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Investigating the Source and Cause of Contamination in The Formation of Blown Packets in Curd Sold in Markets.

Anuraj V.R , K.G.Madhavankutty & Anoop.M[1*]

Central Products dairy, Punnappra, Alappuzha

***KVM College of science and Technology, Kokkothamangalam PO, Cherthala.**

Abstract

The present work holds an efficient attempt to isolate and characterize microorganisms causing excessive gas production and consequent blown packets in the curd obtained in markets. The work figured out source of contamination of the causative organisms and proposed remedial measures based on findings. An efficient and wholehearted effort has been made to include and cover almost all sources that may lead to contamination of the curd .The findings pointed out as air borne contamination as the most common source for the entry of variety of micro organisms. Yeast was found to be the major contaminant upon the analysis of blown packet contaminants.

Keywords:-Curd,Blown Packet,Contamination,Organisms.

Introduction

The importance of fermented food products in our daily diet has been recognized and is being widely accepted by modern man. Indian curd, known as dahi, is a well known fermented food product consumed by large sections of populations throughout the country. Indigenous preparation of dahi or curd is being carried out with the help of homely propagated starter cultures. Sterile procedures cannot be expected for home made products. The micro flora may vary and are quite ignored by the producers. The ability of a started culture to perform its function during manufacture of fermented dairy products depends on its purity and activity.

Yeast appears to be the most common contaminant in dahi.These organisms get entry into the product from atmosphere, utensils and human hands under natural conditions. Due to their ability to tolerate acidic environments, yeast can multiply in dahi producing gassiness and flavor defects associated with lipolysis of milk fat, if the product is held too long for warm ambient temperatures.

Airborne contamination is the most common source for the entry of a variety of microorganisms into packaging materials. Microbial contamination may also occur through contaminated water supplies. Dairy equipments and utensils with which milk products come into contact are the main source of contamination in these products.

Now a days curd is being manufactured and distributed in polythene covers.Polythene packing seems to be a new method practiced in India. In polythene pouches there are no problems in cleaning and sterilization and risk of adulteration is far less than with the glass bottles and cans. The blown packet was common in curd manufactured by

many companies available in the market. Instead of MILMA,the other blown packets available in markets were PENTA and SURYA.These three brands were analyzed for determination of causative organisms with those cause gassiness.

Materials and Methods

Collection of Samples

Skimmed milk, Milk powder, Freeze dried culture, sterilized milk, inoculated milk, curd packet were collected as raw materials from milma,Alappuzha.Blown packet from market, swabs of equipment and packing material (LCPE film),air samples of different stages, samples of cultured milk cut opened from the non weight conforming packets and being repacked were collected.

Culture methods for different microorganisms

Different media are prepared depending on the suspected species of micro organisms in the sample. Plate Count Agar is used for enumeration of micro organisms .Potato dextrose agar is used for cultivating yeast and molds and violate red bile agar is used for coliforms.Special attention has been given to condition of incubations as many different microbes possesses different optimum conditions of growth.

Standard Plate Count

SPC agar media was prepared and an aliquot of 0.1 ml is poured under strict aseptic conditions.The plates after solidification were kept in incubator at 37ºC for 24-48 hours. The bacteria colonies were counted .In order to calculate the number of viable bacteria /gm or ml present .the number of colonies was multiplied by dilution factor.

Presumptive coliform count

Violet red bile agar media was prepared and sterilized. The plates were labeled with many different dilutions .0.1ml of sample is taken in every plates and VRB medium was poured and allowed to solidify. A second layer was added over first layer and the plates were inverted and incubated at 37ºC for 24-48 hours.

Yeast and Mould Count

Petri dish already inoculated with PDA medium were then spread plated .The development of colonies within 5 days at 22 ºC should be considered as a positive evidence of molds and yeasts.

Thermoduric Spore Count

The test determines spores which are heat resistant .A definite measurement of sample were taken in a tube and another tube was occupied with same amount of water. The sample is heated to 80 ºC for 10 minutes. The samples were cooled under a stream of cold water .0.1ml of the samples were directly added into sterile petriplates and sterilized plate count agar plates separately. The plates were incubated further at 37 ºC for 48 hours.

Microbial Analysis of the Samples

Organoleptic test

General appearance that includes phase separation, gas bubbles, foam, granulation, foreign matter, odour and aroma, color, body and texture, taste and flavor were analyzed directly in milma dairy alappuzha.

Gram Stain

Gram stain was performed in accordance with standard protocol.

Negative Staining

Demonstration of actual dimension of living organism and the presence of surface structure like capsule were performed with negative stain procedures under standard protocols.

Micrometry

The size of the micro organism was measured by equipping the microscope with an ocular micrometer which was calibrated against a stage micrometer. The ocular division in micrometer was calculated as number of divisions of stage micrometer/number of divisions of ocular micrometer. The procedure was repeated with high power objective 40 x and oil immersion objective 100x.

Measurement of micro organism using ocular micrometer

The calibration factor for one ocular micrometer was determined for low, high and oil immersion objective .The stage micrometer was removed from the stage and a permanent slide was placed under the objective. The number of outer divisions occupied by the microorganisms was calculated. The size of the microorganism were calculated as the size of number of ocular division*calibration factor of the objective used.

Titrable acidity test

The curd was mixed thoroughly and weighed. An equal volume of freshly boiled and cooled water was added followed by addition of 1 ml of phenolphthalein indicator solution. The contents were titrated against standard sodium hydroxide solution .vigorous stir may be followed to avoid the standard titration time to complete titration not to exceed 20 seconds. The titrable acidity was calculated as volume of standard sodium hydroxide required for titration*0.9/volume of milk in ml.

Sugar Fermentation test

The test was performed with lactose, glucose; maltose and sucrose broths. The culture was inoculated and incubated at 37 ^{o}for 24-48 hours.

Air Count

Standard Plate count and PDA plates were used for air counts. The air samples were taken from different stages of curd production .Petri dishes were commonly 100mm size and the time taken for exposure was 12 minutes.

The colonies were counted as number of organism per sq.ft/min=(144/3.14*r^2*T)*no. of colonies where r was designated as radius of bottom of Petri dishes in inches and T is time of exposure in minutes.

Curd Manufacture

The curd is manufactured as per standard protocol in Milma dairy industries.

Results

Test results of raw materials

Organoleptic tests were found to be good in both in skimmed milk and working culture samples. Analysis of the raw materials, skimmed milk (table 1), milk powder (table 2) and working culture sample (table 3) have desired microbial quality. In working culture sample (table 3) only the desired culture organisms were present.

Table 1:-Cfu/ml in skimmed milk calculated on SPC, VRB and PDA

Media	Dilution	Count	CFU/ml
SPC	10^{-1} 10^{-2} 10^{-3}	62.3 6.1 Nil	623 610 nil
VRB	10^{-1} 10^{-2} 10^{-3}	7 0.5 nil	70 50 nil
PDA	10^{-1}	nil	nil
Spore Count	10^{-1}	2	20

Table 2:- Cfu/ml in milk powder calculated on SPC, VRB and PDA

Media	Dilution	Count	CFU/ml
SPC	10^{-1} 10^{-2}	40 3.24	400 324
VRB	10^{-1}	nil	nil
PDA	10^{-1}	nil	nil
Spore Count	10^{-1}	6	60

Table 3:- Colony count of working culture samples Lactobacillus bulgaricus and Streptococcus thermophilus

media	dilution	count	Cfu/ml
SPC	10^{-1} 10^{-2} 10^{-3}	TNTC TNTC TNTC	TNTC TNTC TNTC
VRB	10^{-1}	Nil	Nil
PDA	10^{-1}	Nil	Nil

Analysis of Sterilized milk and inoculated milk samples

Sterilized milk samples after cooling to inoculation temperature presented the absence of microbes (table 4) that revealed a perfect sterilization process. Analysis of the inoculated milk after 30 minutes of incubation period showed a considerable number of desired culture organisms and complete absence of (table 5) coli forms, yeasts, molds and other micro organisms.

Table 4:-Analysis of sterilized skimmed milk after cooling to inoculation temperature

media	dilution	count	Cfu/ml
SPC	Direct 10^{-1}	Nil Nil	Nil Nil
PDA	10^{-1}	Nil	Nil

Table 5:-Analysis of inoculated milk after 30 minutes of incubation

Media	dilution	count	Cfu/ml
SPC	10^{-1} 10^{-2}	TNTC 198	TNTC 19800
VRB	10^{-1}	Nil	Nil
PDA	10^{-1}	Nil	Nil

Product Analysis

Before and after cooling analysis of the product presented enumerable count of culture organisms and the absence of other microbes (table 6 & 7).The gassiness nature were found to be negative in all the cases (Table 8,9,10).

Table 6:- Analysis of the finished product before cooling

Media	Dilution	Count	Cfu/ml
SPC	10^{-1} 10^{-2} 10^{-3}	TNTC TNTC TNTC	TNTC TNTC TNTC
VRB	10^{-1}	NIL	NIL
PDA	10^{-1}	NIL	NIL

Table 7:-Analysis of the product after cooling from cold store

media	dilution	count	CFU/ml
SPC	10^{-1} 10^{-4} 10^{-5}	TNTC TNTC 316	TNTC TNTC 31600000
VRB	10^{-1}	NIL	NIL
PDA	10^{-1}	NIL	NIL
Spore Count	10^{-1}	NIL	NIL

Table 8:-Cfu/ml in 24 hour incubation in 42ºC

Media	Dilution	count	Cfu/ml
SPC	10^{-3}	TNTC	TNTC
VRB	10^{-1}	NIL	NIL
PDA	10^{-1}	NIL	NIL

Table 9:-cfu/ml after 3 days of incubation in 27ºC

Media	Dilution	count	Cfu/ml
SPC	10^{-2}	TNTC	TNTC
VRB	10^{-1}	NIL	NIL
PDA	10^{-1}	NIL	NIL

Table 10:-cfu/ml after 5 days of incubation in 27ºC

Media	Dilution	count	Cfu/ml
SPC	10^{-2}	TNTC	TNTC
VRB	10^{-1}	NIL	NIL
PDA	10^{-1}	NIL	NIL

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Analysis of the blown packets from market

The three brands showed the presence of enumerable number of microorganisms in plate count and potato dextrose agar .It presented a complete absence of coliforms (Table 11,12,13).These three brands posess frothiness ,increased acidity ,alcoholic smell and taste.The presence of gram positive oval and cylindrical shaped organisms observed instead of the culture organisms.

Table 11:-CFU/ml on analysis of Blown "Milma"brand curd

Media	Dilution	Count	CFU/ml
PCA	10^{-4} 10^{-5} 10^{-6} 10^{-7}	TNTC TNTC 296 39	TNTC TNTC 296000000 390000000
PDA	10^{-4} 10^{-5} 10^{-6} 10^{-7}	TNTC TNTC 151 15	TNTC TNTC 151000000 15000000
VRB	10^{-1}	NIL	NIL
SPORE COUNT	10^{-1}	NIL	NIL

Table 12:-Blown PENTA brand curd

Media	Dilution	Count	CFU/ml
PCA	10^{-2}	TNTC	TNTC
PDA	10^{-2}	TNTC	TNTC
VRB	10^{-2}	NIL	NIL
SPORE COUNT	10^{-1}	NIL	NIL

Table13:-Blown SURYA brand curd

Media	Dilution	Count	CFU/ml
PCA	10^{-2}	TNTC	TNTC
PDA	10^{-2}	TNTC	TNTC
VRB	10^{-2}	NIL	NIL
SPORE COUNT	10^{-1}	NIL	NIL

Isolation of the Microorganisms

Different agar medium have been used to isolate the doubtful microorganisms. The culture consists only bacteria. The presence of yeast and molds in the potato dextrose agar medium were examined. Simple staining revealed the presence of two types of unicellular organisms which were distinguished as oval and cylindrical type. Gram stain has confirmed the presence of Gram positive and Gram negative bacteria. The micrometry studies have shown oval shaped bacteria which are 3-5µm in diameter. The length of the organisms are found to be 5 µm-40 µm.Oval type

organisms fermented sucrose ,lactose and glucose and not maltose where as cylindrical organisms ferment sucrose ,glucose and maltose and no lactose was fermented (table 14).

Table 14:-Sugar fermentations

organism	sucrose	lactose	glucose	maltose
Oval type	Gas & acid	Gas & acid	Gas & acid	nil
Cylindrical type	Gas & acid	nil	Gas & acid	Gas & acid

Analyzing the source of contaminations.

Two air samples and cultured milk sample collected during repacking indicated as source of contamination. The air samples collected from above the sachet filling machine provided doubt ful results .The growth in yeast and mould was more than the permitted limit.

Table 15:-Colony counts on 4 day Analysis of Air samples above steam kettles

media	Time in minutes	Day 1	Day2	Day3	Day4
PCA PDA	12 12	16 6.5	14 8	15 7	12 3.5

Table 16:- Colony count on Analysis of Air samples from storage tanks

media	Time in minutes	count
PCA	5	NIL
PDA	5	NIL

Table 17:- Colony counts on 4 day Analysis of Air samples above sachet filling machine

media	Time in minutes	Day 1	Day2	Day3	Day4
PCA PDA	12 12	64 9	82 12	92 10	108 12

Table 18: Colony counts on hot water used for sterilization of equipments

media	Dilution	Count	CFU/ml
SPC	10^{-1} 10^{-2}	2 NIL	20 NIL
PDA	10^{-1} 10^{-2}	NIL NIL	NIL NIL

Table 19:-Colony counts on pipeline swab

media	Dilution	Count	CFU/ml
PCA	10^{-1}	2	20
PDA	10^{-1}	NIL	NIL

Table 20:-colony counts on steam kettle swab

media	Dilution	Count	CFU/ml
PCA	10^{-1}	NIL	NIL
PDA	10^{-1}	NIL	NIL

Table 21:-Colony count on paper swab

media	Dilution	Count	CFU/ml
PCA	10^{-1}	NIL	NIL
PDA	10^{-1}	NIL	NIL

Table 22:-Colony count on air contaminated cultured milk samples taken from plastic buckets in which the packets were cut opened and collected before repacking

media	Dilution	Count	CFU/ml	organisms
PCA	10^{-1} 10^{-2}	TNTC TNTC	TNTC TNTC	Culture organisms
PDA	10^{-2} 10^{-3}	8 1	800 1000	yeasts

Table 23:-colony counts on sample of blown packets

media	Dilution	Count	CFU/ml	organisms
PCA	10^{-2}	TNTC	TNTC	Culture organisms
PDA	10^{-2}	TNTC	TNTC	yeasts

Discussion

On analysis of the raw materials, it has been found that the raw materials were having desired microbiological quality. It has also been observed that the raw materials do not contribute any microbes that may cause formation of blown packets. Analysis of the sterilized milk sample presented that the sterilization process were perfect. There was no incidence of the presence of any microorganisms in the sterilized milk.

The analysis of the inoculated milk showed the presence of enumerable amount of desired started culture organisms. It is sure that started culture has been prepared properly and it doesn't add any undesirable organisms to the product. Analysis of the curd samples showed the absence of any undesirable organisms. Samples incubated at different incubation temperatures and different incubation periods has shown an acidity increase in level which was more than 1.1.According to IDF (1981), the normal range of acidity is 0.6-1.1 in fermented milks.

Three brands of blown curd packets were collected from market. Out of these, blown packets were frequently observed for the brand SURYA and PENTA .In case of MILMA, only one packet could be collected in a span of three days .The microbial analysis made it clear that yeasts are the organisms that contaminated the curd samples and caused gas production as well as spoilages.

Yadav et al ,1993 reported that the gassiness is primarily due to the production of CO_2 particularly by Leuconostocs during citric acid fermentation. Quite frequently, the rate and timing of gas production may be such that the gas bubbles and even gas ripes appear in the culture.Coliforms contaminated as well as certain lactose fermenting yeast may also cause gassiness. The ovular type of yeasts was found to cause lactose, glucose and galactose fermentations together with gas formations .It is presume that these organisms belongs to the genera Saccharomyces .

The organisms, if contaminated at any stage of production of the curd, can grow after the lactose fermenting bacteria in the culture produce the simple sugars like glucose and galactose and lactic acid. The acidity of final product incubation and cooling usually ranges 0.9-1.2%.

The SURYA and PENTA brands showed the presence of long cylindrical shaped yeast and it may belong to Candida as per further observations. They were found to cause gas formation at low acidity of the curd which is predicted that they might have utilized glucose present in curd as a result of bacterial fermentation to produce gas.

A sincere attempt has been made to include all sources of contaminations to the curd with yeast during the production processes. From the analysis of the samples, collected from dairy only three samples indicated the source of contamination. They are two air samples and the cultured milk samples collected during repacking .Air borne

contamination is the most common source for the entry of a variety of microorganisms in to packing materials.Heldman 1974, explained the concept that packing areas possesses heavy bacterial count $2.8*10^1$,$3.1*10^1$,$2.1*10^1$ per five square feet respectively.

Conclusion

The aim of this study was an attempt to trace out the cause and solution to the problem of spoilage of curd due the development of gas and undesirable flavor change. A short survey conducted at the market has revealed that several brands of curds undergoes spoilage as mentioned while the curd marketed by MILMA has comparatively lower level of such spoilage ,the other brands are found to cause usual spoilage problems. A number of samples were collected from the market and the cause was examined.

The causative organisms identified for the spoilage of curd due to gassiness and flavor change was found to be yeast in all the cases. The types of yeasts differed in different brands of products, but no other causative species could be detected. As the results of the studies pointed out that the source of contamination was definitely the air in the environment and as a remedial measure, the repacking of the cultured milk may be avoided as it leads to slight unsterility in the production processes.

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