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PRESERVATION OF RAW COW MILK USING NON THERMAL TREATMENTS

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ABSTRACT

This study was undertaken to evaluate the feasibility of using non thermal treatments for the preservation of raw cow milk. Samples of cow milk were incubated at 4^{o}C as a control (C), heat treated at 80 ^{o}C for 10min (HT), activated LP-system (LPs), added soluble chitosan (CH, 0.1%), β-glucan (BG, 30mg/L) or glucomannan (GM, 30mg/L). All treated samples were incubated at 25^{o}C, 37^{o}C or 45^{o}C. Ttitratable acidity (T.A%), milk stability, rennet coagulation time, starter activity, total aerobic mesophilic bacterial count (TBC), spore forming bacterial count (SC), coliform (CC) and mould & yeast (M.Y) in all samples were determined during incubation. A significant slowing down in the rate of increase in T.A% was found in all samples. Shelf-life of raw milk was of 19, 13, 12, 11, and 10 hr, during incubation at 25 ^{o}C for samples treated with HT, CH, LP, BG, and GM, respectively, with the same acidity (0.195%) in all samples. The clot on boiling and alchol stability tests of all samples are o0f the same trend of titratable acidity. The starter activity decreased significantly by adding CH, BG, GM and LPs. However HT as well as control treatments had a significant higher starter activity than other treatments. On the other side, all treatments reduced significantly rennet coagulation time, except LPs and HT treatments, which delayed coagulation time. Results showed no significant differences between all samples for TBC at the different incubation temperatures. Spore count in HT and CH treatments was significantly less than other treatments. Moreover, HT treatments had the lowest count for coliform, and M&Y. Coliform count was significantly lower by adding BG than other treatments.

Keywords: LP- system; Chitosan; β-glucan; Glucomannan

INTRODUCTION

The most commonly used method to stop or retard the deterioration of milk on its way from the farmer to the dairy is cooling (Claesson, 1992). However, in many parts of the world especially hot climatic countries in Africa , this is not possible for various reasons, such as lack of available capital, lack of electricity, less developed road systems, high operational costs, frequent break downs of equipment, lack of spare parts and difficulties in repair of equipment in rural areas. The combination of high ambient temperatures and lack of refrigeration facilities are amongst the

leading factors contributing to rapid spoilage of raw cow milk. An alternative method such as non thermal treatments to increase the storage stability of milk at high ambient temperatures has been used.

Lactoperoxidase system (LPS) is antimicrobial system, naturally present in milk that has been used to preserve quality of raw milk during storage and/or transportation to processing plants, when adequate refrigeration is not available. The lactoperoxidase enzyme, present in relatively high amounts in milk can oxidize thiocyanate ions (SCN^-) in the presence of hydrogen peroxide H_2O_2. The products of this reaction bind to sulfhydryl groups of bacterial enzymes, inhibiting their activity (Seifu *et al.,* 2005 and Ellin 2006). In some developing countries, where milk is susceptible to temperature abuse and poor sanitary conditions during transportation and storage, H_2O_2 has been added to raw milk to activate the LP system to preserve the milk during transportation (IDF 1988). The LP system has been recognised as critical in the dairy industry for the preservation of raw milk, pasteurized milk, cheese and yogurt (Touch *et al.* 2004).

Chitosan (poly-β-1-4-glucosamine) is a modified, natural nontoxic carbohydrate polymer derived by deacetylation of chitin, a major component of the shells of crustacean such as crab, shrimp and crawfish (Corbo *et al.* 2009). The positive charge of chitosan grants to this polymer numerous and unique physiological and biological characteristics with great potential in a wide range of industries such as preservative, antimicrobial, coating, antioxidant in food industries (Tayel *et al.* 2010). Chitosan has attracted attention as a potential food preservative of natural origin due to its antimicrobial activity against a wide range of foodborne filamentous fungi, yeast, and bacteria (Sagoo *et al.* 2002). The most feasible hypothesis of the antimicrobial activity of chitosan is a change in cell permeability due to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membranes. This interaction leads to the leakage of proteinaceous and other intracellular constituents (No *et al.* 2007). Other mechanisms are the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of the mRNA and protein synthesis (No *et al.* 2007). Ha and Lee (2001) investigated the effectiveness of water-soluble chitosan to minimize the microbial (bacteria and yeast) spoilage of processed milk. Complete inhibition of microbial growth was observed in the banana-flavored milk containing chitosan, in contrast to that observed in control milk storage for 15 d at 4 and 10◦C. The banana-flavored milk containing chitosan also maintained relatively higher pH than that of control milk during storage for 15 d at both temperatures. Many others observed that chitosan exhibited antibacterial activity against *Corynebacterium michiganense*, *Escherichia coli*, *Micrococcus luteus Staphylococcus aureus and pseudomonads*, which caused food poisoning and food spoilage, also showed chitosan mixture retarded the growth of *Salmonella* species and caused quicker reduction of *Staphylococcus* species in raw milk (Tsai *et al.* 2000; Yang *et al.* 2005 and No *et al.* 2007).

β-Glucans are naturally-occurring polysaccharides found in the cell walls of yeast, fungi, cereal plants and certain bacteria (Chen and Seviour 2007). β - (1,3)-D-glucans with β -(1,6) branches have been reported to have various clinically beneficial effects, such as enhancing the bio-defense activity against bacterial, viral, fungal and parasitic challenge, increasing hematopoiesis and radioprotection, stimulating the wound healing response, decreasing serum lipid levels, protection against infection, inhibition of tumor development, metastasis, and promotion of tumor regression (Hong *et al.* 2004; Yoon *et al.* 2008; Driscoll *et al.* 2009; Shah *et al.* 2009 and Asano *et al.* 2012*). S. cerevisiae* β-glucan showed protective effects against genotoxicity and cytotoxicity of some drugs, such as cyclophosphamide, adriamycin and cisplatin. Such effects have been attributed to the ability of β-glucan to trap free radicals produced in the course of biotransformation of these drugs (Tohamy *et al.,* 2003). In addition, it has been demonstrated that β-glucan-containing products are potent antioxidants, preventing damage caused by H_2O_2 and other reactive oxygen species (Laugier *et al.* 2012).

Mannans represent carbohydrates, especially polysaccharides that contain mannose (sugar) residues. They can be divided into four types; mannan, galactomannan, glucomannan and galactoglucomannan (Tester and Al-Gazzewi, 2013). Mannan polysaccharides are widespread in nature. They are considered to be one of the major components of hemicellulose in the cell walls of plants (Moreira and Filho, 2008). Microbes are a rich source of mannans, so it called 'mannan oligosaccharides' which are derived from the outer layer of the yeast cell walls especially from *Saccharomyces cerevisiae*. Mannan oligosaccharides are often promoted as an alternative to antibiotics in the animal feed industry. This use has evolved from the understanding that certain sugars, particularly mannose, may prevent attachment of the intestinal pathogens such as *Salmonella* species and *Escherichia coli* to the gut mucosa (Benites *et al.* 2008). Corrigan *et al.* (2011) showed that dietary supplementation with mannan oligosaccharides from yeast cells had a significant impact with respect to changing the bacterial ecology in the gut. Others have reported that this source of mannan can improve health and performance of animals by preventing pathogens binding to the gut and by stimulating the immune system (Bozkurt *et al.* 2009; Miguel *et al.* 2004).

Consequently, the present study was designed to preserve raw cows' milk by using different non thermal treatments at different incubation temperatures.

MATERIALS AND METHODS

Fresh raw cows milk were obtained from the herd of the Faculty of Agriculture, Cairo University. Giza ,Egypt.

Yoghurt cultures YC-fast-1 was obtained from Chr. Hansens Laboratories, Copenhagen Denmark. Animal rennet liquid was obtained from local market. Sodium thiocyanate (NaSCN): "Prolabo Adwic Laboratory Chemicals" was used as a source of SCN^-. Sodium percarbonate ($Na_2CO_3.3H_2O_2$) was purchased from BDH chemicals Ltd. Poole England. Chitosan; Mw. 300k Dalton, DA: 85%. Chitosan was obtained from Sigma Chemical Co., USA. Chitosan solution was prepared by adding 1gm of chitosan to 100 ml acetic acid (0.1molar) and the acetic acid in order to reach a final concentration of 0.1% chitosan. β-glucan and Glucomannan were obtained from Alltech Company, USA.

Violet Red Bile Glucose Agar, Oxytetracycline – Glucose -Yeast Extract Agar (OGYE Agar) and Plate Count Agar (Tryptone Glucose Yeast Agar) media were purchased from Oxoid Ltd., Basingstoke, Hampshire, England.

The milk within 2hr after milking was divided into six equal parts. The first part kept at 4^oC without treatment as a control, while the second part was heated at 80^oC／10min. The LP system was activated in the third part of milk according to FAO (1991), where it was added $40mgL^{-1}$ of sodium thiocyanate as a source of thiocyanate (SCN^-) with mixing for 1min. After that sodium percarbonate was added at level $30mgL^{-1}$ as a source of hydrogen peroxide. The Chitosan was added to the fourth part at level 0.1%, β-glucan was added to the fifth part at level $30mgL^{-1}$ and Glucomannan was added to the sixth part at level $40mgL^{-1}$. From the second to the six part milk samples subdivided into three portions and incubated of each at 25^oC, 37^oC or 45^oC until spoilage of samples. The preservative effect of all treatments was evaluated by determine the titratable acidity, clot on boiling, alcohol test every 1 hour until spoilage of samples. Rennet coagulation time, starter activity test and microbial count were analyzed after a titratable acidity reached to 0.195%-0.20% at different times in all samples.

Milk samples were analysed for fat, total protein (T.P), lactose, ash and total solids (T.S), in Milkotronic Ltd, Lactoscan, Milk Anaalyzer,wide LCD display – 4lines × 16 characters.4, Narodni Buditeli Str. 8900 Nova Zagora, Bulgaria.

The acidity of the milk samples was determined according to the method described in the (AOAC, 2005). Clot on boiling and alchol test of the milk samples were done according to Ghatak and Bondyopadhyay (2007).

Yoghurt starter activity in all milk samples as previously mentioned under experimental procedure section was determined according to Rasik and Kurmann (1978), where milk samples were sterilized after the acidity reached to 0.195-0.20% then inculated with yoghurt starter at level 3% and incubated at 37oC for 4hr. The titratable acidity was determined at the end of incubation.

Rennet coagulation time was determined according to Kawai and Mukai (1970)

The total aerobic mesophilic bacterial counts (TBC) were estimated using Plate Count Agar medium as recommended by the American Public Health Association (APHA, 2004). The aerobic spore forming bacterial count

was carried out as described by Luck, 1981. Coliform counts were enumerated using Violet Red Bile Glucose Agar medium as reported by APHA, 2004. Moulds and Yeasts were enumerated using Oxytetracycline-Glucose-Yeast Extract Agar (OGYE Agar) medium according to IDF, 1990.

The experiments were repeated in triplicates and each analysis duplicates and average results were recorded. All statistical analysis was carried out using SPSS- 21. Overall effects of treatments were analyzed conducting a 2-ways ANOVA; statistically different groups were determined by Duncon test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Fresh cow milk sample was chemically analyzed before treatments. It contained 3.15% fat, 3.21% total protein, 4.41% lactose, 0.71% ash and 11.48% total solids.

As can be observed from results presented in Table (1), heat treated milk at 80 ^{o}C/10min, activation of LP-system, adding of chitosan, β-glucan or glucomannan caused a significant slowing down rate of increase in titratable acidity (T.A%) during incubation at 25^{o}C, 37^{o}C and 45^{o}C. This effect was more pronounced upon incubation at 25^{o}C than 45^{o}C. A significant difference ($P < 0.05$) was observed for acidity values between different treatments of milks. In heated sample the T.A value increased slowly at 25^{o}C, compared to all treatments and reached to 0.24% after 19hr. Also, it can be seen from the same Table (1) that the T.A of milk samples treated with heat treatment, activation LP-system or chitosan addition lower than milk samples treated with β-glucan or glucomannan. The acidity of LP-system, β-glucan or glucomannan treatments initially at 25^{o}C was 0.16% and increased slowly until reached to 0.23% after 12, 11 and10 hr, respectively. While the acidity of heat and chitosan treatments was initially at the same temperature was 0.15% and increased slowly to 0.24% after 19 and 13hr, respectively. The acidity of the raw milk without any addition (control) were higher after incubation at 4^{o}C for 70 hr where it was 0.26%.
From the obtained results for activation LP-system, these results in agreement with Stefano *et al.* 1995 who suggest that the activation of LP-system of raw milk leads to a decrease in the rate of microbial growth thus were causing a slower rate in TA increase. Also adding chitosan to the milk at 0.1% slightly decreased the value of T.A, the values of titratable acidity didn't differ significantly at all incubation temperatures The same trend were reported by Lee and Lee 2000 and Seo *et al.* 2011.

The results of clot on boiling (COB) and alcohol stability tests of milk with different treatments had the same trend of titratable acidity results. Data in Fig 1, showed that there was a significant differences between all treatments and control. As for heated milk incubated at 25^{o}C, the COB test remained negative for 18 hr, while it remained negative for 12, 11, 10 and 9 hr in treatments of CH, LPs, BG or GM respectively. Moreover, COB test of all treatments incubated at 37^{o}C was positive after 7 to 11 hr and it was positive in all treatments incubated at 45^{o}C after 5

to 9 hr. In the other hand COB test was positive in control after 69 hr. These results are partly in agreement with Chakraborty *et al.* (1986), who reported that the shelf life of raw milk can be extended to 15 hr by activating LP-system during incubation at 37 ^{o}C.

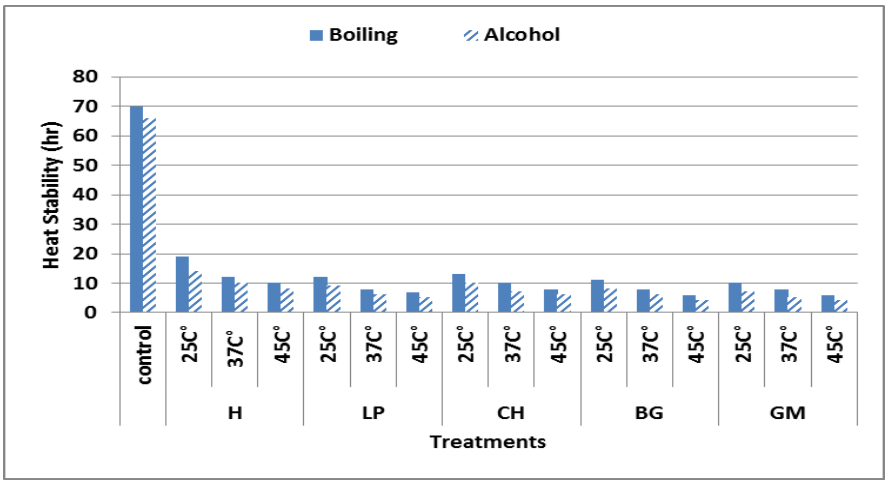

Fig (1): Eeffect of using non thermal treatments of raw cow milk on clotting using boiling (COB) and alchol test.

As shown in the same Fig 1, alchol test was positive after 14, 10, 9, 8 and 7 hr, while it was positive after 8,6,5,4 and 4 at incubation temperatures of 25 ^{o}C or 45 ^{o}C for HT, CH, LP-system, BG or GM respectively. Our data confirmed that the keeping quality (KQ) parameter showed that it can be preserve raw cow milk for several hours before transportation to dairy plant particularly in developing country at high ambient temperatures where refrigeration is not feasible. The same trend was observed by Barrett *et al.* (1999) and Marks *et al.* (2001), who found that LP-system extend the KQ of raw milk.

Data presented in Table (2) showed that there are significant variations in starter activity between and within all treatments. Starter activity reduced significantly in all treatments (ranged from 0.39%-0.76%) except heat treated (HT) sample (0.77%-0.8%). As for samples of LPs, CH, BG or GM incubated at 25^{o}C it could be noticed that there are no significant difference in starter activity between all of them where it was 0.39, 0.52, 0.44 and 0.52% respectively. However, there are no significant differences of starter activity between all samples incubated at 37^{o}C and 45^{o}C. These results could be occurred due to the optimum temperature for LP enzyme activity in milk to be at 25^{o}C. Therefore its effect more pronounced at 25^{o}C than other temperatures (Basaga and Dik, 1994).

T1

Fig (2): Effect of using non thermal treatments of raw cow milk on (A) total bacterial count (B) sporeforming bacterial count (C) coliform count (D) M.Y count (Log $_{10}$ cfu/ml).

Table (2): Effect of using non thermal treatments of raw cow milk on starter activity and rennet coagulation time.

Treatments	Incubation temperatures °C/ time (hr)	Starter Activity* (T.A%)	Rennet Coagulation Time (min) (RCT)
control	4 /62	0.760 ^{B}a	15.00^{D}c
HT	25 /13	0.770 ^{A}c	34.00^{A}a
	37 /10	0.780 ^{A}b	35.00 ^{A}b
	45 /8	0.800 ^{A}b	33.00 ^{A}b
LP	25 /10	0.390 ^{D}c	34.00 ^{B}a
	37 /6	0.480 ^{D}b	30.00 ^{B}b
	45 /5	0.510 ^{D}b	27.00 ^{B}b
CH	25 /11	0.520 ^{C}c	18.00 ^{D}a
	37 /7	0.560 ^{C}b	10.00 ^{D}b
	45 /6	0.480 ^{C}b	12.00 ^{D}b
BG	25 /8	0.440 ^{D}c	17.00 ^{D}a
	37 /6	0.450 ^{D}b	13.00 ^{D}b
	45 /4	0.490 ^{D}b	13.00 ^{D}b
GM	25 /7	0.520 ^{C}c	19.00 ^{C}a
	37 /5	0.500 ^{C}b	19.00 ^{C}b
	45 /4	.0520 ^{C}b	20.00 ^{C}b

***After 4 hr incubation for at 37°C.**
HT: Heat treatment LP: Lactoperoxdiese treatment CH: Chitosan treatment BG: B-glucan treatment GM: Glucomannan treatment
The different capital letters have a significant effect between treatments at the level 0.05.
The different small letters have a significant effect for incubation temperatures at the level 0.05.

As can be observed from the results in the same Table (2) rennet coagulation time increased significantly in LP-system (27-34min) and heated treated (33-35min) samples comparing with other treatments which had a normal rennet coagulation time (15-20min). Regarding the effect of different incubated temperature on rennet coagulation time the same trend of starter activity was observed. Our results were similar to Basaga and Dik (1994) who reported that activation of LP-system delayed the rennet coagulation time . On the other hand the effect of heat treatment on rennet coagulation time may be attributed to milk treated at relatively high temperature (higher than 55 °C) allowed the denaturation of whey proteins on the surface of casein micelles resulting in prolonged rennet coagulation time of milk.

Also the obtained results showed that treatment milk with Chitosan had lower rennet coagulation time (10-17min) than other treatments, this may be due to the addition chitosan to milk causes destabilization and coagulation of casein micelles that takes place without changes in the milk pH or the stability of most whey proteins Ausar *et al.* (2001).

Activated LP system delayed coagulation time and reduced the activity of starter culture, this effect was more pronounced at 25°C compared with 37 °C and 45 °C, these result were in agreement with those reported by Basaga and Dik (1994), Girgis *et al.* (2001) and Seifu *et al.*

(2003) who found that the optimum temperature for LP enzyme activity in bovin milk to be at 25^{o}C followed by a gradual decrease in activity until up to a temperature of 50^{o}C.

The effect of heat treatment on coagulation time was agreement with (Dalgleish, 1992; Lucey, 1995; Hyslop, 2003; Anema *et al.* 2007; Blecker *et al.* 2012), they found that heat treatments of milk at relatively high temperature (higher than 55^{o}C) allowed the denaturation of whey proteins; the percentage of denaturation of whey proteins depends on both the temperature and time. Denatured whey proteins on the surface of casein micelles sterically hinder the aggregation of rennet-altered micelles, resulting in prolonged rennet coagulation time of milk.

The effect of Chitosan treatment on coagulation time was agreement with Ausar et al. (2001) who found that the addition of chitosan on whole or skim milk produces destabilization and coagulation of casein micelles that takes place without changes in the milk pH or the stability of most whey proteins.

As shown in Table (3) and Fig (2A), there is no significant difference either between treatments or the incubation temperature for TBC in all samples which had almost the same acidity of 0.195%. Results shown in Table (3) and Fig (2B) indicated that HT and CH treatment had significantly lower spore forming count (0.150 Log $_{10}$ Cfu/ml at 45 ^{o}C) than other treatments which there is no significant differences between all of them ranged from 1.188-1.676 Log $_{10}$ Cfu/ml. On the other hand the incubation temperature had no effect on spore forming count in all treatment. As can be observed from results in same Fig (2C), coliform counts in both HT and BG treatments were significantly lower (1.499 Log $_{10}$ Cfu/ml at 45 ^{o}C) than other treatments ranged from 2.80 - 3.167 Log $_{10}$ Cfu/ml, while coliform counts were significantly lower at 45^{o}C (1.499 Log $_{10}$ Cfu/ml) than 25^{o}C (3.048 Log $_{10}$ Cfu/ml) and 37^{o}C (3.167 Log $_{10}$ Cfu/ml) incubation temperature in all treatments. The results presented in Fig (2D) revealed that M&Y counts were significantly lower in HT samples (2.057 Log $_{10}$ Cfu/ml) than other treatments ranged from 2.411-3.424 Log $_{10}$ Cfu/ml. However, at 45^{o}C (2.057 Log $_{10}$ Cfu/ml) the significant low of M&Y counts in all treatments were observed compared to at 25^{o}C (3.424 Log $_{10}$ Cfu/ml) and 37^{o}C (3.377 Log $_{10}$ Cfu/ml).

These results may be attributed to the LP-system could be elicit bacteriostatic and bactericidal activity on a variety of susceptible microorganisms including bacteria, fungi and viruses. (Seifu *et al.* 2005). Chitosan was effective in inhibiting the growth of a wide variety of bacteria since it possesses a stronger antibacterial activity (Altieri *et al.* 2005 and Tayel *et al.* 2010**)**. Regarding the results of microbial counts in BG and GM treatments, it's the most likely to be consider them as antibacterial agents.

Table (3): Effect of using non thermal treatments of cow milk on microbial counts (Log $_{10}$ Cfu/ml).

Treatments	Incubation Temperatures °C/ time (hr)	Microbial Counts			
		TBC	SC	CC	M.Y
control	4 / 62	6.190^{Aa}	1.188^{Aa}	2.970^{Aa}	2.969^{Aa}
H	25 /13	5.767^{Aa}	0.951^{Ba}	1.738^{Ca}	2.686^{Ba}
	37 /10	5.866^{Aa}	0.176^{Ba}	1.951^{Ca}	2.389^{Ba}
	45 /8	5.992^{Aa}	0.150^{Ba}	1.499^{Cb}	2.057^{Bb}
LP	25 /10	6.098^{Aa}	1.510^{Aa}	3.048^{Aa}	3.088^{Aa}
	37 /6	6.254^{Aa}	1.573^{Aa}	3.081^{Aa}	2.841^{Aa}
	45 /5	6.302^{Aa}	1.676^{Aa}	3.157^{Ab}	2.669^{Ab}
CH	25 /11	6.027^{Aa}	0.286^{Ba}	2.844^{Aa}	3.149^{Aa}
	37 /7	6.116^{Aa}	0.370^{Ba}	3.040^{Aa}	3.029^{Aa}
	45 /6	6.318^{Aa}	0.520^{Ba}	3.091^{Ab}	2.411^{Ab}
BG	25 /8	6.120^{Aa}	1.366^{Aa}	3.031^{Ba}	3.424^{Aa}
	37 /6	6.449^{Aa}	1.403^{Aa}	3.011^{Ba}	3.327^{Aa}
	45 /4	5.909^{Aa}	1.215^{Aa}	1.849^{Bb}	2.851^{Ab}
GM	25 /7	6.240^{Aa}	1.391^{Aa}	3.044^{Aa}	3.413^{Aa}
	37 /5	6.336^{Aa}	1.516^{Aa}	3.167^{Aa}	3.377^{Aa}
	45/4	6.077^{Aa}	1.301^{Aa}	2.820^{Ab}	2.786^{Ab}

TBC = Total Bacterial Count, SC = Spore forming bacterial Count, CC = Coliform bacterial Count, MY= Mould and Yeast bacterial count.
The different capital letters have a significant effect between treatments at the level 0.05, and the different small letters have a significant effect for incubation temperatures for at the level 0.05.

Conclusion

From obtained results, the present study encourages the uses of LPs, CH, BG and GM for preservation of raw cow milk at 37 °C from 8 to 10hr and at 45 °C from 6 to 8 hr with acidity of 0.195%. These non thermal methods are suitable for rural area which had high ambient temperature and lack of refrigeration facilities.

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حفظ اللبن البقرى الخام بإستخدام معاملات غير حرارية

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تهدف الدراسة الى حفظ اللبن البقرى الخام عن طريق استخدام بعض المعاملات غير الحرارية.

تم فى هذه الدراسة تقسيم اللبن البقرى الخام خلال ساعتين من الحلابة الى ٦ أجزاء . الجزء الاول تم حفظه على ٤°م (عينة المقارنة)، الجزء الثانى تم معاملته بالحرارة على ٨٠°م لمدة ١٠ دقائق، الجزءالثالث تم فيه تنشيط نظام الاكتوبيروكسيديز، الجزء الرابع تم إضافة الكيتوزان بمعدل ٠.١%، الجزء الخامس تم إضافة البيتاجلوكان بمعدل ٠٣. ٠.% والجزء السادس تم إضافة الجلوكومنان بمعدل ٠٣ .٠ % .

تم بعد ذلك تحضين جميع العينات المعاملة ماعدا المقارنة على درجات حرارة ٢٥ °م ، ٣٧ °م ، ٤٥ °م وتم متابعة التغيرات فى الحموضة الكلية ، التجبن بالغليان والتجبن بالكحول كل ساعة فى جميع العينات.

بعد وصول الحموضة فى كل العينات الى حوالى ١٩٥. ٠ % (عند أزمنة مختلفة) تم تقدير كل من وقت التجبن بالمنفحة، نشاط البادئ، عدد البكتيريا الميزوفيلية الكلية، البكتيريا المتجرثمة، بكتيريا القولون والفطريات والخمائر خلال فترة التحضين.

وكانت أهم النتائج المتحصل عليها ما يلى:-

- أدت معاملة اللبن بالمعاملات السا بقة الى حفظه لمدة تتراوح من ٧ – ١٣ ساعة ٢٥ °م و٥ – ٩ ساعات ٣٧ °م و٤ - ٨ ساعات ٤٥ °م مقارنة بعينة المقارنة التى حفظت لمة ٦٢ ساعة على ٤°م وكانت الحموضة لجميع العينات تتراوح مابين ١٩٥ .٠ – ٢٠. ٠ % حيث كانت المعاملة الحرارية اكثر تأثيرا فى مدة الحفظ يليها معاملة الكيتوزان ثم معاملة تنشيط نظام الاكتوبيروكسيديز وأخيرا معاملة البيتاجلوكان والجلوكومنان.
- اختبارى التجبن بالغليان والكحول كانت نتائجهما مؤكدة لنتائج الحموضة السابقة.
- إنخفض نشاط البادئ معنويا فى معاملات الكيتوزان والبيتاجلوكان والجلوكومنان ونظام الاكتوبيروكسيديز مقارنة بالمعاملة الحرارية وعينة المقارنة.
- زاد وقت التجبن بالمنفحة فى كل من المعاملة الحرارية ونظام الاكتوبيروكسيديز بينما لم يتأثر فى باقى المعاملات الاخرى وعينة المقارنة.
- أوضحت النتائج عدم وجود إختلافات معنوية فى عدد البكتيريا الميزوفيلية الكلية على جميع درجات الحرارة المختلفة مقارنة بعينة المقارنة.
- إحتوت معاملة الكينوزان والمعاملة الحرارية على أعداد أقل معنويا فى البكتيريا المتجرثمة مقارنة بباقى المعاملات وعينة المقارنة.
- أعداد بكتيريا القولون كانت أقل معنويا فى المعاملة الحرارية والبيتاجلوكان مقارنة بباقى المعاملات وعينة المقارنة.
- إحتوت العينة المعاملة بالحرارة على أعداد قليلة معنويا فى أعداد الفطريات والخمائر مقارنة بباقى المعاملات وعينة المقارنة.

قام بتحكيم البحث

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Table (1): Effect of using non thermal treatments of raw cow milk on titratable acidity development (T.A%).

Incubation Time (hr)	Treatments															
	Control	HT			LP			CH			BG			GM		
	Incubation Temperatures (^{o}C)															
	4	25	37	45	25	37	45	25	37	45	25	37	45	25	37	45
0	0.16	0.15	0.15	0.15	0.16	0.16	0.16	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.16	0.16
1	0.16	0.15	0.15	0.155	0.16	0.16	0.16	0.15	0.15	0.15	0.165	0.165	0.17	0.165	0.165	0.17
2	0.16	0.15	0.16	0.16	0.165	0.17	0.17	0.155	0.16	0.16	0.17	0.175	0.18	0.17	0.17	0.18
3	0.16	0.155	0.165	0.165	0.17	0.175	0.18	0.16	0.165	0.17	0.175	0.18	0.19	0.175	0.18	0.19
4	0.16	0.155	0.17	0.17	0.175	0.185	0.19	0.165	0.17	0.175	0.18	0.185	0.20	0.18	0.19	0.20
5	0.16	0.16	0.175	0.175	0.18	0.19	0.20	0.17	0.18	0.185	0.185	0.195	0.21	0.185	0.20	0.21
6	0.16	0.16	0.18	0.18	0.18	0.20	0.21	0.18	0.19	0.195	0.19	0.20	0.23	0.19	0.21	0.23
7	0.16	0.165	0.185	0.19	0.185	0.21	0.23	0.18	0.20	0.21	0.195	0.22	*	0.20	0.22	*
8	0.16	0.17	0.19	0.20	0.19	0.23	*	0.185	0.21	0.23	0.20	0.235	_	0.21	0.24	_
9	0.165	0.175	0.195	0.21	0.195	*	_	0.19	0.22	*	0.21	*	_	0.22	*	_
10	0.165	0.18	0.20	0.23	0.20	_	_	0.195	0.23	_	0.22	_	_	0.23	_	_
11	0.165	0.185	0.21	*	0.21	_	_	0.20	*	_	0.235	_	_	*	_	_
12	0.165	0.19	0.23	_	0.23	_	_	0.21	_	_	*	_	_	_	_	_
13	0.165	0.195	*	_	*	_	_	0.23	_	_	_	_	_	_	_	_
18	0.17	0.22	_	_	_	_	_	*	_	_	_	_	_	_	_	_
19	0.17	0.23	_	_	_	_	_	_	_	_	_	_	_	_	_	_
24	0.17	*	_	_	_	_	_	_	_	_	_	_	_	_	_	_

HT: Heat treatment LP: Lactoperoxdiese treatment CH: Chitosan treatment BG: β-glucan treatment GM: Glucomannan treatment

***: Analysis was discontinued due to sample spoilage**