



Colostrum silage: fermentative, microbiological and nutritional dynamics of colostrum fermented under anaerobic conditions at different temperatures

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ABSTRACT. The fermentative, microbiological and nutritional dynamics of bovine colostrum fermented under anaerobic conditions at different temperatures is provided. Colostrum was homogenized and stored in PET bottles in anaerobic conditions and incubated at controlled temperature ($32.5 \pm 1^\circ\text{C}$ or $22.5 \pm 1^\circ\text{C}$) or at room temperature ($17.4 - 21.5^\circ\text{C}$) and opened after 0, 1, 7, 14, 21, 28 and 35 days to determine fermentative and nutritional parameters and bacteria, yeast and mold counts. Further, pH rates showed significant variations during the fermentation period ($p < 0.0001$), with colostrum stored at 32.5°C , exhibiting the lowest rates and significant reduction during the first days. Titratable acidity and lactic acid concentration showed increasing rates and LAB development was intense, especially at high temperatures. After 35 days, about 50% of total nitrogen became non-protein N and casein fraction was reduced to 0.66% of total nitrogen. Lactose decreased during fermentation and fat concentrations were not affected by temperature. Results suggested that the temperature at which the colostrum was fermented directly influenced the speed and intensity of microbial population development and degradation of the main nutritional parameters, such as casein and lactose.

Keywords: dairy calves, feed quality, liquid diet, milk replacer.

Silagem de colostro: dinâmica fermentativa, microbiológica e nutricional de colostro fermentado sob condições anaeróbias em diferentes temperaturas

RESUMO. Objetivou-se caracterizar a dinâmica fermentativa, microbiológica e nutricional de colostro bovino fermentado em condições anaeróbias e diferentes temperaturas. O colostro foi homogeneizado e armazenado em garrafas PET em condições anaeróbias e incubadas em temperatura controlada ($32,5 \pm 1^\circ\text{C}$ ou $22,5 \pm 1^\circ\text{C}$) ou ambiente ($17,4 - 21,5^\circ\text{C}$), e abertas após 0, 1, 7, 14, 21, 28 e 35 dias, para determinação de parâmetros fermentativos, nutricionais e contagem de bactérias, mofo e bolores. Os valores de pH apresentaram variação durante o período de fermentação ($p < 0,0001$), com colostro armazenado a $32,5^\circ\text{C}$ apresentando os menores valores e marcada redução nos primeiros dias. A acidez titulável e a concentração de ácido láctico apresentaram valores crescentes. O desenvolvimento de BAL foi elevado, especialmente em altas temperaturas. Após 35 dias, em torno de 50% do N total estava na forma de N não-proteico e a caseína foi reduzida a 0,66% do N total. A lactose foi reduzida durante a fermentação. A concentração de gordura não foi afetada pela temperatura durante o processo. Os resultados sugerem que a temperatura na qual o colostro é fermentado em condições anaeróbias influencia diretamente na velocidade e intensidade do desenvolvimento da população microbiana e degradação dos principais componentes nutricionais como a caseína e a lactose.

Palavras-chave: bezerros leiteiros, qualidade de alimentos, dieta líquida, sucedâneo lácteo.

Introduction

Colostrum surplus, which is rich in proteins and fats and produced in large quantities by cows, may be an interesting alternative liquid diet for calves (FOLEY; OTTERBY, 1978; DAVIS; DRACKLEY, 1998). However, due to lack in market value, its use is generally restricted to the day of harvest and, in some cases, overproduction is discarded and not used. Although most producers are aware of the potential use

of colostrum during the milk-feeding period, storage difficulties, such as the need of a freezer, causes a lack of interest in its employment in various systems of milk production.

Fermentation may be an alternative to freezer storage and future delivery to animal. Fermentation causes the development of beneficial microorganisms, such as lactic acid bacteria, and pH reduction preserves colostrum at room temperature (OTTERBY et al.,

1980). However, despite the possibility of product conservation, variations in results have been observed when fermentation is carried out under different conditions of storage, especially at room temperature.

Recommendation on the general storage of colostrum during fermentation highlights cool temperatures and always below 25°C (FOLEY; OTTERBY, 1978). Research performed in Europe and the United States showed that in cold weather, colostrum or transition milk may be stored under room conditions for fermentation without any major changes in its physical characteristics or the appearance of undesirable microorganisms. On the other hand, these claims may not be true for periods of the year with higher temperatures (above 30°C) or for weather conditions in tropical countries.

Farmers from southern Brazil have recently succeeded in using a new technique of storage, or rather, anaerobically fermented colostrum or colostrum silage, for the feeding of calves. Although the technique provided good preservation results, the fermentation process could be changed when fermentation was performed at higher temperatures, above 30°C. Furthermore, published studies over the last years described the fermentation and animal feeding of aerobic fermented colostrum, but no studies were extant on the use of anaerobic fermentation as an alternative technique for the conservation of the product. Current investigation characterizes the fermentative, microbiological and nutritional dynamics of bovine colostrum fermented under anaerobic conditions and stored at different ambient temperatures.

Material and methods

The trial was conducted at the Department of Animal Science of the Escola Superior de Agricultura 'Luiz de Queiroz' - USP / ESALQ, Piracicaba, São Paulo State, Brazil. Bovine colostrum from second and third milking of Holstein cows from different private farms was collected, mixed and pooled. The pool was subdivided into sixty-three 500 mL-PET plastic bottles filled and lightly pressed before closure to remove the space with oxygen and thereby establish an anaerobic condition. Throughout the filling process, colostrum pool was mixed to maintain a homogeneous material. After the ensiling process, all bottles were stored in two BOD incubators (model TE-402, Tecnal, Piracicaba, São Paulo State, Brazil) with controlled temperature at $32.5 \pm 1^\circ\text{C}$ (Treatment 1) and $22.5 \pm 1^\circ\text{C}$ (Treatment 2), or in a dark room at room temperature at $17.4 - 21.5^\circ\text{C}$ (Treatment 3). Bottles were opened at 0, 1, 7, 14, 21,

28 and 35 days after ensiling, with three replicates per opening time per treatment.

At opening time, sub-samples of each bottle were collected to determine the fermentative, microbiological and nutritional parameters. The pH measurements were performed on digital pHmeter (Digimed TE-902) and temperature was measured on a digital thermometer; titratable acidity determinations were performed as described by the Federation Internationale de Laiterie (1991); lactic acid was determined by methodology by Pryce (1969).

Microbiological analysis was performed following general procedures (dilutions and plating count) described in 'Standard Methods for the Examination of Dairy Products' (APHA, 1992). For enterobacteria counts, Petrifilm® enterobacteriaceae plates (3M Brazil®, Sumaré, São Paulo State, Brazil) were inoculated and incubated at $32.5 \pm 1^\circ\text{C}$ on BOD incubator (Model TE-402, Tecnal, Piracicaba, São Paulo State, Brazil) for 24 ± 2 hours. After the incubation period, red colonies with yellow edges and/or red colonies with gas bubbles, with or without yellow edges, were considered positive for enterobacteria. Lactic acid bacteria count was performed with Petrifilm® AC plates (3M Brazil®, Sumaré, São Paulo State, Brazil) incubated in anaerobic jars with an anaerobic atmosphere generator (ANAEROBAC, Probac of Brazil Bacteriological Products Ltda., São Paulo, São Paulo State, Brazil) in a BOD incubator at $32.5 \pm 1^\circ\text{C}$ for 48 ± 3 hours. Red colonies were considered positive for lactic acid bacteria regardless of size or color depth. Counts of yeasts and molds were carried out with Petrifilm® YM plates (3M Brazil®, Sumaré, São Paulo State, Brazil) incubated at $22.5 \pm 1^\circ\text{C}$ in a BOD incubator. After 3 days of incubation, small colonies with blue-green edges were considered yeast, whereas the large dark colonies, with fuzzy edges, were counted after 5 days of incubation and considered fungi.

Samples were also analyzed to determine lactose (FEITOSA-TELES et al., 1978), fat by the Bligh and Dyer method (MANIRAKIZA et al., 2001), total nitrogen, non-protein nitrogen and casein by micro-Kjeldahl method. The fermentative (pH, titratable acidity, lactic acid concentration and temperature), microbiological and nutritional (lactose, crude fat and nitrogen fractions) data were analyzed at a completely randomized design by Generalized Linear Models procedure (GLM) of SAS statistical software - version 9.0 (SAS, 1991). Each opened bottle was an experimental unit, with three replicates per opening time. Data were transformed

to \log_{10} and evaluated to analyze microbiological dynamics. For comparison of means between open times, Tukey's test was used at 5% significance level. For graphs, data were estimated by regression equations developed by the REG procedure, and descriptive analyzes were performed by UNIVARIATE procedure of SAS statistical software - version 9.0 (SAS, 1991) for all parameters.

Results and discussion

Higher storage temperature during fermentation resulted in lower average rates and faster pH drop (Figure 1), followed by higher titratable acidity and lactic acid concentration (Table 1, Figures 1 and 2). Results showed that although the initial pH was similar for all treatments due to its retrieval from a single colostrum pool, the pH quickly decreased when ensiled colostrum was stored at a higher temperature (32.5°C) (Figure 1). Muller and Syhre (1975) observed a similar behavior in pH when colostrum fermenting under aerobic conditions was stored at an ambient temperature of 32 or 39°C. According to these authors, pH read between 3.5 and 3.7 in nine days of fermentation, whilst the colostrum, stored at cooler temperatures (21°C), had lower pH decreases and reached its lowest rate at 4.4.

In current study, pH rates close to 3.5 were reached between 7 and 14 days of fermentation when the fermenting colostrum was stored at 32.5°C (Figure 1). The dynamics of pH of the fermenting colostrum stored at a cooler temperature (22.5°C) or room temperature (between 17.4 and 21.5°C) showed higher rates than those in colostrum stored at 32.5°C at the end of the period under analysis (35 days) (Figure 1).

Bush et al. (1980) observed a decrease in pH from colostrum stored under aerobic conditions and suggested that the rapid decline in pH was directly related to a further development of lactic acid bacteria, and consequently, increased production of lactic acid. Although Muller and Syhre (1975) reported that pH decreased faster when colostrum was fermented at temperatures above 32°C, decrease seemed to be unstable. These authors reported that increases in colostrum pH after 21 days of storage were common and related to the initial protein concentration. According to Rindsig et al. (1977), the rapid development of proteolytic microorganisms under

ambient conditions with temperature above 32°C was one of the main factors that caused pH fluctuations.

Changes in the physical characteristics of colostrum could be clearly observed after a few days of fermentation, albeit with differences among treatments. Another important observation was that colostrum fermenting under higher temperatures showed visual separation of solids in fairly distinct layers within a few days of storage. The precipitation of solids to the bottom of the bottle and a separated fat layer at the top above the serum were reported on the first day. In other treatments, a similar separation was observed between 7 and 14 days of fermentation. Another important point dealt with the organoleptic characteristics of colostrum fermented at 32.5°C which, when opened, always had a putrid odor, characteristic of intense proteolytic activity. Jenny et al. (1977) also reported a putrid odor and mold development when colostrum was stored at 27°C or at higher temperatures. This fact was corroborated by Rindsig et al. (1977) with reports on colostrum stored at temperatures between 32 and 39°C. The authors suggested discarding the product under these conditions since its voluntary intake by calves is low, resulting in rejection at the time of delivery.

The decline in pH followed increase in titratable acidity which was greater in treatments at higher temperatures (Figures 1 and 2). Similar dynamics for the acidity data was observed by other authors in studies with fermented colostrum stored between 27 and 39°C under aerobic conditions (OTTERBY et al., 1977).

As expected, a similar effect was observed for the concentration of lactic acid which was higher in the colostrum fermented in warmer conditions (32.5°C). Moreover, when fermented at room temperature or in cooler conditions (22.5°C), rates were very similar despite an increase in fermentation time ($p < 0.0001$) (Figure 2). Other authors also reported rapid increase in lactic acid concentrations according to pH and titratable acidity in colostrum fermented under aerobic conditions (BUSH et al., 1980; OTTERBY et al., 1980).

Table 1. pH, titratable acidity, lactic acid concentration and temperature in bovine colostrum stored at different ambient temperatures during anaerobic fermentation.

Item	Temperature			Range (Min.-Max.)	SEM ⁽²⁾	p < ⁽³⁾ T(°C)
	32.5°C	22.5°C	Room (17.4 - 21.5°C)			
pH	4.28 ^b	4.69 ^a	4.82 ^a	3.47 - 6.27	0.035	<.0001
Titratable acidity ⁽¹⁾	290 ^a	189 ^b	171 ^b	32.3 - 486.4	7.5	<.0001
Lactic acid, mg dL ⁻¹	25.3 ^a	15.6 ^b	14.4 ^b	3.05 - 34.88	0.49	<.0001
Temperature, °C	29.0 ^a	21.3 ^b	19.9 ^b	17.4 - 31.4	0.33	<.0001

⁽¹⁾Values of titratable acidity expressed in °Dornic; ⁽²⁾SEM = standard error of mean; ⁽³⁾T(°C) = effect of temperature, ^{a,b} Different letters on the same row differ at $p < 0.05$

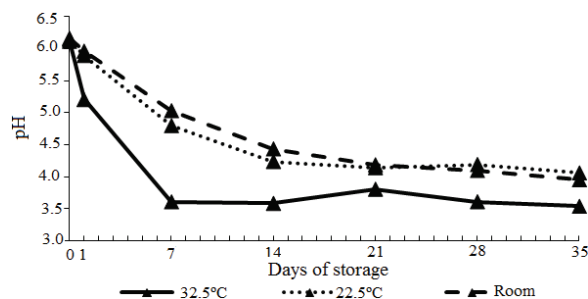


Figure 1. Effect of days of storage on pH of bovine colostrum stored at different ambient temperatures during anaerobic fermentation ($p < 0.0001$).

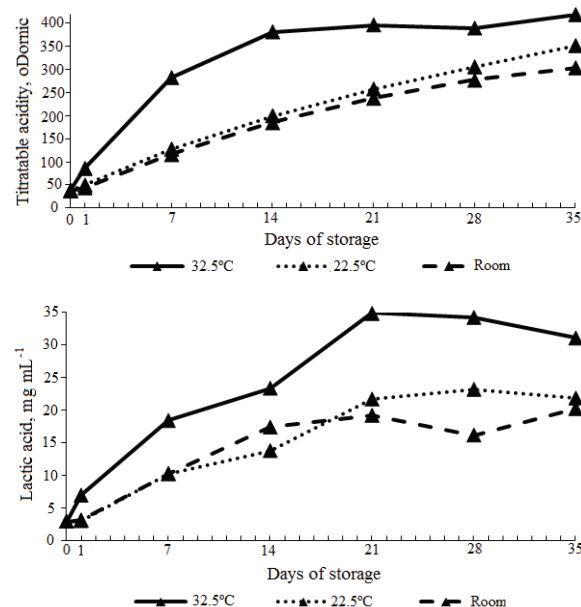


Figure 2. Effect of days of storage on titratable acidity ($p < 0.0001$) and lactic acid concentration ($p < 0.0001$) of bovine colostrum stored at different ambient temperatures during anaerobic fermentation.

Colostrum fermentation at a higher temperature (32.5°C) provided the lowest counts for lactic acid bacteria and enterobacteriaceae, although counts of yeasts were higher when compared to colostrum fermentation at cooler temperatures (Table 2).

Figure 3 shows microorganisms counts as a function of storage temperature and time after ensiling. Most microorganisms' growth occurred during the first days of fermentation (Figure 3). Average counts for colostrum fermenting at 22.5°C or room temperature were very similar (Table 2) and presented

a closed behavior as a function of time after ensiling (Figure 3). Ambient temperature during fermentation directly influenced the development of microorganisms ($p < 0.05$, Table 2), with rapid growth since the first days of fermentation. According to Wouters et al. (2002), intense microbiological development was expected and reported in most research on fermented colostrum.

Total counts of lactic acid bacteria and enterobacteriaceae showed that, in spite of strong development during the first days of fermentation, a decline was observed during the beginning of the fermentation process when ensiled colostrum was stored at 32.5°C (Figure 3). Other studies also reported this behavior on microorganism counts, namely, rapid initial growth, followed by decline, usually after about a month of storage (JENNY et al., 1977). However, as studies in the literature reported microbiological development of colostrum or milk only under aerobic conditions, microbial dynamics observed in this study were quite different since fermentation occurred under anaerobic conditions.

The development of lactic acid bacteria was accelerated when anaerobic fermentation occurred at 20-30°C. Under these conditions and due to fast microorganism development, there was a heavy consumption of the main components of the colostrum or milk, resulting in low concentrations of lactose and protein after a few days of fermentation (ROBINSON; TAMIME, 1999). The consumption of lactose by microorganisms caused the production of lactic acid, and hence a rapid decline in pH with a concomitant increase in titratable acidity (Figure 1). However, most lactic acid bacteria and enterobacteriaceae were sensitive to low pH. Consequently, their development was inhibited, generally when pH reached rates below 4.0, as reported in current study (Figure 3).

The development of molds, albeit small, indicated colostrum contamination, often collected without proper care and hygiene on dirty and contaminated containers. Although storage under higher temperature conditions (32.5°C) inhibited the development of enterobacteriaceae and reduced the population of lactic acid, the yeast counts increased in the early days of fermentation (Figure 3).

Table 2. Means, minimum and maximum rates of total counts of microorganisms observed in bovine colostrum stored at different ambient temperatures during anaerobic fermentation.

Total counts (log ₁₀ UFC mL ⁻¹)	Temperature			Range (Min.-Max.)	SEM ⁽¹⁾	p < ⁽²⁾ T(°C)
	32.5°C	22.5°C	Room (17.4 - 21.5°C)			
Lactic Acid Bacteria	6.52 ^b	7.64 ^a	7.71 ^a	4.60 - 8.85	0.10	<.0001
Enterobacteriaceae	1.38 ^b	2.75 ^a	3.35 ^a	0.0 - 6.38	0.24	0.0007
Yeasts	4.18 ^a	3.77 ^b	3.42 ^c	1.0 - 8.0	0.09	0.0005
Molds	0.25 ^b	1.04 ^a	0.54 ^b	0.0 - 2.48	0.09	0.0007

⁽¹⁾SEM = standard error of mean; ⁽²⁾T(°C)=effect of temperature, ^{abc}Different letters on the same row differ $p < 0.05$.

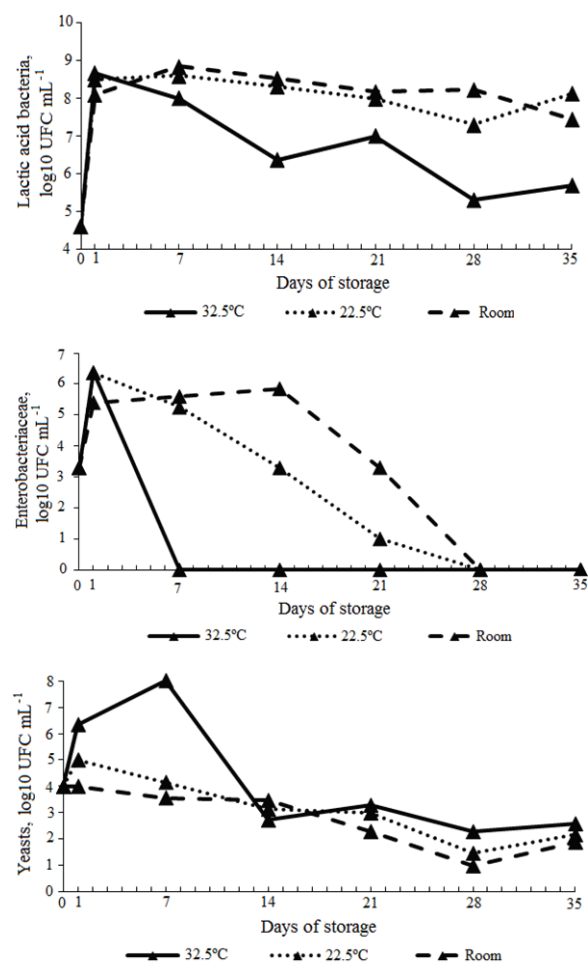


Figure 3. Effect of days of storage on lactic-acid bacteria ($p < 0.0001$), enterobacteriaceae ($p < 0.0001$) and yeasts ($p < 0.0001$) counts of bovine colostrum stored at different ambient temperatures during anaerobic fermentation.

According to Wouters et al. (2002), under pH close to or below 4.0, when most of the bacteria growth was slowed, there was a growth of lactose fermenting yeast. However, the population of non-fermenting lactose yeasts increased. They were effective in the use of the galactose from the lactose hydrolysis and fermentation by bacteria, leading to the formation of various compounds, especially ethanol. Robinson and Tamime (1999) showed that, although present in dairy products, the growth of yeast and mold was slow. However,

when conditions were ideal for their development, such as proper temperature and substrate availability, the fast growth of yeasts led to severe degradation of proteins, especially casein, giving a viscous and dense aspect to the fermented milk product. In current study, when the bottles, stored at 32.5°C, were opened, the aspect of the milk product was viscous and not easily homogenized, as the above authors stated.

All nutrients, except fat contents, were affected by storage temperature during fermentation (Table 3). Higher temperatures decreased lactose ($p < 0.0002$), total nitrogen ($p < 0.0001$) and consequently crude protein ($p < 0.0001$) of anaerobically fermenting colostrum, when compared to effects at cooler temperatures. Casein also decreased as temperature during fermentation increased ($p < 0.0001$). However, temperature at 22.5°C presented intermediate rates for total nitrogen and crude protein when compared to those at higher (32.5°C) and room temperatures (Table 3). Non-protein nitrogen highly increased by higher storage temperature of the fermenting colostrum, resulting in an average rate with a more than two-fold increase when compared to results at other storage temperatures (Table 3).

The most significant changes in nutritional composition occurred during the first days of fermentation, mainly in the case of lactose and casein contents for higher storage temperatures during fermentation (Figures 4 and 5). However, compositional changes during fermentation under cooler temperatures were smoother. Non-protein nitrogen content increased during fermentation time, with a greater increase at higher storage temperatures (Figure 5). Changes in nutritional composition during fermentation time were very similar to those occurring at cooler storage temperature (Figures 4 and 5).

Lactose, the main substrate used by microorganisms for their survival, produced lactic acid as a result of its metabolism. Since storage at higher temperature resulted in a sharp decline in pH and high acidity, lactose concentration had the expected effect, with a fast decline within the first 7 days of fermentation.

Table 3. Nutritional composition of bovine colostrum stored at different ambient temperatures during anaerobic fermentation.

Item (%)	Temperature			Range (Min.-Max.)	SEM ⁽²⁾	p < ⁽³⁾ T(°C)
	32.5°C	22.5°C	Room (17.4 - 21.5°C)			
Lactose	2.60 ^b	3.80 ^a	3.39 ^a	1.08 - 5.50	0.12	0.0002
Fat	5.08	5.12	5.29	4.05 - 5.38	0.06	0.07
Total nitrogen (TN)	0.91 ^c	1.22 ^b	1.34 ^a	0.58 - 1.61	0.03	<.0001
Crude protein	5.83 ^c	7.77 ^b	8.58 ^a	3.7 - 10.3	0.17	<.0001
Casein. % of TN	17.11 ^b	37.84 ^a	35.63 ^a	14.5 - 43.5	0.63	<.0001
Casein	0.96 ^b	2.91 ^a	3.07 ^a	0.66 - 4.64	0.09	<.0001
NPN. % of TN ⁽¹⁾	22.96 ^c	10.12 ^b	8.32 ^b	3.7 - 47.1	1.09	<.0001

⁽¹⁾NPN = non-protein nitrogen; ⁽²⁾SEM = standard error of mean; ⁽³⁾T(°C) = effect of temperature; ^{abc}Different letters on the same row differ $p < 0.05$.

When stored under a cooler temperature (22.5°C or room temperature), reductions occurred gradually during the colostrum fermentation process, although the expected effect of decrease in lactose concentration was observed. Thus, the dynamics observed for concentrations of lactose (Figure 4) corroborated results for pH and titratable acidity (Figure 1) and for lactic acid concentration (Figure 2).

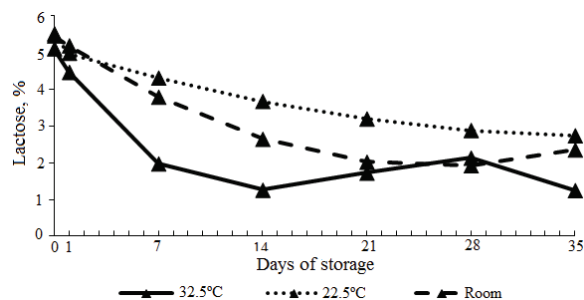


Figure 4. Effect of storage days on lactose concentration of bovine colostrum stored at different ambient temperatures during anaerobic fermentation ($p < 0.0001$).

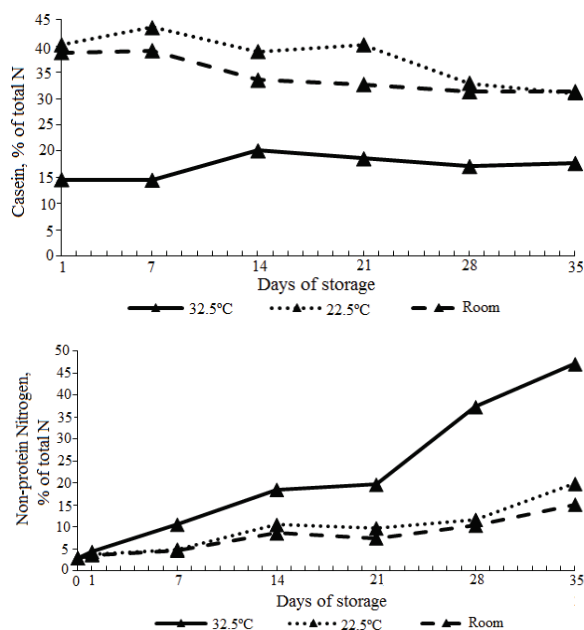


Figure 5. Effect of days of storage on casein ($p < 0.0001$) and non-protein nitrogen ($p < 0.0001$) concentration of bovine colostrum stored at different ambient temperatures during anaerobic fermentation.

Studies on the isolation of microorganisms showed that the reduction of lactose might reach more than 80% of the concentration in colostrum in natura when a high population of lactic acid bacteria was extant (BUSH et al., 1980). Yu et al. (1976) found no significant decrease in lactose concentration during the first seven days of storage. However, there was only 22% of the original concentration of lactose in the colostrum fermented

at 16-24°C, after 35 days. These concentrations were similar to those observed in colostrum and stored at room temperature or 22.5°C in current study.

As expected, due to intense activity and microbial growth, the colostrum's crude protein stored under 32.5°C decreased significantly ($p < 0.0001$), with a reduction of the initial 10% to rates close to 4% after 35 days of fermentation. Effects due to the fermentation period were also reported for the rates of crude protein of colostrum fermented at room temperature or 22.5°C ($p < 0.0001$), albeit with higher rates at the end of 35 days. According to the literature, results with changes in crude protein concentrations were variable and often dependent on environmental conditions in which the colostrum was fermented. In warmer conditions, changes in the concentrations of total nitrogen (TN) and hence crude protein ($TN \times 6.38$) were generally more intense, indicating higher proteolytic activity. Nevertheless, in cooler weather conditions, below 25°C, decreases have been registered, albeit with lower intensity.

Casein concentrations decreased significantly (Figure 5), mainly for the colostrum stored at 32.5°C. This fact corroborated increase in non-protein nitrogen (Figure 5) and reduced crude protein. By favoring the growth of microorganisms at higher temperatures, casein was utilized with greater intensity. Evidence of higher proteolysis was provided by high viscosity observed in colostrum samples stored at 32.5°C when compared to that stored at 22.5°C or at room temperature. Foley et al. (1978) reported similar results with significant casein reduction during the fermentation process. Since casein is easily precipitated in acid, greater microbial growth and high production of lactic acid may have been responsible for further decrease in casein concentrations. Rindsig et al. (1977) suggested that pH decrease had a direct relationship with high casein precipitation, especially in the colostrum fermented at higher temperatures.

Corroborating the significant decrease in crude protein and casein, high storage temperature resulted in an intense proteolysis process, with NPN rates at 47% of total nitrogen at the end of the fermentation period (Figure 5). On the other hand, at lower temperatures, NPN rates lay between 15 and 20% of total nitrogen. NPN increase confirmed the results of yeast and mold count and putrid odor in bottles stored at 32.5°C. Other authors found similar results when the fermentation of colostrum occurred at temperatures above 30°C, with reported cases of rejection of consumption of fermented colostrum in these conditions. Otterby et al. (1977) reported even higher values, reaching 47% of total N

after 28 days of fermentation at 37°C. Although in current study similar values were observed, the temperature was lower (32.5°C) and NPN concentration reached 47% of total nitrogen after 35 days of storage (Figure 5). NPN rates for colostrum ensiled at room temperature or 22.5°C also increased, albeit moderately, exhibiting rates similar to those observed when colostrum was fermented at cooler temperatures (OTTERBY et al., 1977).

The fat concentrations were not influenced by temperature ($p > 0.05$), although effects of fermentation time were observed ($p < 0.0001$). Otterby et al. (1977), in a very similar experiment but developed under aerobic conditions, failed to find any effect on fat concentrations. Foley et al. (1978) did not report any changes in the percentages of fat in fermented colostrum. According to these authors, the fraction is less altered during the fermentation process, with final ratio of 90% or more of the initial percentage observed.

Conclusion

Anaerobic fermentation of colostrum is a good alternative for the storage of surplus colostrum without spoilage. However, the temperature at which the colostrum is stored during the anaerobic fermentation process directly influences the speed and intensity of the degradation of the main nutritional solids, such as casein and lactose, and the growth of undesirable microorganisms. Results show that anaerobically fermented colostrum (colostrum silage) as milk replacer is not appropriate for dairy calves, particularly when fermentation is carried out in warm climates.

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