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A consensus and controversies over the index organism for milk pasteurization: A brief review

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Abstract
Pasteurization, named after French scientist Louis Pasteur, was introduced in milk in the late 1800s. The First law for pasteurization of milk was passed in the year 1908 in Chicago. The batch process of pasteurization (low-temperature-long-time/ LTLT process) was developed in the initial phase to kill *Mycobacterium tuberculosis*. During the early period, some of the countries used *M. tuberculosis* while the others used *Coxiella burnetii* as an index organism for pasteurization of milk, although *C. burnetii* was more heat resistant than *M. tuberculosis*. The current definition given by Codex Alimentarius Commission points towards both the organism. Initially pasteurization conditions were set as 61 ºC for 30 minutes with reference to *M. tuberculosis*, but later it was changed to 62.8 °C for 30 min or 71.6 °C for 15 seconds with reference to *C. burnetii.*

Keywords: Pasteurization**,** *Coxiella burnetii, Mycobacterium tuberculosis*, alkaline phosphatase

Introduction

History of Pasteurization

The value of heat for the preservation has been known for thousands of years, but it was realized in nineteenth century that very mild heat treatment far below boiling point of milk, such as 60 °C made liquid foods, such as milk, improved the keeping quality during storage. The discovery followed the work of French scientist Louis Pasteur on wine and beer and thus the name Pasteurization. Heating at low temperature not only destroyed spoilage organisms, but also preserve the original characteristics of the liquid being treated. Pasteur was the man of inspiration behind Gail Borden who, in 1853, patented a process for heating and condensing milk under vacuum. Pasteur discovered that heating of fermented wine would kill the spoilage microorganisms in it. Similarly, Nicolas Appert, the inventor of in-container sterilization (canning), had already revealed that heat treatment of food could preserve it. Pasteur's determined the exact time and temperature that would kill the harmful microorganisms in the wine without changing its sensory quality. He patented the process as pasteurization. Pasteur is credited with revolutionizing the safety of milk and, in turn, the ability to store and distribute milk well beyond the farm.

The practice of milk pasteurization started in late 1800s. At that time, tuberculosis was usually spread through milk. Batch pasteurization (low-temperature, long-time LTLT process) was first developed to kill *Mycobacterium tuberculosis*. The incidence of tuberculosis from milk significantly reduced and it no longer made CDC list of foodborne disease. In 1908 first law regarding the pasteurization of milk was passed in Chicago.

The first application of pasteurizing heat treatments to milk may have been performed by Soxhlet, who pasteurized bottled milk fed to infants. Gerber and Wieske pasteurized milk in bottles at 65 °C for 1 h as early as 1888 [1]. The first commercial pasteurizer was made in Germany in 1882, using a high-temperature, short-time (HTST) process; pasteurization on a commercial scale quickly became common practice all over the world. This article describes about the history and current status of milk pasteurization; definition, index organism, and verification process.

Definition- Pasteurization

Earlier definition of pasteurization given by CAC [2] is *"*Pasteurization destroys neither the spores nor the toxins secreted in the milk by organisms that lived in it prior to its heat treatment*"*. However, in order to improve readability, to provide a more consistent approach in line with literature and for consistency of format with the other definitions proposed, the definition modified including a reference to the heat treatment necessary to inactivate *M. tuberculosis* with a reasonable safety margin. At that time, some countries use *Coxiella burnetii* as indicator organism, as it is slightly more heat-stable than *M. tuberculosis*.

Further the definition should not refer to the treated product but to the treatment itself and it would be appropriate to include that the treatment also applies to cream. Further recommended definition is-"Pasteurization is a heat treatment aimed at reducing the number of harmful microorganisms in milk and cream to a level at which they do not constitute a significant health hazard. It is intended to result in an extended shelf life of milk and in only minimal chemical, physical and organoleptic changes. Pasteurization conditions are designed to effectively destroy the organism *M. tuberculosis*. Pasteurization of milk and cream should show a negative phosphatase test". Current definition given by CAC [3] after including all suggestions and reports is "Pasteurization is a microbiocidal heat treatment aimed at reducing the number of any pathogenic micro-organisms in milk and liquid milk products, if present, to a level at which they do not constitute a significant health hazard. Pasteurization conditions are designed to effectively destroy the organisms *M. tuberculosis* and *C. burnetii"*.

Index organism of effective pasteurization

A lot of confusion existed about the "index" organisms in milk earlier. As pathogenic microorganisms are readily isolated from raw milk, many State health departments, the USFDA and IDF recommend that unpasteurized milk should not be drunk or used in the manufacture of any dairy product and specifically not in cheese manufacture from raw milk. Pasteurized milk is generally free of pathogen except some spore of *Bacillus cereus*, if present in large numbers. So disease outbreak from pasteurized milk is either due to post-pasteurization contamination or improper processing. To prevent milk borne diseases, the dairy industry and public health regulators should ensure that all steps are taken to prevent the entry and growth of pathogenic micro-organisms during the handling and processing of milk and milk products. IDF has also developed a monograph on pasteurized milk which covers all aspects of pasteurization. Pasteurization destroys about 99% of common bacteria of milk including nearly all which cause spoilage and increase shelf life.

Smith[4] reported that tubercle bacilli which cause tuberculosis (often fatal lung disease) were no more resistant to heat than many other bacilli not producing spores, and complete destruction can be done within 15 to 20 min at 60 °C. Russell and Hastings [5] reported that *M. tuberculosis* was killed in a closed commercial pasteurizer in 10 min at 60 °C and, based on these data, it was earlier recommended that milk should be heated at 60 °C for 20 min to ensure complete destruction. Therefore, the first recommendations for time and temperature combinations for pasteurization were established on this basis.

Earlier, datas were available which confirms *C. burnetii as* most heat-resistant non-sporulating pathogen (the rickettsia responsible for Q fever in raw milk). *C. burnetii* was more heat-resistant than *M. tuberculosis*, and could be isolated from pasteurized milk processed according to minimum standards. Later[6], it was showed that heating *C. burnetii* suspended in whole raw milk for 30 minutes at 143°F (61.7 °C) is not sufficient to eliminate all the viable rickettsiae, while heating at 145°F (62.8 °C) for the same period of time will achieve this. This was confirmed using a vat-type commercial pasteurizer equipped with a space heater. The results of HTST commercial pasteurizer confirms the extrapolation of the laboratory derived data and strongly support the presently recommended standard of pasteurization of 161°F (71.6 °C) for 15 seconds as sufficient to eliminate viable *C. burnetii* from whole raw milk. In table 1 different time and temperature combinations are given for pasteurization of milk.

Table 1: Different time temperature condition for pasteurization of milk

Temperature	Time
63°C (145°F)	30 minutes
72°C (161°F)	15 seconds
89°C (191°F)	1.0 second
90°C (194°F)	0.5 seconds
94°C (201°F)	0.1 seconds
96°C (204°F)	0.05 seconds
100°C (212°F)	0.01 seconds

Source [7] (Grade "A" pasteurized milk Ordinance, 2011)

The recognition of the public health importance of pasteurization prompted the development of regulations that set times and temperatures to control pathogens. Initially pasteurization conditions were devised to inactivate *M. tuberculosis* [8] and were set as 61°C for 30 minutes, but conditions have been changed subsequently to destroy the organism *C. burnetii* which causes Q fever. Pasteurization is designed to achieve at least a 5-log reduction of *C. burnetii* in whole milk. In recent times, however, there has been controversy among scientists about the ability of pasteurization to inactivate the organism *M. avium* subsp. *paratuberculosis*. This organism has become prominent because of its putative link with Crohn's disease in humans, a link which is also the subject of much debate and, currently, lack of consensus. The results regarding survival of *M. paratuberculosis* after heat treatment are very conflicting. A study conducted by the U.S. Department of Agriculture suggested that HTST pasteurization was effective in inactivating *M. paratuberculosis* in raw milk [9]. Another study from UK reported the recovery of viable *M. paratuberculosis* from retail-ready pasteurized milk [10]. In a study regarding inactivation of *M. avium subsp. paratuberculosis* in milk and it was also noticed that survivors in 1.4% (1 of 72) of experiments conducted with the HTST unit [11]. They also concluded that *M. paratuberculosis* would be effectively inactivated by current pasteurization practices in the United States. Till date, there is no definitive answer to the question of whether *M. paratuberculosis* is completely destroyed during pasteurization, but the majority of the research conducted have confirmed a significant log kill during the process [11-13].

In quantitative risk assessment terms the degree of inactivation of a particular organism that pasteurization achieves will be represented by a probability distribution function. The numbers of a pathogen which may survive pasteurization are dependent on the initial load of organisms in raw milk and degree of kill. Hence survivors will also be represented by a probability distribution function. Based on this study Public Health Service recommended to increase pasteurization standards to 145°F (62.8 °C) for 30 min or 161°F (71.6 °C) for 15 seconds. If the product to be pasteurized contain more fat than fluid whole milk, or contain added sugar, there should be additional 5°F (3 °C) added. Today pasteurization standards are based on the destruction of *C. burnetii* and *M. tubuerculosis.* Thus the international definition requires destruction of *C. burnetii* to protect the health of milk consumers.

Process criteria

To ensure that each particle is sufficiently heated, the milk flow in heat exchangers should be turbulent, i.e. the Reynolds number should be sufficiently high. Depending on composition, processing and use of the product the necessary changes to the proper heat treatment should be established and efficiency of the heat treatment should be evaluated. For example, the minimum heat treatment given to cream is greater than milk due to high fat content (minimum 75 °C for 15 seconds).

If the fat content of the milk product is ten percent (10%) or greater, or a total solids of 18% or greater, or if it contains added sweeteners, the specified temperature shall be increased by 3 ºC (5ºF). Thus, the time temperature combination varies with type of milk products (Table 2).

Table 2: Pasteurization conditions for different products

Pasteurization Type	Typical Product	Typical Storage	Temperature	Holding Time
Batch, vat	Milk	Refrigerated	62.8 °C	30 min
"	Viscous products, or products with more than 10% fat or added sweetener	"	65.6 °C	30 min
"	Frozen dessert mixes	"	68.3 °C	30 min
Continuous, high temperature short time (HTST)	Milk	"	71.7 °C	15 sec
"	Viscous products, or products with more than 10% fat or added sweetener	"	74.4 °C	15 sec
"	Frozen dessert mixes	"	79.4 °C	25 sec
"	"	"	82.2 °C	15 sec
Continuous, higher heat shorter time (HHST)	Milk	"	88.3 °C	1 sec
"	"	"	90° C	0.5 sec
"	"	"	93.8 °C	0.1 sec
"	"	"	96.2 °C	0.05 sec
"	"	"	100° C	0.01 sec
Continuous, Ultra pasteurization	Milk and cream	Refrigerated, extended storage	137.8 °C	2 sec
Aseptic, ultra high temperature (UHT)	Milk	Room temperature	135-150 °C	4-15 sec
Sterilization	Canned products	"	115.6 °C	20 min

Source [14] (http://www.milkfacts.info/Milk%20Processing/Heat%20Treatments%20and%20Pasteurization.htm)

Problems associated with assuring the safety of milk and milk products have become extremely complex because of new products, new processes, new materials and new marketing patterns, which must be evaluated in terms of their public health significance. These studies led to the conclusion that effective public health control of milk borne disease requires the application of sanitation measures throughout the production, handling, pasteurization, and distribution of milk and milk products. These early studies were followed by research to identify and evaluate sanitary measures, which might be used to control disease, including studies that led to improvement of the pasteurization process.

The time/temperature combinations for HTST pasteurization were established many years ago on the basis of the hygiene status at that time (quality of raw milk and of hygiene management levels). With time, the hygiene status has increased considerably. However, the tradition to specify the minimum time/temperature combinations in regulatory texts has not enabled the elevation of the hygiene status to be converted into the application of microbiocidal control measures of less intensity. Instead, it has been (and still is) converted into extension of the product shelf life.

Verification of process

Pasteurized milk should be alkaline phosphatase negative immediately after pasteurization. Low residual alkaline phosphatase levels in pasteurized milk (below 10 µg p-nitro-phenol equivalent/ ml) are used as a reference for assurance that the milk has been correctly pasteurized and it has not been contaminated by raw milk. There are other methods available which could also establish that the appropriate heat treatment has been applied. While measurement of residual alkaline phosphatase is considered as being the most appropriate method of verification, still some factors influence the residual levels and should be taken into consideration when interpreting the results e.g., initial concentration in milk (the "pool" of alkaline phosphatase present in milk varies widely between different species and within species e.g., raw cow's milk has higher activity than goats milk), fat content of the milk (Phosphatase is readily absorbed on fat globules, typical concentrations in cow's milk: skim 400 µg/ml; whole 800 µg/ml, and 40% cream 3500 µg/ml). Alkaline phosphatase can also be reactivated in many milk products (cream, cheese, etc.); also, micro-organisms used in the manufacture may produce microbial phosphatase and other substances that may interfere with tests for residual phosphatase. Some researcher also reported that the milk sample showed negative phosphatase test immediately after pasteurization, may yield a positive test after a short period of storage, particularly if the product is not continuously or adequately refrigerated. This phenomenon is known as reactivation. The optimum temperature for reactivation is 34 °C (93°F), although reactivation may occur at temperatures as low as 10 °C (50°F) in HTST pasteurized products after storage. Particularly high fat content products are susceptible to reactivation of phosphatase. Reactivation is greatest in products pasteurized at about 110 °C (230°F) but may occur in products pasteurized at much higher temperatures and as low as 73 °C (163°F). Reactivation can be reduced by increasing holding time during pasteurization. Reactivation is accelerated by the addition of magnesium acetate to HTST processed milk or cream, after pasteurization but before storage. Reactivated phosphatase and residual phosphatase is measured by calculating the difference in activity between an adequately pasteurized sample, stored with and without magnesium, and an inadequately pasteurized sample, stored with and without magnesium.

Conclusions

Initial pasteurization conditions, known as flash pasteurization, were to heat the milk to 155 to 178°F (68.3 to 81 °C) for an instant followed by cooling. Pasteurization conditions were adjusted to 143°F (61.7 °C) for 30 minutes or 160°F (71.1 °C) for 15 seconds to inactivate *M. tuberculosis*, the organism responsible for tuberculosis. However, in 1957 these conditions were shown to be inadequate for the inactivation of *C. burnetii* which causes Q fever in humans. New pasteurization conditions of 145°F (62.8 °C) for 30 minutes for a batch process, or 161°F (71.7 °C) for 15 sec for a continuous process, were adopted in order to inactivate *C. burnetii*, and these conditions are still in use today. Despite the progress that has been made, occasional milkborne outbreaks still occur, emphasizing the need for continued vigilance at every stage of production, processing, pasteurization and distribution of milk and milk products. Milk from different species of milking animals normally contains different levels of alkaline phosphatase. These differences should be taken into account when establishing criteria for phosphatase analysis and when establishing the effectiveness of alkaline phosphatase testing as a means to verify that pasteurization conditions have been properly applied.

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