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**ELECTROPHORETIC PATTERNS OF COW'S AND BUFFALO'S MILK,
YOGHURT AND DOMIATI CHEESE PROTEINS AS AFFECTED BY
THE PRESERVATION OF MILK WITH LP-SYSTEM AND HYDROGEN
PEROXIDE
BY**

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ABSTRACT

Fresh cow's and buffalo's milk were preserved with Lp-system and H_2O_2 . The treated milks were used in the manufacture of yoghurt and Domiati cheese. The electrophoretic patterns of milk protein did not indicate any changes in the various fractions of casein and B-lactoglobulin during the entire period of preservative storage. The electrophoretic patterns of yoghurt protein made from untreated, and Lp-system and H_2O_2 treated cow's and buffalo's milk were almost similar. The electropherograms of Domiati cheese protein manufactured from Lp-system and H_2O_2 treated milks with 5% salt were close to that made by traditional method with 10% salt. This is useful for the manufacture of low salt Domiati cheese with an acceptable quality using Lp-system.

INTRODUCTION

The germicidal properties of hydrogen peroxide have been known many years ago and its use as a milk preservative was thoroughly investigated (FAO 1957 and Cook 1962). The antimicrobial properties of milk has now been known for more than a decade. A method based on a natural antibacterial system in milk, namely, lactoperoxidase system (Lp-system) has been developed to prolong the storage of raw milk at ambient and refrigeration temperatures (Kamau & Kroger 1984, Ewais *et al.* 1985, Ambadkar *et al.* 1991 and Fekry & Metwally 1992). Also Hefnawy *et al.* (1986) studied the effect of Lp-system on the curd and the yield of Domiati cheese. Few studies were carried out to follow the role of Lp-system and H_2O_2 on the proteins of milk and its products.

Therefore, determination of casein fractions would be of great importance in evaluating the effect of preservation with Lp-system and H_2O_2 on milk proteins and to follow their effect on the protein fractions of some products.

such as, yoghurt and Domiati cheese manufactured from Lp-system and H_2O_2 treated cow's and buffalo's milk.

MATERIALS AND METHODS

Materials

Fresh bulk cow's and buffalo's milk were obtained from the herds of Faculty of Agriculture at Moshtohor. Hydrogen peroxide solution of a medicinal quality (Merck Dermschadt, Germany) of 35% by weight strength was used. Potassium thiocyanate (Prolabe-Adowi Laboratory chemicals, France) was used as a source of SCN.

Methods

Each type of milk (cow's and buffalo's) was divided into three parts, the first part was used as a control without additives. For the second part the thiocyanate content of milk was determined according to International Dairy Federation (1988) and increased to be 14 ppm with potassium thiocyanate and 30 ppm H_2O_2 was added followed by another 30 ppm of H_2O_2 after 4 hours. The milk was left for another 12 hours at room temperature (about 32 °C). The third part of milk was treated with 200 and 250 ppm of H_2O_2 for cow's and buffalo's milk respectively and left for 16 hours at room temperature.

Treated and untreated milk were made into yoghurt and stored in refrigerator for 5 and 10 days. Samples were taken from milk as well as from yoghurt for electrophoresis.

Domiati cheese, was made from control, Lp treated and H_2O_2 treated milks. NaCl was added at the rate of 10% to the control and 5% to the treated milk and then made into Domiati cheese by the traditional method according to Fahmi and Sharara (1950). The resultant cheese were pickled in 10% salt whey in sealed containers and stored at room temperature. Samples were taken from cheese when fresh and after 15, 30 and 60 days.

The changes in milk, yoghurt and cheese proteins were followed by slab gel electrophoresis as described by Melachouris (1969) in horizontal polyacrylamide beds).

RESULTS AND DISCUSSION

1- Electrophoretic patterns of milk proteins:

The electrophoretic patterns of cow's and buffalo's milk proteins treated by Lp-system and H_2O_2 are shown in Fig. (1) It is obvious that there is no discernible changes in the electrophoretic patterns of milk protein were encountered for treated and untreated milk. The main protein fractions of cow's milk appeared in these patterns α_s -casein were α_{s1} and α_{s2} -casein (Fox 1981)

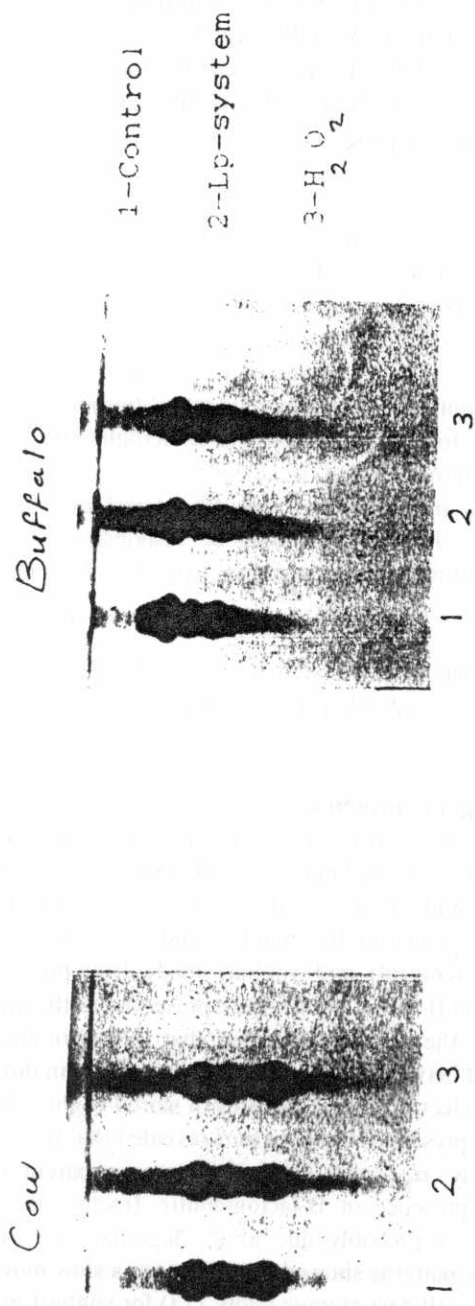


Fig (1) Electrophoretic pattern of cows and buffaloes milk protein as affected by Lp-system and H₂O₂.

B-casein and K-casein as several components with mobilities close to that of buffalo's K-casein (Mehanna *et al.*, 1982). The B-lactoglobulin band was seen in front of the B-casein for all milk samples (Aschaffenberg, 1964). Concerning to buffalo's milk the electrophoretic patterns of preserved and unpreserved milk characterized by the presence of protein fractions with mobilities that differ from those of cow's milk which named α_{sb1} - α_{sb2} - B- and K-casein (Addeo *et al.*, 1977 and Abd El-Salam, 1975).

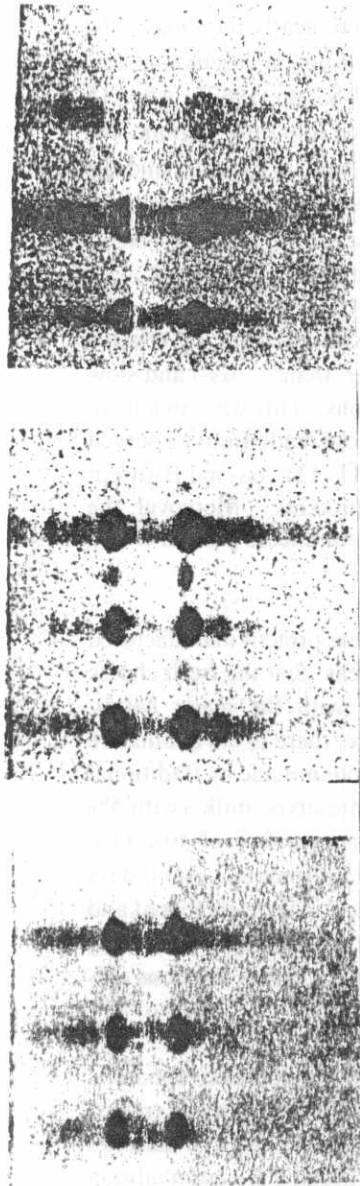
In general, the electrophoretic patterns revealed that the milk protein for both cow's and buffalo's were not affected by preservation with Lp-system and H_2O_2 . This agrees with that reported by Kumar and Mathur (1989a) for Lp-system activated milk. They stated that the casein from preserved buffalo milk samples treated by 25:15 and 70:30 Lp-system stored at 30°C for 16h resolved into similar bands, displaying identical electrophoretic mobilities and relative proportions as that of control. Moreover, the presence of B-lactoglobulin in the electrophoretic patterns of both untreated and treated milks exhibited that the small amount of H_2O_2 employed in Lp-system was utilised preferably in the oxidation of SCN^- (Bjorck *et al.*, 1979). This may be responsible for the resistance of SH-groups of whey proteins to the potential oxidative effect of Lp-system (Kumar and Mathun 1989b).

It may be concluded that the Lp-system (16) could influence the electrophoretic behaviour of casein and whey protein during the period of preservation.

2- Electrophoretic patterns of yoghurt proteins:

Fig. (2A and B) shows the electrophoretic patterns of yoghurt protein made from untreated and treated cow's and buffalo's milk with Lp-system and H_2O_2 as fresh and stored for 5 and 10 days. It is clear that no pronounced changes could be detected with respect to the number and intensity of the different bands of fresh yoghurt (control) and yoghurt made from preserved milk. The α_s - and B-casein and B-lactoglobulin were preserved as the major constituents (Slots 1,2,3). Also, the patterns revealed that K-casein missed detection due to a complex formed between K-casein and B-lactoglobulin during the processing of yoghurt. The electrophoretic patterns of stored yoghurt for 5 and 10 days for both control and preserved milk yoghurt revealed that the main casein fractions of milk namely α_s - and B-casein were apparent in electrophoretic patterns and the presence of B-lactoglobulin fraction of whey protein was obscured and this was probably due to its degradation during storage. Moreover electrophoretic patterns showed that there are a slow moving faint degradation products in 5 or 10 days storage (slots 2, 3) for yoghurt made from cow's preserved milk.

A-Yoghurt made from cows milk.



B-Yoghurt made from buffaloes milk.

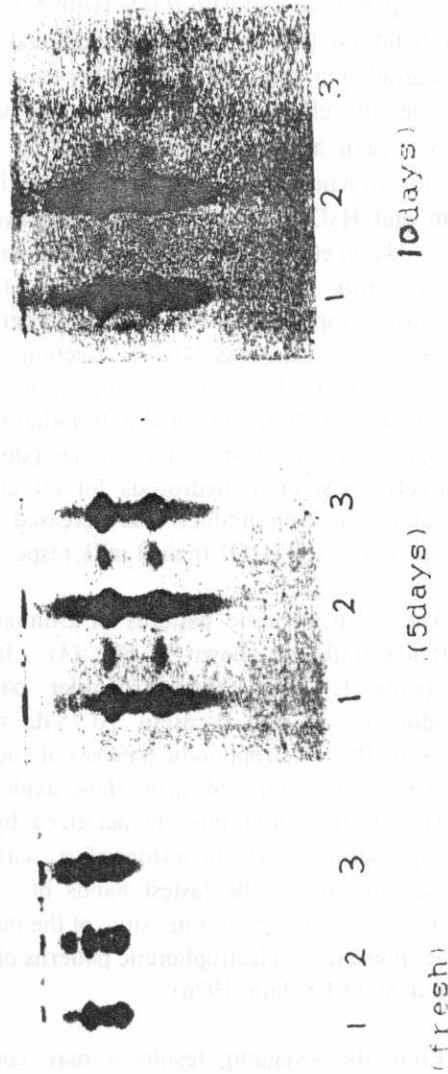


Fig (2 A.B): Electrophoretic pattern of yoghurt protein made of untreated Lp-system and H_2O_2 cows and buffaloes milk during storage.

3- Electrophoretic patterns of Domiati cheese protein:

Fig. (3) illustrates the effect of Lp-system and H_2O_2 on the protein of Domiati cheese manufactured from cows milk and pickled at room temperature for 15, 30 and 60 days. It is obvious that the electrophoretic patterns of fresh cheese made from untreated cows' milk (control with 10% salt) and treated milk with both Lp-system and with H_2O_2 (with 5% salt) were nearly the same. The separated bands (slots 1, 2, 3,) were well defined as α_s -casein, B-casein and slow moving degradation products (El-Shibiny and Abd El-Salam, 1967). After 15 days storage, the electrophoretic patterns can be divided into three regions: the first, is α_s -casein and fast moving degradation; the second, is B-casein and the third, is slow moving products for all cheeses either control or those treated with Lp-system and H_2O_2 . Similar protein bands were observed in electrophoretic patterns by Abdou et al. (1976) for Domiati cheese made from a mixture of cow's and buffalo's milk (1:1) and salted at the rate of 8%. After 30 days storage, the minor fractions appeared in the electropherograms with increase in relative intensity as fast moving degradation fractions (higher than α_s -) and slow mobilities (less than B-casein), moving minor fractions. This was obvious in case of cheese made from untreated milk rather than those made from preserved milk. On the other hand, at 60 days storage (slots 10, 11, 12), α_s -and B-casein still relatively resistant to hydrolysis for all cheese proteins. Moreover, the intensity of degradation products was increased clearly for cheese protein which made of Lp-system and H_2O_2 treated milk respectively.

The electrophoretic patterns of Domiati cheese protein manufactured from buffalo's milk are shown in Fig. (4). It is clear that the fresh cheese samples (slots 1, 2, 3) showed similar patterns with the major bands corresponding to α_s -and B-casein. At 15 days storage, there is no qualitative differences in the electrophoretic patterns of cheese protein made by traditional method (10% salt) compared with those made from preserved milk (with 5% salt). The electropherograms characterized by the appearance of two fast moving degradation products in front of α_s -casein for all samples. At 60 days storage the intensity of the fastest bands of α_s -fraction were increased and accompanied by increase in intensity of the minor slow degradation products beside the presence of electrophoretic patterns of the major casein fractions (El-Shibiny and Abd El-Salam, 1976).

From the foregoing results it may concluded that the electrophoretic patterns of cheese protein made from untreated milk with 10% salt and treated milk with either Lp-system or H_2O_2 with 5% salt for both cows and buffalo's milk reveal that α_s -and B-casein resisted hydrolysis especially B-casein. also, a slight delay in the development of ripening was observed. The high salt content of Domiati cheese may contribute to the slow rate of hydrolysis of the major components of milk casein (Metwally & Moore 1991). Concerning the preserved

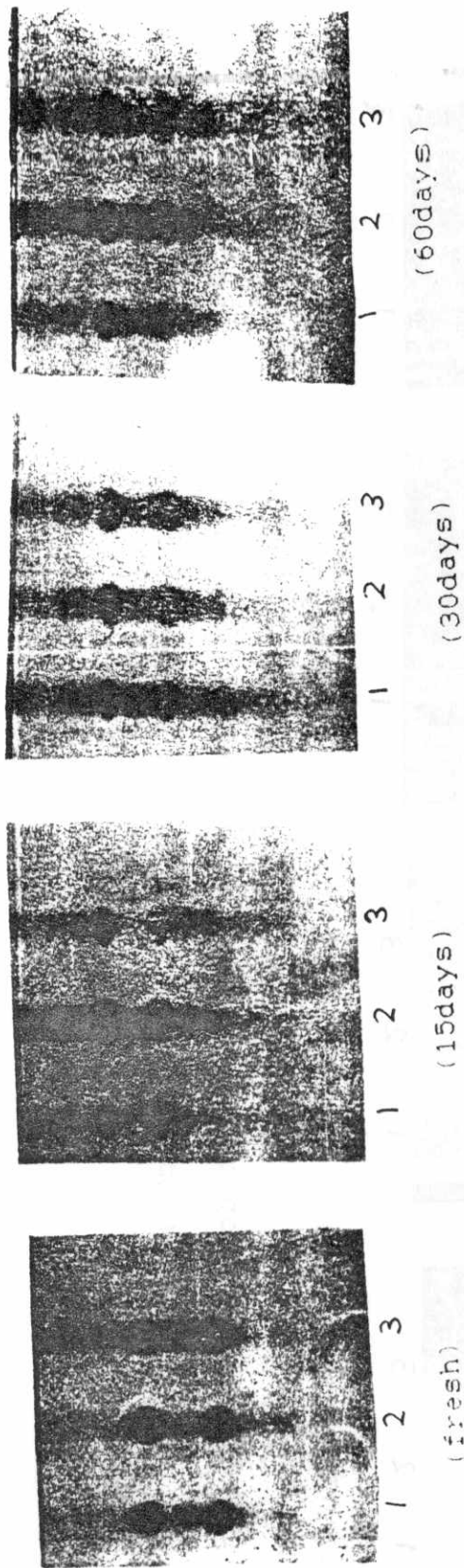
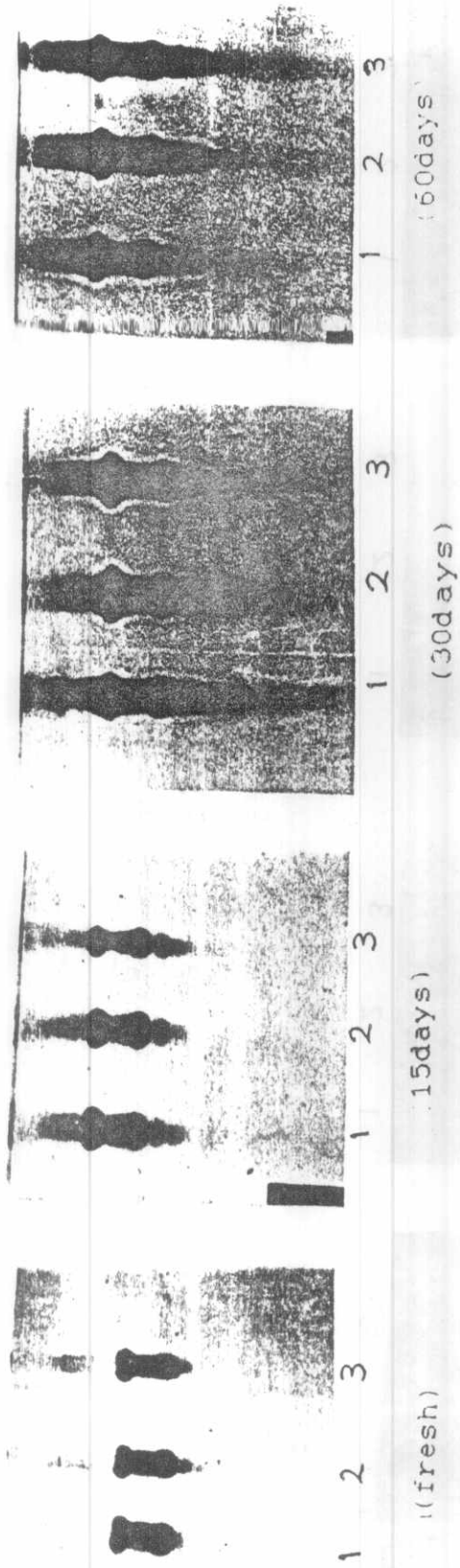


Fig. (3): Electrophoretic pattern of cows Domiati cheese protein made of Lp-system and H_2O_2 during storage

1 -Control
2 -Lp-system
3 - H_2O_2



Fig(4):Electrophoretic pattern of buffaloes Domiati cheese protein made of Ip-system and H₂O during storage.

cheese, the slow degradation of the protein fractions may attributed to either concentration of salt 5% (Fox & Waley 1971) or the preservation of milk with both Lp-system and H₂O₂.

In general, preserving cow's and buffalo's milk with either Lp-system or H₂O₂ had no deleterious effect on the milk protein fractions and such milk can be used satisfactory in the manufacture of Domiati cheese.

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