MICROBIAL QUALITY ASSESSMENT OF CURD PRODUCTION IN AN ORGANIZED DAIRY PLANT*

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Abstract

An investigation on microbial quality assessment of curd produced in a dairy plant was done by collecting 80 samples consisting of eight samples each of pooled raw buffalo milk, pasteurized milk, skim milk (SM), heat treated skim milk, starter culture, starter inoculated milk after 1 and 6 h of inoculation and fresh curd from 10 different batches of production. All the samples were subjected to estimation of total viable count (TVC), coliform count (CC), Escherichia coli count (ECC), psychrotrophic count (PC), faecal streptococcal count (FSC) and yeast and mould count (YMC). Highly significant ($p<0.01$) difference was noted between the TVC of skim milk and starter inoculated milk with that of fresh curd. Coliforms were present in raw, pasteurised and SM, but not in heat treated SM. Yeast and moulds were not detected in heat treated SM, but was observed in starter culture inoculated milk. Fresh curd samples had yeast and mould at the level of 10^3 cfu/g. The mean pH reduced from 6.8 ± 0.01 in heat treated SM to 3.6 ± 0.01 in fresh curd. The TVC of starter culture was 6.4 ± 0.21 $\log_{10}$ cfu/g. Coliforms and faecal streptococci were not detected in the starter culture. The critical control points of microbial contamination of curd was identified and among the CCP assessed (viz., air, water, hand wash, package material and equipment wash) the mean TVC and CC was highest in the hand washings of personnel involved in processing. E. coli was detected only in hand washings at the level of 0.05 ± 0.05 $\log_{10}$ cfu/ml. Faecal streptococci were detected both in hand washings and tap water samples. TVC was highest for cream separator and lowest for the packaging material. Faecal streptococci were detected in all the samples tested and highest count was observed in washings from storage can. Double jacketed vat had the highest coliform count. Air samples collected from the various areas of the production line after processing had high mean TVC and YMC.

Key words: *Curd, microbial quality, CCP*

Curd is an Indian fermented milk product known for its refreshing taste, palatability and therapeutic values and is generally consumed in its original form as an accompaniment to the meal. The quality of curd is influenced by various factors such as type of milk, initial microbial quality of raw milk, proper maintenance of temperature during the heat treatment, proper adherence to hygienic practices during post heat treatment handling and maintenance of cold chain during its transportation and storage.

During the manufacture of curd and its subsequent handling, various types of microbial contaminants gain entry from different sources such as milk, starter culture, water, air, utensils, personnel and others (Singh, 1978). Being a product which is consumed as such, the health hazards originating from consumption of contaminated curd are of immense public health significance. Very little information is available

* *Part of MVSc thesis submitted by the first author to the Kerala Agricultural University, Thrissur*

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regarding the quality assessment of curd production in an organized plant. Hence this work was undertaken to study the microbial quality of curd at various stages of production, processing, packaging and storage. The compiled information will help the producers to prevent the changes in organoleptic quality during refrigerated storage of curd. This in turn will help the traders in preventing economic loss due to product spoilage and also safeguard consumer health.

Materials and Methods

In the present study, from an organized dairy plant, a total of 80 samples which consisted of eight samples each of pooled raw buffalo milk, pasteurized milk, skim milk, heat treated skim milk, starter culture, starter inoculated cooled milk after 1 and 6 h of inoculation and fresh curd were collected from 10 different batches of curd production. Samples of raw milk were collected by random selection of two cans and by mixing the milk thoroughly using a plunger and transferring 250 ml sample from each can to a sterile conical flask. Pasteurised milk samples (250 ml) were collected immediately after pasteurization of milk in a sterile conical flask. Skim milk samples (250 ml) were collected from the skim milk sprout of the cream separator. Before collecting the sample, skim milk was allowed to run for a few minutes and then collected into a sterile conical flask. Heat treated skim milk samples were collected after heat treatment in a vat at 90°C for 15 to 20 min. All the samples were brought to the laboratory in an insulated container and tested immediately.

The various critical points of microbial contamination of curd was evaluated by collecting samples of water as per the procedures described by Indian Standards (IS:1622-1964) and hand washing of personnel in the processing line and packaging material (low density polyethylene sachets of 250 g capacity) following procedure described by Evancho *et al.* (2001). Swab contact method and rinse method described by Evancho *et al.* (2001) was followed to collect samples from the double jacketed vat, cream separator, pasteurization equipment and milk storage can for the estimation of bacterial count. Air sample was also collected before and after the process of pasteurization, cream separation, thermal treatment and at cold storage by sedimentation method as described by Evancho *et al.* (2001) and evaluated for TVC and YMC. To collect samples from pasteurizer and packaging equipment, one litre of water was used for cleaning the equipment before running the milk for pasteurization and then collected in a sterile flask. To collect samples from the double jacketed vat, a sterile cotton swab was moistened with 0.1% peptone water and excess diluent was removed by pressing the swab against the interior wall of the vial with a rotating motion. The swab head was rubbed slowly and thoroughly over 100 cm^2 surfaces, which was marked with a sterile aluminium template (10 x 10 cm^2 interval areas). The swab was rubbed three times reversing the direction between strokes. After swabbing, the head of swab was cut with sterile scissors and transferred into sterile flask containing 100 ml of 0.1% peptone water. The above washings were brought to the laboratory in an insulated container and were processed immediately. The sterile flask with washings from the vat was thoroughly agitated with the help of a cyclomixer at 8000 rpm for three minutes so as to extricate the bacteria attached to the cotton swab into the diluent. The pH of the milk and curd during various stages of production was measured using a digital pH meter (Elico LI 612 pH analyzer). The pH of the starter culture was also determined.

Ten fold serial dilutions were made up to 10^{-7} and selected dilutions of each sample were used for the estimation of various microbial loads per ml or per g of sample. All aseptic precautions were taken during collection and processing of milk samples.

The estimation of TVC (Mortan, 2001), CC (Kornacki and Johnson, 2001), ECC (IS.SP:18.1980), FSC (Anon, 1968), YMC (Beuchat and Consin, 2001) and PC (Cousin *et al.*, 2001) was done and the counts were expressed as $\log_{10}$ cfu/ml or g. The experiment was repeated 10 times and the data obtained in the study were subjected to statistical analysis (Rangaswamy, 1995).

Results and Discussion

Mean microbial count during various stages of production of curd: The mean microbial count during various stages of production of curd is given in Table. Raw milk collected for the production of curd had a mean TVC of

6.0 ± 0.22 $\log_{10}$ cfu/ml. After heat treatment, the count of skim milk was reduced to 2.6 ± 0.34 $\log_{10}$ cfu/ml (Table 1). Starter inoculated skim milk after 1 and 6 h had mean count at the level of 6.0 ± 0.27 $\log_{10}$ cfu/ml and 7.2 ± 0.05 $\log_{10}$ cfu/ml, respectively. Highly significant ($p<0.01$) difference was observed between the mean TVC of heat treated milk and that of curd (zero day). TVC of inoculated milk after 1 and 6 h had a highly significantly ($p<0.01$) difference from that of fresh curd.

The mean CC of raw milk was 1.50 ± 0.27 $\log_{10}$ cfu/ml and the mean CC of pasteurised milk was 0.91 ± 0.41 $\log_{10}$ cfu/ml. Significant ($p<0.05$) difference was observed in the mean count of coliforms in the milk after one hour of inoculation and fresh curd samples. Highly significant ($p<0.01$) difference was noted between the mean count in raw milk and skim milk with that of fresh curd. In heat treated skim milk, the organisms could not be detected. But fresh curd samples had a CC at the level of 0.12 ± 0.2 $\log_{10}$ cfu/g. Starter culture inoculated milk samples, at one and six hour of incubation had coliforms at the level of 0.98 ± 0.23 $\log_{10}$ cfu/ml and 0.75 ± 0.31 $\log_{10}$ cfu/g, respectively. The presence of coliforms in the inoculated milk after one hour of incubation suggests that the contamination of milk with the organism could have occurred from the storage cans, since the heat treated skim milk and starter culture were found to be free from coliforms.

The mean FSC of heat treated skim milk was 0.59 ± 0.38 $\log_{10}$ cfu/ml and the count increased to 1.4 ± 0.63 $\log_{10}$ cfu/ml and 1.6 ± 0.11 $\log_{10}$ cfu/g at one and six hour of starter inoculation. The count of fresh curd was 2.0 ± 0.40 $\log_{10}$ cfu/g. A highly significant ($p<0.01$) difference was observed between the mean FSC of pasteurized milk with that of 6 h starter inoculated milk and fresh curd samples. The mean FSC of heat treated skim milk was significantly ($p<0.01$) lower than that of inoculated milk after one and six hour of incubation. In the present study, it was observed that the FSC increased during the incubation of inoculated milk and in fresh curd samples.

Heat treated skim milk was free of yeast and mould. But after 1 h of starter inoculation, the count was at the level of 3.6 ± 0.27 $\log_{10}$ cfu/ml and the count of fresh curd samples was 3.9 ± 0.11 $\log_{10}$ cfu/g. Highly significant ($p<0.01$) increase in the count was observed between fresh curd, starter inoculated milk after one and six hour with that of raw milk, pasteurized milk and skim milk samples. Yeast and moulds could not be detected in heat treated skim milk, but the organisms were found in starter inoculated milk at one and six hour of incubation. Fresh curd samples had yeast and mould at the level of $\log_{10}$ cfu/g. Starter culture inoculated samples had yeast and mould which indicate that the organisms might be present in the starter culture. Growth of yeast cells in curd is one of the major problems of maintaining starter cultures in pure form under household conditions of preparation of curd. Due to their ability to tolerate acidic environment, yeasts can multiply in curd producing gassiness and flavour defects associated with lipolysis of milk fat, if the product is held too long at warm ambient temperatures. However, they grow slowly in curd and their numbers will be low, unless the inoculum is transferred from old and yeasty curd (Yadav *et al.*, 1993).

Changes in mean pH during production of curd: Raw milk had a pH of 6.6 ± 0.01. Pasteurised and skim milk had pH of 6.7± 0.03 and 6.6 ± 0.03. The pH reduced from 6.8 ± 0.01 in heat treated skim milk to 3.6 ± 0.01 in fresh curd samples. Highly signifiant ($p<0.01$) difference in mean pH was observed between raw milk, heat treated skim milk, inoculated skim milk after one and six hour and fresh curd samples. The findings of the present study are in agreement with the report of Samolada *et al.* (1998) and Birollo *et al.* (2001).

Starter Culture: The starter culture had a mean pH of 3.8 ± 0.01 and the mean TVC was 6.4 ± 0.21 $\log_{10}$ cfu/g. The mean YMC of the starter culture was 3.7 ± 0.21 $\log_{10}$ cfu/g. Aneja *et al.* (2002) stated that yeasts and moulds in starter culture should be <10 cfu/g and a pH of 4.2 is desirable. The YMC of the starter culture in the present study was 3.7 ± 0.21 $\log_{10}$ cfu/g which might have led to rapid fermentation and lowering of the pH of the product of a faster rate and high acidity of the curd can lead to development of wheying off in the curd. The presence of yeasts can also lead to elimination of flavour producing organisms in curd. Coliforms and faecal streptococci were not detected in the starter culture.

Air : The mean TVC of air samples from the area of pasteurisation, cream separation and

thermal treatment, before and after processing were 27.6 ± 1.52, 20.4 ± 2.8 and 70.5 ± 2.3 and 69 ± 1.75, 45.2 ± 4.5 and 96.3 ± 1.65 $\log_{10}$ cfu/ft²/min, respectively (Table). The mean TVC of air samples in the cold room was 10 ± 1.3 $\log_{10}$ cfu/ft²/min. The counts obtained in the present study from various processing areas were well above the standards prescribed by APHA (Hickley *et al.*, 1992). Salustiano *et al.* (2003) also reported that there was considerable increase in the number of indicative organisms during the processing of various dairy products. The yeast and mould count of air samples was maximum in the cold room (19 ± 1.64 $\log_{10}$ cfu/ft²/min). The findings of the present study differed from that of Rajarajan and Nareshkumar (2003) who recorded higher yeast and mould count in the vat section of the dairy plant. Yeast and mould are ubiquitous in the environment and many are psychrotrophic in nature and they are the main spoilage organisms in the production of fermented milk products like curd.

Air samples from the thermal treatment room had the highest mean TVC. The study revealed that microflora of air may contribute to the contamination of product in the processing room.

Water : The mean TVC of water samples from storage tank, tap and hand washings was 3.5 ± 0.10, 4.0 ± 0.25 and 4.5 ± 0.24 $\log_{10}$ cfu/ml as shown in Table. Among the samples, hand washing had the highest mean total viable count whereas storage tank water samples had the lowest count. Water samples from the storage tank did not reveal the presence of coliforms. The samples from the tap water and hand washings had coliforms and the mean counts were 1.0 ± 0.06 and 1.2 ± 0.38 $\log_{10}$ cfu/ml, respectively. *Escherichia coli* were detected only in samples of hand washings (0.05 ± 0.05 $\log_{10}$ cfu/ml). FSC was detected in both tap water and hand washings and the corresponding counts were 1.0 ± 0.19 and 0.49 ± 0.2 $\log_{10}$ cfu/ml. The FSC of tap water was higher than that prescribed by Anon (2001). The high bacterial count in the water samples indicates its unwholesomeness and its role in the contamination of the product.

Hand washings of personnel involved in the production of curd had coliforms as well as *E. coli*. The detection of *E. coli* and faecal streptococci in the hand wash of the workers indicated poor personnel hygiene. Therefore, it may be inferred that these employees might also contribute to the contamination of the curd samples with spoilage and pathogenic organism particularly of intestinal origin.

Equipments: The hygienic status of the equipments used in the processing plant was also evaluated. The mean bacterial count per cm²/ml of equipment is shown in Table. The mean TVC of processing equipments such as pasteuriser, cream separator, double jacketed vat, storage can and packaging material ranged from 3.2 ± 0.18 to 5.0 ± 0.07 $\log_{10}$ cfu/cm² or per ml. Among the equipments, cream separator had the highest TVC and the lowest count was observed in the packaging material. Double jacketed vat had the highest CC followed by storage can. The CC varied between 0.31 ± 0.26 and 1.2 ± 0.16 $\log_{10}$ cfu/cm² or per ml. *E. coli* was absent in all the samples tested. Coliforms and *E. coli* were not detected in any of the packaging material samples. Faecal streptococci were also detected in all the samples tested. Among the equipments involved in the production of curd, the cream separator had a significant role in contributing to total viable count of the product, followed by the double jacketed vat. But the mean TVC of the storage can is important because thermal treatment at the temperature of 90°C for 15 min destroys most of the organisms and the important source of contamination is the storage can on to which milk is transferred for cooling, addition of starter culture and incubation. Packaging material were free of coliforms, but had faecal streptococci at the level of 0.1 ± 0.10 $\log_{10}$ cfu/ml. The TVC in 23% samples of the present study had counts similar to that reported by Singh (1978). Coliforms were also present in dahi containers. Counts of pasteurizer, cream separator and storage can were very much higher than that reported by Yadav *et al.* (1993).

Assessment of critical control points in the production line of curd identified the lack of good quality raw milk, potable water, unsatisfactory personnel hygiene of the workers, improper sanitary conditions of the processing equipments and high bacterial and fungal count of air samples at various sites in the plant (Flow chart). Spoilage under refrigerated storage might be attributed to the high initial microbial load of milk, contamination of the starter culture and contamination

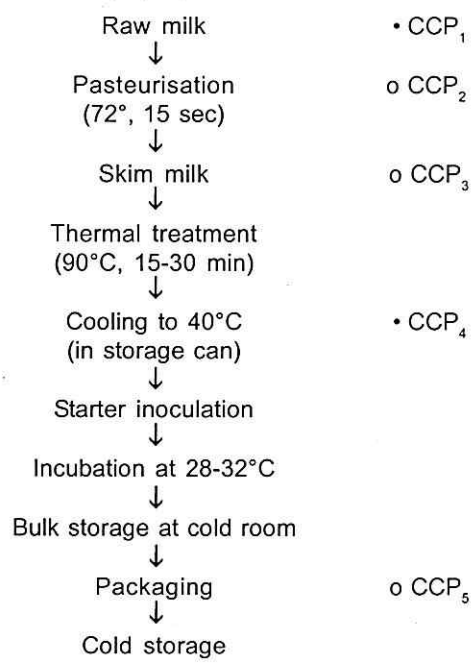
from the processing environment. Curd, being a product consumed as such, an overall improvement in the production, packing, storage and distribution of the product is of great significance to safeguard consumer health. Identification of the critical control points will help the manufacturers to take appropriate remedial measures to curtail the chances of microbial contamination of curd and will help the retailers in preventing economic loss due to product spoilage. The study also reflects the importance of implementing principles of HACCP, which helps to prioritise process steps focusing on hazards that can be reduced or eliminated.

Table. Microbial counts (log_{10}cfu/ml or/g) during various processing steps in a dairy plant

Characteristics	Microbial counts(log_{10}cfu/ml or/g)			
	TVC	CC	FSC	YMC
Raw milk	6.0 ± 0.22^{a}	1.50 ± 0.27^{a}	2.1 ± 0.69^{a}	1.8 ± 0.15^{a}
Pasteurised milk	5.2 ± 0.14^{b}	0.91 ± 0.41^{ab}	0.34 ± 0.21^{b}	1.4 ± 0.16^{ab}
SM	4.7 ± 0.29^{bc}	1.1 ± 0.36^{bc}	1.6 ± 0.42^{ac}	1.3 ± 0.11^{abc}
Heat treated SM	2.6 ± 0.34^{d}	ND	0.59 ± 0.38^{abcd}	ND
Starter inoculated SM after 1 h	6.0 ± 0.27^{abc}	0.98 ± 0.23^{abce}	1.4 ± 0.63^{abcde}	3.6 ± 0.27^{d}
Starter inoculated SM after 6 h	7.2 ± 0.05^{e}	0.75 ± 0.31^{bef}	1.6 ± 0.11^{acef}	3.7 ± 0.22^{de}
Curd (zero day)	8.3 ± 0.16^{f}	0.12 ± 0.12^{f}	2.0 ± 0.40^{acef}	3.9 ± 0.11^{de}
Air samples	Mean ± SE (cfu/ft²/min)			
Pasteurisation room Before After	 27.6 ± 1.52 69 ± 1.75	 – –	 – –	 12.7 ±1.2 13.5 ± 0.93
Cream separation room Before After	 20.4 ± 2.84 45.2 ± 4.5	 – –	 – –	 4.8 ± 1.2 7.83 ± 1.5
Thermal treatment room Before After	 70.5 ± 2.3 96.3 ± 1.6	 – –	 – –	 4 ±0.35 12.3 ± 0.92
Cold storage room	10 ± 1.3	–	–	19 ± 1.64
Water samples	Bacterial count (log_{10}cfu/ml)			
Storage tank	3.50 ± 0.10	–	–	–
Tap water	4.01 ± 0.25	1.03 ± 0.06	1.02 ± 0.19	–
Hand wash of personnel	4.53 ± 0.24	1.19 ± 0.38	0.49 ± 0.23	–
Pasteuriser	4.49 ± 0.22	0.31 ± 0.26	1.01 ± 0.42	–
Cream separator	4.95 ± 0.07	0.36 ± 0.24	1.03 ± 0.32	–
Double jacketed vat (log_{10}cfu/cm²)	4.53 ± 0.15	1.16 ± 0.16	0.46 ± 0.21	–
Storage can	3.92 ± 0.18	1.02 ± 0.09	1.21 ± 0.05	–
Packaging material	3.20 ± 0.18	-	0.10 ± 0.10	–

Values with different superscripts in a column differ significantly ($P<0.01$). Each value is a mean of 10 replicates. TVC: total viable count, CC: Coliform count, FSC: Faecal streptococcal count, YMC: yeast and mould count

Flow chart. Critical control points in the preparation of curd



• Potential site for major contamination

o Potential site for minor contamination

CCP_1 - Use raw milk with good microbial quality

CCP_2 - Ensure proper pasteurization by adhering on to the recommended time-temperature combination

CCP_3 - Periodic cleaning and sanitization of equipments to minimize the bacterial load and strict adherence to personnel hygiene

CCP_4 - Use of properly cleaned and dried cans for storage

CCP_5 - Use of good quality, sterile packaging material

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