Quality of milk protein in dairy farms from Southeastern region of Brazil

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Abstract— *The quality of milk protein is related to the capacity of milk being processed or not, called thermal stability. Unstable Non Acid Milk is characterized by low casein stability that results in precipitation during alcohol test without presence of acidity on milk sample and determines great damages. This study had the objective of diagnosing protein quality and occurrence of Unstable Non Acid Milk on raw milk produced by dairy farms of the Southeastern region of Brazil. For investigation of occurrence, 201 dairy farms were chosen and received a trained technician to collect milk samples monthly. During one year 2,970 milk samples delivered in dairy plants were evaluated for milk quality (fat, protein, lactose, solids non fat, somatic cell count and total bacterial count) and for protein quality in terms of thermal stability using titratable acidity, alcohol test and pH. As a result, it was observed that 7% from 52 million liters of processed milk were classified as Unstable Non Acid Milk. There wasn't relationship between occurrence of Unstable Non Acid Milk and milk production level. However, occurrence displayed a variation throughout months during the year, being the final of fall the highest incidence of the problem. The milk identified as Unstable Non Acid Milk showed changes on milk composition, in special for lactose, what can describe a possible relation between the problem and nutritional practices, more specific related to carbohydrate metabolism, what can help to find the prevention and/or control the problem of thermal stability related to milk protein.*

Keywords— Milk Composition, Thermal Stability, Unstable Non Acid Milk.

I. INTRODUCTION

Protein is currently the most valued component of milk in the majority of countries. The appreciation of this element over the fat can explain the huge increase in cheese consumption and encouraging the reduction of the consumption of animal fat about the relationship to health [1].

The importance of protein levels is directly related to industrial performance, mainly cheeses. Others aspects such as protein quality must be emphasized and also associated to industrial incomes during the processing of milk. Protein quality refers mainly the capacity to withstand thermal processing, in other words, the thermal stability of milk. During this process, several changes occur and the most important thing was associated with the structures of proteins, including casein micelles [2]. So, stability is directly related to milk capacity to resist the coagulation by heat, and thus, its ability to thermal processing.

The estimation of the milk thermal stability can be possible achieved through the alcohol test or alizarol [1]. This test is widely used in dairy as a way to accept or decline milk at the moment of its arrival at the platform in industries or even at dairy farm. The occurrence of milk with thermal instability, diagnosed by this test, is a problem found in various regions of Brazil which results in inappropriate raw materials for the dairy production, representing losses for the entire dairy chain [3].

Most of problems with thermal instability are related to milk with high acidity due to the high bacterial contamination. However, the problem may also be associated to the stability of milk protein, known as Unstable Non Acid Milk. Milk classified as Unstable Non Acid Milk have normal pH and titratable acidity, low bacterial and somatic cell count but, the coagulation is positive on the alcohol test.

The occurrence of this problem is still poorly investigated due to the complexity of factors involved in thermal stability of milk and their random occurrence, encouraging studies for the knowledge in the control and/or preventive measures. In view of the points analyzed in relation to milk protein, the aim of this study was to detect the Unstable Non Acid Milk produced and sent to the dairy industries in southeastern of Brazil. This study was grounded according to the characteristics of milk composition, seasonal variation and occurrence.

II. MATERIAL AND METHOD

The study lasted 12 months for the diagnosis of milk quality and, more specifically, Unstable Non Acid Milk. Almost two hundred dairy farms were chosen according to the routes, and the routes should have at least one farm in the State of São Paulo. So, 59 routes were chosen. During the period October 2005 to September 2006 were evaluated approximately 2,970 samples of bulk tanks milk, divided into two bottles (two types of preservatives) and received by the "Clínica do Leite" Laboratory at ESALQ / USP in Piracicaba, SP. The samples were collected by trained technicians for the dairy industry participant of the project.

Monthly, for one of two milk samples (50 mL) of each farm were conducted the following analyzes: composition (fat, protein, lactose and total solids), pH, titratable acidity and resistance on alcohol test, and these samples were sent by dairy industry conserved with Bronopol®. For the second sample (50 mL), wanted only to total bacterial count, this was conserved with bacteriostatic conservative named Azidiol [4]. Throughout the logistics process, the samples was keep refrigerated at 4 °C from the time of collection until the time of analyzes.

The classification of milk samples was used according CENLAC [5] and samples from tanks with somatic cell count (SCC) above one million cells mL^{-1} and total bacterial count (TBC) above 1,000,000 cfu mL^{-1} were excluded. Were classified as Unstable Non Acid Milk, samples that tested positive in the three tests (coagulation visible on the alcohol test, titratable acidity <18 °D and pH> 6.6).

The samples which showed no visible coagulation in the alcohol test 78% were classified as stable. On the other hand, samples which showed evidence of coagulation visible in 78% in alcohol test, but with a pH below 6.6 and the samples with TBC over 1,000,000 cfu mL^{-1} and SCC over one million cells mL^{-1}, were classified as unstable.

The pH of the samples was measured immediately after the reception, using digital potentiometer. The quantification of titratable acidity was performed by titration with sodium hydroxide (0.1 N), using as indicator alcohol solution of phenolphthalein (1%), according to the National Reference Laboratory for Animal Products [6]. To determine the stability of milk (alcohol test), were placed 2 ml of milk and 2 ml of 72% alizarol in Petri dishes, homogenizing them. The results were scored on a scale of 1 (one) to 5 (five), as follows: 1(one) - milk stable with no precipitation; 2 (two) - milk unstable with light precipitation (lumps similar to sand); 3 (three) - unstable milk with medium precipitation (dense clumps equally distributed) 4 (four) - Milk unstable with heavy precipitation (larger lumps located at some points of the plate) and 5 (five) - Milk unstable with very intense precipitation (clot similar to net) [7].

To calculate the thermal stability was constructed a standard curve based on three values of alcohol concentration: 72, 75 and 78% ethanol. 72% (required by law), 78% (standard used by dairy project participant) and 75% (intermediate value chosen for construction of the standard curve). The standard curve constructed for each combination of the three concentrations of alcohol and so calculated stability (point limit alcohol concentration) to which the sample reached the value coagulation "4" (heavy precipitation sample) of the scale [7]. Achieving this value was then marked the alcohol concentration (percentage) as the stability limit.

The analysis of milk composition (protein, fat, lactose, total solids) were performed electronically by infrared absorption using Bentley® 2000 equipment [8]. Besides, the SCC was performed by electronic counting cytometry using equipment flowmetric Bentley® Somacount 300 [9] in Lactation Physiology Laboratory, "Clinica do Leite" ESALQ/ USP. And finally, the TBC was analyzed by flow cytometry methodology, using equipment Bactocount in Lactation Physiology Laboratory, "Clinica do Leite" ESALQ/ USP.

The characterization of the farms was performed by analysis of milk quality and also by some information provided by the dairy, such as: city, state, route, and daily milk production. It is important to know that daily production of each farm was calculated using the relation between the amounts of milk sold in the month and the number of days in that month.

According to the production, the farm were separated into five groups: group 1(one) - production of less than 180 L day^{-1}, group 2 (two) - production between 180 and 320 L day^{-1}, group 3 (three) - production between 321 and 560 L day^{-1}, group 4 (four)- production between 561 and 1000 L day^{-1} group and 5 (five) - producing greater than 1000 L day^{-1}.

For the economic evaluation of the occurrence of Unstable Non Acid Milk was conducted a research about the average price of milk paid to producers of the State of São Paulo, during the year 2005/2006 at the Center for Advanced Studies in Applied Economics, Department of Economics, Management and Sociology, School of Agriculture "Luiz de Queiroz" at University of Sao Paulo.

The seasonal variation in the occurrence of cases classified as Unstable Non Acid Milk was performed using the Chi-square test. To study the influence of Unstable Non Acid Milk with milk composition and its occurrence related to the farms characteristics was used the completely randomized design, considering as qualifying variable, the classes of thermal stability of milk (stable milk, acid milk and Unstable Non Acid Milk). The dependent variables such as physicochemical characteristics of milk, milk composition, SCC and TBC were submitted to analysis of variance by PROC GLM in SAS version 9.2, least squares, with a significance level of 5%.

III. RESULTS AND DISCUSSION

According to the rule for choosing the routes, were selected 201 farmers to be monitored. The Table 1 shows the milk production average and the number of farms chosen for the experiment by State

TABLE 1
DAILY PRODUCTION AVERAGE AND THE NUMBER OF PARTICIPATING FARMS IN THE EXPERIMENT BY STATE

State	Farms, n (%)	Daily production average (L/day)
Minas Gerais	93 (47)	717.0
Rio de Janeiro	07 (3)	606.0
São Paulo	101 (50)	654.0
TOTAL	**201 (100)**	

The experiment lasted 12 months and there was variation in the number of milk producers analyzed monthly depending on the delivery of milk to the dairy plant. The milk samples classification showed the distribution as 2,004 samples (67%) as a stable, 267 (9%) as Unstable Non Acid Milk and 699 (24%) as unstable, in a total of 2,970 milk samples. In Table 2 shows the number of milk samples analyzed during the experiment, and the volume of milk represented by the samples. The variation in the number of samples over the months can be explained by the change in active milk producers.

TABLE 2
NUMBER OF SAMPLES ANALYZED AND THE OCCURRENCE OF UNSTABLE NON ACID MILK RELATED TO VOLUME AND MONETARY VALUE OVER A YEAR

	Samples	Milk production	Unstable Non Acid Milk		Milk price	Unstable Non Acid Milk Value
Months	**(n)**	**(thousand liters)**	**(thousand liters)**	**(%)**	**(R$/liter)[1]**	**(R$)[2]**
Oct	230	3,701.8	15.5	0	0.485	7,498.1
Nov	224	3,326.2	6.4	0	0.460	2,922.9
Dec	167	3,322.0	157.2	5	0.441	69,386.2
Jan	195	3,371.0	262.2	8	0.426	111,793.6
Feb	311	5,431.0	279.3	5	0.448	125,036.4
Mar	247	4,062.6	541.8	13	0.457	247,759.3
Apr	296	3,354.9	84.6	3	0.491	41,566.1
May	269	3,274.4	200.6	6	0.509	102,182.0
Jun	232	4,162.2	199.6	5	0.514	102,541.3
Jul	275	6,442.7	666.7	10	0.523	348,858.6
Aug	295	6,026.9	850.5	14	0.529	449,650.8
Sep	229	5,587.8	283.7	5	0.529	150,196.6
Total	**2,970**	**52,063.4**	**3,548.0**	**7**		**1,759,392.62**

[1] Average monthly amount paid to producers in States of Minas Gerais and Sao Paulo [10]
[2]Monetary value for the volume of milk classified as Unstable Non Acid Milk.

In Table 2 are also quantized the problem with the quality of protein in the milk produced. In face of the total produced - 52 billion liters of milk received by dairy during the experimental year- represented by 2,970 samples, 7% was linked with the problem of Unstable Non Acid Milk.

To characterize the problem, was considered the average value paid to the producer per liter of milk in the months studied [10], resulting in R$ 1,7 million related to Unstable Non Acid Milk.

Once diagnosed that 7% of total liters received by dairy was as Unstable Non Acid Milk, can be seen in Table 3 the significant influence ($P<0.05$) of the period of the year in the number of samples identified with this problem.

TABLE 3
NUMBER OF MILK SAMPLES CLASSIFIED AS UNSTABLE NON ACID MILK OVER MONTHS

Month	Total Samples	Samples classified as Unstable Non Acid Milk	
		n	%
Oct	230	8 c	3.5
Nov	224	7 c	3.1
Dec	167	14 bc	8.4
Jan	195	13 bc	6.7
Feb	311	21 bc	6.8
Mar	247	44 a	17.8
Apr	296	18 bc	6.1
May	269	25 bc	9.3
Jun	232	27 abc	11.6
Jul	275	29 abc	10.5
Aug	295	31 abc	10.5
Sep	229	30 ab	13.1
TOTAL	**2,970**	**267**	**9**

Means followed by different letters in the column differ by Tukey`s test (P <0.05)

Among the 2,970 samples tested, 267 were identified as Unstable Non Acid Milk and 18% of them were found only in the month of March 2006, this percentage differed from the other months of the year, except for the months June to September. During a study of the occurrence of Unstable Non Acid Milk over 12 consecutive months, found 1,322 samples (55%) with Unstable Non Acid Milk a total of 2,396 samples, analyzed using the same methodology for the classification the samples, however using alcohol concentration of 76% [11]. In this research were monitored, over one year, 200 dairy farms in the region of Panambi - RS, in which was found variation over the months for the occurrence of Unstable Non Acid Milk, correlating mainly to the months of February and March [11]. In the present study, was observed increased incidence of the problem not only in March, but in the months from June to September too.

In Table 3 can be observed significant variations ($P<0.05$) on the occurrence of Unstable Non Acid Milk over the months analyzed. This significant effect of period of the year can be explained by the close relationship of the nutritional animals management, mainly influenced by climatic conditions, availability of forage and feed restriction [12].

Besides climatic and nutritional factors, economic factors that can interfere in milk composition, and thus on its thermal stability [13]. Among these economic factors can be said the cost and availability of inputs involved in the food industry due to the period and place of its distribution. The occurrence of Unstable Non Acid Milk with food shortages or low forage availability at the period of highest occurrence for the region (end of summer and early fall) [11, 14].

The difference between the incidence of *Unstable Non Acid Milk* of this study (7%) and other studies (more than 50%) [11, 14] can be explained for the characterization of the farms. Zanela et al. [14] working in Pelotas - RS and region correlated the incidence of *Unstable Non Acid Milk* with groups of production, finding a decrease according as increased daily milk production. The production groups studied by the authors varied from below 20 $L\ day^{-1}$ up to 500 $L\ day^{-1}$. In the present study, we evaluated producers since below 180 $L\ day^{-1}$ to over 1000 $L\ day^{-1}$. Thus, it is evident the difference profile of the properties studied in the work done in the south, to the present study in the Southeast.

In Table 4 can be seen the distribution of the number of sample classified as *Unstable Non Acid Milk* compared to daily milk production properties. The classification of each property for production group has been done monthly whereas some properties had changes on daily quantity of milk produced during the year.

TABLE 4
DISTRIBUTION OF UNSTABLE NON ACID MILK CASES PER GROUP OF MILK PRODUCTION

Groups	Total of samples	Cases of Unstable Non Acid Milk
Group 1 (less than 180 L/day)	463	60 a
Group 2 (181 – 320 L/day)	478	51 a
Group 3 (321 – 560 L/day)	476	44 a
Group 4 (561 – 1,000 L/day)	450	37 a
Group 5 (more than 1,001 L/day)	472	44 a
TOTAL	**2,339**	**236**

Means followed by different letters in the column differ by Tukey`s test (P <0.05)

In contrast to the results found by Zanela et al. [14], in this study there was no effect on the volume of daily production on the incidence of Unstable Non Acid Milk. This fact may be associated with stratification of farmers because Zanela et al. [14] worked with most samples (95%) from farms with production below 200 liters per day with an incidence of 63% of samples with Unstable Non Acid Milk. In the present study, only 20% of the samples were from farms below 180 liters per day, with 60 samples (13%) classified as Unstable Non Acid Milk. Analyzing the distribution of cases of Unstable Non Acid Milk in the production groups established, in this work was not found relation between effect of group of daily milk production and occurrence of Unstable Non Acid Milk. Zanela et al. [14] supposed that the groups of higher production had the better feeding and management of animals, which reflected in the lower incidence of Unstable Non Acid Milk.

As no relationship was found between Unstable Non Acid Milk and groups of milk production, was not associated also with other characteristics of the farms, such as the geographic location in terms of county and state, and in the logistics of milk collection.

The rejection of the milk with Unstable Non Acid Milk by industry, identified as acid milk, without actually acid, causes losses for farmers. According Zanela et al. [14], when milk is rejected by the industry for being considered acidic, most producers do not know the possibility the occurrence of Unstable Non Acid Milk, and then associated the problem with poor hygiene and acidity by bacterial growth in milk. This fact generates misleading conclusions about the problem of instability of milk and can hamper your adjustment because the real cause of the problem is not being properly identified.

Trying to diagnose the occurrence of Unstable Non Acid Milk, discarding the hypothesis of instability caused by acid milk, was evaluated the change in the composition of milk by comparing the different classifications of the samples (stable, Unstable Non Acid Milk and unstable). In Table 5, can be seen significant variation (P<0.05) in some components in the different classifications of samples.

TABLE 5
COMPARISON OF THE COMPOSITION OF NORMAL MILK IN RELATION TO UNSTABLE NON ACID MILK

	Stable	Unstable Non Acid Milk	Unstable
Degree of alcohol in stability test (%)	82 a	76 b	76 b
Titratable acidity (°D)	16.60 b	15.60 c	17.80 a
pH	6.63 a	6.64 a	6.54 b
Fat (%)	3.56	3.62	3.54
Protein (%)	3.15	3.16	3.16
Lactose (%)	4.44 a	4.40 b	4.43 a
Solids Non Fat (%)	8.54 a	8.50 b	8.55 a
Somatic Cell Count (1000 cells/mL)	570	564	522
Total Bacterial Count (1000 cfu/mL)	84	82	100

Means followed by different letters in the row differ by Tukey`s test (P <0.05)

In the present study was found significant difference between the samples that showed instability (unstable and Unstable Non Acid Milk) and the stable ones only for stability in alcohol test and pH. Same result found by Marques et al. [15] with milk samples from south region of Brazil. For lactose and solids non fat were observed significant differences (P<0.05) between the samples classified as Unstable Non Acid Milk from the other samples. All samples classifications were different in the *titratable acidity*.

According to Zanela et al. [11] there were changes in the composition of Unstable Non Acid Milk in relation of stable milk, showing increase in fat and SCC, decreased of the components lactose and protein, but no change for solids non fat. Zanela et al. [16] also found changes evaluating Unstable Non Acid Milk and stable milk, resulting in a decrease in the levels of lactose and solids non fat, increased fat and no variation in protein. For Oliveira and Timm [17], the highest variation on the milk composition for the samples classified as Unstable Non Acid Milk was the fat, with a significant increase of the levels of this component in the stable milk (3%) when compared casein instability milk (3%). Moreover, stable milk had average contents of lactose (4%) significantly ($P<0.01$) higher than the milk with casein instability (4%).

In the works mentioned above and also in the present study, the lactose content has always been a decrease in its composition in samples classified as Unstable Non Acid Milk. This fact can be explained by the lactose dependence on availability of its precursor, in this case glucose. On the other hand, the main precursor for the synthesis of glucose is the propionate whose origin comes from microbial fermentation [1]. Thus, can be seen the influence of aspects related to nutritional management on the incidence of Unstable Non Acid Milk, as already discussed by Barros [18] and Zanela et al. [14]. Nevertheless, these authors reported the incidence of the problem especially with sudden changes in diet and poor nutrition. One possible explanation can be, perhaps, related to carbohydrate metabolism, according to the reduction of the lactose content in Unstable Non Acid Milk.

IV. CONCLUSION

Finally, the 52 million liters of milk processed, 7% were classified as Unstable Non Acid Milk. This results in about 3,5 million liters of milk involved with low-quality protein, varying its occurrence throughout the months of the year, especially in the early fall and late winter. The problem named Unstable Non Acid Milk has close relation with nutritional management, particularly for the metabolism of carbohydrate that is an important matter to find the prevention and / or control the problem.

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