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Impact of boiling on raw milk – food safety aspects

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ABSTRACT

Goal: Milk is a nutrient of high biological value, consumed in larger quantities and frequencies by sensitive groups of population. This fact makes the monitoring of milk's safety particularly important for the public health. An increasing number of persons consume raw, unpasteurized milk. Superior nutritional value, quality, taste and other health benefits are advocated as reasons for increased interest in the consumption of the raw milk. However, scientifically based data to support such claims are very limited. Almost all of the international advisory and regulatory agencies dealing with food safety strongly support the principle of exclusive consumption of pasteurized milk. This research seeks to verify both the positive and negative impacts of boiling on the safety of the raw milk. **Scope:** This study was conducted on 30 samples of raw milk, selected by a method of random sampling from individual retailers in grocery stores and open markets in the city of Sarajevo. The boiling process was conducted by heating the raw milk to its boiling point during 20 seconds. Each collected sample was divided into two groups (A and B), where sample A was analysed before, and sample B after the boiling process. **Findings:** The study determined that the boiling process improves the safety of raw milk, and is responsible for an exceptional and significant increase of its microbiological safety. The study also revealed that the safety of raw milk in the Sarajevo Canton derogates from provisions in force for the raw milk.

Key words: raw milk, boiling, safety

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INTRODUCTION

Human interest in the consumption of safe food exists from ancient times, which resulted with global demand for production of food that could be used without risks upon health of humans. In addition to the macro and micro nutrients, as well as the non-nutritive natural components of food, often responsible for its organoleptic properties, biological values and admission, the food also contains numerous other substances being more or less harmful to human health. These, primarily, concern the microorganisms and their toxins, additives and residues of contamination. Examination of food's safety rests on evidencing the presence of such substances and their determination [1]. Food safety implies that a food will not cause adverse effects on human health if prepared and consumed in accordance with its intended use [2]. From the perspective of a healthy and balanced diet, milk is a unique food in many different ways. It is of natural origin, and contains all the ingredients needed for proper nutrition of an organism, either of a child or an adult [3].

Milk is a food of high biological value used in larger quantities and frequencies by sensitive population groups (children, pregnant women, elderly persons).

Milk is a food of high biological value used in larger quantities and frequencies by sensitive population groups (children, pregnant women, elderly persons). This fact makes the monitoring of milk's safety particularly important for public health [4]. Specific regulations on microbiological safety of food had set the norms for each food with respect to the type and number of microorganisms allowed for nutrition of humans [1].

Access of individual elements into the food chain can differ, yet predominantly depends on activities of humans. Contamination of food and environment with inorganic pollutants may be of primary or of secondary character. Primary contamination implies that the contamination of the plant foods was conducted through soil, water or air, whereas the contamination of animals was conducted through food or water. Secondary contamination implies that the contamination of food occurred during its processing, packaging or storage due to the migration of toxic metals from equipment, packaging, appliances, utensils or contaminated food additives. Quality of milk depends of its composition and level of hygiene applied during the milking process, i.e. the purity of milking pots, condition of its storage place, mode of transport and cleanliness of each animal's udders. Production of milk and dairy products under non-hygienic conditions and poor manufacturing practices may cause setbacks for both the public health and the economy [4].

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Raw milk can be contaminated by a wide range of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* spp., *Brucella* spp., *Listeria monocytogenes*, *Salmonella* spp. and *Corynebacterium* spp., as well as by a number of moulds and yeasts [5]. In some cases, the milk's infection with viable pathogenic bacteria may cause its contamination and spoilage, which makes the milk unsafe. Main life threatening diseases associated with milk include gastroenteritis, diarrhea, typhoid or tuberculosis of the cattle [6].

The toxic metabolites of aflatoxin may also be found in animal products, milk and meat, in case the cattle feed was previously contaminated with moulds [7]. Aflatoxin M1 is a relatively stable molecule, which cannot be inactivated by the heating treatments like pasteurization and sterilization [8]. Due to these characteristics, Aflatoxin M1 may have adverse effects on health of humans, particularly of children who are the main consumers of milk and dairy products [9].

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MATERIALS AND METHODS

Our analysis was conducted on 30 samples of raw milk, selected by a method of random sampling from individual retailers in grocery stores and open markets in the city of Sarajevo. Criteria for sampling was that the raw milk in question can be found in official grocery stores or at open markets of the Sarajevo Canton. The samples were collected in sterile laboratory containers and kept stored in refrigerating showcases on temperature varying from 4 to 8 °C.

The samples were transported on temperature of 4 °C, and, pending the analysis, kept in storage on temperature of -20 °C.

Collected samples were labelled in accordance with the standard laboratory procedures for receipt and labelling of samples. Each collected sample was split into two groups (A and B), where sample A was analysed before, and sample B after the boiling process. The boiling was conducted by heating the raw milk to the point of its boiling, it most frequently being between 97-102,5 °C, which depended of the milk's composition. Once the boiling point was reached, the milk was heated for additional 20 seconds, by which the boiling treatment was completed [10].

Determination of heavy metals (Lead, Arsenic)

Analysis of samples (that were firstly prepared in a microwave oven) on the presence of heavy metals (lead, arsenic) was conducted in the SHIMADZU AA-6650 atomic absorption spectrophotometer. Even though the SHIMADZU AA – 6650 atomic absorption spectrophotometer is mainly used for determination of concentration of heavy metals in a sample, it may also be used for some other tasks to depend of the software and the needs. The principle of its work is based on atomic absorption and spectrophotometry. The SHIMADZU AA-6650 atomic absorption spectrophotometer combines two correction functions, the D2 method (deuterium lamp method) and the SR method (self-reversal method), thus enabling that the samples are examined under appropriate method.

Determination of Aflatoksin M1

Quantitative analysis of Aflatoxin M1 in the milk samples was conducted through application of the ELISA testing (Enzyme-linked Immunosorbent Assay). The principle was to test the antigen – antibody reaction. The test kit contained reagents for 96 samples (including the calibration curves). Samples of the milk were initially defatted by cen-

trifugation of cold milk in conical tubes at 2000 x g of rotation for 10 minutes. The layer of fat created on the top has been removed with a spatula, and 100 μL of the defatted milk was used for the ELSA testing. The standards were concentrated and needed a dilution with standard diluents prior to the testing. The calibration curve illustrates the ratio of concentration of Aflatoxin M1 in the standard and the value of its absorption at 450 nm, displayed on the reader of the microtiter plate "DAS PLATE READER". All of the milk samples were prepared in accordance with the instructions contained in the ELISA kit.

Microbiological parameters

For evidencing, we used the following microbiological methods: the Horizontal method for detection of *Salmonella* spp (BAS EN ISO 6579:2005); the Horizontal method for detection and counting of coagulase-positive staphylococci – *Staphylococcus aureus* (BAS EN ISO 6888-1:2005); the Horizontal method for detection and counting of *Enterobacteriaceae* (BAS EN ISO 21528-2:2008); the Horizontal method for counting of *Clostridium perfringens* – the colony counting method (BAS EN ISO 7937:2005); the Horizontal method for counting of microorganisms – technique of counting the colonies at 30 degrees Celsius (BAS EN ISO 4833:2006); the Horizontal method for detection and counting of *Listeria monocytogenes* (BAS EN ISO 11290-1:2005).

Statistical data processing

The results of the analysis have been statistically processed through applicable and verified methods – the *Microsoft Excel 2010* computer program and the *IBM® SPSS® Statistics 24.0 software*.

By applying appropriate functions available within the *IBM® SPSS® Statistics 24.0 software*, there have been calculated the basic statistical parameters of: the mean (MEAN) and variability measures – standard deviation (SD) and standard error of the mean (SEM). To acquire a more detailed interpretation and a better understanding of the obtained results, we have also used the Microsoft Excel 2010 computer program to calculate percentages of the average values of certain parameters after the boiling of examined milk samples.

The statistical significance of differences between the measured values of the examined parameters in the raw and boiled milk was evaluated by a dependant (paired) two-tailed t testing, and calculated in the *IBM® SPSS® Statistics 24.0 software*.

RESULTS AND DISCUSSION

Out of 30 samples of the raw cow's milk that were tested, 16 samples (53.3%) proved unsafe. Following the method of boiling that was used, 7 samples (23.3%) remained unsafe.

Table 1: Display of safe and unsafe samples of milk, before and after boiling

Type of sample	Safe	Unsafe
Raw milk	14 (46,6%)	16 (53,3%)
Boiled milk	23 (76,6%)	7 (23,3)

Results of microbiological parameters

Average number of colonies of aerobic mesophilic bacteria in the samples of raw milk was 8.1×10^2, while the samples of boiled milk showed the value of 1×10^1. The average number of colonies of aerobic mesophilic bacteria in boiled milk decreased by 99.33% in comparison to the average number of same bacteria in the raw milk, and t-test (t=7.93) showed statistically significant difference (t=7.93; p <0.0001). The average number of colonies of Staphylococcus aureus in the raw milk samples was 1.7×10^2. Presence of such bacteria was not noted in any sample of the boiled milk, and the t-test showed statistically significant difference (t = 4.79; p <0.0001). The average number of colonies of Enterobacteriaceae bacteria in the samples of raw milk was 1.6×10^2, while their presence in the samples of boiled milk was not found at all. Application of the t-test demonstrated a statistically significant difference (t = 5.22; p <0.0001). Neither of the samples of raw milk demonstrated the presence of Salmonella spp., Clostridium perfringens or Listeria monocytogenes.

Table 2: Comparative review of the number of aerobic mesophilic bacteria (CFU/g), Staphylococcus aureus (CFU/g) bacteria and enterobacteriaceae (CFU/g) in samples of raw and boiled milk

Sample	Aerobic mesophilic bacteria (CFU/g)		*Staphylococcus aureus* (CFU/g)		*Enterobacteriaceae* (CFU/g)	
	Raw milk	Boiled milk	Raw milk	Boiled milk	Raw milk	Boiled milk
Mean	**$8,1\times10^2$**	**1×10^1**	**$1,7\times10^2$**	**0**	**$1,6\times10^2$**	**0**
SD	$5,4\times10^2$	5	$1,9\times10^2$	0	$1,7\times10^2$	0
Min	$6,4\times10^1$	0	$1,2\times10^1$	0	$5,2\times10^1$	0
Max	$1,8\times10^3$	2×10^1	$8,8\times10^2$	0	$6,2\times10^2$	0
p-value	**< 0,0001***		**< 0,0001***		**< 0,0001***	

* t-test

Results of the heavy metals testing (As, Pb)

Average level of arsenic in raw milk samples was 0.01 mg/l, while no evidence of presence of this heavy metal was noted in any sample of the boiled milk. The t-test showed a statistically significant difference (t = 3.42; p = 0.001).

The average level of lead in raw milk samples was 0.03 mg/l, and in the boiled milk samples 0.01 mg/l, thus showing a decrease in the average level of lead in tested samples of the letter category by 73.34%. Application of the t-test demonstrated a statistically significant difference between the samples of raw and boiled milk (t = 3.42, p = 0.02).

Table 3: Comparative review of arsenic (As) and lead (Pb) levels in samples of raw and boiled milk

Sample	Level of As (mg/l)		Level of Pb (mg/l)	
	Raw milk	Boiled milk	Raw milk	Boiled milk
Mean	**0,01**	**0,00**	**0,03**	**0,01**
SD	0,016	0,000	0,057	0,017
Min.	0,000	0,000	0,000	0,000
Max.	0,072	0,000	0,222	0,064
p-value	**0,0019***		**0,0214***	

*t-test

Figure 1: Comparative review of measured levels of Arsenic (mg/l) in raw and boiled milk

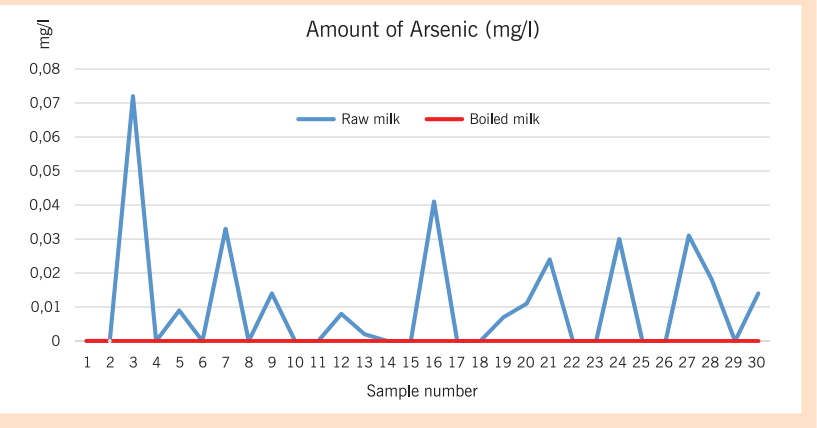
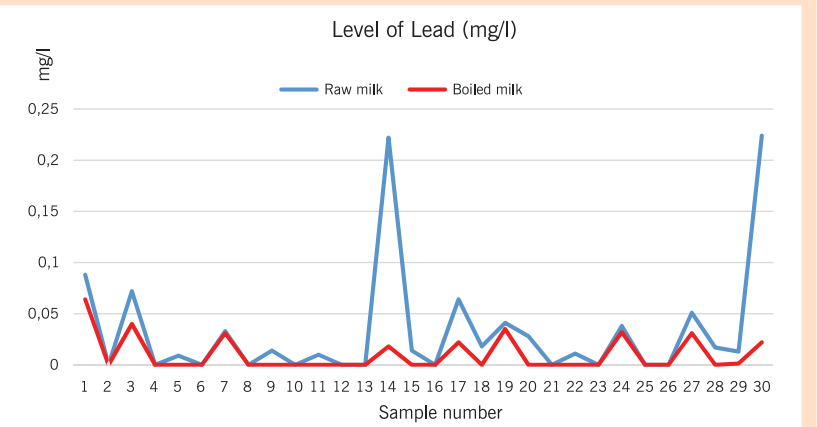


Figure 2: Comparative review of measured levels of Lead (mg/l) in raw and boiled milk



Results of the study relating to presence of Aflatoxin M1

Due to low level of detected concentrations of Aflatoxin M1, the results were displayed in pg/ml instead of μg/l, as required by the Rulebook on maximum permitted levels of certain contaminants in foodstuffs ("The Official Gazette of Bosnia and Herzegovina", No. 37/09).

No significant difference in concentration of Aflatoxin M1 was detected between the samples of raw and boiled milk. The average concentration of Aflatoxin M1 in samples of raw milk was 0.68 pg/ml, and in the samples of boiled milk 0.675 pg/ml. The statistical significance of this difference was additionally evaluated by t-testing, when no statistically significant difference was found (p> 0.05).

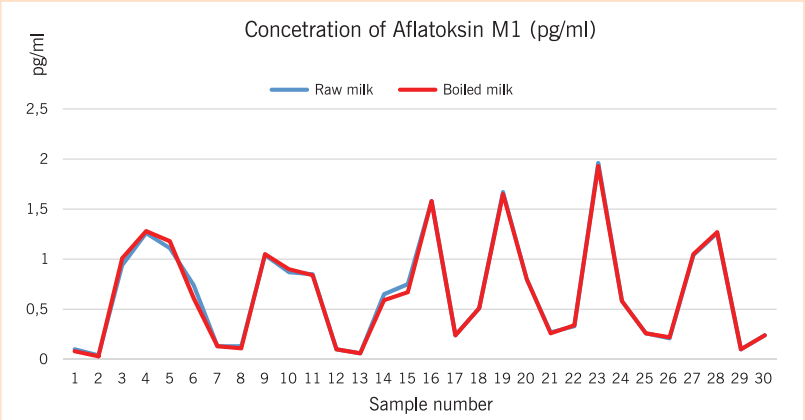


Figure 3:
Comparative review of measured concentrations of Aflatoxin M1 (pg/ml) in raw and boiled milk

The results of the analysis on the raw milk's safety clearly indicate that the raw milk available in the Canton of Sarajevo derogates from applicable regulations on the raw milk. By boiling such milk, its safety improves.

The results of the comparative microbiological analysis of samples of raw and boiled milk clearly show a very pronounced influence of the application of this treatment towards the increase of microbiological stability of the milk by reducing the microbiological contamination of the raw milk. The pronounced decrease in the number of aerobic mesophilic bacteria, as well as the complete destruction of enterobacteriaceae and staphylococci is in line with expected results resting on the data from professional literature, which finds the boiling method a very effective mean of prevention of possible risks for poisoning the raw milk by bacteria [11]. Staphylococcus aureus was found in all of the examined samples of raw milk, where 50% of the tested samples showed the value of $> 10^2$, it being higher than evidenced in a research conducted in the Czech Republic [12]. The data clearly indicate that pore hygiene conditions were applied during the milking, packing and transport of raw milk; as such, the contamination of raw milk is of secondary character.

The results of the quantitative analysis of Aflatoxin M1 show that the boiling of milk does not affect the presence of this mycotoxin. These results are consistent with the literature on the thermostability of its molecule, which cannot be destroyed in the process of pasteurization and sterilization [8]. The obtained results indicate that the boiling does reduce the concentration of lead and arsenic, as unstable metals that could be lost with this treatment. In addition to being non-thermostable at high temperatures, these metals also tend to bind to proteins [13], so their reduced concentration in the boiled milk can also be explained by the fact that they could have been previously removed as tied to coagulated proteins in the surface layer of removed fat. However, the measured concentrations of these metals in raw milk are very low (much lower than their maximum allowed amounts), so no final conclusions can be drawn as to the effect of boiling of milk on its health safety in terms of concentration of these heavy metals. In subsequent researches, this problem could be solved through an experiment in which the raw milks would be contaminated with certified reference materials of Pb and As, and then boiled. The concentrations

The results of the quantitative analysis of Aflatoxin M1 show that the boiling of milk does not affect the presence of this mycotoxin.

The boiling of milk has no effect on the reduction of concentration of Aflatoxin M1.

of the mentioned metals would be measured after the boiling of contaminated milk by the method that was described and applied in this paper.

CONCLUSION

Boiling of raw milk improves its safety and has a very pronounced and significant influence on increase of its microbiological safety. In other words, it is a very effective method for destroying all present pathogens in the raw milk. With respect to chemical contaminants, the boiling of milk proved to be effective in reducing the concentration of arsenic, though not lead. The boiling of milk has no effect on the reduction of concentration of Aflatoxin M1. The safety of raw milk in the Canton of Sarajevo derogates from regulations in force for the raw milk. The results of this work clearly point to the need for educating and informing the population consuming the raw milk in terms of changing their habits as well as removing prejudices related to the boiling of milk.

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