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INFLUENCE OF CO_2 ADDITION TO RAW MILK ON INHIBITION OF PSYCHROPHILIC BACTERIA AND SOME SENSORY CHARACTERISTICS OF PASTEURIZED MILK

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Abstract

Influence of carbon dioxide on the activity of psychrophilic bacteria in raw bovine milk was investigated. Also, some sensory characteristics of pasteurized milk which processed from raw milk preserved by CO_2 were studied. Firstly, effect of CO_2 addition to raw milk was compared with the acidification of raw milk to pH 6.2 with lactic acid and with control milk. The results showed partial inhibition in psychrophilic microorganisms count in CO_2-treated milk compared to both lactic acid-treated and control milks during four days of study. Secondly, the effect of two low temperatures (4 and 10 °C) on reducing psychrophilic bacterial count was evaluated. The storage temperature affected the growth of psychrophilic bacteria in CO_2-treated milk; the lower temperature was the best; samples stored at 4 °C showed better inhibition in psychrophilic microorganisms count in comparison with the samples kept at 10°C. Thirdly, organoleptic characteristics of pasteurized milk (65 °C/30 min) processed from raw milk treated with CO_2 showed insignificant differences ($p \geq 0.05$) in taste, aroma and colour between CO_2-treated and control milks.

Keywords: Co2, Raw Milk, Psychrophilic Bacteria, Pasteurized Milk.

تأثير إضافة ثاني أوكسيد الكاربون الى الحليب الخام في تثبيط نشاط البكتريا المحبة للبرودة وبعض الصفات الحسية للحليب المبستر

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المستخلص

أجري هذا البحث لدراسة تأثير ثاني أوكسيد الكربون المذاب في الحليب البقري الخام في نشاط البكتريا المحبة للبرودة (Psychrophilic bacteria). كذلك درست بعض الخصائص الحسية للحليب المبستر المصنّع من الحليب الخام المعالج بـ CO_2. أولا، درس تأثير ثاني أكسيد الكربون المضاف إلى الحليب الخام في تثبيط البكتريا المحبة للبرودة مقارنة بالحليب المحمض بحامض اللاكتيك الى pH مقداره 6.2 ومعاملة السيطرة. وأظهرت النتائج تثبيطا جزئيا في أعداد البكتريا المحبة للبرودة في الحليب المعالج بـ CO_2 مقارنة مع كل من الحليب المعامل بحامض اللاكتيك خلال أربعة أيام من الدراسة. ثانيا، تم تقييم تأثير درجتين من درجات الحرارة المنخفضة (4 و10م°) في الحد من أعداد البكتريا المحبة للبرودة إذ أظهرت النتائج ان درجة حرارة خزن الحليب المعالج بـ CO_2 تؤثر في نمو البكتريا المحبة للبرودة فكلما كانت أقل، كلما كان ذلك أفضل. إذ ظهرت في العينات المخزنة في 4 درجات مئوية تثبيط أفضل في أعداد البكتريا بالمقارنة مع العينات المخزنّة في 10 درجة مئوية. وثالثا، أظهرت الفحوصات الحسية للحليب المبستر (65 م°/30 دقيقة) والمصنّع من الحليب الخام المعالج بـ CO_2 فروقات غير معنوية ($p\geq0.05$) في الطعم والرائحة واللون بين الحليب المعالج بثاني أكسيد الكربون ومعاملة السيطرة.

كلمات مفتاحية: ثاني أوكسيد الكاربون، الحليب الخام، بكتريا محبه للماء، حليب مبستر.

Introduction

Most of non-sterile dairy products may undergo deterioration within 1 to 3 weeks depending upon the quality of the raw materials, processing and handling conditions (1, 2). Keeping quality of raw milk is very important in the dairy industry in order to provide greater flexibility in milk utilization. Growth of psychrophilic microorganisms (to$\geq 10^6$ CFU/mL) can reduce the quality of pasteurized milk (3). Preservation of raw milk can be stepped up by following higher hygienic procedures at all steps including rapid refrigeration on arrival at the dairy before storage. Storing milk at low temperatures can also expand the shelf-life significantly. The use of antimicrobial additives in order to extend the shelf-life of raw or processed milk is inadequate due to toxicity risks (4).

The eligibility of milk preservation would increase if an innocuous preservative could be adopted. Carbon dioxide CO_2 seems to be an excellent alternative as it is a natural ingredient of milk. The average concentration of dissolved CO_2 in fresh bovine milk is about 220 ppm and ranging from 40 to 1100 ppm and about 88% of this concentration exists as dissolved gas (5, 6 and 7). Carbon dioxide, a low-cost inert and non-toxic gas, when added in adequate concentrations expands the keeping quality of milk and dairy products by controlling microorganisms growth, specifically aerobic bacteria, and probably restricting oxidative rancidity (8 and 9). Sikin *et al.*, (10) found CO_2 processing is a promising alternative for the reduction of microorganisms in mozzarella-type cheese. CO_2 may suppress the growth of microorganisms either directly by its influence on the metabolism of microorganisms and/or indirectly by

displacement of O_2 and decreasing pH (4 and 11). However, King and Mabbitt (12) reported that the influence was directly due to the existence of CO_2.

Psychrophilic gram-negative bacteria specially *Pseudomonas* are responsible for the rapid spoilage through the production of enzymes have the ability to attack milk lipids and proteins which affects the flavour changes of cold raw and pasteurised milk (2, 13, 14 and 15). Cooling of raw milk at 4°C for few days, can supress significantly the bacterial growth, at the same time, it enhances the growth of psychrotrophs and significant numbers, e.g., 10^6 or 10^7 cfu/ml can be reached in 3 or 4 days (15). CO_2 may inhibit the growth of Gram-negative bacteria such as *Pseudomonas* and *Moraxella* by 20% CO_2 (16). Combination between cooling and treating raw milk with low concentration of CO_2 would inhibit the growth of microorganisms (17).

Enterobacteriaceae account about for less than one third of psychrophilic microorganisms existing in raw milk. Their optimum growth temperature is higher than 30 °C, but they adapt well to growth at refrigeration condition (18). The solubility of CO_2 is significantly higher in cold aqueous phase. Consequently, the influence of CO_2 will be greater in refrigerated raw milk (19). However, addition of CO_2 lowers the organoleptic attributes of the pasteurized milk unless the heat treatment includes degassing of the raw milk prior to processing (14 and 20). Sensory evaluation of pasteurised milk post treatment with CO_2, refrigeration and degassing was investigated by many scholars. No significant differences in organoleptic attributes were detected between treated and untreated samples. But, samples that were not degassed showed significantly lower scores than controls (no CO_2) (1 and 21). Milk storage and stability is temperature dependent, and the development of any off-flavours is noticeable in milk because it has naturally mild, slightly sweet flavour (22 and 23).

This work investigated the effect of CO_2 on raw milk preservation for few days before processing. Psychrophilic bacteria were the index in this study because of its influence in cold conditions and its capability to degrade milk components.

Materials and Methods

Ten samples of raw bovine milk were collected from Owainat village in Tikrit Governorate and mixed gently. Bulk milk stored at 4 °C until used for experiments, which was normally within 3-4 h of the time of collection.

Aliquots of milk samples (500 ml) were treated with CO_2 in 1000 ml containers. Food-grade CO_2 was dissolved into the milk samples by bubbling at 4 and 10 °C until pH 6.2 and adjusted daily to retain the same pH. Acidified milk sample was conducted with diluted lactic acid (0.1 N), which was added slowly and mixed gently to pH 6.2. The pH measurements were conducted by portable pH-meter.

The work was carried out in three parts:

1. The effect of milk acidified to pH 6.2 by adding CO_2 and lactic acid on the growth of psychrophilic milk spoilage bacteria at 10 °C in comparison with control milk.
2. The effect of storage temperature (4 and 10 °C / 4 days) on the growth of psychrophilic bacteria in raw milk treated with CO_2.
3. Sensory evaluation of pasteurized milk-treated with CO_2 and kept at 4 °C and 10 °C for 4 days and degassed prior to pasteurization. Batch pasteurisation (65 °C /30 min) was used for all treatments.

Sensory evaluation of pasteurized milk samples was conducted by ten panellists (Tikrit University students, age 20–25 years). Triangle tests were performed to determine if addition of CO_2 contributed to perceivable differences in taste and aroma of the pasteurized milk samples. The treated samples were compared with control milk.

Three samples were presented simultaneously to panellists, two from one formulation (either treated or untreated pasteurized milk) and one from a different formulation (either treated or untreated pasteurized milk) in a balanced random order. Panellists were asked to identify the odd sample according to following form (Figure 1) (24).

TRIANGLE TEST

Name ---------- **Date** ----------

Type of sample ----------

INSTRUCTIONS

Taste samples from to right. Two are identical; determine which is the odd sample. If no difference is apparent, you must guess.

Sets of three samples	**Which is the odd sample?**	**Comments**
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Figure 1: Scoresheet for the Triangle test.

To improve the power of the analysis and to be able to detect true discriminators, duplicate triangle tests were conducted for each batch. The data was analysed using chi-square distribution (24 and 25).

Psychrophilic colony count was done on plate count agar supplemented with 1% (w/v) skim milk. Firstly, 11 ml of raw milk added to 99 ml of distilled water and then other required dilutions were done. Secondly, after pouring the culture medium over

the inoculum, the petri-dishes were incubated at 7±1 °C for 10 d (3, 26). All tests were carried out in duplicate. Data was analysed with XLSTAT copyright 1995-2017, Addinsoft, France.

Results and Discussion

Part 1: The average fat and total protein contents of bulk fresh raw milks before treatment used in this work were 2.95% and 2.24%, respectively. The tests were done by Cryostar (Funke Gerber,) to overview some major contents of the milk samples which were undergone the experiments.

Curves of psychrophilic bacterial growth for the acidified milk to pH 6.2 with CO_2 and Lactic acid separately are shown in Figure 2. The results were compared to control milk (non-acidified milk) through 4 days of storage at 10 °C. In general, differences between acidified and control milks were significant ($p \leq 0.05$). At the first day, the growth showed an increase in lag phase in CO_2 treated milk, but started to show different conduct in day 3. In current study the curve's behaviour of CO_2-treated milk is in agreement with differences reported in the literature (16, 17). The results showed insignificant differences between lactic acid treated and control milk ($p \geq 0.05$), but were significant with CO_2-treated milk ($p \leq 0.05$). These results are in agreement with that reported by some scholars in the literature who confirmed that the reason of the inhibition is directly due to the existence of CO_2 (4, 12).

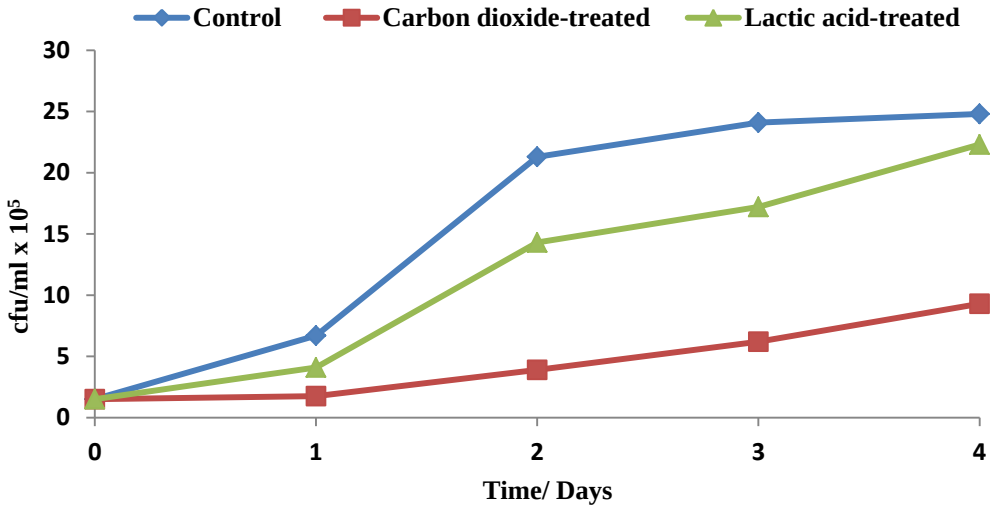

Figure 2 The effect of CO_2 treatment of raw milk in comparison with control and milk acidified with lactic acid (pH 6.2) on the growth of psychrophilic bacteria during storage at 10 °C.

Part 2: The effect of storage temperatures 4 and 10 °C on the growth of psychrotrophs in raw milk samples treated with CO_2 were studied and compared to untreated stored milk samples at the same temperatures 4 and 10 °C during 4 days (Figure 3). Both control milks which kept at 4 and 10 °C showed the same conduct of psychrophilic bacterial growth during the four days of storage. Also, the attitude of the CO_2-treated milks was similar. But, the rates of the growth at temp. 10 °C were higher in

comparison with those at 4 °C in both cases. The differences between the treatments were significant ($p \leq 0.05$) in days 2, 3 and 4. Lag phase in treated milks was longer at both temperatures in comparison with that of untreated milks.

This work revealed that the addition of CO_2 can suppress the growth of psychrophilic bacteria especially at 4°C more than 10°C. The results, also showed that CO_2 affects both lag and log phase of psychrophilic bacterial growth (14).

Part 3: Sensory evaluation for milk-treated with CO_2 and kept at 4 °C and 10 °C for 4 days and degassed prior to batch pasteurization (65 °C /30 min) was compared to control (untreated) pasteurized milk (Table 1). Panellists, who were asked to test both "Aroma" and "Taste" of the milks by "Triangle Test", could not differentiate between all treatments. All samples were statistically insignificant ($p \geq 0.05$). These results may mostly be due to incapability of CO_2 to affect negatively the major constituents of milk especially lipids which its degradation might lead to production of many compounds with low threshold values e.g. aldehydes and ketones. In addition, CO_2 prevents milk microflora especially Pseudomonas and its enzymes to interfere and take parts in enhancing milk spoilage (2, 17 and 27). Panellists preferred milks kept at 4°C rather than 10°C and the former got higher scores.

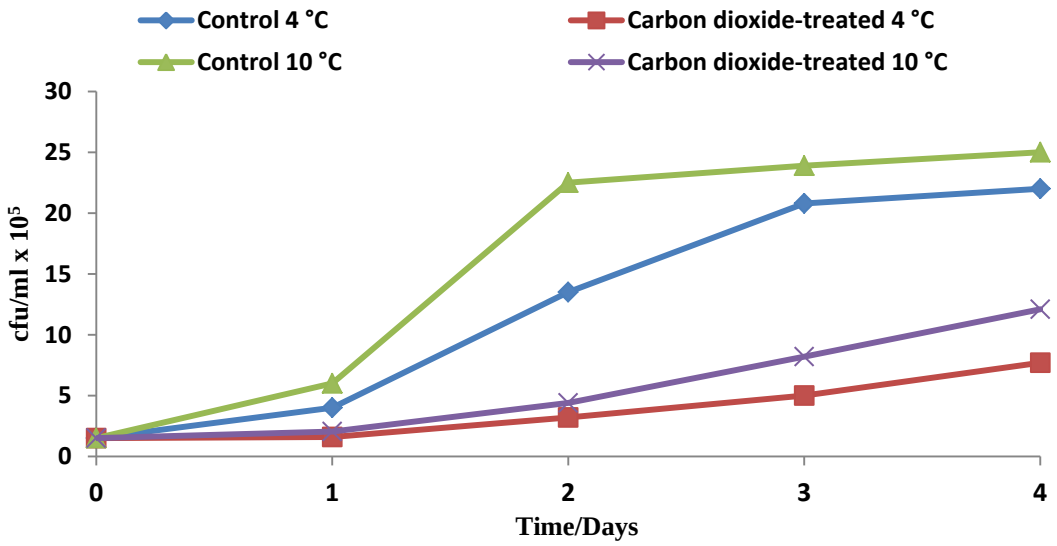

Figure 3 The effect of raw milk preservation with CO_2 on the growth of psychrophilic bacteria during storage at 4 and 10 °C.

Table 1 Panellists' ability to differentiate between controls pasteurized milk and milk treated with CO_2.

Pasteurized milk (65 C° 30 min)	*p-value**			
	Taste		Aroma	
	4 °C	10 °C	4 °C	10 °C
Storage (day 2)	0.67	0.22	0.59	0.15
Storage (day 3)	0.27	0.10	0.54	0.27
Storage (day 4)	0.24	0.16	0.60	0.27

*p-value: Chi-sq (2,0.95) = 5.99146 ; All samples were statistically insignificant.

The addition of carbon dioxide to raw milk and the storage temperature affected the psychrophilic bacterial count. The results showed treating with CO_2 decreased psychrophilic in raw milk especially with low temperature (4°C) which showed better effect on the count reduction. Organoleptic characteristics of pasteurized milk processed from CO_2-treated raw milk were not altered ($p \geq 0.05$) in comparison with control milk. The carbon dioxide addition to raw milk has been demonstrated as an important tool for the reduction of psychrophilic count before processing.

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