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A STUDY ON THE ELECTROPHORETIC PROPERTIES OF MILK PROTEINS DURING STORAGE AS AFFECTED BY THERMIZATION AND CO_2 TREATMENTS

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The attained electrophoretic patterns showed that the patterns of whey proteins from thermized and unthermized milk samples were the same, whereas some changes were noticed with respect to caseins of unthermized milk. CO_2 had no effect on the electrophoretic patterns of whey protein and casein with exception that the highest CO_2 concentration (10.0 g/l) caused some changes in the casein fractions during storage.

Key words: Milk, Thermizations, CO_2.

INTRODUCTION

An increase in the keeping quality of raw milk during storage and/or transportation can be achieved by producing milk of higher hygienic quality at the farm, storing and transporting at lower temperature or thermization – a sub pasteurization heat treatment – of the milk on arrival at the dairy. The undesirability of adding preservatives soon after cooling at the farm would diminish if an innocuous preservative such as CO_2 could be used [6].

Most of the earlier work with respect to using thermization or CO_2 treatments centred on the effect of such treatments on the keeping quality of cold milk. However, storing milk at low temperature favours the growth of psychrotrophic organisms. Speck and Adams [10] and Cogan [2] reported that the lipase and proteinase enzymes produced during the cold storage of milk may give rise to serious flavour defects in products prepared from such milk. So it could be useful to prevent psychrotrophs from growing during cold storage of milk. Zall and Chen [12] and Gilmour *et al.* [5] mentioned that thermization gives a measure of control psychrotrophic species in milk during subsequent cold storage. On the other hand, Gill and Tan [4], Mabbitt [8], King and Mabbitt [6] and Rashed *et al.* [9] reported that milk can be chemically treated with CO_2 to control such bacteria and to increase the keeping quality of milk.

The purpose of this study was to provide some preliminary data on the effect of thermization and CO_2 treatments on the electrophoretic properties of milk proteins during cold storage.

MATERIALS AND METHODS

Fresh cow's milk was obtained from the herd of Faculty of Agriculture, Kafr El-Sheikh. The milk was skimmed and the first portion was immediately thermized (63^{o}/2 min.) and then cooled. CO_2 treatments were carried out as described by Rashed *et al.* [9] to give a calculated CO_2 contents of 5.0, 7.5 and 10.0 g CO_2/l of milk. The treated and untreated milk samples were stored in a refrigerator (5 ± 1^{o}) for 96 hr. and sampled when fresh and after 24, 48, 72 and 96 hr. for preparing acid casein and whey.

Isoelectrically precipitated whole casein was prepared according to the method of Mckenzie [7], whereas whey was decanted from the precipitated casein and filtered through Whatman's No. 40 filter paper. Samples for electrophoresis were prepared by adding sucrose to whey to give a final concentration of 15 % whereas Casein was dissolved in urea (8M). Xylene cyanol FF was used as a tracking dye.

The method of Davies [3] was followed for testing whey proteins with the exception that fixation and staining were carried out using T.C.A (15 %) and Coomassie Blue R 250 solution (0.2 g dye in a 100 ml mixture of glacial acetic acid, methanol and distilled water, 1:5:4 in order). Destaining solution was prepared by mixing methanol (250 ml), glycerol (100 ml), glacial acetic acid (75 ml) and water (420 ml). Testing casein samples was achieved using acrylamide gel (7 %) which was prepared by mixing together 6.75 g acrylamide, 0.25 g bis acrylamide, 27.0 g urea and 10 ml of stock buffer. The mixture was dissolved in distilled water and made to a volume of 100 ml. TEMED

(0.2 ml), 2-mercaptoethanol (0.2 ml) and ammonium persulphate (60 mg) were added. A stock buffer was prepared by dissolving Tris (135.0 g), Na_2EDTA (18.0 g) and H_3PO_3 (10.4 g) in 1350 ml of distilled water. Migration buffer (pH 8.9) was prepared by mixing 1 vol of stock buffer with 8 vol of distilled water. Vertical electrophoresis was carried out at 250 V for 3 hr. Fixation and staining of protein bands as well as destaining were achieved as described above.

RESULTS AND DISCUSSION

It is apparent from the electrophoretic pattern of serum protein from unthermized milk samples (Fig. 1) that all samples when fresh or during storage period had the same electrophoretic patterns of whey protein from thermized milk. On the other hand, Fig. 1 illustrates that some changes occurred in the intensity and properties of some casein fractions during storage. Thus, the αs_2-and β-casein fractions were found to be less resistant to the action of natural milk proteinases. Moreover, the intensity of the bands of the mentioned fractions from thermized milk samples was much stronger than the corresponding bands of the unthermized samples at any given age. This suggests that thermization had pronounced effect on controlling the proteolytic action of psychrotrophic bacteria and their enzymes during storage.

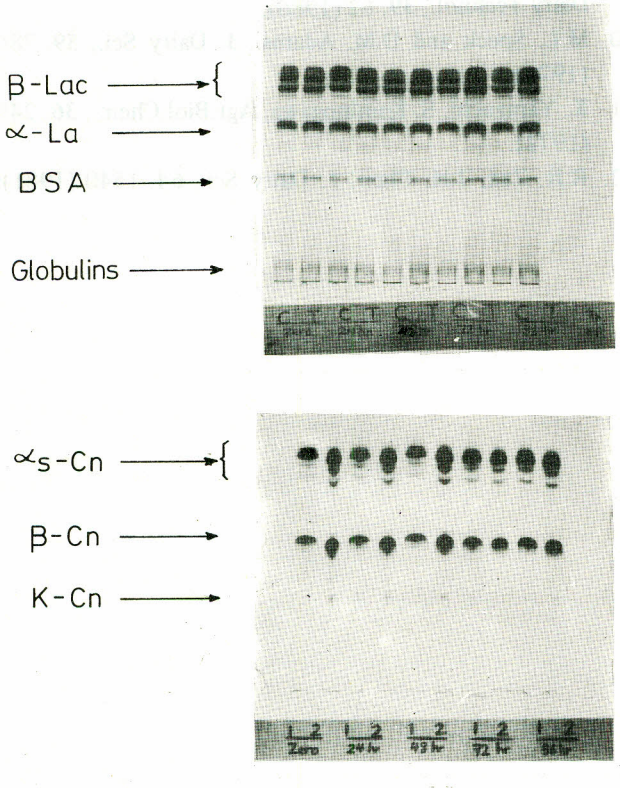

Fig. 1. Effect of thermization on the electrophoretic patterns of whey protein and casein during cold storage. C and 1, unthermized samples; T and 2, thermized samples.

Fig. 2. illustrates the electrophoretic patterns of whey protein obtained from cow's milk before and after treatment with CO_2. It is clear that treated and untreated samples (zero time) had the same electrophoretic pattern, i.e. whey protein in all samples was electrophoretically fractionated into 3 main fractions namely bovine serum, albumin (BSA), α-lactalbumin (α-La) and β-lactoglobulin

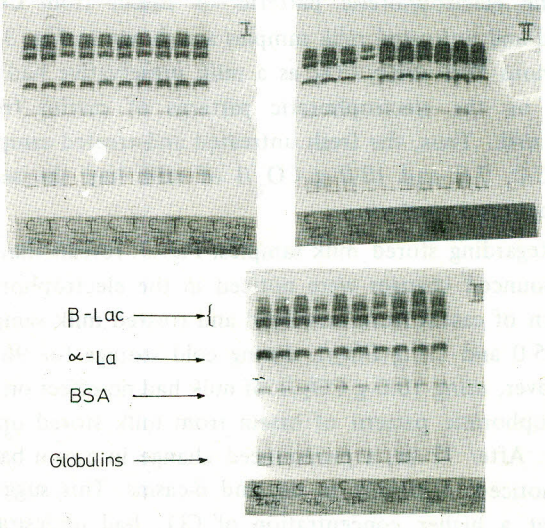

Fig. 2. Polyacrylamide gel electrophoretic patterns of whey protein prepared from skim milk injected with CO_2 (I, II and III; 5.0, 7.5 and 10.0 g CO_2/l milk in order) and stored in refrigerator for 96 hr. C, untreated samples; T, treated samples.

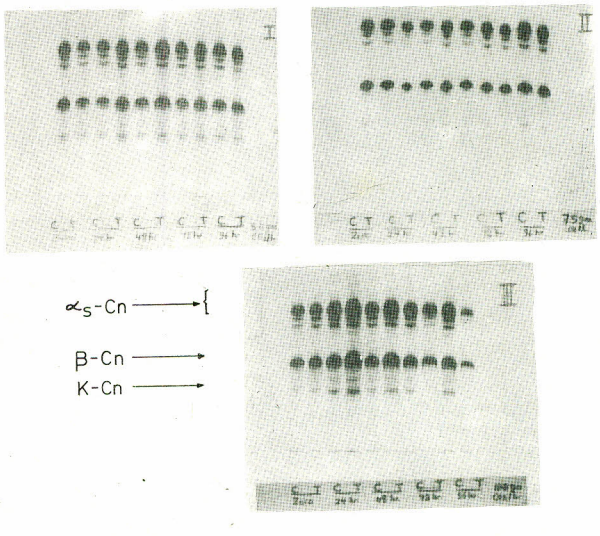

Fig. 3. Polyacrylamide gel electrophoretic patterns of whole casein isolated from untreated (C) and CO_2-treated (T) milk samples during storage for 96 hours in refrigerator. I, II and III, 5.0, 7.5 and 10.0 g CO_2/1 milk respectively.

(β-Lac) respectively as well as immunoglobulin. On the other hand, the electrophoretic patterns of whey proteins were found unaffected by using CO_2 at any given concentration. Concerning the stored milk samples, it is obvious from Fig 2. that storage period and CO_2 treatments had no effect on the electrophoretic pattern of all whey protein fractions. Chen and Ledford [1] and Yamauchi and Kaminogawa [11] reported that the major native whey proteins are relatively resistant to the action of milk proteinase.

The electrophoretic patterns of casein from CO_2-treated and untreated milk samples are shown in Fig. 3. It is obvious that using CO_2 as a milk preservative had no effect on the electrophoretic patterns of caseins from cow's milk. Thus, the fresh untreated and treated samples with 5.0, 7.5 and 10.0 g CO_2/l of milk had the same electrophoretic pattern.

Regarding stored milk samples, Fig. 3. reveals that no pronounced changes were noticed in the electrophoretic pattern of casein from untreated and treated milk samples with 5.0 and 7.5 g CO_2/l, during cold storage for 96 hr. Moreover, using 10.0 g CO_2/l of milk had no effect on the electrophoretic pattern of casein from milk stored up to 48 hr. After 72 hr, a pronounced change in casein bands was noticed, especially in αs_2-and β-casins. This suggests that at a higher concentration of CO_2 had undesirable action on some casein fractions. King and Mabbitt [6] demonstrated that higher concentrations of CO_2 than 30 g/l were found to cause instability of milk proteins with symptoms of bittiness.

From the foregoing results one can conclude that thermization of milk before cold storage had a pronounced effect on controlling protealysis. On the other hand, CO_2 can be used at low concentration as a milk preservative during transportation or storage in cold conditions.

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