Trend Analysis of Developed Acidity in Pasteurized Milk in Aerobic Condition and Establishment of Its Correlation between COB and ALP Activities

Hetvi Patel[1] Abhay Koladiya[2] Divyesh Chauhan[3]
[1,2]Student [3]Consultant
[1,2]Department of Food Processing Technology
[1,2]A.D. Patel Institute of Technology, New Vallabh Vidyanagar, Gujarat, India [3]Vidya Dairy, Anand Agriculture University campus, Anand, Gujarat, Gujarat India

Abstract— This study was aimed to evaluate the microbial and Physico-chemical factor influence on the quality of milk as %Lactic acid. Milk belongs to the category of the highly perishable food products. To know the quality and shelf life of the milk is very important along with the factors affecting milk quality and shelf life is also important. The Lactose present in the milk is the carbohydrate and gets degraded and converts into lactate also known as lactic acid which increases the %Acidity of the milk and cause spoilage of the milk. This study is aimed to know the change occurs in % Acidity of the milk along with its physical parameters like smell, taste, appearance.

Keywords: Lactic Acid, Pasteurized Milk, Titration, Ambient Temperature, ALP Activities

I. Introduction

In dairy industry %acidity plays a critical role and it is considered as a very important tool for quality control and quality assurance. From the udder when the milk secrets there are very minor amount of microbes present but subsequently they are added from other sources like from milker, utensils, vessels, air, water, udder surface etc. The other which will affect the milk is the climatic condition of the region. In India the summer and monsoon season climatic condition affects the milk mostly, like in the summer the climate becomes hot and it provides condition for the growth of the microorganisms while in monsoon there is humidity present in the climate. Milk contains roughly 55% to 57% of lactose so by contamination and ambient temperature there will changes occurs in the milk which affects the quality of the milk. Milk is the highly perishable commodity which results into microbial and enzymatic degradation of lactose which leads to production of lactic acid. As the natural milk also contains casein, Co_2, and minor amount of acidic substances. In addition to those the developed lactic acid also comprises by quality parameters by major industries.

II. Materials, Reagents and Equipments Required

Pasteurized milk samples, Laminar air flow, thermometer (ACETEQ); Acidometer (Metrohm), water bath (D.K scientific technologies, Bunsen burner, Sodium carbonate (Merck specialities private limited, 1.93611.0521), Sodium bicarbonate (RFCL limited, 50680), PNPP (SISCO research laboratories private limited, 88485)

III. Procedure

This study was aimed to evaluate the microbial and equipment factor influence on the quality of milk as %Lactic acid. Total 5 pasteurized milk which contains 1.5 to 6.0% Fat and 8.5 to 9.0% SNF were taken as a sample. They were kept at ambient temperature and studied out various parameters including ALP activities, Temperature, Sensory evaluation, %Acidity and physic-chemical parameters along with variation in body, texture and rheological properties. The samples were analysed at the interval of 2 hours for 24 hours with the standard methodology. The samples were taken in the standard laminar airflow with the help of 10ml pipette. The temperature was measured by the standard thermometer (ACETEQ). The ALP activity was checked by 1ml sample in presterilized calibrated test tube (Borosil, 9830) followed by addition of 5ml AP buffer (PNPP, SISCO research laboratories private limited, 88485) which was earlier prepared in the buffer containing 1.5g of Sodium bicarbonate (RFCL limited, 50680) and 3.5g of Sodium carbonate (Merck specialities private limited, 1.93611.0521) and adjusted its pH value 10.2 .The ALP activities was checked at the 37ºc temperature by incubating test samples in standard water bath (D.K Scientific Technologies) which was previous calibrated before study. %Lactic acid measurement was done by Acidometer (Metrohm) and documented at the rate of 2hours intervals. As time passed the curd which was generated was evaluated by standard sensory evaluation methods. The change in rheological and physic-chemical properties was determined by transferring curd into the sterilized Petri dishes (Borosil, 3160). The clot on boiling test was carried out at 100ºc temperature for 1 minute. The curd particles were considered as positive test. All results as per the action plan were appropriately documented.

IV. Results

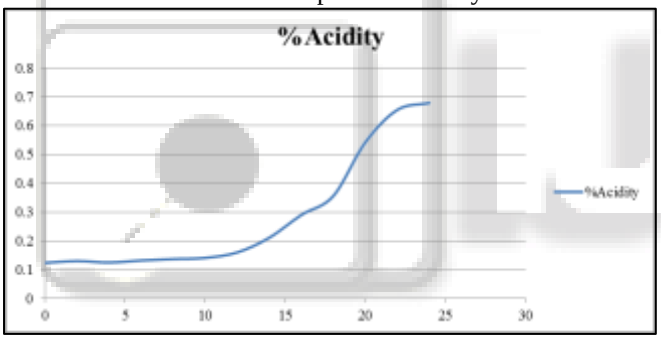
From this experiment we can say that with increase in time the %acidity of the milk is increasing because of the production of the acids in the milk by the bacteria. The following table shows the trend of developed acidity and other metabolites which are proven as spoilage of the pasteurized milk. The post pasteurization contaminants get activated when the packed milk is stored at ambient temperature. It is evidence that when the milk is stored at ambient temperature for initial hours, the microbial load starts degradation of the lactose compound in the milk which is in abundant level into the lactic acid.

Here for this study we have taken five different kind of pasteurized milk sample which contains different amount of %Fat and %SNF so that we can see the effect of ambient temperature onto the acidity of the milk. Here the first sample is Gold which contains 6.0%of Fat and 9.0% of

SNF. In the first sample as we can see from the table that the initial %acidity is 0.124% but after four hours it reaches to 0.125%, then after another four hours it again increase to 0.137%, then after 12 hours it reaches to 0.16%, after 16 hours it increases to 0.29% then again after 20 hours it increases upto 0.542% and lastly after completing 24 hours at ambient temperature we get 0.679% acidity which is the last reading of our observation of the milk sample stored at ambient temperature.

AG				
Hrs.	%Acidity	Temperature	COB	AP activity
0	0.124	12.3	Negative	Negative
2	0.13	20.6	Negative	Negative
4	0.125	25.6	Negative	Negative
6	0.132	26.7	Negative	Negative
8	0.137	23.9	Negative	Negative
10	0.141	23.2	Negative	Negative
12	0.16	22.8	Negative	Negative
14	0.211	25.3	Negative	Negative
16	0.29	28.8	Positive	Negative
18	0.356	29.1	Positive	Negative
20	0.542	29.9	Positive	Negative
22	0.655	30.6	Positive	Negative
24	0.679	30.8	Positive	Negative

Table 1: Milk sample (1) %Acidity, Temperature, COB, and Alkaline Phosphatase activity



For the second sample we have taken SPM variant of the pasteurized milk. The sample is having 4.5% of Fat and 8.5% of the SNF content. In the second sample as we can see from the table that the initial %acidity is 0.14% but after four hours it reaches to 0.144%, then after eight hours it again increase to 0.15%, then after 12 hours it reaches to 0.17%, after 16 hours it increases to 0.184% then again after 20 hours it increases upto 0.265% and lastly after completing 24 hours at ambient temperature we get 0.645% acidity which is the last reading of our observation of the milk sample stored at ambient temperature.

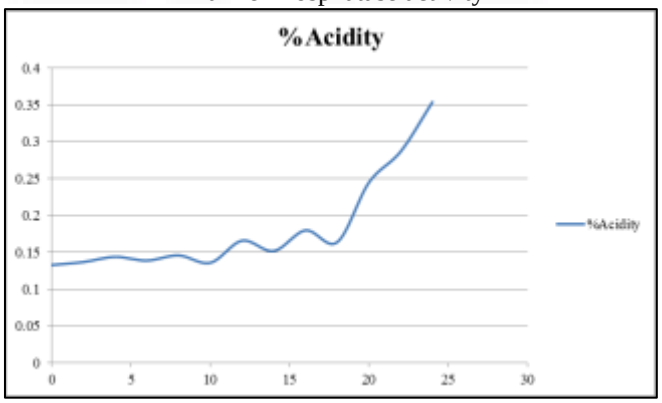
SPM				
Hrs.	%Acidity	Temperature	COB	AP activity
0	0.14	11.3	Negative	Negative
2	0.142	20.2	Negative	Negative
4	0.144	25.3	Negative	Negative
6	0.147	26.4	Negative	Negative
8	0.15	23.8	Negative	Negative
10	0.154	23.3	Negative	Negative
12	0.17	22.9	Negative	Negative
14	0.178	225.6	Negative	Negative
16	0.184	28.5	Negative	Negative
18	0.192	29.1	Positive	Negative
20	0.265	30.4	Positive	Negative
22	0.37	30.7	Positive	Negative
24	0.645	30.9	Positive	Negative

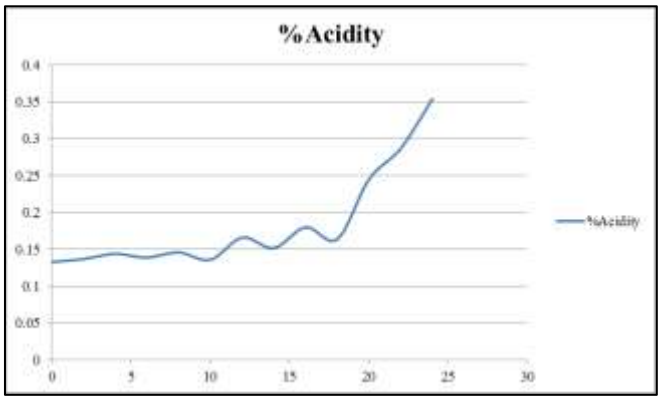
Table 2: Milk sample (2) %Acidity, Temperature, COB, and Alkaline Phosphatase activity

Now we have taken the double toned milk as the third pasteurized milk sample. It contains 1.5% of Fat and 9.0% of the SNF portion into it. In the third sample as we can see from the table shown below that the initial %acidity is 0.133% but after four hours it reaches to 0.144%, then after eight hours it again increase to 0.146%, then after 12 hours it reaches to 0.166%, after 16 hours it increases to 0.18% then again after 20 hours it increases upto 0.245% and lastly after completing 24 hours at ambient temperature we get 0.354% acidity which is the last reading of our observation of the milk sample stored at ambient temperature.

DTM				
Hrs.	%Acidity	Temperature	COB	AP activity
0	0.133	12.5	Negative	Negative
2	0.137	20.3	Negative	Negative
4	0.144	25.7	Negative	Negative
6	0.139	26.2	Negative	Negative
8	0.146	24.0	Negative	Negative
10	0.136	23.5	Negative	Negative
12	0.166	22.7	Negative	Negative
14	0.152	25.7	Negative	Negative
16	0.18	28.5	Positive	Negative
18	0.164	29.2	Positive	Negative
20	0.245	30.8	Positive	Negative
22	0.287	30.7	Positive	Negative
24	0.354	30.8	Positive	Negative

Table 3: Milk sample (3) %Acidity, Temperature, COB, and Alkaline Phosphatase activity

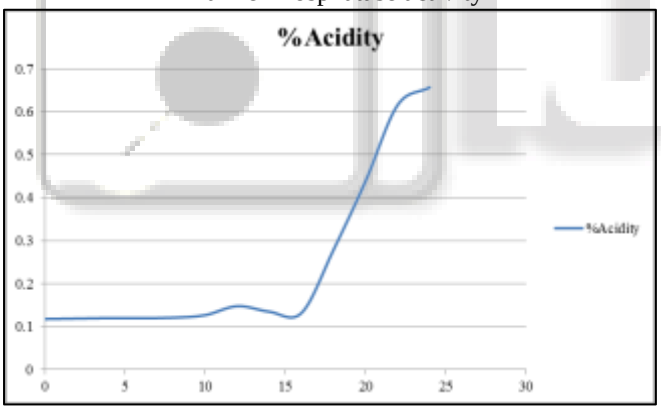




For the fourth sample we have taken tea special variant of the pasteurized milk which contains 4.5% of Fat and 8.5% of SNF content. The fourth sample as we can see from the table that the initial %acidity is 0.129% but after four hours it reaches to 0.137%, then after eight hours it again increase to 0.119%, then after 12 hours it reaches to 0.144%, after 16 hours it increases to 0.153% then again after 20 hours it increases upto 0.163% and lastly after completing 24 hours at ambient temperature we get 0.441% acidity which is the last reading of our observation of the milk sample stored at ambient temperature.

T/SP				
Hrs.	%Acidity	Temperature	COB	AP activity
0	0.129	11.36	Negative	Negative
2	0.128	20.8	Negative	Negative
4	0.137	25.2	Negative	Negative
6	0.131	26.1	Negative	Negative
8	0.119	23.4	Negative	Negative
10	0.161	23.2	Negative	Negative
12	0.144	22.6	Negative	Negative
14	0.133	25.4	Negative	Negative
16	0.153	28.9	Positive	Negative
18	0.147	29.3	Positive	Negative
20	0.163	29.9	Positive	Negative
22	0.223	30.7	Positive	Negative
24	0.441	30.8	Positive	Negative

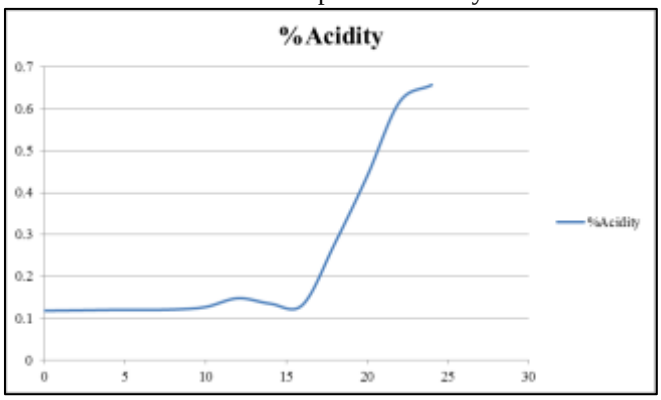
Table 4: Milk sample (4) %Acidity, Temperature, COB, and Alkaline Phosphatase activity



For the fifth sample we have taken Taaza variant of the pasteurized milk. The sample is having 3.0% of Fat and 8.5% of the SNF content. In the fifth sample as we can see from the table that the initial %acidity is 0.118% but after four hours it reaches to 0.112%, then after eight hours it again increase to 0.121%, then after 12 hours it reaches to 0.148%, after 16 hours it increases to 0.132% then again after 20 hours it increases upto 0.1439% and lastly after completing 24 hours at ambient temperature we get 0.657% acidity which is the last reading of our observation of the milk sample stored at ambient temperature.

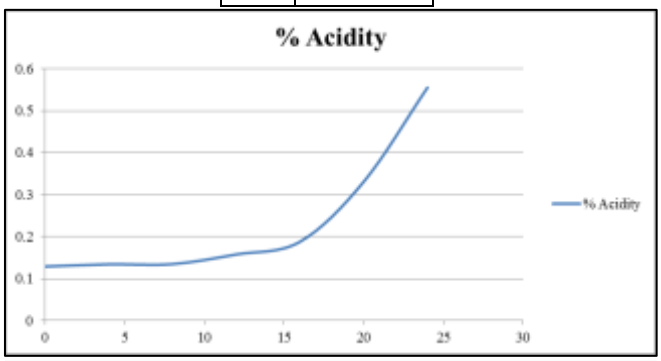
TM				
Hrs.	%Acidity	Temperature	COB	AP activity
0	0.118	11.9	Negative	Negative
2	0.119	20.3	Negative	Negative
4	0.12	25.6	Negative	Negative
6	0.12	26.4	Negative	Negative
8	0.121	23.9	Negative	Negative
10	0.127	23.2	Negative	Negative
12	0.148	22.7	Negative	Negative
14	0.135	25.1	Negative	Negative
16	0.132	29.0	Positive	Negative
18	0.279	29.4	Positive	Negative
20	0.439	29.8	Positive	Negative
22	0.616	30.4	Positive	Negative
24	0.657	30.6	Positive	Negative

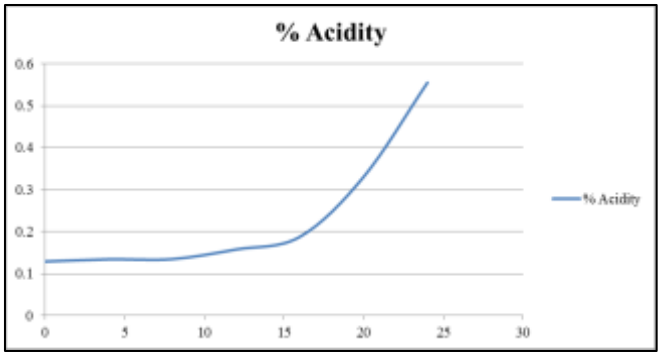
Table 5: Milk sample (5) %Acidity, Temperature, COB, and Alkaline Phosphatase activity



Now from this study we can say that the average of that with increase in the time the % acidity of the variants also increases. Here we have shown the average of the every four hour data which also states that every four hour the %acidity is increasing along with the time.

Hrs.	% Acidity
0	0.1288
4	0.134
8	0.1346
12	0.1576
16	0.1878
20	0.3308
24	0.5552

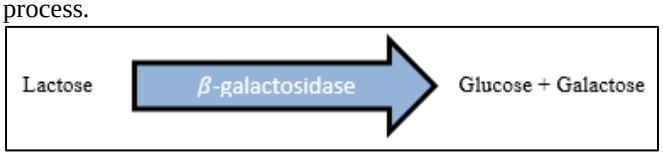




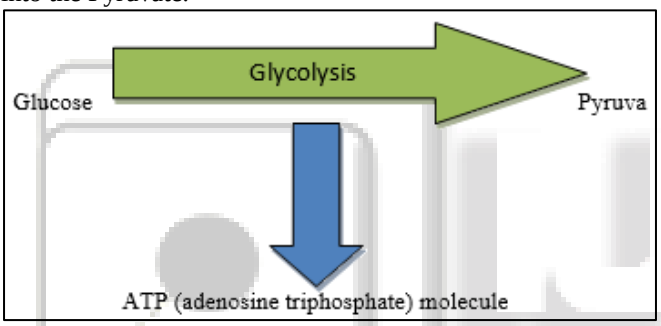
V. DISCUSSION

From this we can conclude that with increase in the temperature the acidity of the milk gets increase by conversion of the lactose. The lactose present in the milk gets converted into lactic acid by Cori cycle also known as lactic acid cycle.

The lactose present into the milk is the disaccharide which is made up of Glucose and Galactose. The β-galactosidase present in the milk degrade the lactose into simpler form and make it easy to do further metabolic process.



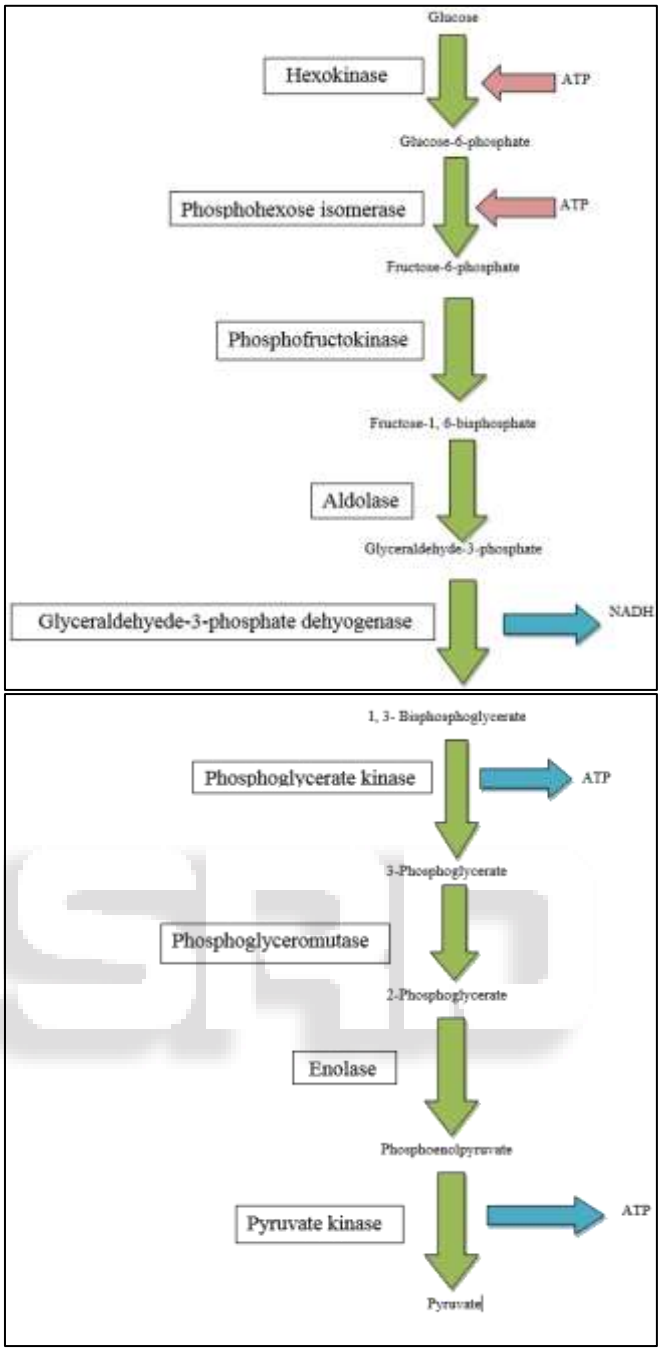
Now from this we get glucose which is monosaccharide or we can also say the simple form of carbohydrate. Now further degradation of the glucose takes place and it gets converted into Pyruvate by glycolysis. As we all know that glucose is also known as the energy coin it also releases ATP molecules during the glycolysis process. After glycolysis process the glucose further gets converted into the Pyruvate.



Here in the Glycolysis metabolic process the Hexokinase enzyme helps in the degradation of the glucose to the Glucose-6-phosphate during this degradation process it requires some energy that is why there is ATP shown in the flowchart. Glucose-6-phosphate gets degraded by the enzyme name Phosphohexose isomerasen. By this degradation it gets converted into Fructose-6-phosphate gets degrade by the enzyme name Phosphofructokinase. After this degradation we get Fructose-1, 6-bisphosphate. Now this Frctose-1, 6-bisphosphate gets degraded by the enzyme name Aldolase and gets converted into the Gleceraldehye-3-phosphate. This Gleceraldehyde-3-phosphate gets converted into the 1, 3-Bisphosphoglycerate by the Gleceraldehye-3-phosphate dehyrogenase enzyme. Now this 1, 3-Bisphosphoglycerate gets converted into the 3-Phosphoglycerate. This 3-Phosphoglycerate further gets converted into the 2-Phosphoglycerate by the Phosphoglyceromutase enzyme. This 2-Phosphoglycerate gets converted in to Phosphoenolpyruvate by the Enolase enzyme. Now this Phosphoenolpyruvate further gets converted into Pyruvate and gives the final end to the glycolysis process with the help of Pyruvate kinase enzyme.

Here in the glycolysis metabolic process each step has its individual catalysing enzyme as shown below in the form of flow chart.

Glucose



Now the reduction of Pyruvate by the NADH lactate or lactic acid which is catalyzed by Lactate dehydrogenase. When the oxygen amount present in the cells is less in this situation this reaction takes place.

It is evidenced that as the amount of oxygen gets decreased with that the amount of the % Developed acidity gets increased.

Here we have also checked the lactose present into the milk by gravimetric method of total solids. In which the amount of lactose present into the milk is 53.7% but after 24 hours the percent amount of the lactose present into the milk is 23.9% which means the amount of lactose gets reduced due to the conversation of lactose to the lactic acid.

Now when we put the milk samples at the ambient temperature it does not only affect the %Acidity of the milk but it also affects the appearance and texture of the milk. As acidity gets increase the curd formation takes place. The acidity causes the curd formation in which the protein present in the milk gets shrieked and this how whey gets separated into the milk and curd formation takes place.

VI. CONCLUSION

The study conducted under the set control parameters including the ambient temperature and milk as the nutritive source. It was observed that due to the actions of microbial diverse population and enzymatic degradation as well as bio-chemical pathways resulting into the conversion of lactose into the lactic acid. The initial value of the lactose is 53.7% by gravimetric method of total solids which has been reduced upto the 23.9% of total solids. Hence it is evidenced that the microbial population and enzymatic action leads to the disturbance in the colour, appearance, taste, sensory, rheological properties and finally curdling of the milk upon incubation the milk at the ambient temperature for the 16 hours. The worst condition in terms of spoilage occurs when milk is further incubating for 24 hours.

So the conclusion came out that in Indian tropical condition during spring season (Feb-March), the tropical climatic condition of India exhibit temperature from 20°c to the 30°c which leads to the shelf life of milk around 16 hours at ambient temperature.

REFERENCES

[1] Frank E. Rice and Alton I. Markley, The relation of natural acidity in milk to composition and physical properties, Department of chemistry, Cornell university, Ithaca, page no.468
[2] Dan W. Steuart, The development of acidity in milk, University college, Cardiff, Wales, page no.52&53
[3] Christina Werner, Michael Schwarzer,, Metabolic pathways and cycles, in the scientist's guide to Cardiac metabolism, 2016
[4] Physical chemistry of milk, Module 8.acids and bases, Lesson 20 Acidity of milk, http://ecourseonline.iasri.res.in/mod/.page/view.php?id=4203
[5] Owen J.Mccarthy, Harjinder Singh, Physico-chemical properties of milk, Book Advanced dairy chemistry, Massey university
[6] Lucius L. Van Slyke and Alfred W. Bosworth, The cause of acidity of fresh milk of cows and a method for the determination of acidity from the chemical laboratory of the New York agricultural experiment station, Geneva, New York
[7] Loretta Gambelli, Milk and its sugar-lactose : A picture of evaluation methodologies, council for Agriculture research and analysis of the agrarian economy, research center CREA-food and nutrition, via ardeatina546,00178, Italy , published on 13 July 2017
[8] R. Waite, J.Mcphillips , The gravimetric measurement of milk total solids in large numbers, international journal of dairy technology/volume 18, issue 1, first published on January 1965
[9] A 18 b- Total lactose in milk and whey powders by Gravimetric, GEA engeering, GEA Nitro Method No.A 18 b, Revised in June 2006
[10] R.T.Marshall and C.C. Harmon, The automated pyruvate method as a quality test for grade a milk, Department of food science and nutrition university of Missouri, Columbia, journal of food protection vol.41 no.3 page 169 , March 1978
[11] Skelte anema, Effect of temperature and rate of acidification on the rheological properties of acid skim milk gels, journals of food processing and prevention 32(6): 2016-1033 november 2008
[12] Hotnida sinaga, Nidhi bansal & Bhesh bhabdari, Effect of milk pH alteration on casein miscelle size and gelation properties of milk, International journal of food properties,8 September 2016
[13] EL-Boraey N.A, IsmailM.M, Hoda F.A>Elashrey, chemical composition, sensory evaluation, rheological properties and starter activity of admixtures of buffalo's, cow's, and soymilk,American journal of food science and nutrition research, vol.2 no.4,2015,page no.122
[14] I.A.GOULD, lactic acid in dairy products. III the effect of heat on total acid and lactic acid production and on lactose destruction,Michigan agriculturalexperiment station, Michigan state college, Eastlansing, Michigan, page no.367
[15] Titrable acidity in milk, http://www.google.co.in/amp/s/www.agrimoon.com/acidity-test/amp

