

## **Changes in the Quality of Recombined UHT Milk Processed by Direct and Indirect Heating System During Storage**

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**Abstract.** The changes in quality of recombined ultra high temperature (RUHT) milk processed by indirect ( $R_1$ UHT and  $R_2$ UHT) and direct ( $R_3$ UHT) systems were investigated during storage at 20° and 35°C. The changes in acidity, pH, fat separation, proteolysis, lipolysis (ADV), viscosity, sedimentation, milk reducing substances (MRS) and sensory characteristics were followed. All RUHT milk samples showed significant changes in most characteristics studied. The extent of changes increased with storage time and temperature. The rates of changes in acidity (T.A) and pH were similar.  $R_1$ UHT and  $R_2$ UHT milks showed lower fat separation which was probably due to higher homogenization efficiency and the quantity of the added stabilizer-emulsifier. The rate of changes in MRS, ADV and viscosity was lower in  $R_3$ UHT milk. However, sedimentation and proteolysis were lower in  $R_1$ UHT and  $R_2$ UHT milks (indirect process). In general, the differences in acceptability might be attributed to the extent of lipolysis and Maillard reaction in  $R_1$ UHT and  $R_2$ UHT milks. The results showed a gradual deterioration of the quality of RUHT milk on storage at high temperature. Therefore, RUHT milk should be stored in air-conditioned storage during the hot months of summer.

### **Introduction**

The dairy industry in Saudi Arabia has expanded significantly in the last fifteen years. Among the problems facing the dairy industry is their location in the far away regions from the dairy centers, which increased the transportation and refrigeration cost. The retailing regulations specify five days for pasteurized milk which results in significant amounts of returned milk. Available solution to these problems is the production of ultra high temperature treated (UHT) milk which offers a very flexible outlet for controlling these problems. UHT milk in Saudi Arabia is made from fresh, but mainly from recombined milk. The major problems facing UHT milk production and distribution are the changes in quality during storage.

The properties of UHT milk when it reaches the consumers are influenced by the effect of temperature. The characteristic of UHT milk that is most susceptible to change during storage is its flavor [1-3]. The effects may reduce the acceptability of the product. Two major defects associated with UHT milk are: cooked flavor followed by the development of the other flavor defects, and destabilization of milk protein resulting in gellation or the formation of fine precipitates [4].

The objectives of this study were to evaluate the quality of recombined ultra high temperature treated milk (RUHT) produced in Saudi Arabia and to follow the changes which result in quality deterioration during storage.

## Materials and Methods

### Milk samples

Samples (one liter brick packs) of RUHT milk were collected from three commercial plants on three occasions at one month intervals. The samples were taken within one week of production and then stored at 20 or 35°C. Sensory, chemical and physical analyses were performed after 0, 5, 10, 15, 20 and 25 weeks of storage.

### UHT milk processing

#### Plant No.1 (R<sub>1</sub> UHT)

R<sub>1</sub> UHT milk was made by reconstituting a mixture of 86% low heat skim milk powder (49.6% lactose, 37.8% protein, 7.8% minerals, 3.8% moisture and 0.8% fat) and 14% buttermilk powder (45% lactose, 34.1% protein, 10.7% fat, 7.1% minerals and 3.8% moisture). Stabilizer-emulsifier, Recodan RS (mono- and diglycerides, sodium alginate, carrageenan and guar gum, grindsted products A/S, Brabrand, Denmark) was added to the water (0.15% of total volume). Reconstituted milk was mixed with 3.0% melted fresh frozen milk fat (FFMF), homogenized (200 bars) and pasteurized at 73°C for 15 sec. It was then stored at 7°C until further processing. The recombined milk was treated in Steritherm (Alfa-laval, Turmba, Sweden), an indirect heating system at 137°C for 4 sec and homogenized after cooling to 76°C using a two stage (200 and 50 bars) aseptic homogenizer. RUHT milk was packaged aseptically immediately after cooling to 30-32°C.

#### Plant No.2 (R<sub>2</sub> UHT)

R<sub>2</sub> UHT milk was made by reconstituting low heat skim milk powder (50.0% lactose, 37.2% protein, 8.0% minerals, 3.8% moisture and 1.0% fat) at 35°C. Melted FFMF was mixed with the milk at 65°C. Then the milk was homogenized at 200 bars and pasteurized at 72°C for 15 sec. The pasteurized milk was kept at 6-7°C overnight (10-16 hr). Stabilizer - emulsifier, Recodan RS was mixed with the fat (0.2% of the total volume of milk). The recombined milk was treated in Steritherm (Alfa - laval, Tumba, Sweden), an indirect heating system at 137°C for 3 sec. Homogenization was carried out after cooling at 70°C via single stage at 150 bars using aseptic homogenizer. RUHT milk was packaged aseptically after cooling to 30°C.

### **Plant No.3 (R<sub>3</sub> UHT)**

R<sub>3</sub> UHT milk was made by reconstituting medium heat skim milk powder (49.8% lactose, 37.6% protein, 7.9% minerals, 3.7% moisture and 1.0% fat) at 30-35°C. Melted FFMF (3%) with added stabilizer emulsifier (0.15), Recondan RS was mixed with reconstituted milk at 65°C. The recombined milk was then homogenized at 150 bars and pasteurized at 72°C for 15 sec. Then it was kept at 5°C overnight. UHT treatment was carried out in a direct heating system at 140°C for 4 sec. The milk was homogenized after cooling to 75°C at 200 bars via a single stage aseptic homogenizer. RUHT milk was packaged aseptically after cooling to 30-32°C.

### **Sensory evaluation**

Milk samples were evaluated by five trained panelists. The color, flavor and overall acceptability of the samples were assigned a score on a descending nine points scale [5, p. 56], a score of 9 indicating the highest possible quality.

### **Physico-chemical properties**

Milk fat (Gerber), protein (Kjeldahl), ash, moisture, pH and titratable acidity were determined according to the Standard Methods for the Examination of Dairy products [6, p. 329]. Lactose content in UHT milk samples was calculated from protein content in the samples using the ratio of protein to lactose in milk powder.

### **Homogenization efficiency**

The homogenization pipet method (Schaap, J.G., Netherlands Institute of Dairy Research, The Netherlands, personal communication) was used. Twenty five ml of milk was centrifuged in specially designed pipet at 40°C and 1200 rpm for 30 min in a Gerber centrifuge. Homogenization efficiency was calculated as the percentage of fat content in the lower 20 ml to the original fat content of milk.

### **Fat separation**

To evaluate fat separation in milk samples, the fat separated from the lower portion (500 ml) of 1 liter packages was calculated as a percentage of the original fat content.

### **Proteolysis**

Proteolysis in milk samples was determined by two methods. Tyrosine value (mg tyrosine/100 ml of milk) was measured according to Hull method, as modified by Citti *et al.* [7], and free amino groups (FAG) were measured by trinitro benzene sulphonic acid method according to McKellar [8].

### **Lipolysis**

Lipolysis was determined by acid degree value, ADV [6, p. 327].

### Browning reaction

Extent of Maillard browning in milk samples was measured indirectly by the change in milk reducing substances (MRS) and was reported as absorbance by ferrocyanide produced from the reduction reaction of ferricyanide by the milk reducing substances according to the method of Abu-Lehia [9].

### Viscosity

Viscosity of milk samples was determined using a Brookfield viscometer (Brookfield Engineering lab. Inc., Storugton, MA, USA), model LVT using spendil ULA and adaptor UL at 60 rpm for 30 sec. at 20°C.

### Sedimentation

After emptying the milk packages, the amount of sediment was evaluated descriptively on a scale from 1 (low) to 5 (high).

### Statistical analysis

The values reported in the results are the averages of the values for the three samples per treatment. Analysis of variance (ANOVA), Duncan's multiple range test and the least significant difference (LSD) were done using the statistical analysis system (SAS) [10].

## Results and Discussion

### Recombined UHT milk composition

Table 1 shows the composition of RUHT milk. The total solids (TS) content were 11.01%, 11.15% and 11.37% for R1UHT, R2UHT and R3UHT milks, respectively. It was noticeable that fat and SNF contents were significantly ( $P = 0.05$ ) different for all three milk samples. Low fat and SNF in recombined milk products in Saudi Arabia have been reported [11]. In addition, all reported measures (Table 1). Conformed to the minimum requirements set by the Saudi Arabian Standards Organization (SASO) for UHT milk [12].

**Table 1.** Composition of recombined ultra-high temperature treated (UHT) milk

Plant	Water %	Fat %	Protein %	Lactose %	Ash %
R <sub>1</sub> UHT	89.06 a (+ 0.05)*	3.13 b (+ 0.05)	3.38 a (0.05)	4.44 ab (+ 0.07)	0.68 b (+ 0.06)
R <sub>2</sub> UHT	88.27 c (+ 0.38)	3.20 a (+ 0.08)	3.25 b (+ 0.08)	4.36 b (+ 0.11)	0.72 a (+ 0.03)
R <sub>3</sub> UHT	88.40 b (+ 0.10)	3.13 b (+ 0.05)	3.44 a (0.11)	4.56 a (+ 0.14)	0.73 a (+ 0.02)

Means in columns not followed by the same letter differ significantly ( $P < 0.05$ ).

\* Standard deviation

### Titratable acidity and pH

There were significant differences ( $P < 0.001$ ) in acidity (T.A) between all milk samples (Table 2). The rate of increase in the acidity of the samples stored at 35°C was higher than that at 25°C (Fig. 1). R<sub>2</sub>UHT milk showed the highest increased in T.A. at both temperatures and after all weeks of storage. In general the pH of all three milk samples decreased significantly after 5 weeks of storage at 2°C and 35°C.

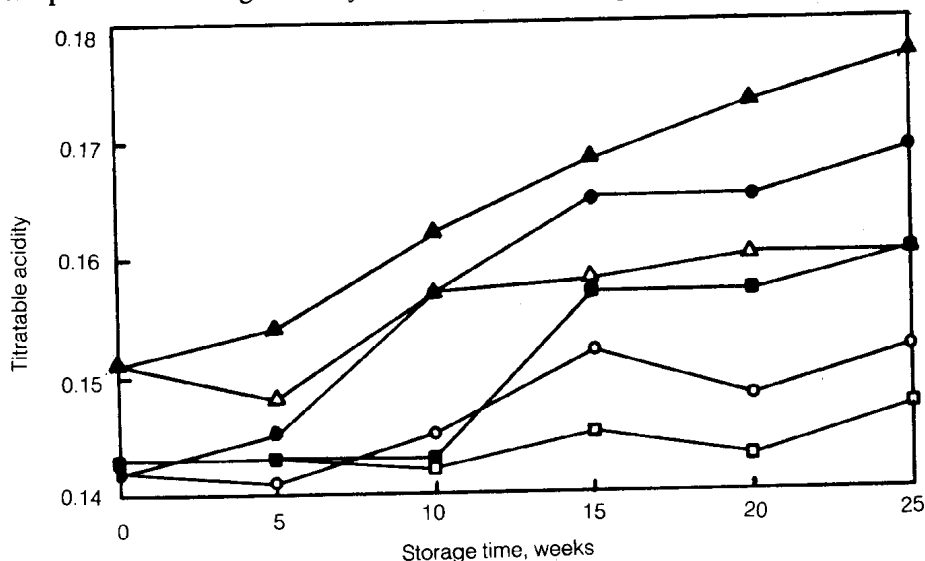


Fig. 1. Titratable acidity of recombined UHT (RUHT) milk during storage. R<sub>1</sub>UHT milk stored at 20°C □ or 35°C ■. R<sub>2</sub>UHT milk stored at 20°C △ or 35°C ▲. R<sub>3</sub>UHT milk stored at 20°C ○ or 35°C ●.

Table 2. Level of significance of the differences in quality parameters between Ultra high temperature treated (UHT) milk samples during storage.

Quality parameter	Plant	Sources			LSD**
		Samples	Storage temp	Storage time	
Titratable acidity	3	2	3	3	0.163
pH	3	3	3	3	0.048
Proteolysis					
Hull	2	2	3	3	0.122
TNBS	3	3	3	3	0.141
Milk reducing substances	3	3	3	3	0.147
ADV	3	NS	3	3	0.255
Viscosity	3	2	3	3	0.006
Fat separation	3	3	3	3	6.48
Sedimentation	3	NS	3	3	0.531
Color	NS	3	3	3	0.402
Flavor	NS	3	3	3	0.414
Acceptability	3	3	3	3	0.415

\* 1 =  $P < 0.05$

3 =  $P < 0.001$

2 =  $P < 0.01$

\*\* Least significant difference.

As shown in Fig. 2, the higher the storage temperature, the greater the fall in pH from the values of 6.80, 6.73 and 6.80 at the beginning of storage to pH values of 6.51, 6.44 and 6.49 for R<sub>1</sub>UHT, R<sub>2</sub>UHT and R<sub>3</sub>UHT milks, respectively. Andrew *et al.* [13] reported similar effects and suggested that the decrease in pH was due to the loss of positive charges on the protein molecule caused by the reaction of free E-NH<sub>2</sub> groups of lysine with lactose in Mailard-type reactions which were more likely to occur at higher temperature. Manji *et al.* [14] found that both direct and indirect processed milks caused change in pH during storage at high temperatures.

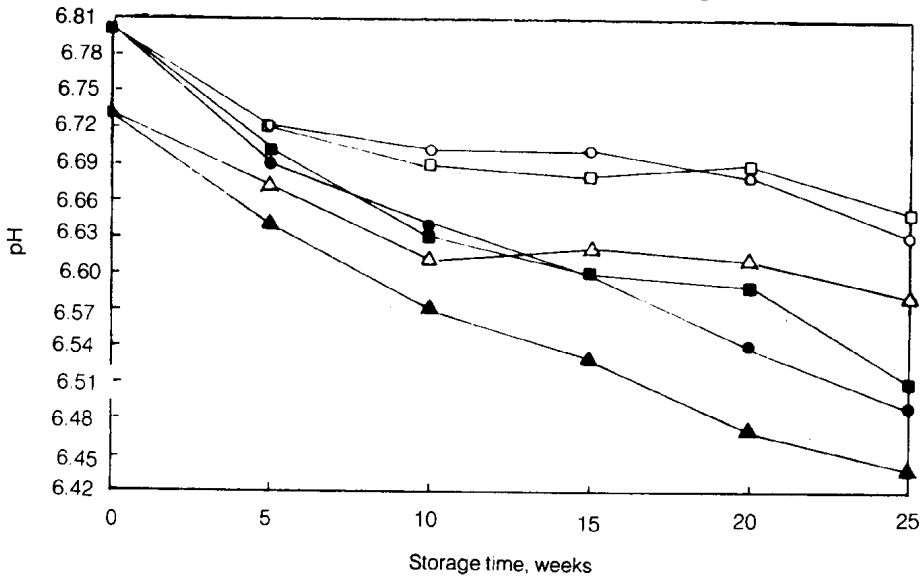


Fig. 2. pH of recombined (RUHT) milk during storage. R<sub>1</sub>UHT milk stored at 20°C □ or 35°C ■. R<sub>2</sub>UHT milk stored at 20°C △ or 35°C ▲. R<sub>3</sub>UHT milk stored at 20°C ○ or 35°C ●.

### Fat separation

Fat separation was zero at the beginning of storage in R<sub>2</sub>UHT and R<sub>3</sub>UHT milks, but it was 2.42% in R<sub>1</sub>UHT (Fig. 3). It was obvious that fat separation increased significantly ( $P < 0.001$ ) with time and temperature in all milk samples (Table 2). The homogenization efficiency were 93.3, 95.3 and 93.4 for R<sub>1</sub>UHT, R<sub>2</sub>UHT and R<sub>3</sub>UHT milks respectively. Efficient homogenization decreased fat separation which occurred in UHT milk on prolonged storage [15]. In addition to UHT processing, the only differences were in homogenization process and quantity of stabilizer-emulsifier added to UHT milk which resulted in different homogenization efficiency and fat separation.

As shown in Fig. 3, on prolonged storage R<sub>1</sub>UHT milk possessed the lowest fat separation and this might be due to the homogenization treatment (two stage, 200-50 bars) at the end of the processing sequence. Similar homogenization treatment was used for R<sub>2</sub>UHT and R<sub>3</sub>UHT milks, but R<sub>3</sub>UHT milk showed higher fat separation and this might be ascribed to the lower percentage of stabilizer-emulsifier which

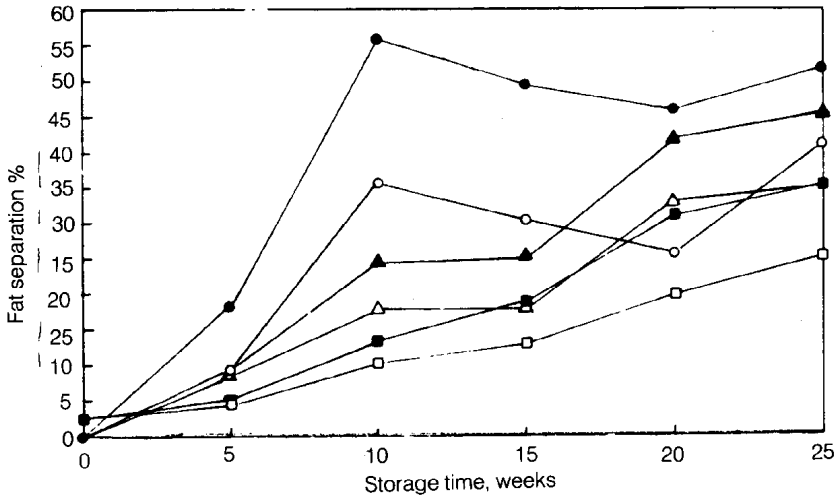


Fig. 3. Fat separation in recombined UHT (RUHT) milk during storage. R<sub>1</sub>UHT milk stored at 20°C □ or 35°C ■. R<sub>2</sub>UHT milk stored at 20°C △ or 35°C ▲. R<sub>3</sub>UHT milk stored at 20°C ○ or 35°C ●.

retard fat separation. According to Kohlman *et al.* [16] fat separation was increased in UHT milk with added plasmin (0.15mg/1) and was due to high proteolysis compared to UHT milk with no detectable proteolysis.

### Proteolysis

There were significant differences in the levels of proteolysis as measured by Hull and TNBS method. In the Hull methods tyrosine and tryptophan were measured but other phenols and reducing compounds present in milk also react with the Folin-phenol reagent [17, 18]. As shown in Tables 3 and 4, the Hull method gave higher values for proteolysis. The TNBS method measures free amino groups (FAG) [19], it gave lower values for proteolysis (Table 4) because some of the amino groups interact with lactose in the browning reaction [20].

The effect of storage time and temperature on the extent of proteolysis of the indirectly (R<sub>1</sub>UHT and R<sub>2</sub>UHT) and directly (R<sub>3</sub>UHT) processed milks are shown in Tables 3 and 4. The rate of proteolysis in all RUHT milk samples increased significantly when stored at 25°C and 35°C. However, proteolysis was greater in the R<sub>3</sub>UHT milk with relatively more proteolysis occurring at 35°C. The rate of proteolysis in R<sub>3</sub>UHT milk was higher due to the direct heating which gave a much more rapid rate of heating that was available with any indirect system [21]. The effect of time and temperature on the extent of proteolysis was in agreement with that found by Manji *et al.* [14] who found that level of plasmin was higher in UHT milk processed by direct heating. Shahin and Fahmy [22] observed significant increase in proteolysis in RUHT milk during storage. Proteolysis occurred in RUHT milk because proteases in milk powder might not be completely inactivated during processing [22] and proteases in UHT milk were not fully inactivated by UHT treatment [23, 8].

### Browning reaction

Maillard reaction is a complex series of reactions in which a condensation reaction first occurs between the carbonyl group of lactose and E-amino group of lysine then lead to the production of brown color of heated milk. In addition, it is well documented that the Maillard reaction occurs during processing and storage of milk powder [21], thus it was expected that RUHT would show high browning reaction. The MRS in  $R_1$ UHT and  $R_2$ UHT milks were higher than in  $R_3$ UHT milk (Fig. 4). MRS increased significantly with time and temperature of storage, however, the rate of increase was lower in  $R_3$ UHT milk. The differences between the samples might be ascribed to heating system. The direct heating gave much less chemical changes [21]. Malatji [14] found an increase in the concentration of hydroxymethyl furfural (HMF) in UHT milk stored at 30° or 40°C. During storage of UHT milk the Maillard reaction would proceed to an appreciable extent only when the storage temperature was above 20°C [20].

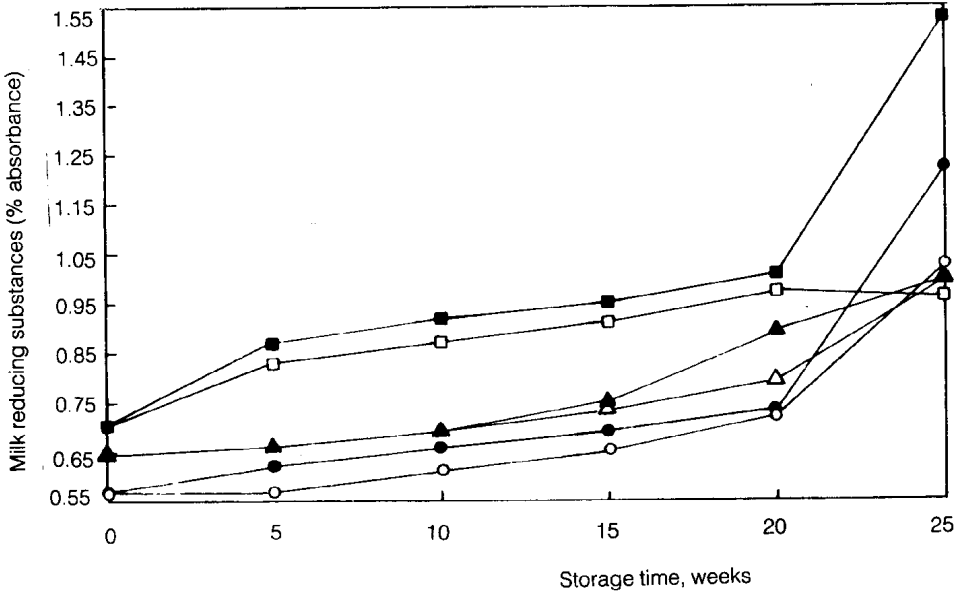


Fig. 4. Milk reducing substances (Measured as absorbance) in recombined UHT (RUHT) milk during storage.  $R_1$ UHT milk stored at 20°C □ or 35°C ■.  $R_2$ UHT milk stored at 20°C △ or 35°C ▲.  $R_3$ UHT milk stored at 20°C ○ or 35°C ●.

### Acid degree value (ADV)

When lipolytic activity occurs during storage of UHT milk the content of free fatty acids (FFA) increases inducing the development of flavors [20]. ADV of  $R_1$ UHT and  $R_2$ UHT milk samples were higher than that of  $R_3$ UHT milk and remained higher through storage (Fig. 5). The rate of ADV of all milk samples increased significantly with time and temperature of storage (Table 2). The increase

was more rapid at high (35°C) storage temperature than at low (20°C). The increase in the ADV of RUHT milk samples during storage were similar to that found by Earley and Hansen [1] for samples stored at 24°C or 40°C. However, Renner [20] Shahin and Fahmy [22] found high rate of lipolysis in RUHT milk, stored at 20°C or above.

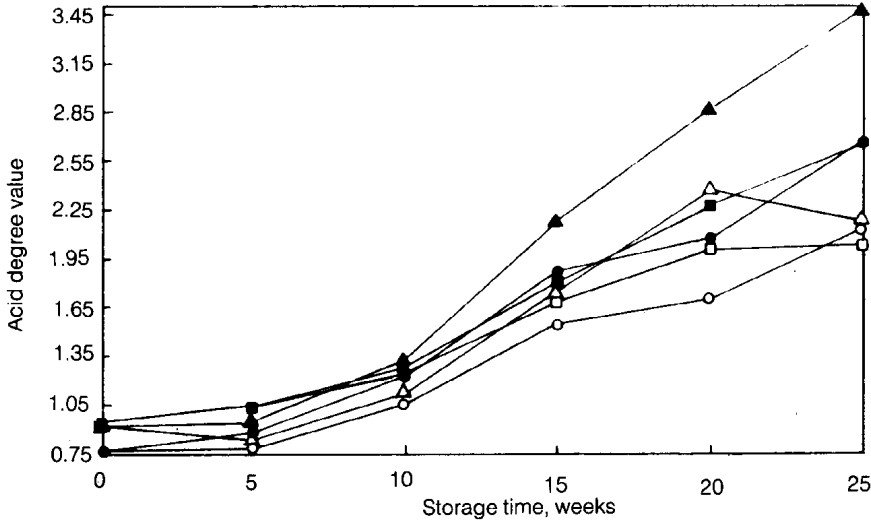


Fig. 5. Acid degree value (ADV) in recombined (RUHT) milk during storage. R<sub>1</sub>UHT milk stored at 20°C □ or 35°C ■. R<sub>2</sub>UHT milk stored at 20°C △ or 35°C ▲. R<sub>3</sub>UHT milk stored at 20°C ○ or 35°C ●.

### Apparent viscosity

The changes in apparent viscosity of RUHT milks are shown in Fig. 6. The initial viscosity of R<sub>1</sub>UHT, R<sub>2</sub>UHT milks were 2.52, 2.38 and 2.31 m Pa.s, respectively. The viscosity of all milk samples increased with time and at all temperatures. However, the increase was higher at high temperature (35°C). The increase in viscosity of UHT milk at high temperatures could be attributed to the effect of the increased Maillard reaction at high temperature [24] or to the proteolytic activity [16]. As shown in Fig. 4 the MRS in R<sub>2</sub>UHT milks were higher than R<sub>3</sub>UHT. Gellation was not observed and the highest viscosity (2.88 mPa.s) was for R<sub>1</sub>UHT milk stored at 35°C for 20 weeks. This might be attributed to the high initial apparent viscosity of R<sub>1</sub>UHT milk and to the raw materials used in the manufacture of R<sub>1</sub>UHT milk. The effect of heat on milk during drying and the changes during storage might increase the inherent viscosity of reconstituted powder [20].

### Sedimentation

The quantities of sediment obtained were very low in all milk samples. The temperature and time of storage affect the process of sedimentation significantly (Table 2). On a 5 point scale the sedimentation levels were 1.33, 1.00 and 2.00 for R<sub>1</sub>UHT, R<sub>2</sub>UHT, and R<sub>3</sub>UHT milks, respectively after 20 weeks of storage. Greater sediment

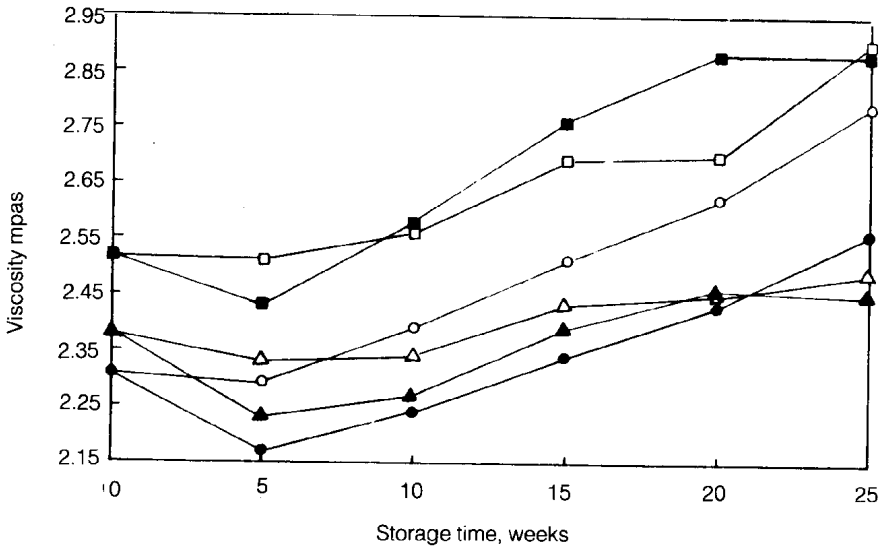


Fig. 6. Viscosity of recombinated (RUHT) milk during storage. R<sub>1</sub>UHT milk stored at 20°C □ or 35°C ■. R<sub>2</sub>UHT milk stored at 20°C △ or 35°C ▲. R<sub>3</sub>UHT milk stored at 20°C ○ or 35°C ●.

occurred in milk stored at 35°C than at 20°C. The observed sedimentation was higher with direct process which was in agreement with Ramsey and Swartzel [25] who found more sediment after direct processing. Sediment would be less after an indirect process than after a direct process, because of the differences in the amount of fouling material retained in the plant [21].

### Sensory evaluation

The shelf life of UHT milk is limited to the period in which it is of acceptable quality. It is well known that milk becomes browner with increased severity of heating. The results (Fig. 7) show that the organoleptic quality of all RUHT milk samples decreased with storage time and temperature. At the beginning of storage, R<sub>3</sub>UHT milk showed a lighter color and better flavor and was more acceptable than R<sub>1</sub>UHT and R<sub>2</sub>UHT milks. RUHT milks remained of acceptable quality during storage for 5 and 10 weeks at 35° and 20°C, respectively.

Cooked flavor is one of the characteristic of UHT milk. The rate of change in flavor are influenced by many variables, including properties of the milk, intensity of heat treatment, type of processing equipment and time and temperature of storage [15]. The differences in acceptability between RUHT milk samples might be ascribed to a higher concentration of lipolysis and Maillard reaction products in R<sub>1</sub>UHT and R<sub>2</sub>UHT milks. Increased levels of Maillard reaction products might cause undesirable flavor [20].

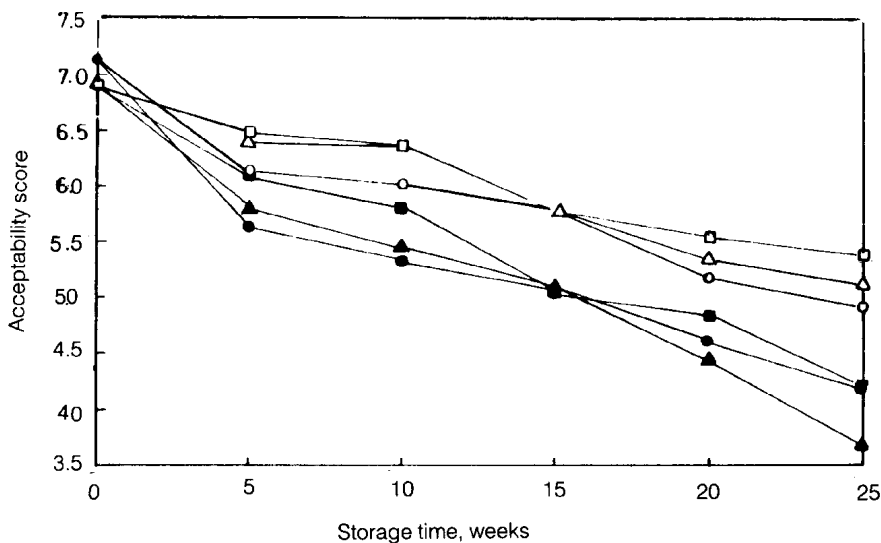


Fig. 7. Acceptability of recombined UHT (RUHT) milk during storage. R<sub>1</sub> UHT milk stored at 20°C □ or 35°C ■. R<sub>2</sub> UHT milk stored at 20°C △ or 35°C ▲. R<sub>3</sub> UHT milk stored at 20°C ○ or 35°C ●.

In general, all milk samples showed significant changes in chemical, physical and sensory characteristics during storage. However, these changes were more pronounced at 35°C than at 20°C. All RUHT milk samples had high level of ADV, MRS and proteolysis. R<sub>3</sub> UHT milk (direct process) had the lowest ADV, MRS and higher acceptability than R<sub>1</sub> UHT and R<sub>2</sub> UHT milks (indirect process).

To decrease fat separation in RUHT milk, homogenization efficiency should be improved and stabilizer-emulsifier should be added to reduce fat separation. During summer months, the decrease in quality of RUHT milk can be overcome by storing RUHT milk in air-conditioned stores. More research is needed to improve the quality of RUHT milk stored at high ambient temperature.

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## التغيرات في جودة الحليب المعاد تجميعه المعامل بالحرارة الفائقة بالطريقة المباشرة وغير المباشرة خلال التخزين

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ملخص البحث. درست التغيرات في جودة الحليب المعاد تجميعه المعامل بالحرارة الفائقة بالطريقة غير المباشرة (المصنع الأول والثاني) والمباشرة (المصنع الثالث) خلال التخزين عند ٢٠ و ٣٥ م. تم تتبع التغيرات في الحموضة المعايرة والأس الهيدروجيني وانفصال الدهن وتحلل البروتين وتحلل الدهن (الدرجة الحامضية للدهن) واللزوجة والترسبات والمواد المختزلة في الحليب والصفات الحسية. وقد أظهرت جميع العينات تغيرات مهمة في معظم الصفات المدروسة وقد ازداد مدى التغيرات مع زيادة زمن ودرجة حرارة التخزين. وكانت درجة الزيادة في الحموضة المعايرة والأس الهيدروجيني متشابهة. وأظهرت عينات المصنع الأول والثاني مقداراً أقل من انفصال الدهن وهذا قد يرجع إلى زيادة كفاءة التجنيس وكمية المواد المثبتة - المستحلبة المضافة. وكانت درجة التغير في المواد المختزلة في الحليب ومقدار الدرجة الحامضية للدهن واللزوجة أقل في عينات المصنع الثالث، ولكن درجة الترسبات والتحلل البروتيني كانت أقل في عينات المصنع الأول والثاني. بشكل عام يمكن أن تعزى الفروق في درجة القبول للعينات إلى مدى التحلل الدهني وتفاعل ميلارد (تفاعل الإسمران) في عينات المصنع الأول والثاني وتظهر النتائج تدهوراً تدريجياً لجودة الحليب المعاد تجميعه المعامل بالحرارة الفائقة خلال التخزين عند درجة حرارة عالية ولذلك يجب حفظ هذا النوع من الحليب في مخازن مكيفة خلال أشهر الصيف الحارة.