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# EFFECT OF FROZEN STORAGE ON THE MICROBIOLOGICAL AND TECHNOLOGICAL PROPERTIES OF BUFFALO'S MILK

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## ABSTRACT

Properties of whole, skimmed, raw, pasteurized, and UF concentrated buffalo milk samples during prolonged frozen storage up to 12 weeks at temperature (-22±2°C) were studied. In order to define the microbiological quality of frozen milk, total viable count, yeasts & moulds and psychrophilic bacterial counts were determined. The numbers of total bacterial count in control raw buffalo, pasteurized whole milk; raw skimmed, pasteurized skim milk decreased during frozen storage of 12 weeks at -22 ºC ±2. Freezing-storage reduced also yeast and mold counts. On the other hand, the number of total bacteria in concentrated buffalo milk also decreased during the freezing storage. Yeasts and molds counts were absent in frozen concentrated milk after-storage. However, results of technological properties, i.e. curd firmness (indicated by curd penetration), coagulation time, syneresis and colour of the resultant curd showed that frozen storage had different influence on these properties depending on the presence of fat (raw and skimmed milk), heat treatment, and concentration of milk. During freezing storage, curd penetration values, were increased. Rennet coagulation time (RCT) of milk samples and its UF retentates increased compared with control milk samples. The change in the RCT was more pronounced in buffalo's milk during the12 weeks frozen period. The syneresis of renneted milk gels is influenced in the present experimental treatments. Curd syneresis decreased by increasing storage period in all treatments, being the lowest after 12 weeks of storage .It could be concluded that suitable frozen conditions for buffalo milk could be successfully obtained at -22 ± 2 °C for 12 weeks.

In raw whole milk the lightness (L-values) decreased, while redness (a-values) and yellowness (b-values) increased during storage period of buffalo milk. Hue and chroma values of raw skim milk were reduced during frozen storage. In pasteurized skim milk, the b-values increased towards the negative side, resulting in an increase in both Hue and chroma of frozen pasteurized skim milk. Hue values of concentrated milk were lower than those of raw milk, while colour intensity (chroma) was remarkably higher than those of raw milk. The increase in chroma is best to be applied as an intensity indicator of Millard's reaction, which is the case in heat concentrated milk.

**Keywords:** Frozen milk, Microbiological analysis, Technological analysis, concentrated milk, Pasteurized milk, curd firmness, coagulation time, syneresis, Colour parameters

## INTRODUCTION

The presence of harmful microorganisms in milk and its products is a great concern for food safety of consumers. Most disease outbreaks from dairy products have been transmitted via raw, improperly pasteurized milk, or post-pasteurization contamination during and after manufacturing of the products (Boor *et al.,* 1998; Johnson *et al*., 1990). Despite of the intense

sanitary precautions , contamination with pathogenic organisms in raw milk may not be completely eliminated.

Freezing is an alternative preservation method, instead of the high energy cost of drying, also for milk, to extend shelf life. Frozen dairy products can be divided into two categories: (i) products frozen for increasing their shelf-life and thawed before consumption or further processing and (ii) products in which the freezing process is responsible for the development of the desired structure and texture and which are consumed in the frozen state. The amount of dairy products fall into the first category, that needs thawing before further processing or consumption is very small relative to the frozen dairy dessert industry.

A reliable alternative mean for detection of frozen milk is colour analysis. When a beam of natural light that consists of an equal amount of every frequency in the visible region of the spectrum reaches a material, some of the frequencies will be absorbed by the material and the rest will be reflected and received by an observer. If all the frequencies are equally reflected, the material will be perceived as white. If the material absorbs – preferentially – some frequencies, the fraction of reflected light will contain predominant frequencies above the others and the material will appear coloured. If the material absorbs all the frequencies, there will be no outgoing light and the material will be perceived as black. This is the basis of colour measurements (Jovanka *et al.,* 2008). Milk is a very turbid medium, with a low absorption coefficient and a high scattering coefficient. When light enters the milk, it will be scattered in all directions and the backwards scattered light will be detected by the sensor. The data are corrected for temperature. Milk has a softly yellowish colour. This implies outgoing light the intensity of red and green will be slightly more noticeable than that of blue, compared to the incident light.

Freezing and frozen storage can have a large effect on the proteins of milk, causing the casein micelles to loose their stability and precipitate on thawing. This manifests itself either as thickened product or as flocks of casein evident either on the sides of thawed glass or plastic containers, or as a precipitate at the bottom. In fact, commercial separation of casein from concentrated milk serum may be accomplished by freezing, storage at -18°C, and thawing to produce a product that has been referred to as cryo-casein. The flocculation of casein from frozen milk is initially reversible with heat and agitation, but becomes irreversible with continued storage. Even minor amounts of casein flocculation can lead to the perception of a chalky texture upon consumption (Douglas 2006).

The raw whole ewe's milk is frozen at -15 or -27°C. Total bacteria decreased at a faster rate in milk stored at -15 than at -27 °C. Acid degree values of milk stored at -15°C were significantly higher than that stored at -27°C. Samples stored at -15°C exhibited protein destabilization after 6 months of storage, whereas those stored at -27 °C were stable throughout the 12-month storage period (Wendorff 2001). The freezing of milk at -19 °C for 48 hrs increased the rennet coagulation time (RCT). These results cleared that the frozen storage had higher effect on dissociation of casein into soluble casein, particularly β-casein (Fox 1969).

These investigations were carried out to measure the color parameters, microbiological and technological properties of frozen buffalo milk samples whole and skim milk during prolonged storage up to 12 weeks at temperature (-22±2°C).

## MATERIALS AND METHODS

Fresh buffalo's milk (6.8% fat and 16.5% total solids) was obtained from the herd of Shalakan experimental Farm, Faculty of Agriculture, Ain Shams University. Calf rennet powder (Ha-La) with strength of (10N) was obtained from CHR- Hansen's Lab. Denmark.

Fresh cooled milk (4°C) was transported to the laboratory within 1h in isothermal container. Raw milk was skimmed using laboratory milk separator at 40 °C. Raw and skim milk were pasteurized (at 75 °C for 15 Sec., followed by cooling to 4°C. The milk was packaged into special plastic bags (300 ml each). Four variants of milk samples were obtained, I- raw whole milk samples, II- pasteurized whole milk samples, III-Raw skim milk samples and IV- pasteurized skim milk samples. All variants were stored in deep freezer at - 22± 2°C for 12 weeks. Samples were taken every two week, thawed throughout 24 h at 5°C., and analyzed for colour, microbiological and technological properties.

For the preparation of frozen concentrated milk, fresh raw milk was divided into two portions; the first portion was treated as whole milk, and the second was skimmed using a laboratory milk fat separator to obtain skim milk. Both fresh whole and skim milk were pasteurized at 75°C for 15 sec, and homogenized at 200 bars. Ultrafiltration of both samples were carried out in a CARBOSEP pilot plant unit (type 2S 151 tubular, France) with Zirconium oxide membrane area of 6.8 $m^2$. The inlet and outlet pressures were 5 and 3 bar, respectively. Milk was concentrated in a batch system to volume concentration factor 3X. The resultant milk retentate was diluted using milk permeate to obtain samples with concentration factor 2X. Retentate types (whole 22-33 %TS and skimmed 18-28% TS) were packaged into PVC cups (300 ml) and frozen at -22 ± 2°C for 12 weeks. The samples were taken when fresh and weekly till the end of frozen storage, and analyzed for colour, microbiological and technological properties.

The total bacterial count was determined according to Houghtby *et al.,* (1992). The count of yeast and molds were determined according to the method described by Frank *et al.,* (1992). Psychrophilic bacterial count was carried out according to the method of Shabani (2003).

Coagulation time was determined according to Joseph and Ashworth (1970).Curd firmness was measured in fresh and stored samples as described by Attaie *et al*., (1996) using a penetrometer (OFD AP 4/3, Germany). Syneresis of the resultant curd was measured according to Lawerence (1959). The results were expressed as the volume of drained whey from 50 ml milk.

Colour parameters of the milk samples were determined according to the tristimulus Colour system described by Francis, (1986) using

spectrophotometer (MOM, 100 D, Hungary). Colour coordinates **X**, **Y**, and **Z** were converted to corresponding Hunter **L**, **a, b** colour coordinates according to formulas given by manufacturer as follows:

$$L = 116\,(Y/Yn)^{1/3} - 16 \quad (1)$$

$$a = 500\,[(X/Xn)^{1/3} - (Y/Yn)^{1/3}] \quad (2)$$

$$b = 200\,[(Y/Yn)^{1/3} - (Z/Zn)^{1/3}] \quad (3)$$

while: **L** indicates lightness or darkness in a scale from 100.0 to 0.0, whiles **a** and **b** represent the coordinates of (red – green and yellow – blue) on a scale from plus 60 to minus 60. Hue angle, which represents the dominating colour, was calculated

$$Hue = \tan^{-1} b/a \quad (4)$$

The colour intensity (chroma) was calculated as follows:

$$C = (a^2 + b^2)^{1/2} \quad (5)$$

**Statistical analysis**

Statistical analysis was performed using the GLM procedure with SAS (2004) software. Duncan's multiple comparison procedure was used to compare the means. A probability of $P \leq 0.05$ was used to establish statistical significance.

## RESULTS AND DISCUSSION

In order to define the microbiological quality of frozen milk, total viable count, yeasts and moulds and psychrophilic bacterial counts were determined. Tables (1, 2 &3) show the average of total viable count, yeasts, moulds and Psychrophilic bacterial counts (log cfu/ml) in the frozen milk samples. As shown in Table (1), log (cfu/ml) of total viable count in control raw whole milk, pasteurized whole milk, raw skim milk, pasteurized skim milk were 7.55, 5.83, 7.18 and 5.53 log (cfu/ml), respectively. Raw whole milk and raw skim milk samples were the highly contaminated samples with bacteria. Such results indicate that the raw milk is accompanied with poor sanitary conditions. Pasteurization reduced the count number by about 1.5 log cycle. After 12 months frozen storage at -22 ºC ±2, total viable count were 6.23, 4.48, 5.90 and 3.85 respectively. As seen, the number of total bacteria count in buffalo milk decreased rapidly during the first 2 months of frozen storage and slightly thereafter. These findings are in agreement with those mentioned by Samuelsson *et al.*, (1957), who reported that during the first 24 h of freezing storage more bacteria are killed than during any later storage. Moreover, Rauschenberger *et al.,* (2000) reported that samples of sheep milk stored at -15 and -27 ºC showed a decrease in coliform and standard plate counts during 6 - 9 months of the experiment. Wendorff (2001) reported similar observations with frozen-stored ovine milk, as the standard plate counts decreased with extended storage time, and microbial storage stability was greater with lower freezing temperatures. Similar trends were observed by Anifantakis *et al.,* (1980), who found that there was no alteration in the

bacterial counts (standard plate counts, coliforms and psychrophiles) of sheep's milk during frozen storage. Young (1987) stated a little change in total bacterial counts and presumptive coliforms in raw sheep's milk throughout the 12 months frozen storage. Freezing-storage reduced yeast and mold counts (Table 2). It coul also be seen, that the combination of freezing and frozen-storage adversely influenced the survival of yeasts and molds in milk samples. These data reveal that there are higher total viable bacterial counts, yeasts and moulds and psychrophilic bacterial counts (Table 3) in raw whole milk samples than in that of pasteurized whole milk, raw skim milk, and pasteurized skim milk. The influence of pasteurization on yeast and mould count was more pronounced in whole milk than in skim milk.

As shown in Table (4), log (cfu/ml) of total viable bacterial count in fresh concentrated whole milk 2X, concentrated whole milk 3X ,skim milk 2X and concentrated skim milk3X were 5.85, 5.69, 5.51and 5.32 log (cfu/ml) and after 12 months storage at -22 ºC ±2, the total viable bacterial count were 4.62, 4.10, 4.04 and 4.02 log (cfu/ml) respectively. The number of total bacteria count in concentrated buffalo milk decreased during the frozen storage period. Yeast and mold counts were absent in frozen concentrated milk after-storage period, which indicates that the combination of freezing and frozen-storage adversely influenced the survival of yeasts and molds in milk samples (Table 5).

The difference in D-value between raw and pasteurized skim milk is more obvious than in the buffalo whole milk due to the role of fat in protecting the microbial cell (Table 6). The difference in D-value of yeasts and molds between raw and pasteurized buffalo milk (whole or skim) are clear due to the damage of the cell wall of the microbial cell during the pasteurizations process. The same trend was observed for the reduction pattern in the number of psychrophilic bacterial. Concerning the concentrated milk, there were no clear differences between the reduction pattern between whole and skim milk, neither for 2X nore for 3X. The difference in D-value of psychrophilic bacterial was clear in concentrated skim milk rather than in the high fat whole concentrated milk. The effect of frozen storage on the survival of the microorganisms in the tested milk samples could be better understand through calculation of the decimal reduction time (D-value) at the freezing temperature (-22 °C). The relationship between storage time and survival microbial numbers for data presented in tables (1, 2, 3, 4 and 5) gave a linear relationship with $R^2$-values higher than 0.92. Therefore the D-values were calculated from the slopes and data are given in Table (6). The decrease in the microbial count during freezing could be referred to the partial plysmosis of the microbial cell wall as a result of the decreasing water activity values of the frozen milk as well as to the damage in cell walls resulting from ice crystal growth during frozen storage.

**Table (1): Total viable bacterial counts (Log cfu/ml) of frozen raw, pasteurized, whole and skim milk during storage at -22 º C ± 2.**

| Storage period (week) | Buffalo whole milk | | Buffalo skim milk | |
|---|---|---|---|---|
| | Raw | Pasteurized | Raw | Pasteurized |
| Fresh | $7.55^{A}$ | $5.83^{A}$ | $7.18^{A}$ | $5.53^{A}$ |
| 2 | $7.30^{B}$ | $5.48^{B}$ | $6.96^{B}$ | $5.11^{B}$ |
| 4 | $7.21^{AB}$ | $5.26^{C}$ | $6.87^{C}$ | $4.70^{C}$ |
| 6 | $7.12^{B}$ | $5.11^{D}$ | $6.80^{D}$ | $4.30^{D}$ |
| 8 | $6.85^{BC}$ | $4.90^{E}$ | $6.45^{E}$ | $4.15^{E}$ |
| 10 | $6.52^{CD}$ | $4.70^{F}$ | $6.20^{F}$ | $4.04^{F}$ |
| 12 | $6.23^{D}$ | $4.48^{G}$ | $5.90^{G}$ | $3.85^{G}$ |

**Means values in column not followed by the same letter are significantly different (P<0.05).**

**Table (2): Yeast and mould counts (Log cfu/ml) of frozen raw, pasteurized, whole and skim milk during storage at -22 º C ± 2.**

| Storage period (week) | Buffalo whole milk | | Buffalo skim milk | |
|---|---|---|---|---|
| | Raw | Pasteurized | Raw | Pasteurized |
| Fresh | $4.18^{A}$ | $2.65^{A}$ | $3.12^{A}$ | $2.54^{A}$ |
| 2 | $4.07^{B}$ | $2.45^{B}$ | $3.05^{B}$ | $2.28^{B}$ |
| 4 | $3.94^{C}$ | $2.23^{C}$ | $2.95^{C}$ | $2.08^{C}$ |
| 6 | $3.83^{D}$ | $2.04^{D}$ | $2.79^{D}$ | $1.95^{D}$ |
| 8 | $3.76^{E}$ | $1.94^{E}$ | $2.68^{E}$ | $1.84^{E}$ |
| 10 | $3.65^{F}$ | $1.84^{F}$ | $2.54^{F}$ | $1.70^{F}$ |
| 12 | $3.48^{G}$ | $1.69^{G}$ | $2.41^{G}$ | $1.54^{G}$ |

**Means values in column not followed by the same letter are significantly different (P<0.05).**

**Table (3): Psychrophilic bacterial counts (Log cfu/ml) of frozen raw, pasteurized, whole and skim milk during storage at -22 º C ± 2**

| Storage period (week) | Buffalo whole milk | | Buffalo skim milk | |
|---|---|---|---|---|
| | Raw | Pasteurized | Raw | Pasteurized |
| Fresh | $4.52^{A}$ | $4.36^{A}$ | $4.41^{A}$ | $4.26^{A}$ |
| 2 | $4.23^{B}$ | $4.04^{B}$ | $4.08^{B}$ | $3.85^{B}$ |
| 4 | $3.90^{C}$ | $3.70^{C}$ | $3.78^{C}$ | $3.54^{C}$ |
| 6 | $3.70^{D}$ | $3.30^{D}$ | $3.60^{D}$ | $3.23^{D}$ |
| 8 | $3.61^{E}$ | $3.25^{E}$ | $3.47^{E}$ | $3.09^{E}$ |
| 10 | $3.51^{F}$ | $3.16^{F}$ | $3.40^{F}$ | $2.92^{F}$ |
| 12 | $3.43^{G}$ | $3.09^{G}$ | $3.32^{G}$ | $2.74^{G}$ |

**Means values in column not followed by the same letter are significantly different (P<0.05).**

**Table (4): Total viable bacterial counts (Log cfu/ml) of frozen concentrated buffalo whole and skim concentrated milk during storage at -22 ºC ± 2.**

| Storage period (week) | Whole milk | | Skim milk | |
|---|---|---|---|---|
| | 2X | 3X | 2X | 3X |
| Fresh | $5.85^{A}$ | $5.69^{A}$ | $5.51^{A}$ | $5.32^{A}$ |
| 2 | $5.64^{B}$ | $5.20^{B}$ | $5.11^{B}$ | $4.90^{B}$ |
| 4 | $5.30^{C}$ | $4.91^{C}$ | $4.78^{C}$ | $4.60^{C}$ |
| 6 | $5.00^{D}$ | $4.78^{D}$ | $4.61^{D}$ | $4.41^{D}$ |
| 8 | $4.85^{E}$ | $4.61^{E}$ | $4.32^{E}$ | $4.27^{E}$ |
| 10 | $4.77^{F}$ | $4.34^{F}$ | $4.17^{F}$ | $4.07^{F}$ |
| 12 | $4.62^{G}$ | $4.10^{G}$ | $4.04^{G}$ | $4.02^{G}$ |

**Means values in column not followed by the same letter are significantly different (P<0.05).**

**Table (5): Psychrophilic bacterial counts (Log cfu/ml) of frozen concentrated buffalo whole and skim milk during storage at -22 ºC ± 2.**

| Storage period (week) | Whole milk | | Skim milk | |
|---|---|---|---|---|
| | 2X | 3X | 2X | 3X |
| Fresh | 4.15$^{A}$ | 3.95$^{A}$ | 3.90$^{A}$ | 3.70$^{A}$ |
| 2 | 3.78$^{B}$ | 3.72$^{B}$ | 3.48$^{B}$ | 3.43$^{B}$ |
| 4 | 3.70$^{C}$ | 3.61$^{C}$ | 3.34$^{C}$ | 3.20$^{C}$ |
| 6 | 3.58$^{D}$ | 3.52$^{D}$ | 3.23$^{D}$ | 3.00$^{D}$ |
| 8 | 3.49$^{E}$ | 3.32$^{E}$ | 3.11$^{E}$ | 2.85$^{E}$ |
| 10 | 3.28$^{F}$ | 3.15$^{F}$ | 3.08$^{E}$ | 2.63$^{F}$ |
| 12 | 3.11$^{G}$ | 2.78$^{G}$ | 3.00$^{F}$ | 2.60$^{F}$ |

**Means values in column not followed by the same letter are significantly different (P<0.05).**

**Table (6): Decimal reduction time (D-values) of microorganism during frozen storage(weeks).**

| | Whole milk | | Skim milk | | Whole milk | | Skim milk | |
|---|---|---|---|---|---|---|---|---|
| | Raw | Pasteurized | Raw | Pasteurized | 2X | 3X | 2X | 3X |
| Total viable bacterial | 9.52 | 9.38 | 9.69 | 6.77 | 9.52 | 8.25 | 8.3 | 9.52 |
| Psychrophilic bacterial | 11.2 | 9.3 | 11.34 | 8.85 | 12.93 | 11.34 | 15.01 | 10.67 |
| Yeast and mould | 17.85 | 9.3 | 11.34 | 8.85 | - | - | - | - |

The penetration values (mm) of curd formed from raw, pasteurized, whole and skim milk during frozen period after 12 weeks at (-22 ºC ± 2) are illustrated in Fig (1). The penetration value for control samples of raw, pasteurized, whole and skim milk were 8.23, 9.12, 7.42 and 8.66 (mm), respectively. From these data, it could be noticed that the control treatment has the highest penetration value. In another meaning, full fat milk is of higher penetration value than the skim milk. This is due to the higher fat content in milk, which affected the weakness of the curd compared with other treatments. Penetration values similarly increased by increasing storage period. The penetration values (mm) of curd made from concentrated whole (2X, 3X) and skim (2X, 3X) milk during frozen period after 12 weeks at (-22 ºC ± 2) are illustrated in Fig (2). The penetration value of concentrated whole and skim milk for 0.0 times were 13.35, 16.78, 10.8 and 7.74 (mm) respectively. From these data, it coul be noticed that the concentrated fresh whole milk has the highest penetration values by prolonging the storage period. In anther meaning, full fat milk has the higher penetration value than the skim milk. This is due to the higher fat content in milk, which affected the weakness of the curd compared with other treatments. Penetration values similarly increased by increasing the storage period.

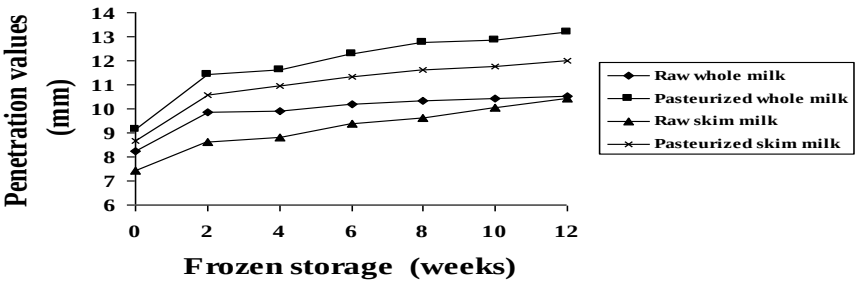


**Fig. 1: Curd penetration values (mm) of raw, pasteurized, whole and skim milk during frozen storage at -22 ºC ± 2.**

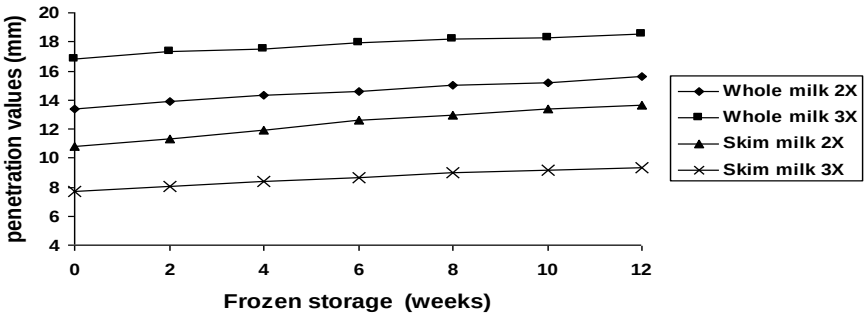


**Fig. 2: Curd penetration values (mm) of frozen concentrated whole and skim milk during storage at -22 ºC ± 2.**

Data in Fig. (3) indicate the rennet coagulation time (RCT, sec.) of raw, pasteurized, whole and skim milk during freezing period of 12 weeks at (-22 ºC ± 2). Rennet coagulation time of pasteurized milk samples increased, compared with raw milk samples. The change in the RCT was more pronounced in buffalo's milk during freezing period of 12 weeks. After 12 weeks of storage, the raw skim milk showed lowest RCT, while pasteurized whole milk exhibited the highest values. Data in Fig. (4) indicate the rennet coagulation time (RCT, sec.) of concentrated whole and skim milk during freezing period of 12 weeks at (-22 ºC ± 2). Concentrated whole milk (3X) showed increase in rennet coagulation time compared with the other concentrated milk samples. The change in the RCT was more pronounced in buffalo's milk during frozen period of 12 weeks. After 12 weeks of storage the concentrated skim milk 3X showed lowest RCT, while concentrated whole milk 3X exhibited the highest values.

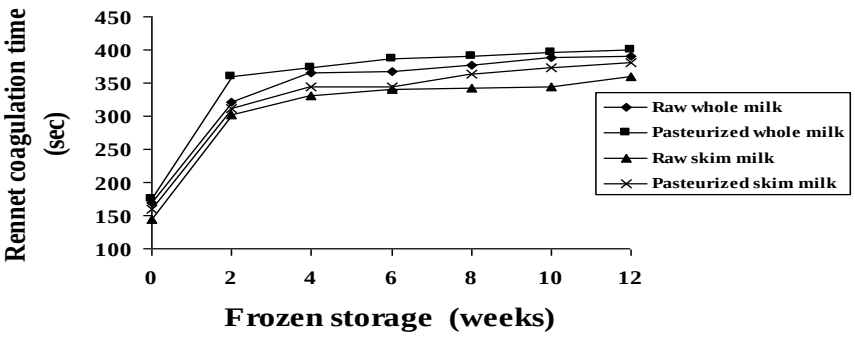


**Fig.3: Rennet coagulation time (Sec) of frozen Raw, pasteurized, whole and skim milk during storage at -22 ºC ± 2.**

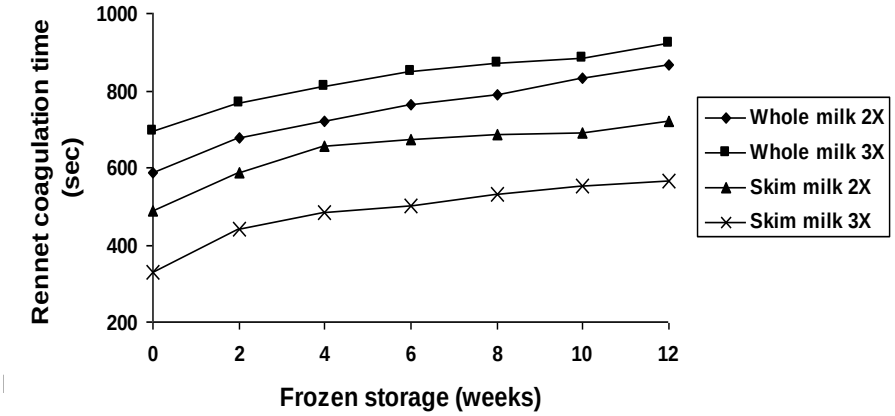


**Fig.4: Rennet coagulation time(Sec) of frozen concentrated whole and skim milk during storage at -22ºC±2**

Data in Figure (5) indicate the curd syneresis (ml) of raw, pasteurized, whole and skim milk during freezing period of 12 weeks at (-22 ºC ± 2). Curs syneresis of pasteurized milk of control samples decreased, compared with raw milk samples. It could also be found that the control treatment has the highest curd syneresis value. In another meaning, full fat milk has the lower curd syneresis value than the skim milk. This is due to the higher fat content in milk, which affects the weakness of the curd compared with other treatments. The changing in curd syneresis was more pronounced in buffalo's milk during frozen period of 12 weeks. The syneresis of renneted milk gels is influenced by milk composition, which in turn is affected by the feed, stage of lactation, and health of the animals from which the milk is obtained. Fat tends to reduce syneresis and increase the water-holding capacity of cheese curd, and increasing the fat content of cheese milk increases cheese yield by about 1.2 times the weight of the additional fat

(Fox *et al.,* 2000). Data in Figure (6) indicate the curd syneresis (ml) of concentrated whole and skim milk (2X, 3X) during frozen period of 12 weeks at (-22 ºC ± 2). The rate of drainage of curd from fresh whole and skim milk (2X, 3X) samples and concentrates was different from that of frozen buffalo's after 12 weeks. The differences were found between the whole concentrated milk and the retentate from skim milk concentrated to a factor 3X. The drainage was faster in the concentrated skim milk than in the other samples. The differences found could be due to the influence of the composition on curd formation. The differences in the relative composition could influence the curd structure and the syneresis. The obtained results indicate that the concentration of milk by UF, and the heat treatment previous to the UF have different influences on the syneresis rate of curd manufacture from milk of different species, when the conditions of cheese manufacture are similar. It could also be noticed that the fresh concentrated sample has the highest curd syneresis value. In another meaning, full fat milk has the lower curd syneresis value than the skim milk. This is due to the higher fat content in milk, which affected the weakness of the curd compared with other treatments. Curd syneresis decreased by increasing storage period in all treatments, being the lowest after 12 weeks of storage. The decrease in curd syneresis during storage period could be correlated to the differences in chemical composition of concentrated milk. The influence of milk concentration on syneresis has been described by Peri *et al*., (1985), who found that the syneresis of milk or milk concentrated up to 5-fold by UF followed a first-order reaction and was independent of protein concentration.

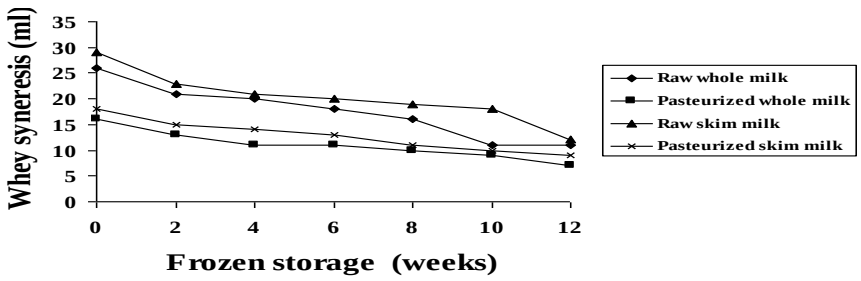


**Fig. 5: Curd syneresis (ml) of raw, pasteurized, whole and skim milk**

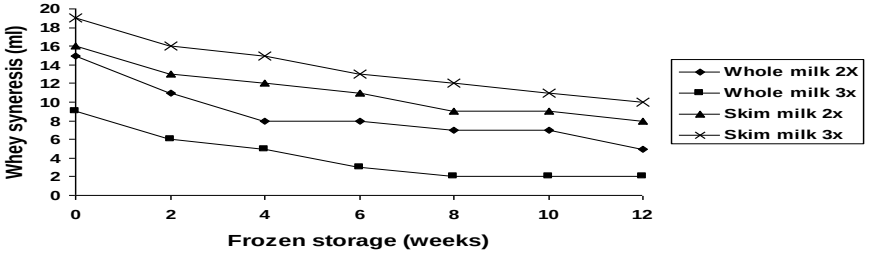

**Fig. 6: Curd synesesis of frozen concentrated whole and skim milk during storage at -22 ºC ± 2.**

The tristimulus colour parameters, lightness (L), redness (a), yellowness (b), were calculated for raw, pasteurized, whole and skim milk during freezing storage at -22 ºC ± 2 during the storage period of 12 weeks, and the data are given in Tables (7&8). L- value of raw whole milk was 90.23. Removal of fat led to a lower L- value of skim milk. Freezing storage did not remarkably influence the lightness values of frozen skim and whole milk. Also, pasteurization was of a minor effect on the lightness values of milk. The raw whole milk showed a redness (a-values) and yellowness (b-value) of 0.55 and 3.5, respectively. The redness values reduced, while the b-values showed a minor change during freezing storage of raw whole milk. The change in a-value resulted in an increase in both Hue and chroma-values of the frozen stored milk. Removal of fat from whole milk resulted in negative a- and b-values, indicating the shift of skim milk colour to be light greenish-blue to some extent. During freezing storage, the greenish colour component increased, while the blue colour tended to decline. Based on these changes, both Hue and chroma-values of frozen skim milk were reduced at the end of frozen storage period.

The effect of pasteurization on colour parameter of whole and skim milk was different. In whole milk, a- and b-values increased upon pasteurization reflecting the extend of heat induced browning reactions. Both Hue and chroma-values of pasteurized whole milk were lower than those of raw milk. In pasteurized skim milk b- value reduced, shifting Hue b-value to be close to the neutral point, while the greenish component (a-value) reduced, indicating the presence of active greenish reaction products induced by pasteurization. As a result, both Hue and chroma values of skim milk were reduced by pasteurization.

Storage of milk samples under freezing conditions affected both a- and b-values. The a-value of raw whole milk reduced, while b-value was more stable during the freezing period, resulting in an increase in both Hue and chroma-values of frozen raw whole milk. For raw skim milk, a- value increased in the negative opposite (greenish) side, while the b-value increased. As a result of such change, Hue and chroma values of raw skim milk reduced during freezing storage. In pasteurized skim milk, the b-values increased towards the negative side, resulting in an increase in both Hue and chroma of frozen pasteurized skim milk. However, Hue values of raw whole milk were higher (twoards white), than those of skim milk (towards more yellow colour shade). According to Espada and Vijverberg (2002), raw milk has a softly yellowish colour (Possitive b- values), because fat is the component that mainly gives to the milk its particular yellowish colour. Thus, as the fat content increases, the colour of milk becomes more yellow. However, red (Possitive a- values) green (negative a- values) and blue (negative b- value) colour shades are present in milk and their ratios changes with milking and processing condition, with blue colour has a tendency to increase slightly at the end of milking. On the other side, Jovanka *et al.,* (2008) reported that lightness (L-value) of UHT milk samples were not significantly changed during storage and L-value increases with increase in

fat content, which agrees with the results of the present work. They also found that chroma (saturation/Purity) did not change up to 60 days of storage. They also reported that L- values of milk with 1.6% fat were lower in all storage period, if compared with the corresponding samples of milk with higher percentual milk fat. The negative sign of a-values of raw skim milk indicating, according to the same author, the presence of components of a green (-) milk colour. They also referred the increase in red colour (+ a-values), as it is the case in pasteurized whole milk, to the degradation of tryptophan and tyrosine, may be as a result of the heat treatment and expossion to light. The change in b-value (as seen in raw and pasteurized skim milk with negative b- value) is probably induced by simultaneous degradation of the yellouish-green coloured riboflavin (vitamin $B_2$), B-carotene and vitamin A molecules.

The colour parameters of concentrated milk Table (9&10) were totally different than those of fresh milk. The concentrated milk becomes lighter, since L-values were close to 100 with concentrated whole milk recording higher L-scores than did concentrated skim milk. The greenish colour component (Negative a- value) and the blue colour component (Negative b-value) were increased for concentrated skim milk compared to those of whole and skim milk samples. Hue values of concentrated milk were lower than those of raw milk, while colour intensity (chroma) was remarkably higher than those of raw milk. The increase in chroma is best to be applied as an intensity indicator of Millard's reaction, which is the case in heat concentrated milk by evaporation in the present work.

**Table (7): Color parameters of whole raw and pasteurized milk during freezing storage at -22 ºC ± 2.**

| Storage period (Week) | Raw whole milk | | | | | Pasteurized whole milk | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | L | b | a | Hue | Chroma | L | b | a | Hue | Chroma |
| resh | 90.23 | 3.50 | 0.55 | 81.04 | 2.69 | 89.03 | 0.96 | 0.89 | 47.17 | 1.31 |
| 2 | 90.19 | 3.48 | 0.39 | 83.60 | 3.50 | 88.97 | 0.97 | 0.72 | 53.41 | 1.21 |
| 4 | 90.12 | 3.39 | 0.52 | 81.28 | 3.43 | 88.94 | 1.02 | 0.51 | 63.43 | 1.14 |
| 6 | 90.02 | 3.32 | 0.76 | 77.10 | 3.41 | 88.91 | 1.05 | 0.47 | 65.89 | 1.15 |
| 8 | 90.00 | 3.38 | 0.31 | 84.76 | 3.39 | 88.89 | 1.04 | 0.35 | 71.40 | 1.09 |
| 10 | 89.88 | 3.23 | 0.40 | 82.94 | 3.25 | 88.86 | 1.05 | 0.34 | 72.06 | 1.10 |
| 12 | 89.83 | 3.17 | 0.15 | 87.29 | 3.17 | 88.80 | 1.03 | 0.23 | 77.14 | 1.055 |

**Table (8): Color parameters of skim raw and pasteurized milk during freezing storage at -22 ºC ± 2.**

| Storage period (Week) | Raw whole milk | | | | | Pasteurized whole milk | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | L | b | a | Hue | Chroma | L | b | a | Hue | Chroma |
| Fresh | 86.80 | -2.48 | -0.88 | 70.46 | 2.63 | 87.50 | -0.42 | -1.64 | 14.36 | 1.69 |
| 2 | 86.87 | -2.25 | -1.21 | 61.73 | 2.55 | 87.42 | -0.51 | -1.66 | 17.08 | 1.74 |
| 4 | 86.91 | -2.04 | -1.24 | 58.71 | 2.39 | 87.30 | -0.58 | -1.69 | 18.94 | 1.79 |
| 6 | 86.98 | -1.77 | -1.67 | 46.66 | 2.43 | 87.26 | -0.69 | -1.73 | 21.74 | 1.86 |
| 8 | 87.11 | -1.34 | -1.78 | 36.97 | 2.23 | 87.15 | -0.78 | -1.71 | 24.52 | 1.88 |
| 10 | 87.20 | -0.99 | -1.89 | 27.64 | 2.13 | 86.93 | -0.85 | -1.70 | 26.57 | 1.9 |
| 12 | 87.28 | -0.64 | -1.63 | 21.44 | 1.75 | 86.87 | -1.02 | -1.74 | 30.38 | 2.02 |

**Table (9): Color parameters of frozen concentrated whole milk during storage at -22 ºC ± 2.**

| Storage period (Week) | Whole milk (2X) | | | | | Whole milk (3X) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | L | b | A | Hue | Chroma | L | b | a | Hue | Chroma |
| **Fresh** | 98.35 | -1.03 | -12.95 | 4.55 | 13.03 | 98.83 | 1.095 | -13.11 | 4.77 | 13.16 |
| **2** | 98.20 | -1.04 | -12.87 | 4.61 | 12.95 | 98.71 | 0.97 | -13.10 | 4.23 | 13.14 |
| **4** | 98.06 | -1.05 | -12.50 | 4.80 | 12.54 | 98.63 | 0.98 | -13.2 | 3.86 | 13.23 |
| **6** | 97.99 | -1.06 | -12.61 | 4.81 | 12.65 | 98.42 | 0.86 | -12.6 | 3.90 | 12.63 |
| **8** | 97.90 | -1.04 | -12.64 | 4.70 | 12.68 | 98.32 | 0.66 | -12.37 | 3.06 | 12.39 |
| **10** | 97.76 | -0.99 | -12.28 | 4.61 | 12.32 | 98.16 | 0.53 | -12.12 | 2.50 | 12.13 |
| **12** | 97.67 | -0.86 | -12.36 | 3.98 | 12.39 | 98.12 | 0.46 | -12.10 | 2.18 | 12.18 |

**Table (10): Color parameters of frozen concentrated skim milk during storage at -22 ºC ± 2.**

| **Storage period (Week)** | **Skim milk (2X)** | | | | | **Skim milk (3X)** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **L** | **b** | **a** | **Hue** | **Chroma** | **L** | **b** | **a** | **Hue** | **Chroma** |
| **Fresh** | 93.93 | -5.6 | -12.54 | 24.06 | 13.73 | 96.75 | -0.92 | -14.34 | 3.67 | 14.37 |
| **2** | 94.19 | -5.58 | -12.65 | 23.80 | 13.83 | 96.86 | -1.0 | -14.29 | 4.0 | 14.32 |
| **4** | 94.69 | -5.06 | -12.82 | 21.53 | 13.78 | 96.89 | -1.09 | -14.27 | 4.37 | 14.31 |
| **6** | 94.74 | -5.04 | -12.90 | 21.36 | 13.85 | 96.94 | -1.17 | -13.90 | 4.81 | 13.95 |
| **8** | 95.07 | -4.50 | -12.89 | 19.24 | 13.65 | 97.0 | -1.13 | -13.57 | 4.76 | 13.62 |
| **10** | 95.16 | -4.30 | -13.1 | 18.17 | 13.79 | 97.07 | -1.15 | -13.09 | 5.02 | 13.14 |
| **12** | 95.20 | -4.26 | -13.2 | 17.89 | 13.87 | 97.18 | -1.02 | -13.03 | 4.48 | 13.07 |

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# تأثير التجميد على الخواص الميكروبيولوجية والتكنولوجية للبن الجاموسى

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تركزت هذه الدراسة على الخواص الميكروبيولوجية والتكنولوجية لعينات اللبن الجاموسى الكامل الخام ، والمبستر ، واللبن منزوع الدسم وعينات اللبن المركز بالترشيح الفائق المجمدة أثناء التخزين لفترة طويلة في درجة حرارة (-٢٢ ± ٢ درجة مئوية) . ولدراسة الخواص الميكروبيولوجية تم تقدير العدد الكلى للبكتيريا الحية، الخمائر & الفطريات والبكتريا المحبة للبرودة ، وقد وجد انخفاضا في هذه التقديرات نتيجة التجميد خلال ١٢ أسبوعا من التخزين المجمد في -٢٢ ± ٢ درجة مئوية .كما انخفضت أيضا الخمائر & الفطريات انخفاضا ملحوظا. ومن ناحية أخرى ، فإن عدد البكتيريا في عينات اللبن الجاموسى المركزة خلال فترة التخزين المجمدة انخفضت انخفاضا ملحوظا واختفت الخميرة والفطر في عينات اللبن المركز المجمدة بعد فترة التخزين. وأظهرت نتائج الخصائص التكنولوجية ( أي صلابة الخثرة المشار إليها بقيمة اختراق الخثرة ، ووقت التحبن وقوة طرد الشرش ) أن التخزين المجمد كان له تأثيرا متباينا على هذه الخصائص طبقا لنوع اللبن ( كامل الدسم والخالي من الدسم) ، والمعاملة الحرارية ( بسترة) ، وكذلك تركيز اللبن. وقد بينت النتائج زيادة قيم اختراق الخثرة، ووقت التجبن لعينات اللبن والمركزات مقارنة مع عينات اللبن المقارنة. التغير كان أكثر وضوحا بعد 12 أسبوع من التخزين على درجة حرارة التجميد . كما تاثرت قوة طرد الشرش من الخثرة نتيجة المعاملات التجريبية بهذه الدراسة. وقد انخفضت قيم قوة طرد الشرش مع زيادة فترة التخزين في كل المعاملات ووصلت إلى أدنى قيمة لها بعد ١٢ أسبوع من التخزين . وتوصى الدراسة بأنه يمكن تخزين اللبن تحت تجميد على -٢٢± ٢ درجة مئوية لمدة لا تزيد عن ١٢ أسبوع.

تم دراسة خواص اللون لكل من اللبن الكامل والفرز المركز والمجمد لهذة العينات بأستخدام جهاز تقدير اللون. أظهرت الدراسة انخفاضا فى قيم درجة سطاعة اللون (L) بينما زادت قيم درجات الاحمرار (a) والاصفرار (b) أثناء فترة التخزين المجمد للبن الجاموسى. كما أنخفضت قيم كل من شدة اللون (الكروما) وزاوية اللون للبن الفرز الخام أثناء فترة التخزين المجمد. وبالنسبة للبن الفرز المبستر فقد زادت قيم الاصفرار فى الاتجاه السالب مما أدى الى زيادة قيم كل من شدة اللون وزاوية اللون للبن الفرز المجمد. وبالنسبة للبن المجمد المركز فقد كانت قيم زاوية اللون له أقل من مثيلاتها فى اللبن الخام بينما ازدادت قيم شدة اللون بدرجة ملحوظة مقارنة بمثيلاته للبن الخام. ويمكن تطبيق درجات الزيادة فى شدة اللون كمقياس لتفاعل ميلارد كما هو الحال فى اللبن المركز سابق التسخين.

**قام بتحكيم البحث**

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