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EFFECT OF CO_2 ADDITION TO RAW MILK ON ITS PROPERTIES DURING STORAGE IN REFRIGERATOR

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ABSTRACT

The aim of this research is to study the effect of using CO_2 on raw cow's milk properties during storage in refrigerator. Fresh raw cow's milk, with low (~100 x 10^3 cells/ml) and high (~700 x 10^3 cells/ml) somatic cell count (SCC) was treated with CO_2 (Food grade) to contain zero, 500, 1000, 1500, 2000 and 2500 ppm. The treatments were analysed organoleptically when fresh and daily for pH and clotting on boiling during storage in refrigerator to choice the best concentration of CO_2 added. The addition of CO_2 up to 1500 ppm had no marked effect on organoleptic properties and extended the shelf-life to 12 and 10 days for low- and high-SCC milks in refrigerator respectively. Effect of using CO_2 with a level of 1500 ppm on chemical composition, sensory evaluation and electrophoretic patterns when fresh and at the end of shelf-life and microbiologically every 2 days during storage was studied. The addition of 1500 ppm CO_2 to low-and high-SCC raw cow's milk decreased the pH of milk and inhibited the microbial growth (total bacterial count, psychrotrophic, proteolytic, lipolytic bacteria and coliforms) and so, decreased the proteolysis and lipolysis compared with control. The proteolysis and lipolysis were more pronounced in the high-SCC milk, probably due to its high proteases and lipases activity. Also, the effect of heat treatment on milk pH treated with 1500 ppm CO_2 was studied. The obtained results reveals that cow's milk treated with 1500 ppm CO_2 can be stored in refrigerator for 10 - 12 days with minimal effects on its properties.

INTRODUCTION

Economic pressures on the dairy industry would be eased if the keeping quality of raw milk could be increased and thus provide greater flexibility in its utilization (King and Mabbitt, 1982). The dairy industry has relied on refrigeration to maintain raw milk quality during storage and transportation. Limited by the growth of psychrotrophic bacteria and the level of somatic cell (SCC), the normal refrigerated storage life of raw milk is usually less than 5 days (Ma *et al.*, 2003).

Enzymes present in refrigerated raw milk are either endogenous (i.e. originating from the cow) or from psychrotrophic bacteria growing in the milk. The two most important endogenous enzymes are plasmin and lipoprotein lipase and can cause slow degradation of milk protein (namely casein) and lipid (namely

triglycerides). Also, enzymes of somatic cell origin in milk increase during mastitis, and the extent of increase depends on the severity of infection (De Rham and Andrews, 1982) and becomes especially significant when SCC is high, above 1 million cells / ml (Saeman *et al.*, 1988). The lipolytic and proteolytic activities contributed by psychrotrophic bacteria (Cousin, 1982).

The dairy industry is interested in expanding the use of CO_2 technology, seeking applications for shelf-life extension in other dairy foods and identifying benefits of CO_2 addition other than its antimicrobial effect (Ma and Barbano, 2003a). The addition of CO_2 to raw milk and dairy products controls the growth of psychrotrophic bacteria at refrigeration temperatures (Ma et el., 2001). The combination of CO_2 and refrigeration has been investigated as a mean of controlling the growth of psychrotrophic microorganisms in raw milk. The lower the initial counts in the untreated milk, the greater was the effect (Hotchkiss *et al.*, 1999). The addition of CO_2 must be carefully controlled so that a sufficient amount to reduce spoilage is added without being detectable by consumers or instability of the milk protein with symptoms 'bitterness' (King and Mabbitt, 1982 and Hotchkiss *et al.*, 1999).

In a series of studies; King and Mabbitt, 1982; Hotchkiss *et al.*, 1999; Ma *et al.*, 2001; Ma *et al.*, 2003 and Ma and Barbano, 2003b about 20 to 40 mM CO_2 were added to untreated whole milk. The results indicated that generation time increased in the presence of CO_2 because of the increase in lag phase time and that the aerobic plate counts in milk was also reduced. There was no evidence that CO_2 increases the growth of anaerobic and facultative organisms. They concluded that the inhibition mechanism of CO_2 is directly associated with CO_2 and is not to the indirect effect of pH reduction or O_2 replacement. Some of the direct effects exerted by CO_2 include its ability to change microbial membrane properties (Rowe, 1988), to lower intracellular pH (Hong and Pyun, 1999), and/or to interfere with cellular enzymatic reactions (King and Nagel, 1975) and it has been suggested that this occurs by a mass action effect on decarboxylating enzymes, and a number of enzymes have been shown to be affected by CO_2 (Gill and Tan, 1979). An alternative theory proposed by Sears and Eisenberg (1961) is that CO_2 adversely affects the permeability of the cell membrane. One point in particular should be noted, the added CO_2 can be easily removed from the milk by warming (King and Mabbitt, 1982).

The objective of this study was to use the proper concentration of CO_2 to increase the shelf-life of raw milk (low and high-SCC) during refrigerated storage by controlling the growth psychrotrophic bacteria, and to study the effect of CO_2 on milk properties.

MATERIALS AND METHODS

Materials:

Milk :

Fresh raw cow's milk was obtained from the herd of the Fac. of Agric. at Moshtohor, Zagazig Univ., Benha Branch and from different farms in Moshtohor.

CO_2:

Carbon dioxide (food grade) was obtained from Air Liquid Misr, Al-Tabia Rashid Road beside Abuqir fertilizer Company, Alexandria, Egypt.

Experimental:

To obtain milk with low-and high-SCC, milks of 20 cow's from different farms in Moshtohor were collected and cooled to 5°C. Milk sample from each cow was preserved using potassium dichromate (0.02% wt/wt) and tested for somatic cell count (SCC). Based on results of milk SCC level of individuals, milks were divided into two portions and the somatic cell for every portion was determined. The first portion, contains low-SCC (~ 100 x 10^3 cells/ml) and the second contain high-SCC (~700 x 10^3 cells/ml). Low- and high-SCC milk samples were treated with CO_2 at 5°C to contain approximately zero (control low-SCC, CoLs and control high–SCC, CoHs), 500, 1000, 1500, 2000 and 2500 ppm. Inlet CO_2 pressure was maintained at 4 psi, and the gas flow rate was 1 L/min. The desired carbonation time was determined from a preliminary experiments, which established the relationship between the addition time and the levels of CO_2 dissolved in a fixed amount of milk. Milk samples were stored in glass containers in refrigerator and sensory evaluated (after pasteurization) in zero time and tested every day for pH and clot-on boiling (COB) until the end of shelf-life, in order to select the suitable concentration of CO_2 which can be increase the self-life of low-and high-SCC milks during refrigerated storage without effect on sensory properties. The effect of the proper concentration of CO_2 on low-SCC and high-SCC milks ($LsCO_2$ and $HsCO_2$) during refrigerated storage on milk composition, electrophoretic patterns of milk protein and sensory evaluation (after pasteurization) were studied when fresh and at the end of shelf life, while the microbiological examination was conducted every two days. In addition, the effect of heat treatments on milk pH after addition of CO_2 was determined at 20°C.

Chemical analysis:

Milk pH was measured using a digital pH meter JENCO electronics LTD model 1671. Fat, total nitrogen (TN) and casein nitrogen (CN) (Ling, 1963). FAA (meq. of FFA/100 ml) in milk were determined by a modified copper soap solvent extraction method (Anderson *et al.*, 1991).

Somatic cell counts (SCC):

The number of somatic cells in milk was determined using integrated milk testing. The Fossomatic 5000, Type 71300, Operators Manual, Food First in Food Analysis, 69 Slangerupgade, DK 3400 Hillerod.

Microbiological analysis:

Milk samples were microbiologically examined for total bacterial count (TBC), psychrotrophic bacterial count (PBC), proteolytic bacteria, lipolytic bacteria and coliforms counts according to the methods described by the American Public Health Association (APHA, 1992).

Sensory evaluation:

Sensory evaluation of milk (liquid milk score card) was determined according to Degheidi *et al.* (1992).

Statistical analysis:

Statistical analysis of the results was followed by LSD (< 0.05) according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect of CO_2 concentrations and somatic cell levels on pH, shelf-life and sensory evaluation of raw cow's milk :

The preliminary trials were carried to choose the suitable concentration of CO_2 added to raw milk to inhibit the microbial growth during refrigerated storage without noticeable changes in the sensory properties (Table 1).

The results clear that the milk pH decreases when CO_2 dissolved in milk. The extent of pH reduction is related to the amount of CO_2 dissolved, hydrated and protonated in the aquous phase of milk, thus depends on the intrinsic properties of the aquous phase, such as buffering capacity and initial pH (Devlieghere *et al.*, 1998 and Ma and Barbono, 2003a). The hydrated form of dissolved CO_2 gas, carbonic acid (H_2CO_3) dissociates to give H^+, HCO_3^- and CO_3^{2-}. The increase in hydrogen ion concentration causes milk pH to decrease. With the lowering of milk pH, micelle calcium-phosphate begins to dissociate, contributing to increase concentrations of calcium and phosphate ions in the soluble serum phase (Gevaudan *et al.*, 1996; De la Fuente *et al.*, 1998 and Ma *et al.*, 2001).

Table (1): Effect of CO_2 concentration on pH, shelf-life and sensory evaluation of raw cow's milk at refrigerated temperture.

Properties	Concentration of CO_2 (ppm)					
	Low somatic cell					
	0	500	1000	1500	2000	2500
PH	6.82	6.71	6.59	6.49	6.34	6.25
Shelf-life (day)	2	4	8	12	12	9
Sensory evaluation						
Flavour (40)	39	39	39	38	34	32
Consistency (40)	39	40	39	38	35	30
Appearance (20)	20	20	20	20	16	14
Total (100)	**98**	**99**	**98**	**96**	**85**	**76**
	High somatic cell					
PH	6.84	6.72	6.56	6.49	6.36	6.28
Shelf-life (day)	2	4	7	10	9	7
Sensory evaluation						
Flavour (40)	39	39	38	38	34	31
Consistency (40)	39	38	38	37	33	32
Appearance (20)	19	19	18	17	15	13
Total (100)	**97**	**96**	**94**	**92**	**82**	**76**

N.B. The shelf-life of milk was the day before milk clotting with heating (COB test).

Regarding to shelf-life, it was observed that the shelf-life increased significantly ($P < 0.05$) by increasing CO_2 concentration. Dissolved CO_2 in refrigerated milk inhibits the growth of bacteria especially psychrotrophic by increasing both the lag phase and the generation time in the growth cycle of microorganisms (King and Mabbitt, 1982 and Daniels *et al.*, 1985). The concentration of CO_2 required to have a significant effect on shelf-life would detract from the sensory quality of milk. Sensory panelists found no noticeable differences between the control (untreated) and treated milks up to 1500 ppm CO_2. Increasing the CO_2 concentration more than 1500 ppm causes some defects in sensory properties. However, the highest CO_2-treated samples above 1500 ppm scored significantly ($P < 0.05$) lower than the untreated milk. These results are in agreement with Hotchkiss *et al.* (1999), who reported that, the addition of CO_2 must be carefully controlled, so that a sufficient amount to reduce spoilage is added without being detectable by consumers or instability of the milk proteins (King and Mabbitt, 1982 and Hotchkiss *et al.*, 1999).

From the preliminary trials, it could be concluded that the concentration of CO_2 at 1500 ppm is the best one which have a significant effect on shelf-life without noticeable changes in sensory properties. So, it was interest to study the effect of adding CO_2 with a level of 1500 ppm to raw milk on chemical and microbiological properties during storage in refrigerator.

Chemical composition:

Table (2) cleared the chemical composition of raw cow's milk (low and high somatic cell) treated with 1500 ppm CO_2 during storage in refrigerator. For both the low and high SCC control milks, pH was similar (~pH 6.8) in the start of storage, but it decreased significantly ($P < 0.05$) during storage period. The decrease in milk pH in control milks was probably due to the microbial growth through storage period (Guinot *et al.*, 1995). The pH values of low and high SCC milks treated with 1500 ppm CO_2 were 6.49 and 6.50 in the start and decreased during storage to reach 6.25 and 6.20 respectively at the end of storage.

Table (2): Effect of 1500 ppm CO_2 on milk composition of low- and high-SCC milks.

Properties	Low somatic cell				High somatic cell			
	Control		Treated		Control		Treated	
	Zero	End of storage	Zero	End of storage	Zero	End of storage	Zero	End of storage
pH	6.78	6.14	6.49	6.25	6.80	6.12	6.50	6.20
Fat	3.45	3.35	3.45	3.35	3.35	3.20	3.35	3.15
TN %	0.55	0.54	0.55	0.53	0.51	0.50	0.49	0.49
CN %	0.45	0.41	0.45	0.43	0.40	0.35	0.39	0.38
CN/TN %	82.15	76.41	82.22	81.11	79.10	70.32	79.17	77.81
FFA (meq. /100 ml)	0.15	0.36	0.15	0.20	0.21	0.48	0.21	0.26

It was noticed that the decrease in pH values of CO_2 treated milk was slightly higher than the controls. This may be due to the addition of CO_2 which decreased the milk pH (Ma *et al.*, 2001; Ma and Barbano, 2003a and Ma and Barbano, 2003b). The extent of pH reduction is related to the amount of CO_2 dissolved in the aquous phase of milk (Devlieghere *et al.*, 1998).

Proteolysis:

Decrease in CN/TN (Table 2) was used as an index of proteolysis. Decrease in CN/TN can have important economic impacts. Enzymatic damage of CN can be directly reflected in the percentage decrease of CN/TN. In the high SCC control milks, significant proteolysis occurred and CN/TN decreased during storage. The extent of decrease was slightly higher in high SCC milk than low SCC milk. Enzymes of somatic cell origin in milk increase during mastitis. The extent of increase depends on the severity of infection (De Rham and Andrews, 1982). The addition of 1500 ppm CO_2 to milk had no significant proteolysis, probably due to the inhibition of bacteria that produce proteolytic enzymes (Ma *et al.*, 2003).

Lipolysis:

The fat content of low-and high- SCC milks were nearly similar. At the end of the shelf-life, the fat slightly decreased as a result of lipolysis. Increase in FFA was used as an index of lipolysis. Comparing lipolysis in the control and treated low-and high-SCC milks with 1500 ppm CO_2 are shown in Table (2). Milk FFA concentration increased during storage in both low-and high-SCC milks. The FFA increase during storage in refrigerator was greater in the high SCC than low SCC, this may be due to the increase of active somatic cells lipases and its effect on milk-fat globule membrane (Downey, 1980 and Murphy *et al.*, 1989). Treated milk with CO_2 in low and high SCC milks showed no significant increase ($P < 0.05$) in FFA during storage. Thus, the addition of 1500 ppm CO_2 to milk retarded lipolysis in low-and high-SCC raw milks during storage.

Effect of heat treatments on the pH of milk treated with 1500 ppm CO_2.

Table (3) shows the effect of different heat treatments on pH of milk treated with 1500 ppm CO_2 to determine whether the effect of CO_2 on milk pH was reversible.

Table (3): Effect of heat treatments on the pH of milk treated with 1500 ppm CO_2.

Treatments	Heat treatments					
	Before heating	Warming to 60°C	63°C/ 30 min	72°C/ 15 min	90°C/ 5Sec.	100°C/ 15 min.
Control	6.78	6.77	6.72	6.70	6.69	6.67
Treated milk	6.49	6.72	6.68	6.67	6.65	6.64

The results cleared that the heating of treated milk to 60°C with stirring was enough to return the pH of milk to nearly the control value and subsequently the advanced heat treatments above 60°C lead to return the pH nearly similar the control after the same heat treatments.

The decrease in milk pH as affected by CO_2 added is reversible, CO_2 is simply removed from milk by applying a heating or during further processing without detrimental effects (King and Mabbitt, 1982 and Ma and Barbano, 2003a).

Removal of dissolved CO_2 gas reduces the concentration of carbonic acid, and thus its derived hydrogen ion. This decrease shifts the milk pH to its precarbonation level. With this return of milk pH, the soluble calcium and phosphate concentrations in the serum phase also shifted back to levels similar to those observed in milk without carbonation (Gevaudan *et al.*, 1996 and Ma *et al.*, 2001).

Electrophoretic patterns:

The electrophoretic patterns of $LsCO_2$ and $HsCO_2$ milks (after the end of shelf-life) as well as untreated milks (CoLs and CoHs as controls) when fresh and after the end of shelf-life are illustrated in Fig. (1).

There is no discernible changes in the electrophoretic patterns of milk protein were encountered for two controls when fresh (slots 1,4). The main protein fractions of cow's milk protein appeared in these patterns as α_s-casein (Fox and Waley, 1981 and Abdou *et al.*, 1994). β-casein and K-casein was seen in all milk samples (Mehanna *et al.*, 1982 and Abdou *et al.*, 1994).

The electrophoretic patterns of CoLs and CoHs milks after the end of shelf-life (slots 2,5) revealed that pronounced changes could be detected with respect to the number and intensity of the different bands. This may be due to its degradation during storage. Enzymes present in refrigerated raw milk either endogenous or from psychrotrophic bacteria (grown in the milk) able to produce extracellular enzymes. These enzymes are active at refrigeration temperatures and can cause slow degradation of milk proteins (Law, 1979; Cousin, 1982 and Ma *et al.*, 2003).

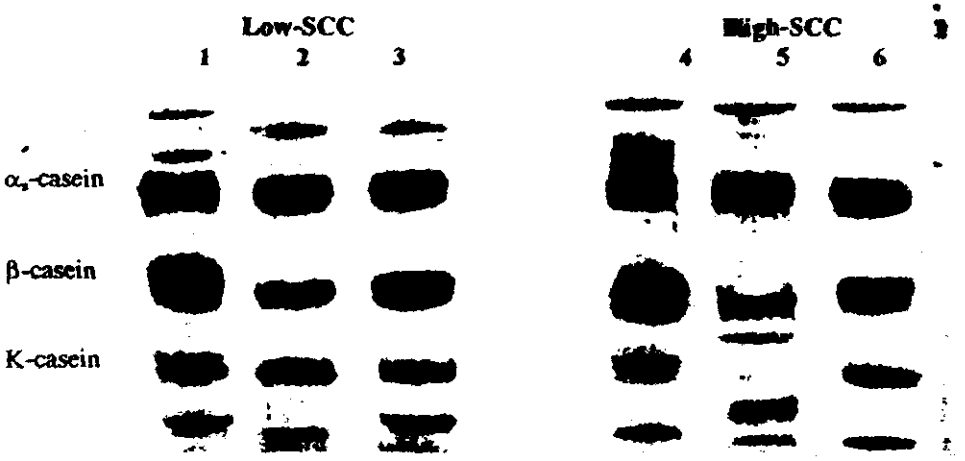

Fig. (1): Electrophoretic pattern of low- and high-SCC milks protein as affected by CO_2.

On the other hand, electrophoretic pattern showed that there are a slow moving faint degradation products were indicated after 12 and 10 days of storage for $LsCO_2$ and $HsCO_2$ milks respectively (slots 3,), but it was nearly similar with control. This suggests that native enzymes of milk tend to remain dormant during the preservative influence of CO_2. Addition of CO_2 can effectively control growth of psychrotrophic bacteria in raw milk during refrigerated storage (King and Mabbitt, 1982; Rashed *et al.*, 1986; Hotchkiss *et al.*, 1999; Werner and Hotchkiss, 2002 and Ma and Barbano, 2003a).

In general, the electrophoretic patterns indicated that the high-SCC milk protein was higher in proteolysis than low-SCC milk protein. This may be attributed to that the increase in milk SCC increased the activity of proteolysis (Ali *et al.*, 1980; Grandison and Ford, 1986; Verdi and Barbano, 1991; Santos *et al.*, 2003 and Albenzio *et al.*, 2004). Kelly *et al.* (2000) found that an elevated SCC can alter the protein fractions distribution and decrease casein in milk.

Microbial analysis :

Microbial growth curves for two controls (CoLs and CoHs) and milk treated with 1500 ppm CO_2 ($LsCO_2$ and $HsCO_2$) are shown in Figure (2). At the first day, for both CoLs and CoHs milks, TBC (approximately 10^4 cfu/ml) was higher than PBC (approximately 10^3 cfu/ml). During storage, TBC and PBC became similar for all treatments and increased by storage. This indicated that during 4°C storage, the microorganisms present in milk had become predominantly psychrotrophic bacteria (Ma *et al.*, 2003). The proteolytic bacterial count was 30×10^2 and 50×10^2 cfu/ml for CoLs and CoHs milks respectively, while the lipolytic bacterial count was 290 and 370 cfu/ml for CoLs and CoHs milks in the same order. On the other hand, coliform count were less than 100 cfu/ml for two controls (data not shown). In general, the microbial counts were slightly higher in high-SCC milks than low-SCC milks. The addition of CO_2 to low- and high- SCC milks caused a significantly decrease in the growth rate of bacteria compared with controls. King and Mabbitt, 1982 mentioned that the dissolved CO_2 at 10°C or below reduced the growth rate of these psychrotrophic bacteria and so increased the shelf-life of milk. The only major difference was that the CO_2-treated milk had lower counts of coliforms, psychrotrophic, proteolytic and lipolytic bacteria than the untreated raw milk when fresh and during storage (Ruas-Madiedo *et al.*, 1996 and Hotchkiss *et al.*, 1999).

$LsCO_2$ and $HsCO_2$ milks stored at 5 ± 1°C took 12 and 10 days respectively longer than the controls (2 days) for TBC or PBC to reach 10^6 cfu/ml. The storage time of raw milk or raw milk with CO_2 was dependent on initial numbers of bacterial count in the milk (King and Mabbitt, 1982 and Ma *et al.*, 2003).

Sensory evaluation:

Table (4) showed that the sensory scores of low- and high-SCC were high when fresh. The scores slightly decreased at the end of shelf-life. The rate of decrease was slightly higher in high-SCC than low-SCC as a result of some proteolysis and lipolysis occurred during storage. The acceptability of low-and

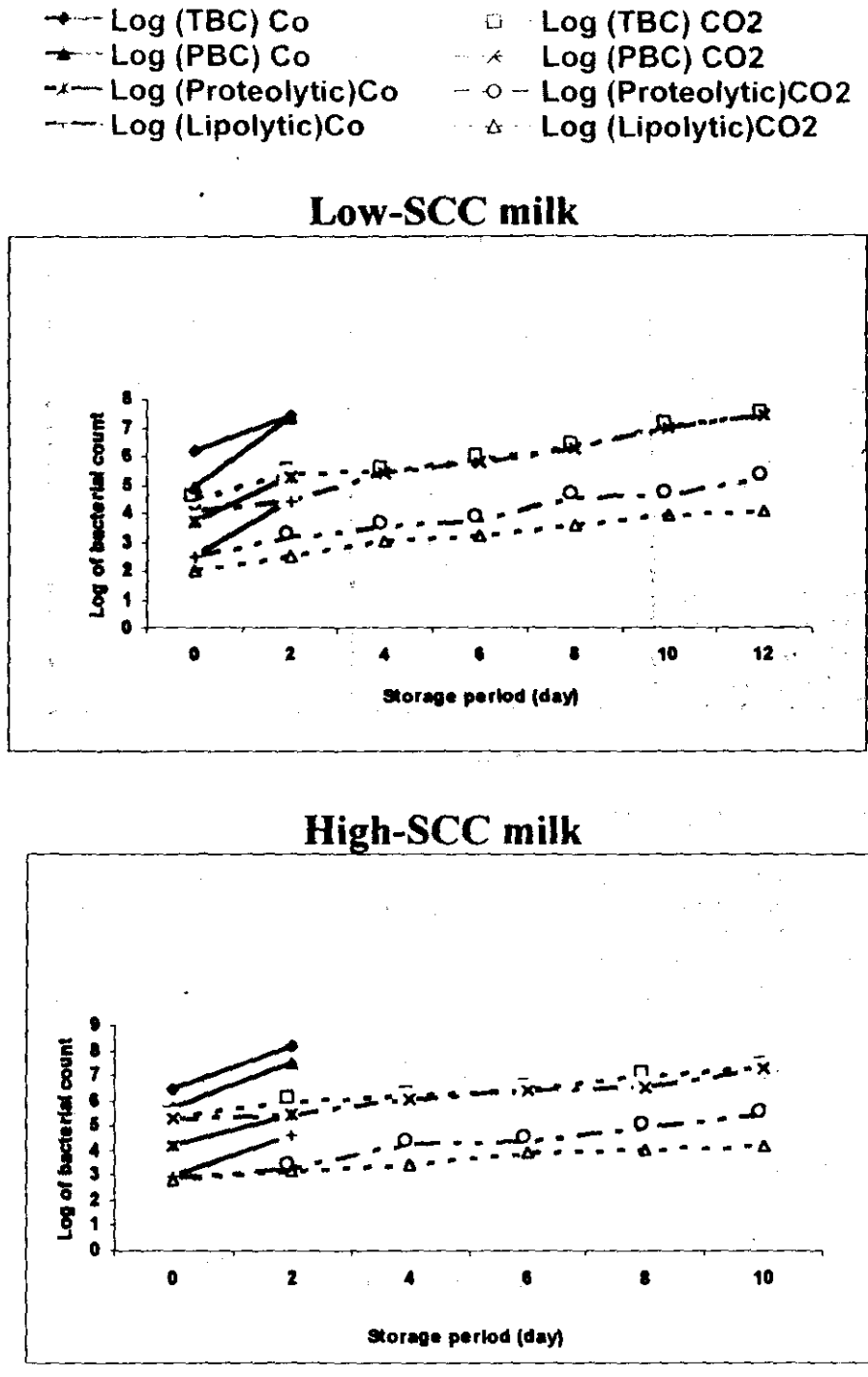

Fig. (2): Effect of 1500 ppm CO_2 on microbial growth of low- and high-SCC milks.

high-SCC milks at the end of shelf-life was significantly ($P < 0.05$) good by sensory panelists. These results agree with the preliminary trials and also with King and Mabbitt (1982)and Hotchkiss *et al.* (1999).

Table (4): Sensory evaluation of low- and high-SCC milk treated with 1500 ppm when fresh and shelf-life end.

Properties	Low somatic cell			
	Control		Treated	
	Zero	Shelf-life end (2 days)	Zero	Shelf-life end (12 days)
Flavour (40)	39	38	39	37
Consistency (40)	38	35	38	35
Apperance (20)	20	18	20	18
Total (100)	97	91	97	90
	High somatic cell			
	Zero	Shelf-life end (2 days)	Zero	Shelf-life end (10days)
Flavour (40)	38	36	38	35
Consistency (40)	37	34	37	33
Apperance (20)	20	17	20	16
Total (100)	95	87	95	84

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تأثير إضافة ثانى أكسيد الكربون إلى اللبن الخام على خواص اللبن أثناء الحفظ بالتبريد

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يهدف هذا البحث إلى دراسة تأثير استخدام ثانى أكسيد الكربون على خواص اللبن البقرى الخام أثناء الحفظ بالتبريد:

١- تم معاملة اللبن البقرى الخام المنخفض (١٠٠ × ١٠^{٣} خلية/مل) والمرتفع (٧٠٠ × ١٠^{٣} خلية/مل) فى محتواه من الخلايا الجسمية بثانى أكسيد الكربون (درجة غذائية) بحيث يحتوى على صفر ، ٥٠٠ ، ١٠٠٠ ، ١٥٠٠ ، ٢٠٠٠ ، ٢٥٠٠ جزء فى المليون. ثم تحليل المعاملات حسيا وهى طازجة ويوميا بالنسبة للـ pH ، نقطة التجبن بالغلى أثناء فترة التخزين فى الثلاجة وكان أفضل تركيز من ثانى أكسيد الكربون دون تغيرات حسية غير مرغوبة هو تركيز ١٥٠٠ جزء فى المليون حيث أطال فترة التخزين إلى ١٢ يوم للبن المنخفض فى الخلايا الجسمية و ١٠ أيام فى المرتفع فى الخلايا الجسمية.

٢- تم دراسة تأثير استخدام ثانى أكسيد الكربون بتركيز ١٥٠٠ جزء فى المليون على التركيب الكيماوى وحسيا وتحليل البروتين بالإلكتروفريسيس فى بداية ونهاية الحفظ فى الثلاجة وكذلك ميكروبيولوجيا كل يومين اثناء الحفظ حيث وجد أن إضافة ثانى أكسيد الكربون بتركيز ١٥٠٠ جزء فى المليون فى اللبن البقرى المنخفض والمرتفع فى الخلايا الجسمية أدى إلى خفض الـ pH وتثبيط البكتريا (العدد الكلى – البكتريا المحبة للبرودة – البكتريا المحللة للبروتين – البكتريا المحللة للدهن وبكتريا القولون) وبالتالى تقليل تحلل البروتين وتحليل الدهن بالمقارنة بالكنترول. وكان التحلل فى البروتين والدهن ملحوظا فى اللبن ذو المحتوى العالى من الخلايا الجسمية لزيادة محتواه من إنزيمات التحلل البروتينى والدهنى. كما تم دراسة تأثير المعاملات الحرارية على pHاللبن المعامل بثانى أكسيد الكربون بتركيز ١٥٠٠ جزء فى المليون.

وبصفة عامة يمكن أن نوصى بإمكانية حفظ اللبن البقرى الخام باستخدام ١٥٠٠ جزء فى المليون من ثانى أكسيد الكربون لمدة ١٠ – ١٢ يوم داخل الثلاجة حيث لا يؤثر ذلك بدرجة ملحوظة علي خواصه.