Milk contamination in different points of the dairy process. ii) mesophilic, psychrotrophic and proteolytic microorganisms

Contaminação do leite em diferentes pontos da produção leiteira: ii) microrganismos mesófilos, psicrotróficos e proteolíticos

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Abstract

Psychrotrophics microorganisms are able to multiply under refrigeration temperatures. They can produce heat-resistant extracellular enzymes that cause organoleptic and structural changes in milk and dairy products. With the objective of determining the frequency of microorganisms in the dairy process, four farms were evaluated in Londrina city – Brazil and different points were assessed. Mesophilic (48hs at 35ºC), psychrotrophic (25hs at 21ºC) and proteolytic (72hs at 22ºC) microorganisms were researched, and the proteolytic predominant microflora were determined by using the Gram stain technique. The main psychrotrophic contamination points were the residual water on milk cans, on bulk tank and bad cleaned teats, and milk cans and teats were the most important sources of proteolytics. Gram negative bacilli represented 69% of the microorganisms in the refrigerated milk that means 45% of the total proteolytics microorganisms in the dairy process. Gram negative bacilli is most frequent on bad cleaned teats, teatcups, residual water in milk cans and bulk tank. The psychrotroph count in the refrigerated milk was more than the mesophilic count, showing that that psychrotrophics are the best indicatator for refrigerated milk contamination. The psycrotrophics count at all points assessed were greater than the ideal limit (10% of the mesophilic count). Thus as refrigeration at 4ºC is not sufficient to control the multiplication of the psycrotrophics group, good practices should be associated to avoid or control milk contamination by psycrotrophics.
Key words: Milk, quality, psychrotrophics, proteolytics, refrigeration.

Resumo

Os psicrotróficos são microrganismos que têm capacidade de multiplicação em temperaturas de refrigeração. Produzem enzimas termorresistentes como proteases e lipases que promovem alterações organolépticas no leite e comprometem a produção de derivados. Com o objetivo de determinar a freqüência desses microrganismos a produção do leite, foram estudadas 04 propriedades da região de Londrina, PR, analisando diversos pontos do processo. Determinou-se a contagem de microrganismos aeróbios

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mesófilos (48hs a 35ºC), psicrotróficos (25hs a 21ºC), psicrotróficos proteolíticos (72 hs a 22ºC) e as características morfotintoriais da microbiota psicrotrófica proteolítica predominante. Os principais pontos de contaminação por psicrotróficos foram a água residual de latões, tanques de expansão e tetos higienizados inadequadamente, sendo que os psicrotróficos proteolíticos predominam nos latões e tetos. Bacilos Gram negativos representaram 45% da microbiota proteolítica de todo o processo de produção, no leite refrigerado corresponderam a 69% dos psicrotróficos proteolíticos encontrados e ainda predominaram nos tetos higienizados, teteiras, água residual de latões e tanques de expansão. As contagens de psicrotróficos superaram as de mesófilos em leite refrigerado, mostrando que a pesquisa de psicrotróficos é ideal para avaliar a real carga microbiana do leite refrigerado. Como a refrigeração a 4ºC não inibe o crescimento deste grupo de microrganismos, deve-se evitar sua incorporação ao leite durante a produção, havendo necessidade da adoção de boas práticas de manejo em todo o processo produtivo.
Palavras-chave: Leite, qualidade, psicrotróficos, proteolíticos, refrigeração.

Introduction

The psychrotrophs are a group of microorganisms that are able to grow at low temperatures, causing spoilage of fluid milk and some other dairy product (OLIVERIA; PARMALEE, 1976). According to Smithwell and Kailasapthy (1995) contamination with psychrotrophs is one of the most important points in determining milk quality.

The psychrotrophs found in milk are environmental, originating in the soil, water, vegetation, teat/udder and improperly cleaned milking equipment. (COUSIN, 1982; SOLER; DE PAZ; NUÑEZ, 1995). They are mainly Gram-negative organisms and while the *Pseudomonas* genus is the most common (STADHOUDERS, 1975; COUSIN, 1982; MUIR, 1996; SORHAUND; STEPANIAK, 1997), *Achromobacter, Aeromonas, Escherichia, Flavobacterium, Proteus, Xanthomonas* e *Cytophagei* are also found (LAW, 1979). Gram-positive psychrotrophic organisms, such as species of *Micrococcus*, *Bacillus* and *Arthrobacter* are usually present in smaller numbers than the Gram-negative bacteria (COUSIN, 1982; AMERICAN PUBLIC HEALTH ASSOCIATION, 1992). There are also psychrotrophic moulds and yeasts, which can present quality problems, especially cream and butter (COUSIN, 1982; FONSECA; SANTOS, 2000).

The Gram negative psychrotrophic microorganisms are easily destroyed by heat processes such as pasteurization, but produce proteolytic and lipolytic heat-resistant extracellular enzymes that remain active in the milk and cause flavor defects such as bitter and soapy taste, rancidity, gelation of UHT and reduced cheese yield (LAW, 1979; MAHIEU, 1991; SMITHWELL; KAILASAPATHY, 1995). Gram-positive microorganisms present low proteolytic action and limited lipolytic action compared with the Gram-negative microorganisms (MUIR, 1990). However, Washam, Olson and Vedamuthu (1977) associated the *Bacillus* spp. genus to a series of organoleptic alterations in milk refrigerated 14 days at 7.2ºC .

Alterations in milk and its products become perceptible when the psychrotrophic counts are above 10^6 CFU/mL (PUNCH; OLSON; THOMAS, 1966; SANTANA et al., 2001). However, Thomas (1966) considered that knowledge of the grade of psychrotroph microorganism present was more important than the microbial load, as each microorganism has its own multiplication and enzyme production rates. According to the Industrial and Sanitation Inspection Regulations for Products of Animal Origin: Brasil – RIISPOA (1976) milk should present at most 10% psychrotrophic microorganisms at total mesophilic aerobic count. Extracellular enzyme production by psychrotrophs is related to temperature, microorganism growth phase, oxygen availability and culture medium composition (NUÑEZ; NUÑEZ, 1983). *Pseudomonas* spp. synthesizes heat stable enzymes at refrigeration temperature, mainly at the end of the log cell growth stage (SORHAUND; STEPANIAK, 1997).

This study was carried out to determine the main points of contamination by mesophilic, psychrotrophic and psychrotrophic proteolytic microorganisms in the dairy production, and to determine the classification of proteolytic microorganisms by Gram staining.

Material and Methods

Farm characteristics and practices

The study was carried out from May 2000 to June 2001 on four dairy farms. One farm producer grade A milk, one grade B milk and the two farms producer grade C milk (C1, C2) and all were located in the Londrina region, Northern Paraná state, Brazil. These farms were selected by a local cooperatives to represent the management, production and installation characteristics most frequent in milk production in the region. In Brazil, these classification of milk in grade A, B and C is based in the milk farm installations and in the milk microbiology quality. The better conditions of production is showed by milk grade A.

Tables 1 and 2 show the management and production characteristics and the practices used in the milking and pasteurization equipment on the farms studied.

Table 1. Characterization of the dairy farms studied (grades A, B, C1 and C2) from Londrina region, Paraná, may 2000 to june 2001.

Features	A	B	C1	C2
1. Animals	60	93	80	145
2. Livestock	*Tie stall*	*Free stall*	pasture	pasture
3. Production/day	02	02	02	02
4. Mean days yield	900 L	1.700 L	960 L	*920 L/ **240 L
5. Milking	Closed circuit	Closed circuit	Use of milk cans	* **
6. Calf presence	N	N	Y	Y* N**
7. Teat cleaning (water and paper)	Y	Y	Y	Y
8. Pre dipping (Chlorine solution)	Y	N	N	N
9. Post dipping	Chlorine solution	Iodine Solution	N	N
10. Teatcup cleaning during milking	Water spout	Chlorine solution	N	N
11. Reject firth 3 jets	Y	Y	N	N
12. CMT	Y	Y	N	N
13. Bulk tank at 4ºC	2.000L	4.000 L	2.000 L	2.000L
14. Plate cooled	Y	N	N	N
15. Frequency of milk collection	Pasteurization at farm	Daily / 48 hs	48 hs	Pasteurization at farm
16. Frequency of pasteurization	Each 02 days	N Pasteurization	N Pasteurization	Daily

*Mechanic milking with closed circuit in cattle shed with calf.
**Mechanic milking with milk cans
Y= yes N= No
CMT= California Mastitis Test

Table 2. Characterization of milking and pasteurization cleaning of the dairy farms studied (grades A. B. C1 and C2) from Londrina region. Paraná, may 2000 to june 2001.

Properties	A	B	C1	C2
Milk cans cleaning	N	N	Detergent lightly alkaline	Soap
Bulk tank cleaning	Manual with Neutral detergent	Closed circuit	Detergent lightly alkalin	Soap
Teatcups cleaning	Closed circuit	Closed circuit	Detergent alkaline chlorine	Detergent alkaline chlorine
Pasteurization equipment	NaOH and Nitric acid at 1.5%	N	N	NaOH and Nitric acid at 1.5%
Bulk pasteurized tank	Manual with Neutral detergent. Circulation with Alkaline solution	N	N	Manual with soap
Packed holding tank	Manual with Neutral detergent Passage of alkaline solution	N	N	Manual with soap

N: Do not exist these equipment/utensils in this farm

Collection Points

Microbial contamination was assessed at various points in the milk production process. In the grade A milk farm 38 points were analyzed and 59 points in the B grade milk farm. In the grade C milk farms, 38 points were analyzed on C1 and 69 points on C2.

All the animals in production on the farms were tested for mastitis using the California Mastitis Test (CMT), according to Silva et al (2000 apud SCHALM; NORLANDER, 1957). Ten percent of the animals tested were selected from each farm, 50% were positive and 50% negative in the CMT. (Table 1). These procedure was carried out to evaluated the influence of infections on the milk contamination. Swabs were taken from the dirty and cleaned teats of these animals. The first three jets of milk were also collected and after CMT a second sample was collected, representative of the milk in the udder cistern.

Teatcups, individual and collective balloons, milk cans and bulk tanks were analyzed before and after milking. Swabs were taken from these points and samples of milk and residual water were collected.

Swabs

There are no indications in the literature on the area to be sampled in some situations so we determined the areas to simplify taking the swabs at the different points assessed. An area of 3 cm^3 was used on the teats, teatcups, hoses and tubing, compatible with the diameter of the tubing and equipment and with the mean teat length. An area of 25 cm^2 was used in the tanks and milk cans as indicated by the literature (BRAMLEY; MCKINNON, 1990).

The areas were limited by a model of sterile, flexible plastic developed and manufactured in the Inspection Laboratory for Products of Animal Origin (LIPOA) of the Universidade Estadual de Londrina (UEL). The swabs were taken using flexible sterile sticks with ryon tips and Letheen broth for transport and to neutralize the action of cleaning residues. The colleted material was immediately transported in a thermal box with ice to LIPOA, where the analyses were performed. The results of the samples obtained by swabs were converted into CFU/cm^2.

Microorganism count

Petrifilm™ AC plates were used to count the aerobic mesophilic microorganisms in raw milk and residual water from the points studied. The samples were incubated at 35°C for 48 hours and the red colonies resulting from the reduction in the triphenyltetrazolium chloride (TTC) indicator were counted.

Plating on Plate Count Agar (PCA) (Biobras, Montes Claros, MG, Brazil.) incubated at 35°C/48 hours was used (BRASIL, 1991) for pasteurized milk, that contains a higher percentage of heat resistant non TTC reducer microorganisms (BELOTI et al., 1999).

To count psychrotrophs, the samples from all the circuit were sown on surface of PCA and incubated at 21°C/25 hours following methodology described by International Dairy Federation Standard (1991) and Marshall (1992).

Milk Agar at 10% (Skim Milk Powder, Oxoid, Basingstoke, Hampshire, England) was used to determine the proteolytic psychrotroph microorganisms. Plates of psychrotrophs with counts that varied from 10 to 100 CFU/plate were selected and all of the colonies were transferred from these plates to Milk Agar, using a cylindrical wooden stamp covered with sterile velvet. The samples were incubated at 22°C for 72 hours and the colonies that presented a proteolyis halo were considered proteolytic (BRASIL, 1991).

Proteolytic psychrotrophs microbiota classification

Ten percent of the colonies that grew in Milk Agar and formed the proteolysis halo were randomly selected, and submitted to Gram staining.

Results and Discussion

The main points of contamination by psychrotrophs and proteolytic microorganisms in the milk production chain on the farms studies were the improperly cleaned milk cans, bulk tanks and/or the presence of residual water, and improperly cleaned teats (Tables 3 and 4). Fonseca and Santos (2000) considered that more than 95% of the problems of high bacteria count in milk originate from deficiencies in the washing and cleaning of utensils and the milking system.

Table 3. Enumeration of mesophilic aerobic (M) psycrotrophics (P) and proteolytic (PR) microorganisms from the main contamination points from 2 dairy farms (grade A and B) from Londrina region, Paraná, May 2000 to June 2001.

	Grade A milk			Grade B milk		
Points	M	P	PR	M	P	PR
1. Unclean teats *	519,667	63,933	8,695	54,500	25,417	2,333
2. Cleaned teats *	106,6	17	17	4,983	4,075	725
3. Firth three jets **	10,315	5,198	NR	2,780	3,825	804
4. Second milk sample**	2,722	37	NR	632	758	344
5. Teatcups (before milking)*	3,423	571	354	167	66	47
6. Teatcups (during milking)*	2,943	327	327	143	06	0
7. Collection balloon *	408	200	28	267	517	323
8.Bulk tank (lateral)*	NR	NR	NR	04	600	300
9. Bulk tank (Residual water) **	10,375,000	2,070,000	NR	980,000	161,500	70,656
10. Milk before cooling in the bulk tank**	80,500	13,000	13,000	2,450	700	632
11. Refrigerated milk (around 12 h)**	1,990,000	2,800,000	97,160	91,000	763,250	97,849
12. Pasteurized milk**	5.320	30	30	NR	NR	NR

NR = not realized
* CFU/cm² **CFU/mL.

Table 4. Enumeration of mesophilic aerobic (M) psycrotrophics (P) and proteolytic (PR) microorganisms from contamination points from 2 dairy farms (grade C1 and C2) from Londrina region, Paraná, May 2000 to June 2001.

	Grade C milk (C1)			Grade C milk (C2)		
Points	M	P	PR	M	P	PR
1. Unclean teats *	89,205	23,183	969	7,237	6,573	3,407
2. Cleaned teats *	8,559	3,690	334	6,534	4,421	1,299
3. Firth three jets **	37,300	1,090	520	700	60	06
4. Second milk sample**	8,137	2,778	878	425	555	285
5. Teatcups (before milking)*	11,6	8,3	01	3,884	3,584	390
6. Teatcups (during milking)*	210,000	199,400	12,024	33,042	6,104	3,793
7. Milk cans (residual water)**	78,000,000	920,000	920,000	24,800,000	780,000	428,454
8. Cans milk**	171,500	13,500	7,500	800,000	510,000	265,608
9. Bulk tank (lateral)*	36,880	16,460	27	81	88	80
10. Bulk tank (Residual water) **	NR	NR	NR	1,115,000	1,100,000	176,880
11. Milk before cooled in the bulk tank**	171,500	13,500	7,500	160,667	93,967	29,017
12. Refrigerated milk (around 12 h)**	820,000	4,982,000	221,201	2,065,000	4,875,000	702,975

NR = not realized
* CFU/cm^2
**CFU/mL.

Milk cans were the main source of psychrotrophic microorganisms and proteolytic psychrotrophs on the farms where cans are used (C1 and C2) (Table 1), mainly because of the presence of residual water (Table 4). Prabha and Shankar (1994) analyzed rinsing water from milking utensils such as buckets and cans, and found mean psychrotrophic counts of 4.09 $\log_{10}$CFU/mL and 79,39% of proteolytic psychrotrophics. We found on C1 and C2 farms 5.96 $\log_{10}$CFU/mL and 5.89 $\log_{10}$CFU/mLin the buckets and cans, and the proteolytic psychrotroph was 77%.

When the mean psychrotrophic count of 850,000 CFU/mL (Table 4) is considered in the residual water in milk cans of 50 liters and a mean volume of water of 80 mL/milk can, there will be 2.7 x 10^3 CFU psychrotrophs/mL in milked milk. When the same calculation is performed for a mean proteolytic psychrotrophic count of 6.7 x 10^5 CFU/mL, the residual water in a milk can contribute with 2.1 x 10^3 CFU proteolytic psychrotrophs/mL milk.

The bulk tanks are also an important source of psychrotrophs either because of the residual water on farms A and C2 (Table 1 and 2) or because of contamination of the equipment surface, as on farm C1 (Table 2). The importance of residual water in the bulk tanks as contamination source is directly related to the volume incorporated in the milk. The proportion of proteolytic psychrotrophs in the residual water compared to the psychrotrophic count showed a variation between 43.75% on the farm that producer grade B milk and 16.08% on farm C2. On farm C1, although there was an insufficient volume of residual water for analysis, swabs from the side of bulk tank showed counts around 1.6 x 10^4 CFU psychrotrophs/cm^2, higher values than those from the other bulk tanks analyzed but the percentage of proteolytic psychrotrophs was only 0.2%. Thus it can be said that in these samples, the equipment surface compared with the residual water was not the most important source of proteolytic psychrotrophs.

According to Bramley and Mckinnon (1990) contaminated or untreated water can be a source of a great variety of saprophyte microorganisms originating from the soil or vegetation, such as *Pseudomonas spp*, coliforms and other Gram-negative bacteria. Prabha and Shankar (1994) analyzed water rinsing from milking utensils such as pail and cans and founded 79.39% proteolytic psychrotrophs of the total microorganism count.

Bad cleaned teats were an important source of psychrotrophs and proteolytic psychrotrophs in the milk production process. Considering farms B, C1 and C2, where a mean count of 4.0 x 10^3 CFU psychrotrophs/cm^2 and 789 CFU proteolytic psychrotrophs/cm^2 on the cleaned teats was obtained, and estimating the mean teat surface at 50.8 cm^2 (FONSECA; SANTOS, 2000), each animal could contribute with 8.5 x 10^5 CFU psychrotrophs and 1.6 x 10^5 CFU proteolytic psychrotrophs.

Santana et al. (2001) compared the microorganism counts from cleaned teats before and at the end of milking and observed a 86.0% and 96.0% reduction in the mean mesophilic and psychrotrophic values, respectively, indicating that a great percentage of the teat microorganisms is incorporated in the milk. Prabha and Shankar (1994) analyzed contaminated teat swabs and reported 75.21% proteolytic psychrotrophs of the total microorganism count and 64.64% of these microorganisms in soil samples, an important source of teat contamination. According to Garg (1990) the soil is a major source of microorganisms of the *Arthrobacter* genus and Gram-negative bacteria as *Alcaligenes, Achromobacer, Pseudomonas* and *Flavobacterium* genera. Fonseca and Santos (2000) found mean psychrotrophic counts of 2.0 x 10^5 CFU/teat and 4.1 x 10^4 CFU proteolytic psychrotrophs /teat. For these authors, practices such as pre-dipping decrease the incidence of mastitis in the herd and are also very important instruments in improving milk quality. In the present study, the grade A milk farm, the teats presented a decrease of 99.9% in the proteolytic psychrotrophs counts after pre-dipping (Table 3).

On farms C1 and C2, where the teatcups are never cleaned during milking (Table 1), there was a mean ten-thousandfold increase in the psychrotrophic count and a six thousandfold increase in the proteolytic psychrotrophic count (Table 4). The increase in the counts of the teatcups during milking may be attributed to the improperly cleaned teats that form a real source of contamination. On farms A and B, where some teatcup cleaning practices are performed (Table 1) the microorganism counts during milking were reduced. On farm B, the immersion of the teatcup in a chlorinated solution reduced the psychrotroph and proteolytic psychrotrophic count by 90.9% and 100%, respectively, and was more efficient than rinsing the cups with jets of water, as performed on farm A where the reduction was 42.7% and 7.3%. For the mesophilic count, the practices adopted on the two farms presented a mean reduction of 14% (Tables 3 and 4).

In the milk samples refrigerated after 12 hs, the psychrotroph showed a variation between 140.7% and 997.7%, when compared with the mesophilic count. These results differ from those found in the literature, where the psychrotrophic frequency does not surpass that of the mesophilic (POFFÉ; MERTENS, 1988; BRAMLEY; MCKINNON, 1990; PRABHA; SHANKAR, 1994; VILLAR et al., 1996). When the milk was analyzed, the psychrotrophic counts varied from 7 x 10^5 to 4.9 x 10^6 CFU/mL after 12 hours refrigeration, and the proteolytic psychrotrophic frequency varied from 3.47% on the grade A milk farm to 14.42% on farm C2. Poffé and Mertens (1988) found in refrigerated milk a mean count of 5.55 $\log_{10}$ CFU proteolytic psychrotrophs/mL, that corresponds a frequency of 8% from the total psychrotrophic count. These data were close to those detected in this study (Table 3 and 4) where proteolytic psychrotrophic counts showed a variation between 4.99 $\log_{10}$ CFU/mL to 5.85 $\log_{10}$ CFU/mL, indicating a proteolitic average of 8.77% from the total psychrotrophic count. Villar et al. (1996) reported a mean proteolytic count of 4.53 $\log_{10}$ CFU/mL in refrigerated milk.

According to Tinuoye and Harmon (1975) the optimum temperature for psychrotrophic microorganisms to produce enzymes is lower than the optimum temperature for cell growth. Thus organoleptic alterations can be found in refrigerated milk with the presence of a lower number of microorganisms than necessary to cause these alterations at higher temperatures.

The results of the present study show that raw milk after 12 hours refrigeration presented a sufficient psychrotrophic count to cause organoleptic and structural alterations in the milk and especially in its products. In the case of the grade A milk producing farm, proteolytic psychrotroph microorganisms were found in the pasteurized milk indicating recontamination and/or the presence of heat resistant psychrotrophs.

Regarding the Gram staining of the proteolytic psychrotrophs it was observed that milk samples after 12 hours refrigeration presented a 69.24% frequency of Gram-negative bacilli that is in line with results in the literature that points to the Gram-negative bacilli as the main proteolytic psychrotrophs (SHAH, 1994).

The frequency of Gram-negative proteolytic psychrotrophs increased from 54.44% to 69.24% after 12 hours refrigeration showing their capacity to multiply well in refrigeration temperatures. It should further be considered that the frequency of the organisms will certainly increase if the milk is kept refrigerated on the farm for more than 24 hours until collection by tanker trucks.

Bad cleaned teats and residual water in milk cans and bulk tanks milk are important sources of Gram-negative bacteria incorporation and contribute to the development of a proteolytic psychrotrophic microbiota in refrigerated milk (Table 5).

Table 5. Morphology of proteolytic psycrotrophics microorganisms characterization (Gram staining) from main points of contamination from 4 dairy farms in Londrina region, Paraná from May 2000 to June 2001.

Points analized	Gram + cocci	Gram – cocci	Gram + bacilli	Gram – bacilli	Gram+ coccibacilli
1. Unclean teats	40.00%	5.00%	35.00%	20.00%	0
2. Cleaned teats	32.35%	6.46%	16.13%	38.60%	6.46%
3. Firth three jets	50.00%	0	0	50.00%	0
4. Second milk sample	30.00%	0	25.00%	35.00%	10.00%
5. Teatcups (before milking)	9.09%	0	27.27%	63.64%	0
6.Teatcups(during milking)	36.84%	0	21.05%	31.58%	10.53%
7. Collection balloon	0	0	0	66.66%	33.34%
8. Milk cans (Residual water)	0	0	0	100.00%	0
9. Milk cans	0	0	25.00%	75.00%	0
10. Bulk tank (Residual water)	0	0	0	100.00%	0
11. Milk before cooled in the bulk tank	36.36%	0	9.09%	54.55%	0
12. Refrigerated milk (around 12 h)	7.69%	769%	15.38%	69.24%	0

Considering the microbiota as a whole, 45% of the proteolytic psychrotrophs were Gram-negative bacilli in the milk production process on the farms studied that were the major isolated group of microorganisms. However, the Gram-positives microorganisms were more frequent (52%) (figure 1) when we added together 28% cocci, 19% bacilli and 5% coccibacilli. According to Muir (1990) the Gram-positive microorganisms present low proteolytic action compared with the Gram-negative microorganisms. However, some authors attribute alteration in the flavor and quality of dairy products to the *Bacillus* genus, the main heat sporulated resistant microorganisms (WASHAM; OLSON; VEDAMUTHU, 1977; MUIR, 1990; SORHAUND; STEPANIAK, 1997). In the literature, Gram-positive cocci are not quoted as important psychrotrophic microorganisms or proteolytic psychrotrophs but in this study their frequency was significant, and they were the second largest proteolytic group found in the milk production process, originating on teats and teatcups (Table 5). The same results were founded in Brazil by Andrade, Ajao and Zottola (1998), that described *Enterococcus faecium* as a frequent heat

resistant psychrotroph in refrigerated milk and although results were not presented on its proteolytic activity, it was associated with alterations in milk.

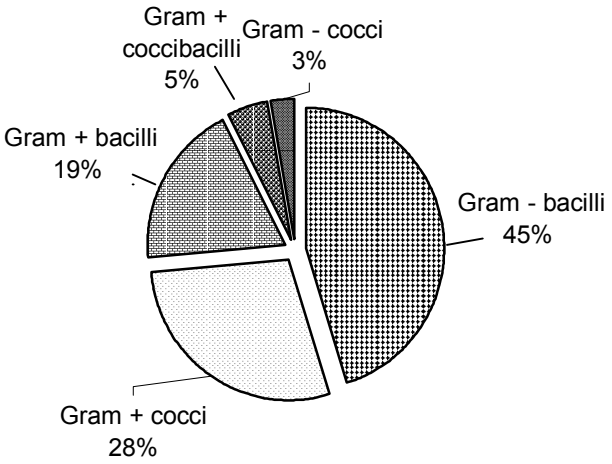

Figure 1. Distribution of proteolytic psycrotrophics according to morphology from 4 dairy farms, Londrina region, Paraná, may 2000 to june 2001.

Conclusions

Residual water in milk cans, cooling tanks, improperly cleaned teats and the surface of milk equipments were the main points of contamination by psychrotrophs and proteolytic psychrotrophs on the farms studied.

The psychrotrophic counts in the milk after 12 hours refrigeration are considered sufficient to alter milk quality except on the farm that produces grade B milk.

The group of Gram-positives microorganisms are the most frequent proteolytic psychrotrophs in the milk production process although the Gram-negative bacilli representing 45% of the proteolytic psychrotrophs detected.

Gram positive cocci, with a 28% frequency, represent an important group of proteolytics in milk on the farms studied.

Refrigerating milk at 4ºC is more efficient when the milk is less contaminated by psychrotrophs.

The psychrotrophic counts were higher than the mesophilic showing that the mesophilic counting technique for refrigerated milk quality control underestimates the true microbial load.

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