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Microbiological Considerations: Pasteurized Milk

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ABSTRACT

Milk is an ideal medium for the growth and multiplication of diverse microorganisms resulting in its early deterioration. Consumption of raw milk should be discouraged, as numerous epidemiological outbreaks even death have been recorded. Amongst various methods, pasteurization is the widely adopted technology to render milk safe for human consumption. Microbiological quality of pasteurized milk is resultant of various factors including quality of raw milk, heat-treatment employed, storage conditions and extent of post-pasteurization contamination. In the present endeavor, attempts have been made to highlight microbiological considerations for the safety of pasteurized milk. Endeavour has been made to explore various factors affecting the microbiological quality of pasteurized milk and hygienic practices to be implemented for quality improvement. Reviewed literature indicated that to ensure safe pasteurized milk, an improvement in the microbiological quality of raw milk, proper pasteurization and prevention of post-pasteurization contamination is important. Introduction of microfiltration prior to pasteurization is suggested to ensure complete removal of spores thereby enhancing the microbiological safety of pasteurized milk.

Key words: Pasteurized milk, hygiene, microbiology, safety

INTRODUCTION

Microbial load and incidence of the bacterial pathogens in foods are indicators of food quality (Rosmini *et al*., 2004), as well as the sanitary conditions of its production (Guerreiro *et al*., 2005). Reviewed literature indicated poor microbiological quality of raw milk due to bacterial contamination (Ahmed and Abdellatif, 2013), inadequate packaging system (Singh *et al*., 2012) and improper temperature control (Moussa *et al*., 2013), which favour microbial growth and metabolism and brings in undesirable changes thereby shorten the shelf life of milk (Fromm and Boor, 2004). Diverse processing techniques like thermization, Low Temperature Long Time (LTLT) pasteurization, High Temperature Short Time (HTST) pasteurization, sterilization, ultra high temperature treatment (Gedam *et al*., 2007), ultraviolet treatment (Reinemann *et al*., 2006; Matak *et al*., 2007), microwave treatment (Tremonte *et al*., 2014), membrane processing (Eckner and Zottola, 1991) and microfiltration (Elwell and Barbano, 2006) can be adopted to treat raw milk to render it safe for human consumption by reducing or eliminating pathogenic microorganisms and to enhance the shelf-life by inactivating spoilage microorganisms. Amongst various techniques, pasteurization is widely adopted but it is not designed to sterilize milk and microbiological quality of pasteurized milk is governed by the initial flora of raw milk, the processing conditions and post-heat treatment contamination.

Adequate food control programme must be implemented in all countries around the world to ensure safe food with acceptable nutritional attributes at an affordable price. Dairy products must

comply with the microbiological criteria laid down by the regulatory authorities, which could be achieved through pasteurization or more severe heat treatments and prevention of post-heat treatment contamination (NRC, 1985). In the present endeavor, attempts have been made to highlight microbiological considerations for the safety of pasteurized milk.

MICROBIOLOGICAL QUALITY OF PASTEURIZED MILK

Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder (Tolle, 1980) and may be contaminated during milking and handling with equipment, personnel and environmental sources and may contain pathogens (ICMSF, 1998). Pasteurization is the widely adopted milk process to ensure completely destruction of all pathogenic and spoilage microorganisms, commonly found in milk and inactivation or reduction of other non pathogenic spoilage bacteria and certain undesirable enzymes to safeguard the food value of milk (Teka, 1997).

FAO/WHO (2004) defined pasteurization as "A microbiocidal heat treatment aimed at reducing the number of any pathogenic microorganisms 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 *Mycobacterium tuberculosis* and *Coxiella burnetii*". Initially, pasteurization conditions were devised to inactivate *M. tuberculosis* (North and Park, 1927) but subsequently, *C. burnetii* appeared as the most heat-resistant organism present in the milk and therefore, pasteurization was redesigned to achieve at least a 5-log reduction of *C. burnetii* in whole milk (Hudson *et al*., 2003). The HTST pasteurization kills 99.999% of pathogens (FDA, 2009) and is effective in reducing the viable population of *Mycobacterium avium* subsp. *paratuberculosis* (4-5 log) but its efficacy depends on the total viable concentration (Okura *et al*., 2012). Stabel and Lambertz (2004) noted greater thermal destruction (7.7 vs. 5.0 log) due to pasteurization of Ultrahigh-temperature milk inoculated with higher concentrations (10^8 vs. 10^5 CFU mL^{-1}) of *M. paratuberculosis*, regardless of time-temperature combinations adopted. During pasteurization the initial rapid decline in population of *M. paratuberculosis* in cow's milk is not due to thermal death but attributed to aggregation of cells into clumps (Grant *et al*., 1996). Various recommended temperature-time combinations applicable for different pathogenic organisms have been delineated in Table 1. Pasteurization efficiency can be determined by Phosphatase test. Alkaline phosphatase, an enzyme naturally present in milk of all mammals have a thermal resistance greater than that of the most heat resistant non-spore-forming pathogens commonly found in milk (Sharma *et al*., 2003) and hence, its destruction confirms proper pasteurization (Ludikhuyze *et al*., 2000). Positive Phosphatase activity is the indicative of inadequate pasteurization or contamination of pasteurized milk with raw milk or post-process bacterial contamination (Vega-Warner *et al*., 1999).

Microbiological analysis of pasteurized milk indicated presence of pathogens like *Staphylococcus* sp., *Salmonella* sp. (Singh *et al*., 2011), coliform (Aglawe and Wadatkar, 2012) from India, *Salmonella* (Okpalugo *et al*., 2008) from Nigeria, *Enterobacter* spp., *Escherichia coli* from Jamaica (Anderson *et al*., 2011), *Staphylococcus aureus* from Brazil (De Oliveira *et al*., 2011), coliform, *B. cereus* from Kuwait (Al-Mazeedi *et al*., 2013) and *E. coli* and *S. aureus* from Iran (Vahedi *et al*., 2013). Silva *et al*. (2010) noted complete deactivation of phosphatase and *Salmonella* sp. but presence of coliform in 57.5% samples of pasteurized milk from Brazil. Incidence of pathogens in pasteurized milk (Ryan *et al*., 1987; Upton and Coia, 1994; Silva *et al*., 2010) and food-borne outbreaks due to inadequate pasteurization or post-pasteurization contamination

Table 1: Decimal reduction time of pathogens in milk

Pathogens	Temperature (°C)	D time	References
Bacillus spp.	95.0	1.2-36.0 min	Wong *et al*. (1988)
	100.0	2.0-5.4 min	Wong *et al*. (1988)
Brucella abortus	61.5	23 min	Foster *et al*. (1953)
	72.0	12-14 sec	Foster *et al*. (1953)
Campylobacter spp.	60.0	0.12-0.14 min	Sorqvist (1989)
Clostridium botulinum	100.0	240 min	Jay (1986)
	125.0	5 sec	Collins-Thompson and Wood (1993)
Coxiella burnetii	62.2	30 min	Enright *et al*. (1957)
	73.4	15.2-17 sec	Enright *et al*. (1957)
Escherichia coli O157:H7	63.0	16.2 sec	D'Aoust *et al*. (1988)
Listeria monocytogenes	63.3	33.3 sec	Bunning *et al*. (1986)
	68.9	7.0 sec	Bunning *et al*. (1986)
Mycobacterium avium subsp. *paratuberculosis*	63.0	12.2-17.8 sec	Pearce *et al*. (2001)
	66.0	5.2-6.3 sec	Pearce *et al*. (2001)
Mycobacterium bovis	64.0	6.6 sec	Kells and Lear (1960)
	69.0	0.6 sec	Kells and Lear (1960)
Pathogenic *Streptococcus*	66.0	0.1-0.2 min	ICMSF (1996a)
Salmonella spp.	62.8	0.11 min	Doyle and Mazzotta (2000)
	71.7	0.004 min	Doyle and Mazzotta (2000)
Staphylococcus aureus	65.0	0.2 min	ICMSF (1996b)
	75.0	0.02 min	ICMSF (1996b)
Yersinia enterocolitica	62.8	0.7-17.8 sec	Francis *et al*. (1980)

D-values (the time required to reduce the number of microorganisms by one log cycle)

(Da Silva *et al*., 1998; ICMSF, 1998) have been reported. Presence of *Salmonella* in pasteurized milk due to improper pasteurization resulting from malfunctioning of a pasteurizer valve (Bergquist and Pogosian, 2000) and post-pasteurization contamination of pasteurized milk with *Bacillus cereus* from packaging paper and board (Vaisanen *et al*., 1991; Pirttijarvi *et al*., 1996) and filling machine (Eneroth *et al*., 2001) have also been reported. Murphy (1997) attributed unclean equipment, improper sanitizing practices and milkstone deposition for higher total viable count in laboratory pasteurized milk. Therefore, to ensure safe pasteurized milk proper pasteurization and prevention of post-pasteurization contamination is important.

FACTORS AFFECTING MICROBIOLOGICAL QUALITY OF PASTEURIZED MILK

Shelf life of pasteurized milk is influenced by the quality of the raw milk (Rysstad and Kolstad, 2006), duration of storage of raw milk prior to processing, the heat-treatment employed, concentration of heat-resistant microorganisms, extent of post pasteurization contaminants, packaging system adopted, post-pasteurization storage conditions (Cromie, 1991) and effect of light (Rysstad and Kolstad, 2006).

Type of milk: Type of milk influences the shelf-life of milk due to diverse chemical composition and enzyme activity. Significantly, lower shelf-life of skim milk than whole milk during storage at 4.5°C or 7°C may be attributed to relatively higher protease activity in the skim milk or inhibition of protease or protection of protein from enzymatic proteolysis due to fat in whole milk (Janzen *et al*., 1982a).

Microbiological quality of raw milk: Microbiological quality of pasteurized milk is dependent on the microbial load as well as the type of organisms present in the raw milk. Raw milk may contain heat-resistant bacterial spores of different genera such as *Bacillus* spp. and *Paenibacillus* spp. (Ralyea *et al*., 1998; Fromm and Boor, 2004; Huck *et al*., 2007b; Ranieri and

Boor, 2009) and serve as the major source of *Bacillus cereus* spores in pasteurized milk (Lin *et al*., 1998) whereas only *Paenibacillus* spp. can outgrowth at refrigeration temperatures and represents major factor for limited shelf-life (Huck *et al*., 2007a,b; Ranieri and Boor, 2009; Ranieri *et al*., 2009). Amongst psychrophilic, thermoduric and thermophilic organisms, psychrotrophs are the major contributor of total microbial flora in raw milk (98.1, 1.4 and 0.5%, respectively) and the corresponding figures in pasteurized milk are 53.0, 39.5 and 7.5%, respectively (Mahari and Gashe, 1990). *Bacillus cereus,* a gram-positive, aerobic or facultatively anaerobic spore-forming, motile, rod-shaped bacterium are thermally resistant and can survive milk pasteurization. Sutherland *et al*. (1996) denoted that based upon growth temperature strains of *B. cereus* can be divided into two groups as psychrotrophic (grow at 5°C and relative rapidly at 10°C) and mesophilic (fail to grow below 8°C and only grow slowly at 10°C). Pasteurization will actually induce spore germination by eliminating competing flora (Granum and Lund, 1997) and psychrotrophic spores will thus germinate and grow during refrigerated storage (Kramer and Gilbert, 1989).

Heat-treatment employed: Heat treatment applied to any food induced reduction in the number of organism present and the bactericidal effect is influenced by following factors (Hudson *et al*., 2003):

- Properties of the organism
- Variation in the heat susceptibility of different strains of the organism
- Physiological state of the organism prior to treatment
- Chemical composition of the food

Milk is generally subjected to High Temperature Short Time (HTST) pasteurization at 71°C/15 sec (Linton, 1982) or the Low Temperature Long Time (LTLT) pasteurization at 63°C/30 min (Teka, 1997) and the selection criteria should be based on the type and initial bacterial concentration in milk (Dumalisile *et al*., 2005; Hanson *et al*., 2005; Ranieri *et al*., 2009). Jayamanne and Samarajeewa (2010) noted that both HTST and LTLT pasteurization of milk were efficacious in destroying *L. monocytogenes*, when present in lower concentration (10^2 CFU mL^{-1}) but not at higher concentration (10^7 CFU mL^{-1}). Ranieri *et al.* (2009) encountered lower microbial population in pasteurized milk heated at 60ºC followed by a thermal treatment at 72.9°C/25 sec than those subjected to 85.2 ºC/25 sec. Pasteurization of milk at lower temperature (76.1°C vs. 79.4°C) induced significantly lower bacterial count (log CFU mL^{-1}) in pasteurized milk (1.39 vs. 1.58), which remained lower (3.74 vs. 4.82) even after 21 day post-processing storage at 6°C (Martin *et al*., 2012) due to residual natural antibacterial activity of lacto-peroxidase system. Complete destruction of the lacto-peroxidase enzyme in milk at 80°C/15 sec (Griffiths, 1986) but retention upto 90% activity at 72°C/2 min and 36% activity at 76°C/40 sec (Marin *et al*., 2003) have been reported.

Heating of milk at a temperature range of 72.9-85.2°C did not exhibit any variance in the lethal effect on the different isolated bacterial genera but the endospore-forming psychrotolerant bacteria present in milk grow more effectively in pasteurized milk (Ranieri *et al*., 2009) and has emerged as a key hurdle to extending product shelf life beyond 14 day (Meer *et al*., 1991; Fromm and Boor, 2004; Durak *et al*., 2006). Optimum temperature for spore generation is 65-75ºC (Coghill and Juffs, 1979) and an elevated pasteurization temperature of 80-90°C resulted in a decline in the shelf life of milk attributable to growth stimulate of spores, decline in the affectivity of antimicrobial compounds and production of growth factors (Vatne and Castberg, 1991). Milk

processed at 76°C had the lowest bacterial growth rate and longest shelf life and no improvement in the shelf life could be achieved at elevated pasteurization temperatures of 84.0 and 92.2°C as maximal bacterial growth was observed at 86.0°C (Simon and Hansen, 2001).

Storage conditions: Pasteurized milk has a shelf life of 2-20 days and is dependent on quality of raw milk, processing method, hygienic conditions during filling and maintenance of temperature during the entire cold chain (Rysstad and Kolstad, 2006). Janzen *et al*. (1982b) reported no significant effect of age of raw milk (0-6 days at 4.5°C) or duration of storage of the pasteurized milk (0-20 days at 4.5°C) on microbiological quality of pasteurized milk with a initial bacterial population of <1000 and <100 mL^{-1} coliform. Storage temperature has a greater influence on microbiological shelf life of pasteurized milk (Petrus *et al*., 2010) and refrigerated pasteurized milk has a shelf-life of approximately 10-20 day when stored at 6.1°C (Labuza, 1982). Burdova *et al*. (2002) denoted a decrement in shelf life of full cream pasteurized milk (31-11 days) and skimmed pasteurized milk (32.57-10.71 days) with an elevation in storage temperature from 4-10°C due to more enhanced proteolytic and lipolytic activities of psychrotrophic microorganisms after 2-3 days at 10ºC in contrast to 4-6 days 4ºC. Minimum bacterial growth at 4-7°C but 15 times more activity at an elevated temperature of 15°C was noted during storage of milk (Calderon *et al*., 2006). Schroder *et al*. (1982) noted a decline in shelf life of the commercial pasteurized milk from 13-5 days with an elevation in storage temperature from 5-11ºC. Zahar *et al*. (1996) observed that storage of pasteurized milk at a higher temperature (25°C) induced rapid enhancement in microbial growth (CFU mL^{-1}) after 20-24 h (10^7-10^8) in contrast to those held at lower temperature (7°C) after 5 days (10^5-10^6) or 7 days (10^7-10^8).

During refrigerated storage psychrotrophic strains appear as the most important organisms limiting the shelf life of pasteurized milk (Griffiths, 1992; Ternstrom *et al*., 1993) and even though mesophilic strains do not grow at low temperatures, they serve as a breeding ground for the colonization of other bacteria in biofilms (Kumar and Anand, 1998). Further, storage studies of pasteurized milk at 6 ºC indicated dominance of genus Bacillus (>85%) upto 7 days followed by a shift genus *Paenibacillus* (92%) upto 21 days (Ranieri *et al*., 2009).

Post pasteurization contaminants: Microbial spoilage of processed fluid milk is due to Gram (+) ve organisms surviving pasteurization temperatures or post-pasteurization contamination from Gram (-) ve bacteria (Ternstrom *et al*., 1993; Boor and Murphy, 2002). Detection of *L. monocytogenes*, *E. coli* (Hosein *et al*., 2008), *M. avium* subsp *paratuberculosis* (Paolicchi *et al*., 2012), *Pseudomonas* spp. (Samet-Bali *et al*., 2013) and bacterial phosphatase (Moshoeshoe and Olivier, 2012) in pasteurized milk was attributed to a faulty pasteurization process (Hosein *et al*., 2008; Samet-Bali *et al*., 2013), post-pasteurization contamination (Paolicchi *et al*., 2012; Moshoeshoe and Olivier, 2012) or improper post-pasteurization storage (Hosein *et al*., 2008). Mesophilic aerobic counts of pasteurized milk (7×10^5 CFU mL^{-1}) enhanced 2-4 fold increase as it left the pasteurizing unit due to its contamination with utensils used for holding and the plastic sheets used for packaging of pasteurized milk (Mahari and Gashe, 1990).

Post-pasteurization contaminations of milk products are mainly due to the filling machines (Dogan and Boor, 2003; Waak *et al*., 2002) and gaskets with biofilms (Austin and Bergeron, 1995). Biofilm formation on milk post-pasteurization contact surfaces (Chmielewski and Frank, 2003; Dogans and Boor, 2003) and isolation of *Bacillus cereus* from the post-pasteurization equipment

surfaces of a dairy processing unit indicated that the equipment surfaces can act as reservoirs for milk recontamination (Salustiano *et al*., 2009), thereby reducing the efficiency of pasteurization and sanitation treatments (Malek *et al*., 2012). Biofilms are matrix-enclosed bacterial population adherent to each other and/or to surfaces or interfaces (Costerton *et al*., 1995) and may have a bacterial count upto 10^8 CFU cm^{-2} (Marques *et al*., 2007). Biofilms are difficult to eradicate employing conventional cleaning and disinfection regimens due to their resistant phenotype (Simoes *et al*., 2010) and disinfectants do not penetrate the biofilm matrix (Simoes *et al*., 2006). Amongst different sanitizer chlorine (Trachoo and Frank, 2002) and ozone (Dosti *et al*., 2005) were effective for inactivating biofilm microflora. Nada *et al*. (2012) reported a decline in total viable count (3.11±0.30-2.18±0.54) in pasteurized milk and suggested additional investment for automated cleaning and disinfection system.

Process innovations for extended shelf life of pasteurized milk: An extension in the shelf-life of raw milk could be achieved with the addition of CO_2 (King and Mabbit, 1982; Rajagopal *et al*., 2005) and N_2 (Murray *et al*., 1983) or N_2 flushing through the headspace of the milk-containing vessel (Munsch-Alatossava *et al*., 2010) due to decline in bacterial count and reduction of proteolytic and lipolytic activities (King and Mabbit, 1982; Rajagopal *et al*., 2005).

The HTST pasteurization was ineffective in destroying spore-forming bacteria (Tomasula *et al*., 2011). Spores from milk can be removed employing Bactofugation or Microfiltration and latter technology is more efficient than former. Te Giffel and van der Horst (2003) reported greater removal of aerobic spores from milk employing Microfiltration (99.1-99.9%) than Bactofugation (94-98%). Microfiltration of milk for 10 min employing 0.8 μm membrane, capable of removing 5.91±0.05 log 10 spores/mL prior to HTST pasteurization (72°C/18.6 sec) is recommended and the pasteurized milk obtained showed no growth of spore-forming bacteria up to 7 days when stored at 4°C (Tomasula *et al*., 2011). Schmidt *et al*. (2012) reported that introduction of microfiltration induced a decline in microbial loads (5-6 log10 units to <1 CFU mL^{-1}) and spoilage occurred during storage (4-10°C) when microbial load reached >6 log10 CFU mL^{-1}.

CONCLUSION

Consumption of raw milk may pose health hazards as milk is highly prone to microbial growth and may harbors pathogens. Pasteurization is the widely adopted and most effective method to ensure completely destruction of all pathogenic and spoilage microorganisms, commonly found in milk and inactivation or reduction of other non pathogenic spoilage bacteria and certain undesirable enzymes to optimal levels to safeguard the food value of milk. Proper pasteurization, storage of processed milk at lower temperature and avoidance of post-pasteurization contamination are the keys to produce safe pasteurized milk. Good hygiene practices during milking and subsequent handling of milk are essential to reduce the risk of contamination on the farm and in the milk processing plant. Introduction of microfiltration prior to pasteurization is suggested to ensure complete removal of spores thereby enhancing the microbiological safety of pasteurized milk.

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