milk heated at 80, 90 and 100°C for 15s, respectively. The destruction effect was temperature dependent. The 80ºC resembles ultra-pasteurization treatment.

Lethality values of above 6 $\log_{10}$ cfu/ml which obtained by boiling treatment, was greater than the minimum microbial inactivation of 5 $\log_{10}$ required for pasteurization. Boiling milk for 15s reduced microbial count from 2 or 3 × 10^9 cfu/ml into few hundreds about 900 cfu/ml. The 15s of heating resembles the boiling flow-over end point used by regular consumer at home.

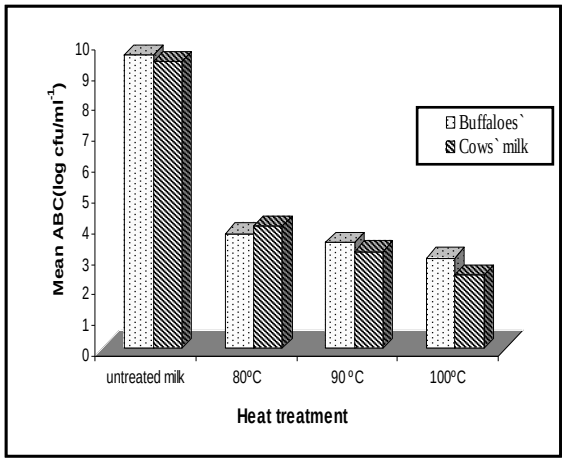


Fig (1): Change in aerobic bacteria counts (ABC) of cow's & uffaloes` milk heated at different temperatures for 15 second.

Table (1) shows the effect of increasing the period of boiling into 0.5, 1, 2, 5 and 10min.on milk microbial contents. The lethality rate increased by increasing boiling period to reach 6.76, 7.059, 7.012, 7.15 and 7.59 in cow's milk and 6.53, 6.77, 7.301, 6.76 and 7.44 $\log_{10}$ cfu/ml in buffaloes´ milk at 0.5, 1, 2, 5 and 10min., respectively. Boiling for 0.5, 1.0 and 2min reduced total bacterial count from 7.8×10^9 to 2.26×10^3, 1.3×10^3, 3.9×10^2 in buffaloes´ milk and from 3.6×10^9 to 6.3×10^2 and 3.2×10^2 to 3.5×10^2 in cows` milk. Increasing the holding period beyond the 0.5 min progressively reduced the numbers, but insignificantly. Lethality values of greater than 67 were obtained by boiling milk for 1 and 2 min for buffaloes´ and cow's milk. Boiling periods beyond

2min did not show any increase in the lethality rate. Therefore, boiling milk need not to exceed than more 2min period.

Table (1) Total bacterial count in for milk boiled for different period.

Period of boiling, min[1].	Type of milk[2]	
	Cow's	Buffaloes´
	Bacterial counts, × 10^9 cfu/ml	
Raw milk (untreated)	3.6±26.46	7.8±31.22
	Bacterial counts, × 10^3 cfu/ml	
0.5	0.63±0.03	2.26±0.26
1.0	0.32±0.05	1.3±0.13
2.0	0.35±0.07	0.39±0.04
5.0	0.25±0.06	0.28±0.03
10.0	0.25±0.07	0.29±0.04

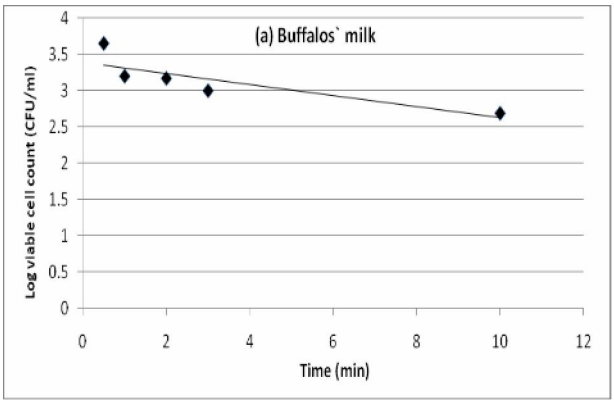
1- $LSD_{(0.05)}$ = 2.5 2- LSD (0.05) = 2

Microbial lethality at boiling temperature was higher in cow's milk than in buffaloes´ milk. This is may be due to buffaloes´ milk contains more fat and T.S than cow's milk, this high T.S reduces heat rate of exchange and the high fat content protects the microorganisms against heat. This is expected to increase bacterial heat resistance in buffaloes´ than cow's milk. Some workers (Nasr, 2008) found that a temperature of 75°C for 25 seconds was required to kill *L.monocytogenes* in buffaloes´ milk while the 72ºC/15s was enough for cows` milk. But still boiling for both milks could be for 1 or 2 min at the most.

Therefore, regular boiling practices at home regardless the period used reduces the microbial load into a level considered to be safe for human consumption, particularly
that all pathogens are also destroyed. The bacterial counts were reduced by boiling to levels significantly lower than the 2o.ooo cfu/ml limit required for grade (A) pasteurized milk in pasteurized milk ordnance (Ranieri, *et al.*, 2009).

To determine the safety of boiled milk for human consumption, the survival of *Listeria monocytogenes* and *Bacillus cereus,* two pathogens usually found in milk, through boiling was studied. The D-value of both microorganisms at 100ºC was determined. *L. momocytogenes* (7.5×10^6 cfu/ml) was completely destroyed at 100ºC for all periods starting from the first instant of boiling, therefore, its D- value could not be determined. This means that non- spore former pathogens pose no danger in boiled milk. The D-value of *B.cereus* was determined in both milks to be 7.5min & 10.4 min for cow's and buffaloes` milk, respectively (Fig 2). These results are in the agreement with that obtained by El-kholy, 1993 who reported that D-value of *L. momocytogenes* was 1.4 sec. at 70ºC. So, 100ºC is more than enough for destroying the pathogen.

This means that *B.cereus* as spore formers would tolerate the boiling treatment; however, storage cold temperature would prevent its germination and growth.



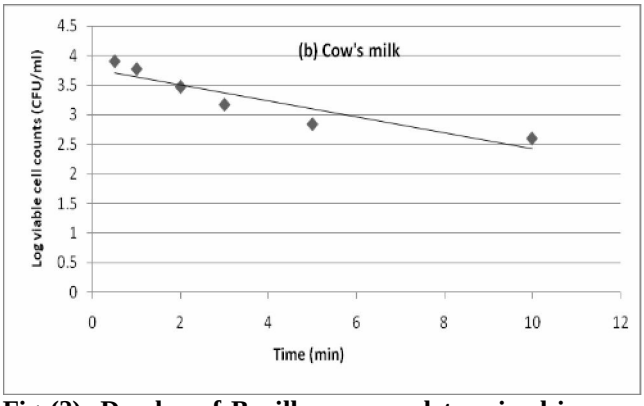


Fig (2): D-value of Bacillus cereus determined in boiled milk.

Actually boiling destroys all vegetative cells leaving behind spores of sporeformers. This was found when the ecology of boiled milk was studied; results are presented in Table (2). Of the 15 gram-positive spore forming bacteria isolated in the present

study from boiled milk for 10min, 5 isolates were *Bacillus cereus* and the rest were characterized to be of the genus Micrococcus. Five isolates were *M. leteus*, 7 isolates were *M. varians* and one was *M.roseus*.

Table (2) Bacterial isolates obtained from milk boiled for 10min.

Bacterial isolates	Number of isolates	Isolates (%)
Bacillus cereus	5	38.4
Micrococcus leteus	5	38.4
Micrococcus varians	2	15.3
Micrococcus roseus	1	7.6

Non –of these isolates were psycrotolerant endospore since after 10 days of cold storage the counts insignificantly increased as in Table(3) which presents the keeping quality of boiled milk. Milk samples boiled for 1, 2 and 5min were stored for 10 days in household refrigerator to determine the keeping quality.

Table (3): Boiled milk keeping quality

Cold Storage[2], days	Cows' Milk			Buffaloes' Milk		
	Boiling[3] Period (min).					
	1	2	5	1	2	5
			$\times 10^4$cfu/ml			
0	2.3±0.05	1.4±0.23	1.3±0.21	2.4±0.21	2.8±0.21	0.9±0.2
5	2.9±0.09	1.6±0.21	1.2±0.2	2.22±0.26	3.6±0.44	0.20±0.10
7	0.65±0.02	0.65±0.02	0.6±0.18	0.8±0.09	0.2±0.03	0.2±0.02
10	1±0.26	1.55±0.03	0.75±0.04	0.33±0.03	0.2±0.20	2.2±0.02

1- LSD $_{(0.05)\ (overall)}$ = 2 2- LSD $_{(0.05)}$ = 1.2 3-LSD $_{(0.05)}$ = 2

There was insignificant count decrease after the fifth day of storage. This was followed by a significant decrease in the 7th day and the counts insignificantly changed on the 10th day of cold storage. The counts were of a range between 2×10^3 and 7×10^3 cfu/ml on seventh day of storage, which means that boiling milk has a good keeping quality under refrigeration. Actually, the samples remained in good condition after 20 days of storage. It was found that spore formers are the major spoilage bacteria of heat treated milk .The bacterial ecology of high temperature short time pasteurized milk in the US for example was found to be gram positive endospore-forming bacteria (i.e *Bacillus* and *Paenibacillus*). During cold storage the predominant spoilage genera shifted from *Bacillus spp* to *Paeinbacillus sp*, some of these strains were psycrotolerant endospores and their growth caused milk spoilage, (Ranieri and Boor,2009).

To compare the effect of heat treatment on milk quality a UV- method was use to discriminate between boiling & UHT treatments and the results are in Fig (3). Boiling up to 5 min developed less than half of 0.D- values of other heat treatments. The extended shelf life treatments was of intermediate 0.D–value between boiling & UHT-milk. These 0.D-values correlate with furosine contents and according to the above reference, furosine formation at 100ºC is a straight line relationship with 0.D- values. This means that boiling is a more delicate treatment than all kinds of UHT treatment by forming far less amount of furosine.

A better flavor and causing less nutrition deterioration than UHT. The formation of furosine reduces the nutritive value of milk by decreasing protein availability and may behave as chelating agents for metal cations affecting their bioavailability. Over heating such as in bottle sterilization which sometimes is used in UHT production may result in decreasing of food intake (Seiquer, *et al*. 2010). It was found that UHT milk produce more furosine than boiling, boiling for 5min produced 116.7 while commercial UHT contained 142 mg furosine/100g protein (Sun and Wang, 2009). Also, UHT-milk was found to contain 0.181% free fatty acids, 0.453 mEq of O_2/kg of fat peroxides and thiobarbituric acid (TBA) values of 0.019 as compared to 0.118% Free fatty acids, 0.296 mEq of O_2/kg of fat peroxides and 0.018 TBA in 5min boiled milk (Meshref and Al-Rowaily,2008)

In conclusion, milk boiling for periods less than 2min whether boiling was carried out in an oil bath or on direct flame provides the consumer the required safety which lasts for a reasonable shelf life

under at cold temperature life. The method though is simple and inexpensive for regular consumers preserves more of milk nutritive value and flavor compared to UHT treatments which is develops old or stale flavor. However, on boiling continuous stirring is essential particularly at boiling temperature to be sure that the formed foam is exposed to boiling temperature.

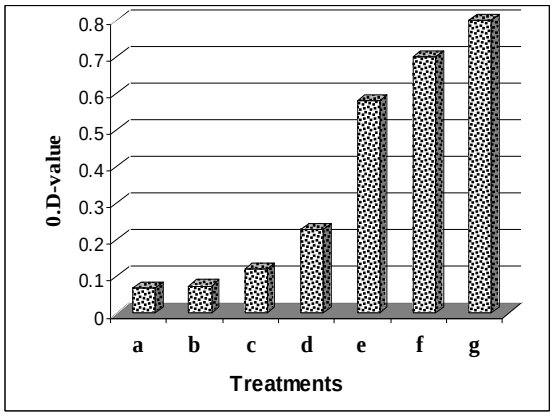


Fig (3): Discrimination between the effects of milk heat treatments using an UV-Absorption method

a: Boiling for zero period b: boiling for 1min
c: Boiling for 2min d: boiling for 5min
e: Extended shelf life f&g: UHT of different brands

Corresponding author
Nadia M.A.Dabiza
Dairy Science Department, National Research Center
Dokki, Giza, Egypt
nadiadabiza@yahoo.com

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