CONTROLLING GROWTH OF PSYCHROTROHS AND ITS EFFECTS ON The PROPERTIES OF COLD STORED BUFFALOE'S MILK

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ABSTRACT

Glucono-delta-lactone (GDL), lactic acid bacteria (LAB) and their metabolites (LABM) were added to buffalo's milk before cold storage for 72 h. Numbers of total bacteria (TBC), psychrotrophs (Ps.BC), prteolytic (PBC) and lipolytic (LBC) bacteria were counted during storage period. All milk samples were analysed for pH, acidity, TN and NPN and for stability to ethanol (AS) and to rennin (RCT). The resultant curd was tested for curd tension (CT) and curd syneresis (CS).

Results showed that the prementioned bacteria gradually increased during storage of the untreated milk whereas the applied treatments decreased the numbers of TBC, PsBC, PBC and LBC during storage period and the best results were achieved using LABM.

Acidity and NPN/TN gradually increased during storage period but with different rates. AS was the highest in the control milk and the lowest in LABM-treated milk, whereas RCT was longer in the first case and shorter in the second one. GDL slightly increased CT and LABM decreased it. All the applied treatments increased slightly CS when compared to the untreated milk.

INTRODUCTION

Psychrotrophs are defined as the organisms which are able to grow at 7°C or less, regardless of their optimal growth temperature. Such bacteria are heat-sensitive and killed easily by the mild heat treatments (Fairbairn and Law, 1986) but they produce heat resistant lipases and proteinases (Kohlmann *et al.*, 1991 ; Shah, 1994 ; S rhaug and Stepaniake, 1997). Presence of the prementioned enzymes adversely influences the quality and shelf-life of most dairy produces including gelation of UHT milk and development of off-flavour in pasteurized milk and other dairy products. Besides they decrease the yield of cheese (Cousins, 1982 ; Shah, 1994 and Burdova *et al.*, 2002).

Controlling the growth and multiplication of the psychrotrophs was the aim of numerous studies. Zall and Chen (1981) and Dzurec and Zall (1982) revealed the importance of applying thermination, whereas Wolfson and Sumner (1993) and Shah (1994) suggested activation of the LP-system before cold storage of milk.Juffs and Babel (1975) and Champagne *et al.* (1990) showed the inhibition action of lactic acid bacteria (LAB) on psychrotrophs.

Some local studies were carried out on buffalo's milk in this respect. These include carrying of thermization, activation of the LP-system, adding LAB or making combination from these treatments (Moussa *et al.*, 2000 ; Mehanna *et al.*, 2001 ; Saleh, 2001 and El-Ghandour, 2002).

The present study was a trial to control growth and multiplication of psychrotrophs during cold storage of buffalo's milk via direct acidification using GDL, adding LAB or using LAB metabolites (LABC). Impact of such

trials on chemical composition and some properties of milk were taken into consideration.

MAERTAISLS AND METHODS

1- Fresh buffalo's milk used in the present study was collected from the herd belongs to Mehalet Moussa Station, Animal Production Research Institute, Kafr El-Sheikh.
2- Glucono-Delta-Lactone (GDL) was obtained from Roquette, France.
3- Lactic acid bacteria (LAB), thermophilic DVS yoghurt culture (YC-XII) was obtained from Chr. Hansen Lab., Denmark.
4- Lactic acid bacteria metabolites (LABM) were prepared by adding the prementioned LAB to sterile liquid skim milk (12%) at the rate of 0.04 g/L in conical flasks. The flasks were incubated at 40±0.5°C for 6 hours until complete coagulation. The coagulum after mixing was then pasteurized at 72°C for few minutes, mixed well and the filtrate was collected using Whatman No. 42 filter paper. This filtrate represents LABM.

The collected milk samples were divided into 5 equal portions to give the following treatments:

The first portion was kept without any additives and served as a control sample. The second and third portions were treated with GDL at the rate of 0.02 and 0.05% (w/w) to give treatments I and II respectively. For treatment III LAB was added at the rate of 0.04 g/L, whereas LABM was added to milk to decreased the original pH of milk to 6.65±0.01 representing (treatment IV). About 20 ml was required in this respect.

The treated and untreated milk samples were stored in refrigerator (5±1°C) and analysed at the beginning an after 24, 48 and 72 h of storage.

Total bacterial count (TBC) and count of psychrotrophs (Ps.BC) were carried out using nutrient agar medium (Oxoid) as described by American Public Health Association (APHA, 1992). Poteolytic bacterial count (PBC) was counted using nutrient agar medium (Oxiod) with adding sterile milk (12%) to the plates before pouring the melted medium (Chalkmers, 1962). On the other hand, lipolytic bacterial count (LBC) was enumerated using the same medium of PBC but with adding butter fat at the rate of 5% (Berry, 1933) instead of sterile milk.

All milk samples were chemically analysed for pH, acidity, NPN and TN as described by Ling (1963), whereas stability of milk to ethanol was done according to method of White and Davies (1958).

Rennet coagulation time (RCT) was measured using the same amounts of rennin units (Fahmi and Amer, 1962). Curd tensions (CT) was determined at room temperature (25-30°C) as described by Chandrasekhara *et al.* (1957), whereas curd syneresis (CS) was determined at the same room temperature according to Mehanna and Mehanna (1989).

The collected data were statistically analysed for anlaysis of variance and Duncan's test as well as averages and standard error as given by Steel and Torrie (1984).

RESULTS AND DISCUSSION

Table (1) reveals that total bacterial count (TBC) and psychrotrphic bacterial count (Ps. BC) were almost in an insignificant increase during cold storage of control milk and the maximum counts (log CFU/ml) were recorded at the end of storage period. GDL was added at the higher ratio (0.05%) and such effects was significant with respect to Ps.BC.

The same trend of results was observed with respect to proteolytic bacterial count (PBC) and lipoytic bacterial count (LBC) which tended to increase – in general – in control milk and decrease in GDL-treated milk during the storage period. Again, the higher was the amount of GDL added, the lower were PBC and LBC at any given storage time. Such action of GDL might be due to its hydrolysis to gluconic acid after dissolving in milk and subsequently increased acidity and decreased pH.

Although lactic acid bacteria (LAB) were added in treatment III to milk prior to its cold storage, the TBC was lower at any given storage time when compared to the corresponding count of the control. This might be due to the unfavorable conditions for their growth and multiplication. This impact of LAB agrees with the finding of Mehanna *et al.* (2001). They found that in LAB-treated milk, TBC gradually increased during cold storage of milk and the rate of increase was inversely correlated with the number of LAB added. On the other hand, adding LAB (Treatment III) considerably decreased the PsBC during storage of milk and their values were considerably different than those of the control milk after 24, 48 and 72 h of storage. These are in accordance with the results of PsBC given by Mehanna *et al.* (2001). They gave values ($x10^4$) of 2.75, 2.85, 2.95 and 3.13 for the untreated milk stored in refrigerator for zero, 24, 48 and 72 h respectively. The corresponding counts in LAB-treated milk were 2.75, 2.63, 2.51 and 2.37 respectively.

Concerning PBC and LBC, it was found that LAB had the same inhibitory action on their counts. Their numbers in LAB-treated milk (Treatment III) gradually decreased during storage and considerably differed in most cases than the corresponding counts of the control milk at any given storage time. In LBC gradually increased during cold storage of untreated milk and gradually decreased during cold storage of LAB-treated milk.

It may be of interest to note that adding LAB metabolites (Treatment IV, Table 1) greatly decreased TBC and considerably decreased PsBC, PBC and LBC during cold storage of milk. Such impact was more effective when compared to those of using LAB (Treatment III) or GDL (Treatments I and II).

The role of LAB or their metabolites (LABM) might be due to LAB still active during cold storage of milk (Mehanna *et al.*, 2001) and can lower the redox potential to prevent growth of psychrotrophs (Shah, 1994). Also, LAB can produce a variety of substances with antibacterial activity such as H_2O_2 and bacteriocins (Desmazeaud, 1992 and Shah, 1994). Such substances may be more concentrated in LABM and cause more effectiveness.

Table (1): Bacterial count (log CFU/ml) of untreated and treated milk samples stored in refrigerator for 72 h (Means±SE of 3 replicates).

Property / Storage time (h)	Treatments				
	Control	I	II	III	IV
Total count					
0	6.43±0.18^{Aa}	6.52±0.14^{Aa}	6.49±0.05^{Aa}	6.23±0.17^{Aa}	6.15±0.14^{Aa}
24	6.68±0.23^{Aa}	6.65±0.14^{Aa}	6.13±0.19^{Aa}	6.57±0.12^{Aa}	6.08±0.22^{Aa}
48	6.56±0.27^{Aa}	6.53±0.26^{Aa}	6.34±0.28^{Aa}	6.43±0.37^{Aa}	5.98±0.41^{Aa}
72	6.89±0.05^{Aa}	6.06±0.46^{ABa}	5.96±0.12^{Ba}	6.31±0.30^{ABa}	5.72±0.24^{Ba}
Psychrotrophic					
0	5.00±0.18^{Aa}	5.07±0.23^{Aa}	5.09±0.04^{Aa}	4.96±0.23^{Aa}	5.02±0.05^{Aa}
24	5.25±0.02^{Aa}	4.87±0.07^{BCa}	5.02±0.05^{Bab}	4.94±0.07^{BCa}	4.81±0.04^{Cb}
48	5.33±0.14^{Aa}	4.72±0.07^{BCa}	4.88±0.05^{Bbc}	4.82±0.04^{Ba}	4.52±0.06^{Cc}
72	5.43±0.15^{Aa}	4.72±0.07^{Ba}	4.83±0.03^{Bc}	4.87±0.04^{Ba}	3.40±0.03^{Cd}
Proteolytic					
0	3.44±0.07^{Ba}	3.69±0.04^{Aa}	3.46±0.09^{ABa}	3.49±0.07^{Ba}	3.28±0.06^{Ba}
24	3.55±0.08^{ABa}	3.61±0.04^{Aa}	3.37±0.07^{BCa}	3.33±0.05^{Ca}	3.25±0.06^{Ca}
48	3.81±0.24^{Aa}	3.56±0.03^{ABa}	3.29±0.03^{BCa}	3.38±0.15^{Ba}	2.91±0.05^{Cb}
72	3.40±0.09^{Aa}	3.29±0.08^{Bb}	3.18±0.13^{Ba}	3.38±0.05^{Ba}	2.86±0.06^{Cb}
Lipolytic					
0	3.23±0.08^{Aa}	2.98±0.48^{Aa}	2.81±0.49^{Aa}	3.05±0.18^{Aa}	3.05±0.09^{Aa}
24	3.28±0.10^{Aa}	2.94±0.29^{ABa}	2.90±0.33^{Aba}	2.54±0.25^{ABab}	2.79±0.14^{Bbc}
48	3.35±0.18^{Aa}	2.65±0.11^{Ba}	2.56±0.34^{Ba}	2.45±0.17^{Bab}	2.58±0.15^{Bb}
72	3.44±0.14^{Aa}	2.43±0.28^{Ba}	2.37±0.11^{Ba}	2.24±0.41^{ABb}	1.94±0.08^{Cc}

*** Control means milk without any additives, treatments I and II represent milk treated with GDL at the rate of 0.02 and 0.05% respectively. Treatment III represents milk treated with lactic acid bacteria (LAB), whereas treatment IV represents milk treated with LAB metabolites (LABM).**

- Averages in the same row (A, B... etc) or in the same column (a, b... etc) with different superscripts differed significantly (P<0.05).

As a results of applying the previous treatments, pH of milk decreased and the acidity increased (Table 2). At zero storage rime, these changes were more pronounced in case of using LABM (Treatment IV). During storage of treated and untreated milk samples, the pH gradually decreased and the acidity gradually increased but with different rates. Such changes were insignificant and only significant in case of acidity values of the control milk. The same trend of results was observed by Saleh (2001) with respect to effect of LAB and storage period.

The proteolysis expressed as NPN/TN was observed in the control and all treated milk samples with exception of LABM-treated milk. Table (2) shows that the control milk possessed from the greatest proteolysis (6.98-8.11%), whereas adding GDL greatly decreased such proteolysis (Treatment I and II). The higher was the amount of GDL added, the lower was the rate of proteolysis. Moreover, treatment (II) showed the lowest NPN/TN values when compared to the corresponding values for the control and treated samples at any given storage time. This suggests that adding GDL at the rate of 0.05% greatly controlled the proteolysis during cold storage of milk, but the differences in NPN/TN of all samples were statistically insignificant. Action of

LAB on controlling proteolysis during cold storage of milk was more pronounced in the studies given by Saleh (2001) and El-Ghandour (2002). This might be due to differences in composition and microbiological quality of milk.

Table (2): pH, acidity and NPN/TN of untreated and treated milk samples stored in refrigerator for 72 h (Means±SE of 3 replicates).

Property / Storage time (h)	Treatments				
	Control	I	II	III	IV
PH					
0	6.80±0.038^{Aa}	6.76±0.049^{Aa}	6.69±0.055^{Aa}	6.80±0.0110^{Aa}	6.65±0.001^{Aa}
24	6.80±0.026^{Aa}	6.76±0.029^{ABa}	6.68±0.035^{Ba}	6.84±0.17^{Aa}	6.75±0.029^{ABa}
48	6.80±0.022^{ABa}	6.76±0.029^{BCa}	6.66±0.04^{Ca}	6.89±0.047^{Aa}	6.75±0.029^{BCa}
72	6.72±0.014^{ABa}	6.74±0.038^{ABa}	6.63±0.040^{Ba}	6.84±0.039^{Aa}	6.75±0.046^{ABa}
Acidity (%)					
0	0.17±0.001^{Bb}	0.18±0.005^{ABa}	0.19±0.001^{Aa}	0.18±0.001^{ABa}	0.19±0.006^{Aa}
24	0.18±0.006^{Aab}	0.19±0.006^{Aa}	0.20±0.006^{Aa}	0.18±0.006^{Aa}	0.19±0.006^{Aa}
48	0.18±0.004^{Aab}	0.19±0.006^{Aa}	0.20±0.006^{Aa}	0.19±0.009^{Aa}	0.20±0.009^{Aa}
72	0.19±0.005^{Aa}	0.19±0.006^{Aa}	0.21±0.006^{Aa}	0.19±0.009^{Aa}	0.21±0.006^{Aa}
NPN/TN (%)					
0	6.98±0.447^{Aa}	6.96±0.400^{Ab}	7.07±0.454^{Aa}	7.57±0.531^{Aa}	8.07±0.329^{Aa}
24	7.33±0.381^{Aa}	7.55±0.254^{Aab}	6.42±0.403^{Aa}	7.80±0.576^{Aa}	8.07±0.329^{Aa}
48	7.33±0.381^{Aa}	7.55±0.254^{Aab}	7.14±0.280^{Aa}	7.81±0.626^{Aa}	8.07±0.329^{Aa}
72	8.11±0.283^{Aa}	8.00±0.078^{Aa}	7.80±0.182^{Aa}	8.26±0.644^{Aa}	8.07±0.329^{Aa}

* See legend to Table (1) for details.

Concerning ethanol stability, Table (3) shows that at zero storage time the control samples had positive results with 92% ethanol, whereas the weakest ethanol concentrations required were 91, 89, 92 and 87% for treatments I, II, III and IV, respectively. This might be to the corresponding acidity and pH values. During storage all samples were coagulated by lower ethanol concentrations suggesting lower AS. Samples from treatments II and III showed the lowest AS among all the applied treatments ($P<0.05$), whereas the control was significantly more stable at any storage time followed by samples from treatment III.

Rennet coagulation time (RCT) had a wide range of variations at any given storage time indicating that the applied treatments had significant effect in this respect. Table (3) reveals that the control sample was the slowest one in this respect. This was only true at the beginning, but during storage a gradual decrease in RCT was recorded in the control and all treatment samples with exception of treatment III.

The impact of GDL might be ascribed to increase solubility of calcium salts in milk which by its turn accelerated RCT. Abd El-Salam *et al.* (1996) reported the same finding and mentioned that GDL showed changes similar to that of enzymatic coagulation of milk in which two phases can be distinguished i.e. the onset of gelation and development of fine curd.

Concerning effect of LAB, our results agree – in general- with those given by El-Ghandour (2002). He mentioned that the changes in RCT as affected by adding LAB were insignificant but were highly significant as affected by cold storage of milk.

The differences in curd tension (CT) due to the applied treatments were not clear enough to make conclusion. At the beginning of storage, treatment IV had the lowest CT value, whereas the other treatments had nearly the same values. This was also true at 48 and 72 h of storage. However, GDL caused an increase in CT when compared to the control samples. This agrees with the finding of Naeim *et al.* (2003), whereas action of LAB agrees – in general- with the trend given by El-Ghandour (2002).

Table (3): Changes in stability to ethanol and rennet clotting time (min) of buffalo's milk during storage in refrigerator for 72 h (Means±SE of 3 replicates).

Property	Treatments				
Storage time (h)	Control	I	II	III	IV
AS**					
0	92±0.001^{Aa}	91±0.577^{Aa}	89±0.577^{Ba}	92±0.001^{Aa}	87±0.577^{Ca}
24	92±0.001^{Aa}	91±0.577^{Aa}	88±1.15^{Ba}	91±0.577^{Aa}	87±0.058^{Ba}
48	90±1.15^{Aab}	87±0.577^{Bb}	80±1.15^{Db}	91±0.577^{Aa}	84±0.001^{Cb}
72	86±1.15^{Ab}	84±1.150^{A}c	75±2.39^{Bb}	86±2.89^{Ab}	78±1.15^{Bc}
RCT					
0	6.19±1.28^{Aa}	4.30±0.655^{ABa}	2.83±0.491^{Ba}	5.83±1.02^{Aba}	4.95±1.209^{ABa}
24	6.58±1.23^{Aa}	4.35±0.811^{ABa}	2.58±0.387^{Ba}	6.59±1.643^{Aa}	2.82±0.274^{Ba}
48	6.03±1.079^{Aa}	4.15±0.754^{ABa}	2.53±0.372^{Ba}	6.05±1.703^{Aa}	2.99±0.517^{Aba}
72	4.86±0.785^{ABa}	3.37±0.644^{Ba}	2.04±0.349^{Ba}	7.51±2.589^{Aa}	2.77±0.424^{Ba}

*** See legend to Table (1) for details.**

**** Expressed as the weakest ethanol concentration which when added to an equal volume of milk caused clotting.**

In most cases (Table 4) increased GDL increased curd syneresis (CS) at both 10 and 120 min of holding time, and their CS values were similar or slightly higher than those of the control samples. This agrees with the results given by Naeim *et al.* (2003). This trend was also recorded for the effect of LAB or LABM, since their CS were almost higher than those of the control samples. These results are in accordance with those given by El-Ghandour (2002).

From the foregoing results it could be concluded that using GDL or adding LAB or LABM controlled growth of psychrotrophs and their proteolytic and lipolytic bacteria and decreased RCT without adverse effect on general quality of cold stored milk. GDL or LAB slightly improved firmness and tension of the resultant curd, whereas the changes in curd syneresis were insignificant. Choice of one of the given treatments depends on cost of application and suitability of the treated milk for making the required dairy product.

Table (4): Curd tension (CT, g) and curd syneresis (CS, g/15 g) after 10 min or 120 min of untreated and treated milk stored in refrigerator for 72 h (Means±SE of 9 determinations from 3 replicates).

Property	Treatments				
Storage time (h)	Control	I	II	III	IV
CT					
0	39 ± 0.577^{Aab}	38.67 ± 0.882^{Aa}	39.33 ± 0.330^{Aa}	39.00 ± 0.577^{Aab}	37 ± 1.73^{Aa}
24	38 ± 2.814^{Abb}	40.67 ± 0.882^{Aa}	39.00 ± 1.154^{Aa}	33.33 ± 0.330^{Bb}	36 ± 2.31^{Aba}
48	41 ± 1.134^{Aab}	45.00 ± 3.464^{Aa}	47.33 ± 5.487^{Aa}	40.33 ± 0.882^{Aab}	38 ± 2.03^{Aa}
72	44 ± 0.577^{Aa}	45.00 ± 5.20^{Aa}	46.33 ± 0.882^{Aa}	45.00 ± 4.63^{Aa}	40 ± 2.89^{Aa}
CS, 10 min.					
0	3.36 ± 0.326^{Aa}	3.14 ± 0.078^{Aa}	2.88 ± 0.090^{Aa}	3.27 ± 0.289^{Ab}	3.40 ± 0.297^{Aa}
24	3.29 ± 0.433^{Aa}	3.13 ± 0.361^{Aa}	3.51 ± 0.248^{Aa}	3.25 ± 0.248^{Ab}	3.95 ± 0.142^{Aa}
48	3.11 ± 0.142^{Ba}	3.24 ± 0.494^{Ba}	3.38 ± 0.300^{Ba}	3.96 ± 0.268^{Ab}	3.28 ± 0.280^{ABa}
72	3.12 ± 0.061^{Aa}	3.69 ± 0.140^{Aa}	3.05 ± 0.113^{Aa}	4.24 ± 0.271^{Aa}	3.65 ± 0.165^{Aa}
CS, 120 min					
0	4.46 ± 0.084^{Ab}	6.55 ± 0.008^{Ac}	6.47 ± 0.069^{Ac}	7.10 ± 0.346^{Aa}	6.99 ± 0.055^{ABb}
24	6.92 ± 0.335^{Aab}	6.79 ± 0.243^{Abc}	6.98 ± 0.107^{Ab}	7.14 ± 0.133^{Aa}	7.13 ± 0.205^{Aab}
48	7.03 ± 0.118^{ABab}	7.18 ± 0.196^{ABab}	7.37 ± 0.095^{Aa}	6.79 ± 0.031^{Ba}	7.38 ± 0.245^{Aab}
72	7.15 ± 0.055^{Aa}	7.55 ± 0.075^{Aa}	7.39 ± 0.162^{Aa}	7.63 ± 0.41^{Aa}	7.79 ± 0.251^{Aa}

* See legend to Table (1) for details.

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دراسة على التحكم فى نمو الميكروبات المقاومة للبرودة وتأثير ذلك على خواص اللبن الجاموسى اثناء حفظه مبرداً

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اهتمت الدراسة بمعاملة اللبن الجاموسى قبل حفظه مبردا بمركب جلوكونو-دلتا-لاكتون (GDL) او ببكتريا حمض اللاكتيك او نواتج التمثيل الغذائى لهذه البكتريا وقد أدت المعاملات المذكورة الى خفض العدد الكلى للبكتريا واعداد البكتريا المقاومة للبرودة والمحللة للبروتين والمحللة للدهن ، بينما زادت تلك الاعداد فى اللبن غير المعامل طوال فترة التخزين هذا وقد انخفضت قيم الرقم الهيدروجينى وزادت قيم الحموضة ومعدل التحلل البروتينى فى كل عينات اللبن المعامل وغير المعامل ولكن بمعدلات مختلفة طوال فترة التخزين... وكان ثبات اللبن للكحول اعلى ما يمكن فى اللبن غير المعامل واقل ما يمكن عند المعاملة بنواتج التمثيل الغذائى لبكتريا حمض اللاكتيك بينما كان وقت التجبن اطول ما يمكن فى الحالة الاولى واقصر ما يمكن فى الحالة الثانية ، هذا ويمكن القول ان المعاملة بمركب (GDL) ادت لزيادة قيم الجذب الخثرى بينما ادت نواتج التمثيل الغذائى الى خفض هذه القيم فى حين زادت قيم ومعدل طرد الشرش من الخثرة فى اللبن المعامل مقارنة باللبن غير المعامل.