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# INFLUENCE OF SOME PRESERVATIVES ON CAST UF-WHITE SOFT CHEESE PROPERTIES DURING COLD STORAGE

BY

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## ABSTRACT

The aim of this work was planned to study the effect of formaldehyde (FA) and hydrogen peroxide ($H_2O_2$) on the properties of UF cheese made without whey drainage. Cast UF-white soft cheese was made using precheese spiked either with FA or $H_2O_2$ at the level of nil or 0.3% whether further heat treated at 72°C for 20 sec. after preservative adding or not. The resultant cheese was kept at 5°C for 4 weeks (W).

The obtained results indicated that, neither dry matter (DM), fat/DM, protein/DM nor ash content of cheese was significantly affected by the kind of the preservative added. However, the fat/DM as well as the ash contents raised and that of protein/DM decreased due to the further heat treatment of the precheese after preservative adding prior cheesemaking. The water soluble nitrogen/total nitrogen and pH value of cheese lowered by FA than $H_2O_2$. While, the titratable acidity of cheese increased in the presence of FA than $H_2O_2$. Considerable depressing in the absorbancy of most proteins fractions especially β-lactoglobulin, (β-Lg) gained by Sephadex G100 due to precheese treating either by $H_2O_2$ adding or further heating, opposite to that occurred when FA was added, which inhibited the heat-induced dissociation of casein (Cn) contributed to the further heating of precheese. While, $H_2O_2$ retarded but did not completely prevent the heat-induced association between k-Cn and β-Lg. Except of $\alpha_{s1}$-Cn, the level of $\alpha_{s2}$-Cn, β-Cn, k-Cn and γ-Cns of cheese protein fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were increased by FA and decreased by $H_2O_2$ as compared with the control. However, the level of lactoferrin (placed also with the cheese ripening proteinous product of $\alpha_{s1}$-I-fragment), immunoglobulins (Igs), β-Lg and α-lactoalbumin (α-La) of UF cheese protein were raised by adding $H_2O_2$ to precheese as compared either with those containing FA or even the control. The precheese further heating led to peak diminishing of most whey protein fractions (except of those of Igs and α-La), $\alpha_{s2}$-Cn as well as β-Cn and increasing in both of k-Cn and γ-Cns peaks. The prolonging of cheese cold storage period was associated with decrement in all

casein fractions except of γ-Cns, those increased similarly as exhibited by β-Lg and α-La fractions. Cheese firmness weakened due to preservatives adding, regardless their kind, and strengthened by the further heating of precheese. The behaviour of cheese firmness during cold storage period tended to weaken after 2W and restore after 4W. The addition of 0.3% preservatives regardless their kind to the precheese or/and further heating of precheese led to obtained cheese with decreased total viable bacterial count, which gradually raised by prolonging the cold storage period. All cheeses, those made from preservative-containing precheese, were free from the coliform bacteria as well as yeasts and moulds.

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**Key words:** Formaldehyde, Hydrogen peroxide, Precheese heating, Gross composition, Sephadex G100, SDS-PAGE, Cheese firmness, Ripening indices, Microbiological quality.

## INTRODUCTION

In spite of the sanitary precautions that are standard practice in the field of food production, large quantities of high valuable foods are ruined every year throughout the world as a result of attack by undesirable microorganisms (Jager, 1994). In this respect, milk is a highly perishable commodity and difficult to handle, especially in countries with relatively high ambient temperature. Besides, unhygienic conditions under which the animals are milked, small quantity of milk which is delivered by individual producer, long distance between the production and market areas, poor transportation, insufficient or non-availability of milk cooling and chilling system are the main problems of milk collection. Therefore, some milk processors illegally, whether qualitatively or quantitatively add some chemical preservatives to milk to destroy and/or inhibit the microflora, and hence to avoid the probable lacking in its keeping quality. Preservatives most commonly used are formalin (40%) and hydrogen peroxide for their high efficiency against many microorganisms, cheap prices and easy to obtain. Although they usually are used with small quantities, they have bad effects on physical, chemical technological and nutritional milk properties. For their hazardous effect on human health, for those reasons, many countries considered using of preservatives illegal (Brunn and Klostermeyer, 1983; Pellegrino and Resmini, 1996 and Mohamed, 2002).

Formalin is a strong antiseptic and disinfectant for various kinds of bacteria. When formalin is added to cheese milk the amount of free formaldehyde (FA) which remains in cheese after ripening is very low (below 0.5 ppm) and is not detectable by the official method in some countries for evaluation of additive residues (Resmini *et al.*, 1980 and Restani *et al.*, 1989). After a period of time, the bacterial cells presumably produce necessary enzymes capable to metabolize formaldehyde to $CO_2$, consequently, its concentration decreased to a critical level, with increase in cell population (Neely, 1963). Also, formaldehyde is a highly reactive chemical which readily combines with deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins and amino acids (Siomin *et al.*, 1973; Chaw *et al.*, 1980 and Hemminki, 1982).

Hydrogen peroxide ($H_2O_2$) is considered as a safe preservative for milk and legitimately be employed at levels between 100-800 ppm (FAO, 1957). Some

reports recommended that, at these allowed concentrations certain types of pathogenic microorganisms are not destroyed (Gregory *et al.*, 1961). Using higher amounts than are legalized means adding negative effect on flavor and possibly being risk effective on health (Kosikowski, 1977 and Jha, 1984). Also, some loss in biological value of proteins and availability of sulphur containing amino acids were recorded by El-Magdoub (1971); Shehata *et al.* (1975) and Deodhar and Mehta (1980).

For that in view, this study was planned to study the effect of formaldehyde (FA) and hydrogen peroxide ($H_2O_2$) on the properties of UF cheese made without whey drainage, regardless whether their residues stilled present to be qualitatively detectable or not.

## MATERIALS AND METHODS

**Materials:**

Fresh buffalo's milk was obtained from the herd of the Faculty of Agriculture, Ain Shams University. Maxiren 1800, (100% chymosin purified from *Kluyveromyces maxrianous* biovar. *lactis*) made by DSM Jist, France, fine cooking salt produced by El-Nasr Salines Co., commercial formaldehyde solution (40%) and hydrogen peroxide solution (35%) made by Pfizer Co. Inc. USA were obtained from the local market at Cairo.

**Experimental procedures:**

Standardized buffalo's milk (3.0% fat) was heat treated at 72°C for 2 min and ultrafiltrated (UF) at 50°C as recommended by Moubois *et al.* (1971) using a CARBOSEP UF-unit (Type 2S 37, France) with Zirconium-oxide membrane area of 1.68 $m^2$ to about 35% total solids at the Agriculture Secondary School at Giza. UF-retentate samples each with 4 Kg were spiked with 0.3% FA or $H_2O_2$ and gently agitated for one min. Besides, another UF-retentate part was remained preservative-free for control cheesemaking. The UF-retentate samples were either further heat treated or not at 72°C for 20 sec. Then the UF-retentates were adjusted at the coagulation temperature (about 42°C). The manufacturing procedure was carried out according to the method of the cast UF-enzymatically made Kariesh cheese without whey drainage described by Fayed (1986). Where the precheese was prepared by salting the UF-retentate to 3.0% NaCl. Then Maxiren solution (4%) was added at the rate of 2.5 ml/10 Kg UF-retentate. The precheese was casted into plastic containers, where every container had about 0.5 Kg precheese. The containers were incubated at 42°C for 30 min., through which the complete coagulation took place. The containers were thereafter made up with a given volume of pasteurized salted brine (5%) and air tightly closed. The resultant UF-white soft cheese samples were cold stored at 5°C for 4 weeks. Three replicates were carried out for every batch.

**Analytical methods:**

Formaldehyde and hydrogen peroxide were qualitatively detected in milk samples according to the methods of Chalmers (1962) as mentioned by EOSQC (1974). Dry matter (DM) and fat content were determined according to AOAC (1998). Ash was determined as mentioned by Pearson (1973). Total as

well as water soluble nitrogen, and titratable acidity contents were determined according to Ling (1963). The gel filtration procedure described by Davies (1974) was adapted using Sephadex G-100 for protein fractionation. Protein fractionation using sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) was carried out as described by Weber and Osborn (1969) for protein extraction and by Laemmli (1970) modified by Studier (1973) for SDS-PAGE preparation. Moreover, SDS-PAGE patterns were identified according to Eigel *et al.* (1984) and Basch *et al.* (1985). The firmness of UF-soft cheese was measured using penetrometer model SUR, BERLIN, PNR6 as described by Bourne (1982). Total viable and coliform bacterial counts (Houghtby *et al.*, 1993), yeasts and molds count (Koburger, 1970) were determined in cheese. The obtained data were statistically analyzed as in SPSS (1998).

## RESULTS AND DISCUSSION

**Qualitative detection of preservatives:**

The description given in Table (1) for the qualitative detection reveals that, FA remain detectable along the cold storage period for 4 weeks in UF-cheese made from precheese spiked with it at the level of 0.3% whether the spiked precheese was further heated or not. On the contrary, $H_2O_2$ was detected only in the fresh UF-cheese made from not further heated precheese spiked previously with $H_2O_2$. While, $H_2O_2$ added to the precheese was not detectable in the analogous cheese made from that further heated. That could be attributed to the decomposing effect of the heat treatment on the added $H_2O_2$. This fact was previously confirmed by Lück (1956). On the other hand, $H_2O_2$ has completely disappeared from 2 weeks-and 4 weeks-cheeses regardless whether their precheese was further heated or not. Lück (1956) reported that, theoretically $H_2O_2$ should be unable to preserve milk for any long period. Milk, like other biological substances, contains catalase which is originally present in the milk and additionally so from cell and bacterial catalase. This enzyme decomposes $H_2O_2$. After a certain period of time all $H_2O_2$ should therefore be split and no further preserving effect could exist.

It is worthy to mention that, although both control samples made whether from heated or further heated precheese are FA free, coloured rings fluctuated from violet to brown were observed in 4 weeks-cold stored samples. That means, some aldehydes other than FA might be formed during storage and caused, of course, doubted qualitative results.

The foregoing descriptive results, whether those were negative or positive needed to be accurated rather by following up the chemical and physical changes contributed to cheese adulteration with such preservatives namely formaldehyde and hydrogen peroxide even when they became itself undetectable.

**Gross composition:**

The dry matter (DM), fat/DM, protein/DM, and ash contents of fresh UF-cheese were not significantly influenced either by the kind or the level of the preservative added ($p>0.05$; Table, 2). Although, the slight increase in the dry matter content of cheese due to the further heating of precheese was not

significant ($p>0.05$), the increments occurred by the same reason in both of fat/DM and ash contents as well as the decrement in protein/DM were significant ($p<0.05$). Moreover, except of the protein content on dry basis, all other compositional changes occurred along cold storage period were significant, where the progressing of cold storage period of UF-cheese more than 2 weeks was associated with significant increment in both of dry matter and fat/DM contents (Table, 2). However, the gradual increase in the ash content of cheese took place from the beginning of cold storage period and continued until the end of the experimental period (4 weeks). The trends of these results agree with those reported by Fayed (1986) in UF-Kariesh cheese during cold storage period.

**Table (1): Qualitative results of the descriptive preservative detection in cast UF-white soft cheese during cold storage at 5°C as a function of precheese treating either by adding 0.3% formaldehyde or hydrogen peroxide or/and further heating at 72°C/20 sec. after preservative adding.**

| Cold Storage period (weeks) | Kind and level of preservative % (V/V) | | | | | |
|---|---|---|---|---|---|---|
| | Nil | | 0.3% | | | |
| | Control | | FA | | $H_2O_2$ | |
| | Without further heating | With further heating | Without further heating | With further heating | Without further heating | With further heating |
| Fresh | - | - | + | + | + | - |
| 2 | - | - | + | + | - | - |
| 4 | + (violet ring) | + (Brown ring) | + (violet ring) | + (violet ring) | - | - |

**Ripening indices:**

The presence of FA led to delay the formation and liberation of the proteolytic substances and consequently making UF-cheese contained lower level of water soluble nitrogen / total nitrogen (WSN/TN%) during cold storage as compared with the control or those containing $H_2O_2$ (Table, 3). Aoki and Kako (1984) reported that, the addition of FA depressed the formation of soluble casein upon heating concentrated milk, where, no soluble casein was formed by the addition of 20 mM formaldehyde.

They also explained that, FA may introduce cross-links among the casein components and prevent the formation of soluble casein accompanying the release of k-casein (k-Cn) from micelles, thus stabilizing the casein micelles. On the contrary, $H_2O_2$ had opposite trend to that done by FA (Table, 3). Uraz and Yildirim (1995) reported that, $H_2O_2$ treatment of milk with levels expanded from 0.05 to 3.00% resulted in higher total nitrogen and non-casein nitrogen values in whey of renneted milk, i.e. promoted the formation of soluble nitrogen. The further heating of precheese resulted in decrease in the releasing rate of WSN during cold storage period of UF-cheese. That could be attributed essentially to heat-denaturalizing effect on the whey proteins besides, the negative effect of the heat treatment on the cheese flora and proteolytic enzymes.

**Table (2): Gross compoaition of cast UF-white soft cheese during cold storage at 5°C as a function of precheese treating either by adding 0.3% formaldehyde or hydrogen peroxide or/and further heating at 72°C/20 sec. after preservative adding.**

| Cold Storage Period (weeks) | Kind and level of preservative % (V/V) | | | | | |
|---|---|---|---|---|---|---|
| | Nil Control | | 0.3% FA | | 0.3% $H_2O_2$ | |
| | Without further heating | With further heating | Without further heating | With further heating | Without further heating | With further heating |
| **Dry matter (DM) %** | | | | | | |
| Fresh | 37.20 | 37.30 | 37.13 | 37.33 | 36.95 | 37.13 |
| 2 | 37.55 | 37.41 | 37.53 | 37.59 | 37.08 | 37.59 |
| 4 | 37.84 | 37.61 | 37.73 | 37.94 | 37.87 | 37.75 |
| **Fat/DM %** | | | | | | |
| Fresh | 28.15 | 29.30 | 27.86 | 29.62 | 28.28 | 29.23 |
| 2 | 28.33 | 29.29 | 28.46 | 29.58 | 28.46 | 29.13 |
| 4 | 28.71 | 29.33 | 29.26 | 29.68 | 29.14 | 29.57 |
| **Protein/DM %** | | | | | | |
| Fresh | 47.04 | 46.11 | 46.86 | 45.81 | 46.82 | 47.13 |
| 2 | 47.14 | 46.24 | 46.63 | 46.02 | 46.92 | 47.35 |
| 4 | 47.30 | 46.53 | 46.91 | 45.86 | 46.47 | 47.68 |
| **Ash %** | | | | | | |
| Fresh | 4.81 | 4.87 | 4.83 | 5.00 | 4.88 | 4.91 |
| 2 | 4.92 | 4.99 | 4.96 | 5.03 | 4.91 | 4.94 |
| 4 | 5.05 | 5.02 | 5.00 | 5.07 | 5.04 | 5.07 |

Concerning the titratable acidity (TA%) and pH value of cheese, data exhibit that, FA-containing cheese was characterized with higher TA% and hence lower pH value than those containing $H_2O_2$. Similar findings were reported for Domiati cheese by Hamdy *et al.* (1973). Moreover, as the further heating of precheese delayed the liberation rate of WSN, the development rate of acidity was also harmed making the resultant cheese to have lower TA% and hence higher pH value. Furthermore, the prolonging of cold storage period of cheese was associated with gradual increase in WSN/TN% as well as TA% and hence decrease in pH value (Table, 3). Similar observations were reported by Fayed (1986) and Farahat *et al.* (2004).

**Chromatogramic properties of cheese proteins:**

There are considerable depressing in the absorbancy of most proteins fractions as a result either of precheese treating with $H_2O_2$ or further heating (Figure, 1). These facts remained remarkable along the cold storage period (4 weeks) of cheese. Metwally (1985) found that, when the skimmilk was heated to 80°C/10 min, two out of three components appeared through gel filtration on Sephadex G100 were decreased. Uraz and Yildirim (1995) reported that, $H_2O_2$ treatment of the milk with levels expanded from 0.05 to 3.00% resulted in higher total nitrogen and non-casein nitrogen values in whey of renneted milk. The largest decrease was observed in the case of component three β-lactoglobulin

fraction (β-Lg). On the contrary, FA containing samples were characterized with protein fractions gained higher absorbancy compared with the control samples which made from preservative-free precheese. Aoki and Kako (1984) fractionated acid casein containing 10 and 20 mM FA through Sephadex G200 and found that, the formation of soluble casein was depressed by the addition of FA. They also described that, an increase in the fast eluting fractions was observed for the acid casein from concentrated whey protein-free (WPF) milk containing 10 mM FA. The fast eluting components appeared in the reduced acid casein from heated concentrated WPF milk containing 10 mM FA. Moreover, they elucidated that, cross-links among the casein components were formed in heated concentrated WPF milk containing 10 mM FA and reported that, gel filtration was not carried out of the acid casein from heated concentrated WPF milk containing 20 mM FA because it was poor in solubility and only 41% was solubilized in 6.6 M urea after stirring overnight.

**Table (3): Ripening indices of cast UF-white soft cheese during cold storage at 5°C as a function of precheese treating either by adding 0.3% formaldehyde or hydrogen peroxide or/and further heating at 72°C/20 sec. after preservative adding.**

| Cold storage period (weeks) | Kind and level of preservative % (V/V) | | | | | |
|---|---|---|---|---|---|---|
| | Nil Control | | 0.3% | | | |
| | | | FA | | $H_2O_2$ | |
| | Without further heating | With further heating | Without further heating | With further heating | Without further heating | With further heating |
| **Water soluble nitrogen / Total nitrogen %** | | | | | | |
| Fresh | 10.37 | 9.95 | 8.45 | 8.36 | 11.70 | 11.68 |
| 2 | 11.81 | 10.64 | 9.44 | 8.77 | 16.45 | 14.62 |
| 4 | 20.44 | 19.42 | 12.72 | 11.00 | 25.36 | 22.31 |
| **Titratable acidity %** | | | | | | |
| Fresh | 0.32 | 0.28 | 0.40 | 0.36 | 0.28 | 0.24 |
| 2 | 0.36 | 0.32 | 0.44 | 0.40 | 0.32 | 0.28 |
| 4 | 0.96 | 0.56 | 0.52 | 0.50 | 0.40 | 0.40 |
| **pH value** | | | | | | |
| Fresh | 6.53 | 6.55 | 6.38 | 6.45 | 6.56 | 6.58 |
| 2 | 6.36 | 6.45 | 6.36 | 6.33 | 6.49 | 6.45 |
| 4 | 6.21 | 6.40 | 6.33 | 6.32 | 6.35 | 6.40 |

Concerning the heat-induced behaviour of protein in the presence of FA or $H_2O_2$, data illustrated in Figure (1) elucidated that, FA inhibited the heat-induced dissociation of casein. Similar observations were reported by Metwalli *et al.* (1995). While, $H_2O_2$ retarded but did not completely prevent the heat-induced association between milk proteins especially the interaction between β-Lg and k-Cn. Similar finding were reported by Fish and Mickelsen (1967) and Metwally (1985).

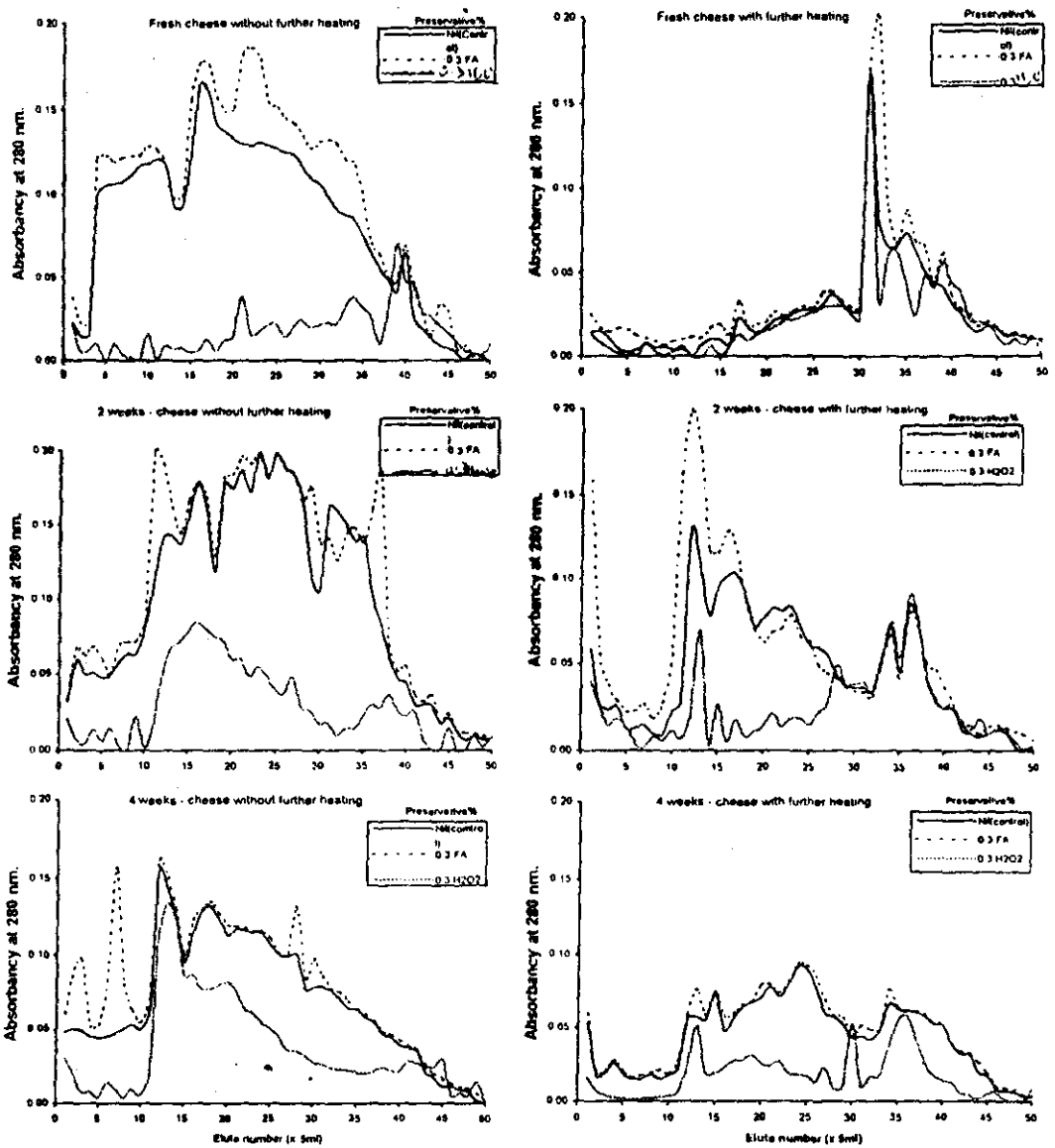


Figure (1) : Partitation chromatograms on Sephadex G100 gel filtration of cast UF-white soft cheese proteins during cold storage period as a function of precheese treating either by adding 0.3% of formaldehyde (FA) or hydrogen peroxide ($H_2O_2$) and / or further heating at 72° C /20 sec. after preservative adding

FA : Formaldehyde $H_2O_2$ :Hydrogen peroxide UF : Ultrafiltration

Furthermore, although the cold storage period led to alter the kind and sequence of cheese protein fractions, the $H_2O_2$ treated samples stilled being distinguished with smaller patterns of most protein fractions (i.e. lower absorbancy), while FA treated samples remained possessing the larger ones.

**Electrophoregramic properties of cheese proteins:**

Regarding the gel scan sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in Table (4), all milk proteins fractions of fresh cast UF-cheese namely, in order according to their velocity, lactoferrin (Lf) replaced by $\alpha_{s1}$-I-fragment due to cheese ripening, serumalbumin (SA), immunoglobulins (Igs), $\alpha_{s2}$-Cn, $\alpha_{s1}$-Cn, β-Cn, k-Cn, γ-Cns, β-Lg and α-Lactalbumin (α-La) were evidently appeared exactly as described by Basch *et al.* (1985). It is worthy to mention that, the sum level of whey proteins fractions of UF cheese proteins were as high as those known in liquid buffalo's milk. That was, indeed, because of the complete trapping of whey proteins into precheese due to UF retention and cheesemaking without whey drainage. The precheese treating with 0.3% FA led to depress, while $H_2O_2$ at the same level, exalted the patterns of most whey proteins fractions namely Lf besides + $\alpha_{s1}$-I-fragment (which began to appear among cheese ripening), Igs, β-Lg and α-La. With regard to caseins fractions, the levels of $\alpha_{s2}$-Cn, β-Cn, k-Cn and γ-Cns were significantly higher in the FA containing cheeses than those containing $H_2O_2$. The level of the fraction of $\alpha_{s1}$-Cn of FA-samples did not significantly vary from those containing $H_2O_2$. Korolczuk (1984) reported that, the SDS-PAGE patterns for caseins reacted with different amounts of FA indicated that, the polymerizing effect of the FA was low as α-Cns ($\alpha_{s1}$, $\alpha_{s2}$ and k-Cns) and β-Cn bands were only slightly diminished with the addition of 16% FA (approximetely10-folds excess of reacting carbonyl groups over available amino groups). Data also stated that, the further heat treatment of precheese after preservative adding resulted in decrease in the levels of Lf + $\alpha_{s1}$-I-fragment, SA, $\alpha_{s2}$-Cn, β-Cn as well as β-Lg and increase in the levels of k-Cn and γ-Cns of cheese proteins. The increment in the level of k-Cn and the decrement in β-Lg contributed to heat treatment may be, indeed, because of their interaction leading to bind together and aggregate at the expense of the latter. Similar observations were reported by McKenzie (1971); Metwally (1985); Marshall (1986) and Gaafar (1992). However, the levels of Igs, $\alpha_{s1}$-Cn and α-La were not influenced thereby. The levels of $\alpha_{s2}$-Cn, $\alpha_{s1}$-Cn, β-Cn and k-Cn gradually decreased while, those of γ-Cns, β-Lg and α-La proportionally raised as the cold storage period of cheese prolonged. These results indicate that, whey proteins fractions were less able to be proteolyzed during cold storage of cheese compared with casein fractions. De-Koning *et al.* (1981) showed that whey proteins resisted breakdown in Gouda cheese during maturation. Moreover, Furtado and Partridge (1988) reported that, no significant breakdown was detected in the whey proteins nitrogen of UF-soft cheese cold stored at 8°C for 3 weeks. Qvist (1989) and Orme *et al.* (1994) have attributed the slow development of flavour and texture in UF-cheese to the increased retention of whey proteins, which inhibit proteolysis. Similar findings were also reported by Fayed (1986) and Farahat *et al.* (2004). No responses were recorded in this respect with regard to the fractions of Lf + $\alpha_{s1}$-I-fragment, SA and Igs. The liberation increasing of $\alpha_{s1}$-I-fragment during cheese ripening led to disappear the reduction occurred in Lf fraction.

Table (4): SDS-PAGE proteins fractions of cast UF-white soft cheese during cold storage for 4 weeks (W) at 5°C as a function of precheese treating either by adding 0.3% formaldehyde or hydrogen peroxide or/and further heating at 72°C/20 sec. after preservative adding

| Fractions | Kind and level of preservative % (V/V) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nil Control | | | | | | 0.3% FA | | | | | | 0.3% $H_2O_2$ | | | | | |
| | Without further heating | | | With further heating | | | Without further heating | | | With further heating | | | Without further heating | | | With further heating | | |
| | Fresh | 2W | 4W | Fresh | 2W | 4W | Fresh | 2W | 4W | Fresh | 2W | 4W | Fresh | 2W | 4W | Fresh | 2W | 4W |
| Lactoferrin + $\alpha_{s1}$-I-fragment | 2.30 | 2.30 | 2.20 | 1.30 | 1.30 | 1.20 | 1.72 | 1.96 | 2.38 | 0.80 | 1.00 | 1.40 | 2.93 | 2.45 | 2.48 | 2.50 | 2.00 | 2.00 |
| Serumalbumin | 1.10 | 1.60 | 1.70 | 0.80 | 0.80 | 0.70 | 0.95 | 1.08 | 1.14 | 0.70 | 1.10 | 1.30 | 1.61 | 1.35 | 1.18 | 1.50 | 1.20 | 1.00 |
| Immunoglobulins | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.43 | 0.49 | 0.52 | 0.40 | 0.40 | 0.40 | 0.93 | 0.61 | 0.54 | 0.70 | 0.60 | 0.50 |
| $\alpha_{s2}$-casein | 14.40 | 13.00 | 11.80 | 14.00 | 12.50 | 10.80 | 14.83 | 11.76 | 10.85 | 13.20 | 11.00 | 9.80 | 13.54 | 10.41 | 9.32 | 13.50 | 10.00 | 8.80 |
| $\alpha_{s1}$-casein | 24.50 | 22.50 | 20.20 | 24.00 | 22.50 | 20.20 | 25.26 | 21.56 | 18.62 | 25.00 | 21.00 | 18.00 | 23.30 | 20.12 | 19.45 | 23.00 | 20.00 | 19.00 |
| β-casein | 21.00 | 19.40 | 17.50 | 20.00 | 18.40 | 16.50 | 21.72 | 20.58 | 18.08 | 21.00 | 20.00 | 17.50 | 20.20 | 19.15 | 17.91 | 19.80 | 19.00 | 17.00 |
| k-casein | 12.80 | 11.20 | 10.00 | 15.80 | 13.80 | 12.00 | 13.27 | 12.15 | 10.33 | 16.30 | 15.20 | 17.00 | 12.37 | 10.05 | 8.55 | 13.80 | 13.00 | 11.50 |
| γ-caseins | 7.60 | 12.20 | 17.60 | 8.30 | 12.70 | 19.40 | 7.02 | 13.93 | 18.18 | 8.80 | 15.00 | 19.50 | 7.27 | 12.05 | 16.02 | 9.20 | 13.00 | 17.00 |
| β-lactoglobulin | 10.40 | 11.40 | 12.30 | 8.90 | 10.90 | 12.00 | 9.96 | 11.55 | 13.08 | 8.00 | 10.00 | 10.50 | 9.94 | 13.68 | 14.20 | 9.00 | 12.00 | 13.00 |
| α-lactalbumin | 5.40 | 5.90 | 6.20 | 6.40 | 6.60 | 6.70 | 4.84 | 4.94 | 6.82 | 5.80 | 5.30 | 4.60 | 7.91 | 10.13 | 10.35 | 7.80 | 9.20 | 10.20 |

SDS-PAGE : Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

**Rheological profile:**

The firmness of cheese was significantly weakened ($p<0.001$) by precheese spiking with 0.3% preservatives regardless their kind, i.e. the difference between FA and $H_2O_2$ in this criterion was not significant ($p>0.05$) as inversely indicated from penetration values given in Table (5). Moreover, due to all milk proteins present in the UF-retentate in a concentrated form, the further heating of precheese made it more viscous as a result of whey proteins denaturation and consequently the resultant cheese became more firm, therefore, the statistical analysis exhibited that, the further heating of precheese yielded cheese with lower penetration values, i.e. strengthened matrix firmness ($p<0.05$). Similar observations were reported by Antoniou (1986). Moreover, all cheese samples appeared the highest penetration value, i.e. lowest firmness at the first two weeks of cold storage. This could be attributed to the water adsorption as well as the proteolysis occurred in the cheese matrix. Thereafter, the cheese resistance to be penetrated was significantly restored after 4 weeks of cold storage period. This could be ascribed to the acidity development and consequently the relative cheese shrinkage which causing some firmness in the cheese matrix. Jong (1977) as well as Wium and Qvist (1998) found in cold stored Feta cheese that, the firmness of cheese during ripening was the result of both an initial increase in firmness due to changes the physicochemical conditions and a decrease in firmness caused by the breakdown of $\alpha_{s1+2}$-Cn by the rennet enzymes. Moreover, Desouky *et al.* (2002) reported that, penetration value of UF-Goat soft cheese increased (i.e. the firmness weakened) during cold storage at 4°C till the 4$^{th}$ week and then decreased (i.e. the firmness restored). Furthermore, these findings are in complete agreement with those reported by Farahat *et al.* (2004) in UF-white soft cheese during cold storage period for 4 weeks.

**Microbiological properties:**

Data in Table (6) show that, the addition of any experimented preservative to precheese, whether it was FA or $H_2O_2$ ($p>0.05$) yielded in considerable reduction in the total viable bacterial count (TBC) of the resultant cheese ($p<0.001$). The bacteriostatic and bactericidal effects of such preservatives were previously confirmed and reported by Neely (1963 and 1966) and Tawfek (1977) for FA and by Lück (1956); Foster *et al.* (1957); Björck and Rosen (1976) and Robinson (1981) for $H_2O_2$. Likewise, the further heating of precheese led its cheese to have TBC lower than those found in cheese made without further heat treatment. The results also seem that, the TBC of UF-cheese raised as the period of cold storage progressed until the end of the experimental period ($p< 0.001$). Similar findings were reported by Farahat *et al.* (2004).

Concerning the coliform bacterial count of UF-cheese, the results presented in Table (6) also indicate that, the fresh UF-cheese made from not further heated precheese was the sole sample that contained low level of coliform, which disappeared from the beginning of the 2$^{nd}$ week of cold storage period. Moreover, UF-cheeses, whether made from further heated precheese, or made from preservative spiked precheeses were found to be coliform free whether when cheese was fresh or during cold storage along 4 weeks. That could be attributed to the high sanitary conditions offered as well as to the further heating or the

preservative spiking of precheese. Similar trends were found in cold stored UF-white soft cheese made from heated precheese by Farahat *et al.* (2004).

**Table (5): Penetration values (mm) indicating inversely the matrix firmness of cast UF-white soft cheese during cold storage at 5°C as a function of precheese treating either by adding 0.3% formaldehyde or hydrogen peroxide or/and further heating at 72°C/20 sec. after preservative adding.**

| Cold storage period (weeks) | Kind and level of preservative % (V/V) | | | | | |
|---|---|---|---|---|---|---|
| | Nil Control | | 0.3% | | | |
| | | | FA | | $H_2O_2$ | |
| | Without further heating | With further heating | Without further heating | With further heating | Without further heating | With further heating |
| Fresh | 222 | 217 | 236 | 225 | 230 | 223 |
| 2 | 243 | 238 | 250 | 247 | 246 | 240 |
| 4 | 225 | 221 | 241 | 242 | 236 | 232 |

**Table (6): Microbiological properties of cast UF-white soft cheese during cold storage at 5°C as a function of precheese treating either by adding 0.3% formaldehyde or hydrogen peroxide or/and further heating at 72°C/20 sec. after preservative adding**

| Cold storage period (weeks) | Kind and level of preservative % (V/V) | | | | | |
|---|---|---|---|---|---|---|
| | Nil Control | | 0.3% | | | |
| | | | FA | | $H_2O_2$ | |
| | Without further heating | With further heating | Without further heating | With further heating | Without further heating | With further heating |
| **Total viable bacterial count (CFU* × $10^6$/g)** | | | | | | |
| Fresh | 25.10 | 0.37 | 1.30 | 0.11 | 7.00 | 0.16 |
| 2 | 211.00 | 8.20 | 1.50 | 0.50 | 22.00 | 0.29 |
| 4 | 290.00 | 9.00 | 2.20 | 1.25 | 23.00 | 2.00 |
| **Coliform bacteria (CFU* × $10^1$/g)** | | | | | | |
| Fresh | 6 | N.D | N.D | N.D | N.D | N.D |
| 2 | N.D** | N.D | N.D | N.D | N.D | N.D |
| 4 | N.D | N.D | N.D | N.D | N.D | N.D |
| **Yeasts and moulds (CFU* × $10^1$/g)** | | | | | | |
| Fresh | N.D | N.D | N.D | N.D | N.D | N.D |
| 2 | 4.00 | N.D | N.D | N.D | N.D | N.D |
| 4 | 6.20 | N.D | N.D | N.D | N.D | N.D |

*CFU: Colony forming unit ** N.D: not detected

Regarding the count of yeasts and moulds of cheese, although, all fresh samples were completely free from them, little counts were enumerated from the beginning of the 2nd week of cold storage in the UF-cheese made from not further heated precheese. Moreover, yeasts and moulds stilled present in this cheese sample until the end of the experimental period (4 weeks). While, the yeasts and

moulds in UF-cheeses made whether from further heated or/and preservative containing precheese were not detected whether at the beginning or along the cold storage period. Again, that could be attributed to the high sanitary conditions offered either due to the further heating or the preservative spiking of precheese. Similar trends were recorded in some varieties of cold stored UF-white soft cheese by El-Zayat and Osman (2001); Mehanna *et al.* (2002) and Farahat *et al.* (2004).

Finally, the considerable changes in the properties of cheese are making it possible to design measured procedures for preservative detection based essentially on those occurred in the cheese protein fractions and properties.

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## تأثير بعض المواد الحافظة علي خواص الجبن الطري المصنع بإستخدام تقنية الترشيح الفائق بدون تصفية شرش أثناء التخزين بالثلاجة

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الهدف من هذا البحث هو دراسة تأثير الفورمالدهيد وفوق أكسيد الهيدروجين علي خواص الجبن الطري المصنع بإستخدام تقنية الترشيح الفائق بدون تصفية شرش.

ولهذا الغرض تم تصنيع جبن ابيض طري بإستخدام مركز ترشيح فائق معامل إما بالفورمالدهيد أو فوق أكسيد الهيدروجين بنسبة صفر أو ٠,٣% علاوة علي المعاملة الحرارية الإضافية علي ٧٢°م لمدة ٢٠ ثانية بعد إضافة المادة الحافظة أو بدون. تم حفظ الجبن الناتج علي ٥°م لمدة ٤ اسابيع.

ولقد أوضحت النتائج أنه لم يحدث تأثير معنوي علي محتوي الجبن من المادة الجافة ، الدهن/المادة الجافة، البروتين/المادة الجافة والرماد بنوع أو نسبة المادة الحافظة المضافة . مع ذلك حدث زيادة في محتوي الجبن من الدهن/المادة الجافة وأيضاً الرماد ونقص في البروتين/المادة الجافة راجع للمعاملة الإضافية للمركز بالحرارة بعد إضافة المادة الحافظة وقبيل تحويله إلي الجبن. كما أدي الفورمالدهيد إلي

إنتاج جبن بقيم نيتروجين ذائب/النيتروجين الكلي وقيم الـ pH أقل مما نتج في الجبن المحتوية علي فوق أكسيد الهيدروجين. وكانت حموضة الجبن المحتوية علي فورمالين كانت أعلي مما في المحتوية علي فوق أكسيد الهيدروجين. كما حدث نقص كبير في قراءة الإمتصاص المقاس لمعظم شقوق البروتين وخاصة البيتالاكتوجلوبيولين بإستخدام السيفادكس G100 وذلك في بروتين جبن المركز المعامل بفوق أكسيد الهيدروجين أو المعامل إضافياً بالحرارة وهذا عكس ما حدث عند إضافة الفورمالدهيد الذي ثبط تفكك الكازين بالحرارة وذلك في الجبن الناتج من مركز معامل إضافياً بالحرارة. بينما لم يمنع فوق أكسيد الهيدروجين بصورة كلية الإرتباط بين الكابا كازين والبيتالاكتوجلوبيولين. وبإستثناء الفا س١ كازين زاد تركيز كل من ألفا س٢ كازين ، البيتاكازين ، الكابا كازين والجاما كازينات لبروتين الجبن مع الفورمالين وقل مع فوق أكسيد الهيدروجين مقارنة بالكنترول بإستخدام التفريد الكهربي بنظام الصوديوم دودوكسيل سلفيت لجل بولي الأكريلاميد. مع ذلك زاد تركيز اللاكتوفررين الذي ظهر معه شق ناتج من تحلل $\alpha_{s1}$ كازين، جلوبيلينات المناعة ، والألفا لاكتالبيومين لبروتين الجبن وذلك بإضافة فوق أكسيد الهيدروجين للمركز مقارنة بتلك المحتوية علي فورمالدهيد أو الكنترول. أدي التسخين الإضافي للمركز بعد إضافة المواد الحافظة إلي إنخفاض منحني معظم شقوق بروتينات الشرش (بإستثناء جلوبيلينات المناعة والألفا لاكتالبيومين) و ألفا س٢ كازين والبيتاكازين وزيادة في كل من الكابا كازين والجاماكازينات. كما أدت إطالة فترة التخزين بالثلاجة للجبن إلي نقص كل شقوق الكازين بإستثناء الجاماكازينات التي زادت وبصورة مماثلة شقوق البيتالاكتوجلوبيولين والألفا لاكتالبيومين. كما ضعفت صلابة الجبن نتيجة إضافة المواد الحافظة بغض النظر عن نوعها في حين أن صلابة الجبن إزدادت نتيجة للمعاملة الإضافية بالحرارة للمركز بعد إضافة المادة الحافظة. كما لوحظ أيضاً ضعف صلابة الجبن بعد اسبوعين من التخزين بالثلاجة بينما زادت عند نهاية فترة التخزين (٤ أسابيع). أدي إضافة المواد الحافظة بنوعيها إلي المركز أو تسخينه إضافياً بعد إضافة المادة الحافظة إلي الحصول علي جبن بها قيم منخفضة من العدد الكلي للبكتيريا الحية والتي زاد تدريجياً بإطالة مدة التخزين بالثلاجة. كما كانت كل الجبن المصنع من مركز مضاف له مواد حافظة خالياً من بكتيريا الكوليفورم وكذلك الخمائر والفطريات.

وختاماً ، فإنه يمكن من التغييرات المعنوية التي حدثت في الجبن نتيجة إضافة المواد الحافظة إستنباط وسائل جديدة للكشف عنها وذلك بتوظيف التغيرات التي حدثت وخاصة المتعلقة ببروتينات الجبن.