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Research Article

COMPARATIVE STUDY OF MICROBIOLOGICAL AND CHEMICAL CONTENT OF SOME FERMENTED MILK PRODUCTS FROM NASIK REGION

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A B S T R A C T

A heterogeneous population of mesophilic, thermoduric, thermophilic, psychrophilic and psychrotrophic organisms constitute the microflora of milk and fermented milk products. These organisms grow and multiply in dairy products producing one or more defects due to growth and cause organoleptically detectable undesirable changes. The majority of undesirable changes includes off-flavors, change in colour, ropiness, rancid odours, proteolysis resulting in bitter flavour etc. The rate at which defect develops will depend on initial number of organisms present, rate at which organisms grow at holding temperature used and an ability of organisms to cause the organoleptically detectable change in product. Dairy products are mostly preserved by storage at refrigeration temperature by wholesale dealers and retailers but at the refrigeration temperature, milk and milk products allow the growth of psychrotrophic organisms. To study microbiota and chemical composition of these fermented milk products, five samples of curdand nine samples of shrikhand were collected in different seasons from nine different dairies of Nasik region of Maharashtra in India. Enumeration of bacteria from these samples showed the presence of mesophilic as well as psychrotrophic bacteria in each of the sample and there was a considerable variation in mesophilic and psychrotrophic bacterial content amongst the samples. Chemical examination was carried out to decide the nutritive quality of fermented milk products. The chemical examination of fermented milk products includes determination of pH, fat, total solids, SNF, proteins, sucrose and ash contents. Chemical contents of all fermented milk products were within the range mentioned by Bureau of Indian Standards.

INTRODUCTION

The problem of maintaining good shelf life or achieving extended keeping quality has always been one of great concern to milk processors. Keeping quality or shelf life of perishable dairy products depends on the initial quality of the products, on care and techniques employed in processing and distribution and on storage temperatures. Temperature control is generally considered the key to extended shelf life for dairy products. Raw milk serves as an ideal medium for the growth of a broadspectrum of bacteria due to its high nutritional value and can beeasily contaminated mainly with bacteria during handling, transportation and processing (Yuan *et al*.,2017). The growth of bacteria depends on the environment which includes substrate microenvironment and the outside conditions (of which temperature is most important).The microorganisms growing optimally at 30° to 32°C are designated as 'mesophilic'. Psychrophiles are those bacteria which have an optimal temperature for growth at about 15°C or lower, a maximal temperature for a growth at about 20°C or lower and a minimal temperature for a growth at 0°C or lower. Psychrotrophic bacteria grows at temperature such as 7°C or lower but had higher optimal growth temperature.Most psychrotrophic bacteria can form biofilms on various milk storage and processing equipment, which serve as persistent sources of microbial contamination due to their biotransfer potential(Lei *et al*.,2019). The microbial changes in milk products are produced as a result of fermentation of one or more of the milk constituents by the causative micro-organisms. These milk fermentations may be of normal or abnormal type. For example, curdling of milk that occurs due to lactic acid production is normal fermentation. The changes like gassiness, ropiness, proteolysis, sweet curdling, lipolysis, etc are abnormal fermentations. The microorganisms which may gain entry into milk or its products can multiply and bring about either spoilage of these products or render them unsafe due to potential health hazards. The extent of contamination and subsequent microbial multiplication directly determine the microbiological quality of the product.

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Objectives of the study

1. To find out the chemical composition of fermented milk products collected from different dairies of Nasik Region.
2. To find out the microbial content of fermented milk products collected from different dairies of Nasik Region.

MATERIALS AND METHODS

Collection and coding of Samples

In present work samples constituting curd and shrikhand were collected in different seasons from nine different dairies in Nasik region of Maharashtra and were appropriately coded which includes five samples of curd namely WSC, RSC, SGC, SAtC and SSC and nine samples of shrikhand namely WTS, WShS, WAtS, WSS, WVS, RTS, RRS, RSS and RVS.

Chemical Examination of samples

Determination of pH: The pH value of all samples was determined electrometrically with a pH meter (Toshcon Industries Private Ltd.). The pH meter was standardized with standard buffer solutions of pH 4 and pH 7 before use.

Determination of Fat Content: Fat content of all the samples was determined by Gerber's method by using Gerber's butyrometer(FAO, 1977).

Determination of Total Solids: Total solids present in samples were determined by Gravimetric method using thermostatically controlled oven set at 100+1^{o}C(FAO, 1977).9. All samples were analyzed in triplicates.Solids not fat (SNF) content was determined using the following formula:SNF content (%) = TS (%) - Fat (%)

Determination of Sucrose Content: Sucrose content was determined by Lane-Eynon (1923) Method(A.O.A.C., 2005).

Determination of Protein Percentage: Percentage of proteins present in all samples were determined by Kjeldahl's method (A.O.A.C. 2000). All samples were analyzed in triplicates

Determination of Total Ash: The total ash was determined gravimetrically by igniting the dried milk samples in a muffle furnace in which the temperature was slowly raised to 550^{0}C. The sample was ignited until carbon (black color) disappears or until the ash residue becomes white(Richardson, G. H.1985).

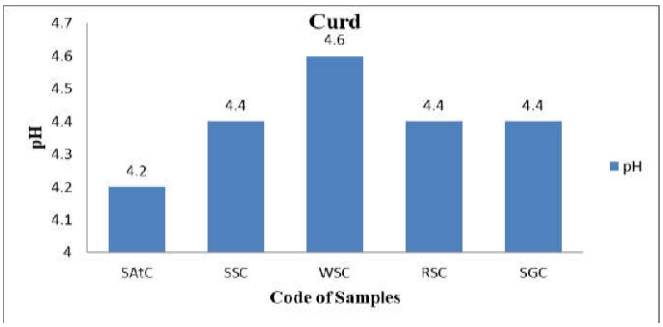
Enumeration of Psychrotrophic and Mesophilic bacteria Fromcurd and Shrikhand

Serial dilution technique was used for the enumeration of psychrotrophic and mesophilic bacteria present in samples as described by standard methods of the American Public Health Association (Vanderzant *et al*., 1992). Samples were diluted (10^{-1} to 10^{-10}) using normal saline as a diluent and 0.1 ml of each diluted sample was plated on sterile 10% milk agar plate. Pour plate technique was used for plating and plates were incubated at 30^{o}C for 24 hours and at 7^{o}C for 10 days for growth of mesophilic and psychrotrophic bacteria respectively. After incubation appropriate plates were selected and results were expressed as $\log_{10}$ cfu/ml.

RESULTS AND DISCUSSION

Chemical Examination of samples

Curd: The chemical examination of curd samples includes determination of pH, fat, total solids, SNF, proteins and ash contents.

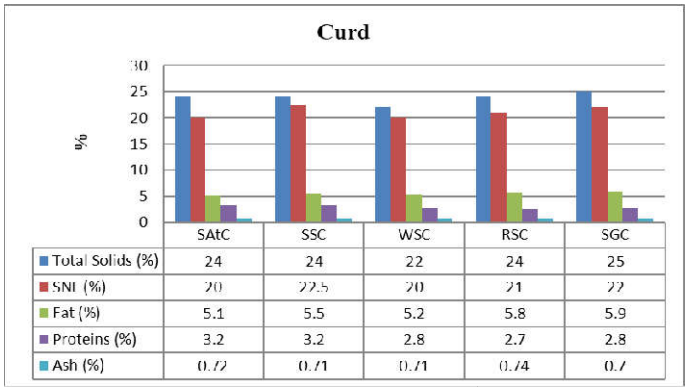


Graph 1

It can be seen from the **Graph 1** that the pH of curd samples was in a range between 4.2 to 4.6. The lowest pH value i.e. 4.2 amongst the samples was reported for a sample namely SAtC and the highest pH value i.e. 4.6 was reported for a curd sample namely WSC. Remaining three samples namely SSC, RSC and SGC showed a pH value of 4.4. Jha *et al*., (2011) reported that the pH values of the fermented dairy products are of great importance as it determines the extent of fermentation. Complete fermentation is required to get the optimum quality product. They reported that the pH of traditional dahi was 4.10. Padmanabha R.V. and Reddy S.I., (2010) studied the changes in pH, microbial counts and sensory characteristics of dahi during refrigerated storage. They reported that the pH of dahi samples dropped to values ranging from 4.51 to 4.55 during the first 3 days of storage and stayed between 4.32 and 4.43 at the end of 2 weeks of storage. Rybka S. and Kailasapathy K., (1995) reported that the drop in pH during first few weeks of storage could be due to continued fermentation during cooling of the product.

It can be seen from the Graph 2 that the total solids content in curd samples was in a range between 22% to 25%. Higher percentage i.e. 25% of total solids content was found in a curd sample namely SGC and lower percentage i.e. 22% of total solids content was found in curd sample namely WSC. However, remaining three samples namely SAtC, SSC and RSC showed total solids content was 24%. SNF content in curd samples was in a range between 20% to 22.5%. Higher percentage i.e. 22.5% of SNF content was found in curd sample namely SSC while lower percentage i.e.20% of SNF content was found in two curd samples namely SAtC and WSC. However, remaining two samples namely RSC and SGC showed SNF content as 21% and 22% respectively. These results are in accordance with the values reported by Yadav *et al*., (1993). The fat content of curd samples was in a range between 5.1% to 5.9 %. Higher percentage i.e. 5.9% of fat content was found in the curd sample namely SGC while curd sample namely SAtC contained least percentage i.e.5.1% of fat content. Remaining three samples namely SSC, WSC and RSC showed fat content of 5.5%, 5.2% and 5.8% respectively. The protein content in curd samples was in range between 2.7% to 3.2 %. Higher percentage i.e.3.2% of protein content was found in two curd samples namely SAtC and SSC while lower percentage i.e.2.7% of protein content was found in curd sample namely RSC. Remaining two curd samples namely

WSC and SGC showed protein content of 2.8%. Protein content reported in this work agrees with those reported by Yadav *et al*., (1993) who reported that the total solids,fat and protein content of curd samples in a range between 24%,5% to 8% and 3.2% to 3.4%respectively.Similarly,Sukumar D., (1999) reported the fat and protein content of curd samples is in a range between 5% to 8% and 3.2% to 3.4% respectively. Ash contents in curd samples was in a range between 0.70% to 0.74%. Higher percentage i.e. 0.74% of ash contents was found in curd sample namely RSC while lower percentage i.e. 0.70% of ash contents was found in curd sample namely SGC. However, two samples namely SSC and WSC showed ash contents of 0.71% and a sample namely SAtC showed ash contents of 0.72%. Ash contents in a range between 0.70% to 0.75% was reported by Yadav *et al*., (1993).

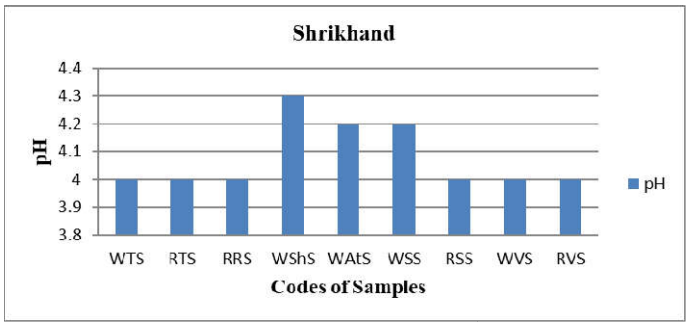


	SAtC	SSC	WSC	RSC	SGC
Total Solids (%)	24	24	22	24	25
SNF (%)	20	22.5	20	21	22
Fat (%)	5.1	5.5	5.2	5.8	5.9
Proteins (%)	3.2	3.2	2.8	2.7	2.8
Ash (%)	0.72	0.71	0.71	0.74	0.7

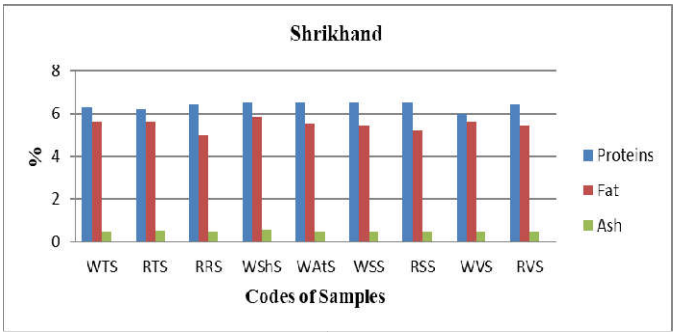
Graph 2

Shrikhand: The chemical examination of shrikhand includes determination of pH, fat, total solids, SNF, proteins, sucrose and ash contents.

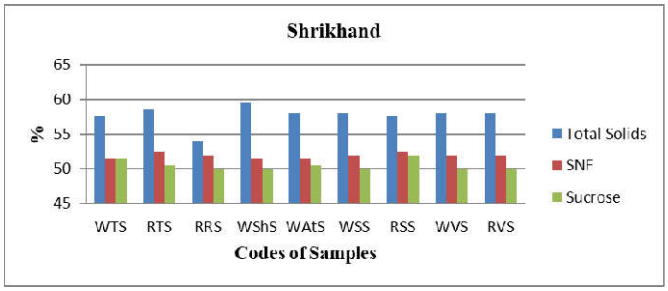
It can be seen from the Graph 3, pH values of shrikhand samples was in a range between 4 to 4.3. The lowest pH value i.e. 4 amongst the samples was reported for six samples namely WTS, RTS, RRS, RSS, WVS and RVS while the highest pH value i.e. 4.3 was reported for a sample namely WShS. Remaining two samples namely WAtS and WSS showed pH value of 4.2. The results are in agreement with the results reported by Salunke *et al*., (2006) who found that the pH of shrikhand samples was 3.9 to 4.3. Similarly, Kulkarni *et al*. (2006) as well as Boghra and Mathur (2000) reported that pH of shrikhand was in a range of 4.2 to 4.4.



Graph 3



Graph 4



Graph 5

It can be seen from the **Graph 4** that the protein content of shrikhand samples was in a range between 6.0% to 6.5%. Higher percentage i.e. 6.5% of protein contents was found in four shrikhand samples namely WShS, WAtS, WSS and RSS while lower percentage i.e. 6.0% of protein content was found in a sample namely WVS. Samples namely WTS and RTS contained 6.3% and 6.2% proteins respectively. Remaining two samples namely RRS and RVS contained 6.4% proteins. The fat content in shrikhand samples was in a range between 5.0% to 5.8%. Higher percentage i.e.5.8% of fat content was found in the sample namely WShS while shrikhand sample namely RRS contained lower percentage i.e. 5.0% of fat content. Remaining seven samples showed the fat contents in between this range. Three samples namely WTS, RTS and WVS and two samples namely WSS and RVS showed 5.6% and 5.4% of fat content respectively. Two samples namely RSS and WAtS showed 5.2% and 5.5% fat content respectively. The results are in agreement with the results of Salunke *et al*., (2006) who reported that the protein and fat content in shrikhand samples was in range between 4.6% to 6.7% and 3.0% to 8.9% respectively. According to PFA and BIS standards the fat content in shrikhand is 5.015% and 5.31% respectively. The ash contents found in shrikhand samples was in a range between 0.45% to 0.55%. Higher percentage i.e. 0.55% of ash contents was found in a sample namely WShS while lower percentage i.e.0.45% of ash contents was found in four shrikhand samples namely WTS, RRS, WAtS and RSS. RTS showed 0.52% of ash contents while remaining three samples namely WSS, WVS and RVS showed 0.46% ash contents. Yadav *et al*., (1993) reported the ash content of shrikhand sample in a range between 0.49 to 0.55%. Salunke *et al*., (2006) reported the ash content of shrikhand sample in a range between 0.30% to 0.45%. Kulkarni *et al*. (2006) as well as Boghra and Mathur (2000) reported that the ash content of shrikhand as 0.5%.

It can be seen from the Graph 5, the total solids content found in shrikhand samples was in a range between 57.5% to 59.5%. Higher percentage i.e. 59.5% of total solids content was found in shrikhand sample namely WShS while lower percentage i.e.

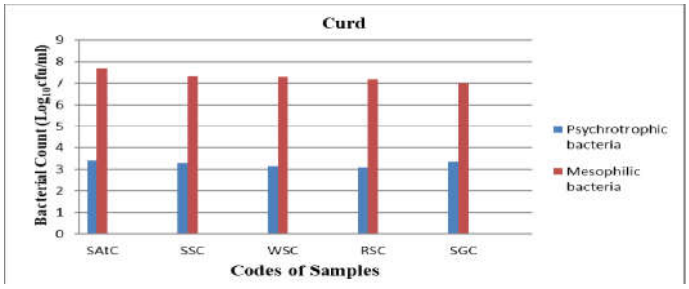
57.5% of total solids was found in two shrikhand samples namely WTS and RSS. Remaining six samples showed total solids content in between this range. Four samples namely WAtS, WSS, WVS and RVS showed 58% total solids content. Samples namely RTS and RRS showed 58.5% and 54% total solids content respectively. Sonawane *et al*., (2007) reported the total solids content of shrikhand was in a range between 36.53% to 46.87%. The SNF content in shrikhand samples was in a range between 51.5% to 52.5%. Lower percentage i.e. 51.5% of SNF content was found in three shrikhand samples namely WTS, WShS and WAtS while higher percentage i.e.52.5% of SNF content was found in two shrikhand samples namely RTS and RSS. Remaining four samples namely RRS, WSS, WVS and RVS showed SNF content was 52%. Sonawane *et al*., (2007) reported the SNF content in shrikhand was in a range between 50% to 55%. The sucrose content in shrikhand samples was in a range between 50% to 52%. Higher percentage i.e.52% sucrose content was found in shrikhand sample namely RSS while lower percentage i.e. 50% sucrose content was found in five shrikhand samples namely RRS, WShS, WSS, WVS and RVS. Two samples namely RTS and WAtS showed 50.5% of sucrose content and a sample namely WTS showed 51.5% of sucrose content. Salunke *et al*., (2006) reported the sucrose content in shrikhand samples was in a range between 44.5% to 51%.

Enumeration of psychrotrophic and mesophilic bacteria

Enumeration of psychrotrophic and mesophilic bacteria from Curd

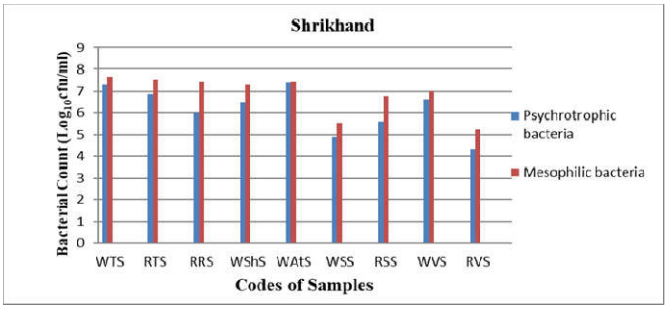
It can be seen from the **Graph 6** that the number of psychrotrophic bacteria present in curd samples was in a range between 3.10 to 3.40 $\log_{10}$cfu/ml. The higher number i.e. 3.40 $\log_{10}$cfu/ml of psychrotrophic bacteria was found in curd sample namely SAtC and the least number i.e. 3.10 $\log_{10}$cfu/ml of psychrotrophic bacteria was found in curd sample namely RSC. The number of psychrotrophic bacteria found in remaining three samples namely SSC, WSC and SGC was 3.30, 3.15 and 3.34 $\log_{10}$cfu/ml respectively. Sahan *et al*., (2004) reported that the number of psychrotrophic bacteria present in yoghurt significantly decreased after 60 days in the samples stored at refrigeration temperature and to a smaller degree decreased in the samples stored at room temperature. They reported that storage time of yoghurt had a significant effect on the growth of aerobic mesophilic bacteria, aerobic spore-forming bacteria ($P<0.01$), yeast, mould and psychrotrophic bacteria counts.

Number of mesophilic bacteria present in curd samples was in a range between 7.0 to 7.69 $\log_{10}$ cfu/ml. Higher number i.e. 7.69 $\log_{10}$ cfu/ml of mesophilic bacteria was present in curd sample namely SAtC while less number i.e. 7.0 $\log_{10}$ cfu/ml of mesophilic bacteria was present in curd sample namely SGC. The number of mesophilic bacteria found in remaining three samples namely SSC, WSC and RSC was 7.35, 7.3 and 7.17 $\log_{10}$ cfu/ml respectively. Sahan *et al*., (2004) also reported that total aerobic mesophilic bacteria counts in yoghurt decreased during storage at room and refrigeration temperatures. These results indicate that variable number of psychrotrophic and mesophilic bacteria were present in curd samples from five dairies.



Graph 6

Enumeration of psychrotrophic and mesophilic bacteria from shrikhand



Graph 7

It can be seen from the Graph 7 that the average number of psychrotrophic bacteria present in shrikhand samples was in a range between 4.30 $\log_{10}$ cfu/ml to 7.39 $\log_{10}$ cfu/ml. It was found that the sample namely WAtS showed higher number of psychrotrophic bacteria i.e. 7.39 $\log_{10}$ cfu/ml and sample namely RVS showed less number of psychrotrophic bacteria i.e. 4.3 $\log_{10}$ cfu/ml. The average number of psychrotrophic bacteria found in remaining samples was in this range. These results indicate that the measurable number of psychrotrophic bacteria was present in all shrikhand samples. The average number of mesophilic bacteria present in shrikhand samples was in a range between 5.25 $\log_{10}$ cfu/ml to 7.65 $\log_{10}$ cfu/ml. It can be seen from the table that sample namely WTS showed higher number of mesophilic bacteria i.e.7.65 $\log_{10}$ cfu/ml while sample namely RVS showed less number of mesophilic bacteria i.e.5.25 $\log_{10}$ cfu/ml. The number of mesophilic bacteria found in remaining samples was in this range. The number of mesophilic bacteria i.e.7.65 and 7.53$\log_{10}$ cfu/ml were present in sample namely WTS and RTS respectively. The number of mesophilic bacteria i.e.6.77 and 5.53 $\log_{10}$ cfu/mlwere present in sample namely RSS and WSS respectively. Number of mesophilic bacteria i.e.7 and 5.25 $\log_{10}$ cfu/ml were present in shrikhand sample namely WVS and RVS respectively. Mane *et al*., (2010) reported that average numbers of thermoduric psychrotrophs present in five shrikhand samples were 33.30×10^3, 34.48×10^3 and 33.06×10^3 in summer, rainy and winter seasons respectively. Yadav *et al*., (1993) reported that the microflora of shrikhand is like that of dahi.

CONCLUSION

Chemical contents of fourteen different samples constituting five samples of curd and nine samples of shrikhand were within the range mentioned by Bureau of Indian Standards. Enumeration of bacteria from these samples showed the presence of mesophilic as well as psychrotrophic bacteria in each of the samples and there was a considerable variation in mesophilic and psychrotrophic bacterial content amongst the

samples. Thus, growth mesophilic and psychrotrophic bacteria may result in development of undesirable flavours and spoilage of milk and milk products. Spoilage of milk and milk products may be avoided or prevented by controlling mesophilic and psychrotrophic organisms in milk and milk products.

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