

Effect of Milk Quality and Stabilizers on Some Physicochemical Properties of UHT-Milk

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ABSTRACT

Milk quality and stabilizers were investigated to reverse their effects on undesirable changes in sensory of UHT milk as influenced by storage at 25°C or 37°C. The obtained results showed that the total microbial count, psychrophilic bacteria, mesophilic & thermophilic spore forming bacteria were higher in grade B (GB) milk than in grade A (GA) milk. Seven treatments were carried out with GA, GB, reconstituted full cream milk powder (FCMP), FCMP mix with GA and GB milk, meanwhile, two commercial stabilizers were separately evaluated. Mesophilic and thermophilic spore forming bacteria exhibited high scores and were found in standardized pasteurized GB milk comparing with GA milk. The type of stabilizer has no any effect on the UHT milk quality. The treatments of UHT milk made using GB milk received low levels of sensory properties (color & flavor), as well as, fat separation and sedimentation could be noted during storage when compared to that made from GA milk or FCMP. The fat separation and sedimentation were more pronounced in treatments stored at 37 °C when compared to that stored at 25°C. Results of SDS-PAGE and RP-HPLC did not show any significant differences among all treatments in proteolysis during storage period. Accordingly, the fat separation and sedimentation were not related only to proteolysis but were related to quality of milk used in processing.

Key words: UHT milk, sedimentation, fat separation, milk stabilizers.

INTRODUCTION

The manufacture of almost all types of milk and its products involves one or more heat treatments. The aim of heat treatments is to kill microorganisms and inactivate enzymes “partially or fully” dependent on type of heat treatment. This is done to secure safety of consumer along with extension of shelf life of the dairy product. Heat treatment can, however, also cause certain undesirable changes like production of brown pigments, development of cooked flavour and loss of nutrients (Walstra *et al.*, 1999, Kessler 2002). UHT treatment is a technological process used to produce drinking milk that is microbiologically safe and its shelf life ranges between 6-12 months at room temperature and stability of casein micelles during their storage (Deeth, 2010, Baglinière *et al.*, 2012). Normally, UHT treatment is carried out at a range of 135–150°C for 1–10 s as a holding time required to achieve ‘commercial sterility’ (Chen *et*

al., 2015). UHT milk is stable for long-term storage at ambient temperatures if microbiological sterility has been achieved by the UHT treatment and maintained by aseptic packaging (Kelly & Fox 2012). The UHT milk processed by indirect systems show low sediment formation than its counterpart from direct systems, but sedimentation increased with elevating heat treatment and temperature of storage in direct UHT-milk (Datta, *et al.*, 2002). Age gelation is a main factor reduces the shelf-life of UHT milk. It can be explained by a two-stage process involving formation of a β -lactoglobulin-k-casein complex during heating which cross-links after partial or complete release from the micelle of casein to forming a protein network gel. Proteolysis, by native milk proteinase (plasmin) or bacterial proteinases, increase gelation as a result of facilitating release of the complex from the micelle of casein-. Plasmin is sufficiently heat-stable enzyme to play a key role in term of age gelation of UHT milk, especially with direct UHT processing

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(McMahon, 1996). The activators of plasmin are not affected by pasteurization and are only slightly inactivated by ultra-high temperature processing conditions (140°C/ 32 s) (Deharveng & Nielsen 1991). Addition of polyphosphates retard gelation by inhibiting formation of the protein network. Gelation can be minimized by using high quality (low somatic cells and bacterial count) raw milk, inactivating proteinases, increasing the severity of heat treatment, storing the UHT milk at temperatures lower or higher than room temperature, and or adding polyphosphates (Datta & Deeth, 2001). UHT-milk proteolysis causes the development of bitter flavors and leads results in increase the viscosity, with formation a gel. These changes are caused, or at least accelerated, by hydrolysis of caseins, releasing the β -lactoglobulin-k-casein complex (β k-complex) from the micelle. The released protein complex aggregates and forms a three-dimensional network of cross-linked proteins to cause a formation a gel (McMahon, 1996).

The UHT milk demand is increasing worldwide. It possesses many advantages regarding milk include distribution and storage at ambient temperature without needs cooling system. But undesirable changes could be occurred in UHT milk such as sedimentation, gelation, fat separation, cooked flavor and browning to limit its shelf life. In Egypt, about 20% of milk is produce from animals under good hygienic conditions. Milking machine is used, and the milk is cooled directly after milking, this milk is classified under grade A milk. The other 80% are produced from small herds composed of 1 to 5 lactating animals milked tow times a day. The obtained milk contains microorganisms' extremely high numbers due to poor hygienic practices during hand milking in the outdoors and milk handling practices, this milk is classified under grade B milk. Notwithstanding, raw milk used in processing UHT milk in Egypt is insufficient. So, reconstituted milk is used at 100% or mixed with raw milk. The present study aimed to evaluate the effect of milk quality on physicochemical and sensorial properties of UHT milk produced under the common conditions.

MATERIALS AND METHODS

Materials

Raw cow's milk GA was obtained from the good dairy farm located in the governorate of Gharbia, Egypt, while raw cow's milk GB was obtained

from the milk collection center located in the governorate of Gharbia, Egypt. Full fat milk powders (FFMP) and skimmed milk powders (SMP) were obtained from Fonterra Company, New Zealand. Super Midagel "milk stabilizer" contains mono & di-glyceride of fatty acids was obtained from Misr Food Additives (MIFAD) Company, Cairo. Lacta 760 R "milk stabilizer" contains mono & di-glyceride of fatty acids and carrageenan was obtained from Misr Food Additives (MIFAD) Company, Cairo. Trifluoroacetic acid for HPLC grade and Acetonitrile for HPLC grade were obtained from SDS, Peypin, France and Scharlau, Barcelona, Spain, respectively. Acrylamide (2X) was obtained from SERVA Feibiochemia, New York, USA. Methanol and TMED were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ammonium persulphate and Glycine were obtained from Oxford, India.

Methods

Experimental and UHT- milk processing

Raw milks either GA, GB were passed throw *Bactofuge (Tetra Pack)* at 60°C, then standardized to 3.2% fat and 8.5% SNF by adding SMP, while 100% reconstituted milk was prepared by reconstitution of FFMP at level of 12.5% to get 3.2% fat and 8.5% SNF. Some treatments of mixing raw milk and milk powder were conducted by mixing standardized raw milk and reconstituted milk at level of 1:1. The milk stabilizers were added at level of 125 g/ton for Super Midagel and 700 g/ton for Lacta 760R (Table 1). All standardized milks were pasteurized at 75°C/15 sec and kept at 4°C until UHT processing in not more than 30 hr.

UHT of milk was processed using direct heating (infusion, APV, USA) at 142°C for 6 sec and

Table 1: Mixed treatments to produce UHT milks

No.	Treatments	Stabilizer
1	Grade A Raw milk (100%)	Midagel
2	Grade B Raw milk (100%)	Midagel
3	Full fat milk powder (100)	Midagel
4	50% GA + FFMP	Midagel
5	50% GB + FFMP	Midagel
6	50% GA+ FFMP	Lacta
7	50% GB + FFMP	Lacta

GA: Refer to milk which grade A, **GB:** Refer to milk which grad B, **FFMP:** refer to full fat milk powder

homogenized at 200 Pa. UHT milk was packed in tetra pack paper under aseptic conditions.

Chemical analysis of UHT product

Fat, protein, lactose and SNF contains were determined using Milko Scan FOSS FT2 (FOSS, Denmark). The pH value was determined using a digital pH meter (Mettler Toledo 320) at room temperature ($20\pm 1^\circ\text{C}$) according to the AOAC (2007). The Titratable acidity was determined according to the AOAC (2007).

Rheology analysis of UHT milk

Viscosity was measured using oscillatory viscometer (VR 3000M YR Viscometers, Spain), using spindle 1 at speed of 60 rpm at 10°C .

Microbiological analysis

Total viable count

All samples of milk were serially diluted in peptone saline solution (0.85 %). The enumerations were done on nutrient agar (NA) medium. Plates were incubated at 30°C for 48 hr under aerobic conditions (ISO 4833: 2003).

Aerobic spore-forming bacteria

Typical spore count tests involve the milk heating samples at 80°C for 10 min then cooled suddenly to the room temperature before transferring one ml aliquots into petri dishes. The enumerations were done on plate count agar. The plates were incubated at $32^\circ\text{C}/48\text{hr}$ (Standard Methods for the Examination of Dairy Products 2010).

Thermo-Spore Forming bacteria

The enumeration of colony-forming units (CFU) of resistant spores of thermophilic bacteria in UHT milk samples by using a colony-count technique at 55°C for 72 hr after heating the sample at 106°C for 10 min (ISO/TS 27265:2009).

Psychrophilic bacteria count

The enumerations were done on nutrient agar (NA) medium. Plates were incubated at 7°C for 7 days.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE (12.5%) technique was conducted using the discontinuous buffer system described by Laemmli (1970), and mentioned by Hames & Rickwood (1990). The data were analyzed by total lab software (V1.11). To extract the total milk proteins, 25 ml of cold ace-

tone (stored overnight at -20°C) were added to 5 ml of milk and stirred for 15 min by magnetic stirrer, then filtrated through filter paper and dried. While, about 5 g of milk gel that found at inner surface of the package wall was used of extraction to present the gel proteins.

Preparation of UHT milk extracts (12% TCA)

Soluble extracts (12% TCA) of UHT milk were prepared by adding TCA (24%) to an equal volume of milk and mixing by vortexing for 5 min and then the mixture keeping at room temperature (30°C) for 60 min. The mixture was centrifuged at 8,000 xg for 25 min at 4°C and the supernatants were filtered through filter paper Whatman 102 (Datta & Deeth 2003).

Analysis of peptides by RP-HPLC

A HPLC system (Agilent Technologies 1260) at 40°C and a binary solvent gradient system at a flow rate of 1 ml/min and detection at 210 nm to analyse UHT milk peptides according to the method described by Datta & Deeth (2003). Solvent A was 0.1% of trifluoroacetic acid in water (v/v) and solvent B was 0.1% of trifluoroacetic acid in acetonitrile (v/v). The proportion of solvent B was increased from 20% to 35% during the first 20 min and after 5 min raised to 65% in 20 min and finally to 100% in 5 min. Samples were filtered through a 0.2- μm membrane filter (Millipore Corp., Bedford, MA). Injections of 50 μl of filtrates samples were made by auto injector. Between samples the column was washed by increasing solvent B to 65% over 15 min and holding for 15 min and returning to 100% solvent A.

Sensory evaluations

UHT milk samples were evaluated for sensory quality during the storage period at 0, 30, 60 and 90 days by a panel of 10 members of researchers and postgraduate students at Dairy Science and Technology Department, Faculty of Agriculture (El-Shatby), Alexandria University. Samples were scored on a hedonic scale of 0-5 for colour, taste, fat separation and overall acceptability, according to the recommended protocol proposed by the ISO 22935-2:2009 (IDF 99-2:2009) for the sensory evaluation of milk products..

Statistical analyses

Data were subjected to analysis of variance by using Statistical Package for the Social Sciences (SPSS) program version 21.

RESULTS AND DISCUSSION

Physicochemical and microbiological properties of raw ingredients

The results in Table (2) showed that GB milk was significantly ($P \geq 0.05$) higher than GA milk in protein and SNF contents. There was no significant ($P \leq 0.05$) difference between GA and GB in fat%. While the pH was significantly ($P \geq 0.05$) lower in GB than GA. Meanwhile, both milks (GA and GB) were negative for 75% Ethanol stability and the both milks were stable for UHT processing. There was a significant ($P \geq 0.05$) difference between GA and GB milks in total bacterial count, spore forming count and Psychrophilic bacteria (Table 3). Spore forming micro-organisms, mainly *Bacillus* spp caused microbial spoilage of heat treated milk (Mayr *et al.*, 2004). So, raw milk grade affected on the microbial quality of used milk for processing UHT milk. The Physicochemical and microbiological specifications of GA milk were within the rang recommended by Egyptian Standard (2010), while the physical, chemical specification of GB milk were within the rang recommended by Egyptian Standard (2010) but it has higher level of total bacterial count (Table 2 and 3). Egyptian Standard (2010) recommended the fat and SNF should not

less than 3 and 8.25% respectively, and the total bacteria count should be less than 300,000 CFU/ml. The microbiological and chemical analyses of milk powder were within the range that recommended by Codex Alimentarius (2007) and Egyptian standard (2005). It's recommended the protein in dry basis should be not less than 34%, moisture not higher than 5%, and the total bacterial count should be less than 10,000 CFU/g. The stabilizers that used in the present study have a good microbiological quality.

Physicochemical and microbial analysis of milk and milk mixtures before UHT process (after standardization, pasteurization) and after UHT process.

The results of physicochemical properties of milk after pretreatments (standardization, pasteurization) and after UHT process (Table 4) showed that there were no significant ($P \geq 0.05$) differences could be figured out in fat, lactose, SNF and acidity % among all treatments after standardization, pasteurization of milk. After UHT process, protein and fat contents were significantly, ($P \leq 0.05$) decreased when compared to the values before UHT process in the same treatments. The obtained results not agree with that reported by Burton (1994), who stated that during UHT milk production, the fat in milk does

Table 2: Physicochemical analyses of raw ingredients

Raw ingredients samples	Mean*				
	pH value	75% Ethanol stability	Fat %	Protein ($N \times 6.38$) %	SNF %
Raw milk GA	6.74 ^c ± 0.01	-	3.63 ^b ± 0.01	3.32 ^a ± 0.01	8.72 ^a ± 0.01
Raw milk GB	6.72 ^b ± 0.01	-	3.74 ^b ± 0.02	3.34 ^b ± 0.05	8.84 ^b ± 0.02
Full fat milk powder	6.68 ^a ± 0.01	-	25.8 ^c ± 1.81	24.37 ^c ± 0.01	71.1 ^c ± 0.1
Skimmed milk powder	6.67 ^a ± 0.01	-	0.54 ^a ± 0.01	33.78 ^d ± 0.03	95.67 ^d ± 0.01

*Means with the same letter(s) in the same column are not significant, but different letters are significant ($P < 0.05$).

Table 3: Microbiological analysis of raw ingredients

Raw ingredients samples	Mean of Microbial count (\log_{10} cfu)			
	Total bacterial count	Meso-Spore Forming bacteria	Thermo-Spore Forming bacteria	Psychrophilic bacteria
Raw milk GA	4.67d	2.08a	1.30a	2.90a
Raw milk GB	6.54e	2.86e	1.73d	4.04b
Full fat milk powder	3.41b	2.54c	2.07e	N.D.
Skimmed milk powder	3.57c	2.67d	1.81d	N.D.
Super Mida gel	3.08a	2.14a	1.32b	N.D.
Lacta 760 R	3.38b	2.39b	1.54c	N.D.

*Means with the same letter(s) in the same column are not significant, but different letters are significant ($P < 0.05$).

Table 4: Physicochemical analysis of milk before and after UHT process

Milk Sample NO.	Protein% before	After	Fat%		Lactose%		SNF%		pH		Acidity%	
			before	after	before	after	before	After	before	after	before	after
1	3.2 ^{cde} AB	3.1 ^{2a} AB	3.29 ^{bcd} A	3.20 ^{ab} A	4.75 ^c A	4.64 ^{abcd} A	8.73 ^{ca}	8.60 ^{ab} AB	6.73 ^{cde} AB	6.71 ^{bcd} B	0.152 ^{abc} A	0.15 ^{ab} A
2	3.16 ^{bca}	3.08 ^a A	3.27 ^{abcd} A	3.19 ^{ab} A	4.67 ^{bcd} A	4.57 ^{ab} A	8.71 ^{ca}	8.56 ^{ab} A	6.71 ^{bca}	6.68 ^{aa} A	0.153 ^{bca}	0.151 ^{abca}
3	3.25 ^e AB	3.15 ^{bcd} B	3.34 ^{da}	3.25 ^{abc} A	4.74 ^{de} A	4.64 ^{abcd} A	8.72 ^{ca}	8.58 ^{ab} AB	6.72 ^{bcd} AB	6.71 ^{bcb}	0.152 ^{abc} A	0.149 ^{aa}
4	3.25 ^e B	3.16 ^{bcd} B	3.32 ^{cd} A	3.19 ^{ab} A	4.74 ^{de} A	4.62 ^{abc} A	8.74 ^{ca}	8.61 ^{ab} AB	6.74 ^{cb}	6.72 ^{bcd} B	0.151 ^{abc} A	0.15 ^{ab} A
5	3.203 ^{cde} AB	3.13 ^{abc} AB	3.31 ^{bcd} A	3.23 ^{ab} A	4.68 ^{cde} A	4.56 ^{aa}	8.70 ^{ca}	8.55 ^{aa}	6.73 ^{deb}	6.71 ^{bcd} B	0.154 ^{ca}	0.152 ^{abc} A
6	3.21 ^{de} AB	3.12 ^{ab} AB	3.30 ^{bcd} A	3.21 ^{ab} A	4.68 ^{cde} A	4.58 ^{abc} A	8.71 ^{ca}	8.59 ^{ab} AB	6.73 ^{cde} AB	6.70 ^{bb}	0.152 ^{abc} A	0.151 ^{abca}
7	3.23 ^e AB	3.15 ^{bcd} B	3.29 ^{bcd} A	3.19 ^{ab} A	4.71 ^{de} A	4.64 ^{abcd} A	8.75 ^{ca}	8.62 ^{bb}	6.73 ^{deb}	6.71 ^{bcd} B	0.150 ^{ab} A	0.149 ^{aa}

1: UHT milk made from grade A raw milk 100% by using Midagel stabilizer; 2: UHT milk made from grade B raw milk 100% by using Midagel stabilizer; 3: UHT milk made from full fat milk powder (FFMP) 100% by using Midagel stabilizer; 4: UHT milk made from grade A raw milk 50% + 50% FFMP by using Midagel stabilizer; 5: UHT milk made from grade B raw milk 50% + 50% FFMP by using Midagel stabilizer; 6: UHT milk made from grade A raw milk 50% + 50% FFMP by using Lacta stabilizer and 7: UHT milk made from grade B raw milk 50% + 50% FFMP by using Lacta stabilizer

* Means with the same small letter(s) are not significant between before and after UHT process in the same treatment, but different letters are significant ($P < 0.05$).

Means with the same capital letter(s) in the same column are not significant among treatments, but different capital letters in the same column are significant ($P < 0.05$).

not change physically or chemically, so it cannot have adverse nutritional consequences. Such an effect could be related to the direct heating used in the present study rather than indirect heating used by Burton (1994). In addition, no significant ($P \leq 0.05$) differences were found in fat %, lactose % and acidity % after UHT process among all treatments. From these results, it can be concluded that milk or stabilizer types have no any effect on the physical and chemical properties of UHT milk products.

The source of raw milk used in this study has a significant ($P \leq 0.05$) effect on the count of mesophilic and thermophilic spore forming bacteria (Table 5). In general, all treatments used GB, either 100% or 50%, have significant ($P \leq 0.05$) high level of mesophilic and thermophilic spore forming bacteria when compared with treatments used GA milk. Sample 7 (50% GB with lacta) has the highest count of mesophilic spore forming bacteria but sample 4 (50% GA with super medagel) has the lowest count. The log counts of thermophilic spore forming bacteria were 1.86 in sample 3 (100% full fat milk powder). Whereas the log counts of thermo-spore forming bacteria in sample 1 (100% milk grade A with super medagel) was 1.61. No significant ($P \geq 0.05$) differences were found between sample 2 (milk grade B with super medagel), sample 5 (50% GB with super medagel) and sample 7 (50% GB with lacta) in mesophilic and thermophilic - spore forming bacteria count. Stabilizer and full fat milk powder have no effect on the milk quality. Using of milk grade B and lacta stabilizer in mixture cause not significantly ($P \geq 0.05$) increasing in mesophilic

and thermophilic spore forming bacteria. The only factor effects the microbial quality of milk samples was raw milk grade. After UHT process, all samples were sterilized and there were no any microbiological defects after streak plates from incubated samples for 5 days at 32 or 55°C, the pH was not changed in samples incubated for 8 days at 32 or 55°C. These results recommended that the mesophilic and thermophilic spore forming bacteria (max log 2.63 of mesophilic and log 1.9 of thermophilic) in used milk has no any effect on sterility of product by direct heating at 142°C for 6 sec.

Changes of sensory evaluation and some physicochemical properties of UHT milk during storage

The changes of fat separation, gelation, color, flavor, pH and viscosity during the storage at ambient temperature showed significant ($P \geq 0.05$) differences among samples made from Grade A milk (sample 1 and 6) and Grade B milk (sample 2 and 7) in sensorial evaluation (Table 6). There are no significant ($P \geq 0.05$) differences among samples made from GA milk and reconstituted milk (1:1) and GB milk and reconstituted milk (1:1) using Medagel stabilizer in all sensorial evaluation expect flavour. The sedimentation was not noticed in samples made from GA milk during the 90 days of storage, but it was noticed in samples made from GB milk after only 30 days and the sedimentation increased during storage. The sedimentation was noticed after 90 days of storage in samples made from 100% reconstituted milk or GA and reconstituted milk (1:1) with Lacta stabilizer, but it was noticed after 30 days in samples made from 100% GB or GB and reconstituted milk (1:1).

The behavior of fat separation was similar with sedimentation as when the sedimentation increased or noticed the fat was also separated. The sedimentation and fat separation were affected by source of milk used in this study. There was a reduction in acceptability of colour in all treatments, but the reduction was much noticed in samples made from GB milk than in those made from GA milk. There were no significant differences ($P \geq 0.05$) in flavour acceptability in fresh samples made from GA or GB milk, but the flavor acceptability was lower after 30 days till 90 days in samples made from GB milk than in samples made from GA milk. Milk made by 100% reconstituted milk or mixing of reconstituted milk with raw milk received low score of flavour acceptability when compared to samples made from

Table 5: Microbiological analysis of milk after pre-treatments (after standardization, pasteurization)

Milk Samples NO.	*Mean of Microbial count (\log_{10} cfu)	
	Meso-Spore Forming bacteria	Thermo-Spore Forming bacteria
1	2.28 ^a	1.61 ^{ab}
2	2.56 ^{ab}	1.84 ^{bc}
3	2.62 ^b	1.90 ^c
4	2.25 ^a	1.59 ^a
5	2.49 ^{ab}	1.84 ^{bc}
6	2.50 ^{ab}	1.86 ^c
7	2.63 ^b	1.83 ^{bc}

*Means with the same letter(s) in the same column are not significant, but different letters are significant ($P < 0.05$)

Table 6: Sensory evaluation of UHT milk during storage at ambient temperature

UHT Milk samples	Time of storage (days)	Sensory evaluation				
		Sedimentation (5 degrees)	Fat separation (3 degrees)	Colour (5 degrees)	Flavour (5 degrees)	Appearance (5 degrees)
1	0	0 ^a	0 ^a	4.76 ^p	5 ^e	4.83 ^h
	30	0 ^a	0 ^a	4.59 ^{no}	5 ^e	4.79 ^h
	60	0 ^a	0 ^a	4.40 ^{jk}	5 ^e	4.70 ^h
	90	0 ^a	0 ^a	4.36 ^{hij}	4.36 ^{cd}	4.36 ^g
	Mean	0 ^D	0 ^E	4.50 ^A	4.84 ^A	4.67 ^A
2	0	0 ^a	0 ^a	4.32 ^{ghi}	5 ^e	4.66 ^h
	30	1 ^b	1 ^b	4.08 ^e	4 ^{bc}	3.54 ^c
	60	2 ^c	2 ^c	3.95 ^{cd}	4 ^{bc}	2.975 ^b
	90	2 ^c	3 ^d	3.89 ^{bc}	3 ^a	2.445 ^a
	Mean	1.25 ^A	1.5 ^A	4.06 ^E	4 ^D	3.405 ^D
3	0	0 ^a	0 ^a	4.58 ^{no}	4 ^{bc}	4.29 ^g
	30	0 ^a	0 ^a	4.45 ^{kl}	4 ^{bc}	4.225 ^{fg}
	60	0 ^a	1 ^b	4.24 ^f	3 ^a	3.62 ^{cd}
	90	1 ^b	1 ^b	3.84 ^{ab}	3 ^a	2.92 ^b
	Mean	0.25 ^C	0.5 ^D	4.27 ^C	3.5 ^E	3.76 ^C
4	0	0 ^a	0 ^a	4.49 ^{lm}	5 ^e	4.74 ^h
	30	1 ^b	1 ^b	4.26 ^{fg}	4.5 ^{cde}	3.88 ^{de}
	60	1 ^b	1 ^b	4.01 ^{de}	4.5 ^{cde}	3.75 ^{cde}
	90	2 ^c	2 ^c	3.82 ^{ab}	4 ^{bc}	2.91 ^b
	Mean	0.916 ^B	1.083 ^C	4.14 ^D	4.5 ^B	3.82 ^C
5	0	0 ^a	0 ^a	4.53 ^{mn}	5 ^e	4.76 ^h
	30	1 ^b	1 ^b	4.31 ^{ghi}	4 ^{bc}	3.82 ^{cde}
	60	1 ^b	2 ^c	4.01 ^{de}	4 ^{bc}	3.50 ^e
	90	2 ^c	2 ^c	3.79 ^a	4 ^{bc}	3.06 ^b
	Mean	0.83 ^B	1.1667 ^C	4.16 ^D	4.25 ^C	3.78 ^C
6	0	0 ^a	0 ^a	4.620 ^p	5 ^e	4.81 ^h
	30	0 ^a	0 ^a	4.50 ^{lm}	5 ^e	4.75 ^h
	60	0 ^a	0 ^a	4.38 ^{ijk}	5 ^e	4.69 ^h
	90	1 ^b	0 ^a	4.29 ^{fgh}	4.33 ^{cd}	3.81 ^{cd}
	Mean	0.25 ^C	0.083 ^E	4.45 ^B	4.83 ^A	4.51 ^B
7	0	0 ^a	0 ^a	4.45 ^{kl}	5 ^e	4.72 ^h
	30	1 ^b	1 ^b	4.26 ^{fg}	4.66 ^{de}	3.96 ^{ef}
	60	1 ^b	2 ^c	4.02 ^{de}	4.33 ^{cd}	3.67 ^{cde}
	90	2 ^c	2 ^c	3.83 ^{ab}	3.66 ^b	2.91 ^b
	Mean	0.916 ^B	1.33 ^{AB}	4.14 ^D	4.41 ^{BC}	3.82 ^C

1:UHTmilk made from grade A raw milk 100% by using Midagel stabilizer;2: UHTmilk made from grade B raw milk 100% by using Midagel stabilizer; 3: UHTmilk made from full fat milk powder (FFMP) 100% by using Midagel stabilizer ; 4: UHTmilk made from grade A raw milk 50% + 50% FFMP by using Midagel stabilizer; 5: UHTmilk made from grade B raw milk 50% + 50% FFMP by using Midagel stabilizer;6: UHTmilk made from grade A raw milk 50% + 50% FFMP by using Lacta stabilizer and 7: UHTmilk made from grade B raw milk 50% + 50% FFMP by using Lacta stabilizer

Means with the same letter(s) in the same column are not significant, but different letters are significant (P <0.05).

Capital letters refer to differences among treatments, Small letters: refer to differences among storage times in the same column.

raw milk. The milk stabilizers used in this study has no effect on color and flavor acceptability of final UHT milk's made from GB milk. Using of lacta stabilizer has improvement flavor and color acceptability of samples made from GA and reconstituted milk (1:1). The change of UHT milk sensorial during storage referred to some reaction such as Maillard reaction, proteolytic activity (Renner 1988; Valero *et al.*, 2001), lipid oxidation (Contarini *et al.*, 1997). Thermal treatments of UHT milk cause aggregation of protein, fat and inorganic salts. These aggregates are sediment or/and clump on the surface of packaging. The sediment depends on some factors such as quality of raw milk, type and temperature/time of heat treatment, homogenizing pressure, homogenizer position and storage temperature. Sedimentation could be created after processing and during storage. Datta, *et al.* (2002) reported that direct UHT heating system usually case more sedimentation when compared to indirect heating system. Perkin, *et al.* (1973) reported that directly heated of UHT milk produced tow folds as much as sediment of indirectly processed milk. So, the direct heating system used in processing UHT milk in this study case increasing of the sedimentation of UHT GB milk which is mainly related to the low quality of raw milk and the storage temperature.

Viscosity of UHT milk samples was increased during storage (Fig.1). Fresh UHT milk made from

GA milk had lower viscosity than fresh samples made from GB. There were no significant differences ($P \leq 0.05$) in viscosity of samples made from GA and GB milk after 30 days till 90 days. Viscosity changes may a direct phenomenon to age gelation and shelf stable of sterilized milk (Clare *et al.*, 2005). Casein micelles play a key role in determining viscosity parameters of skim milk (Walstra & Jenness 1984); therefore, any factor that affects the aggregation state of the micelle such as ionic strength, pH, or heat would influence resistance to flow. pH values of UHT milk were slightly decreased during storage time (Fig. 2). UHT milk samples at 1st day of storage has pH 6.68, while the lowest pH value (6.61) for was observed in sample 3 after 90 days. So, this may be one of factors affected on increasing viscosity during storage.

Changes of some physicochemical properties and sensory evaluation of UHT milk during storage at different temperature

There were significant differences ($P \leq 0.05$) among samples in colour, flavour and appearance acceptability during storage at different temperature 25° and 37°C (Table 7). The reduction of colour was higher in samples stored at 37°C than that stored at 25°C especially with that made from GB milk. The reduction of colour was the highest in samples made

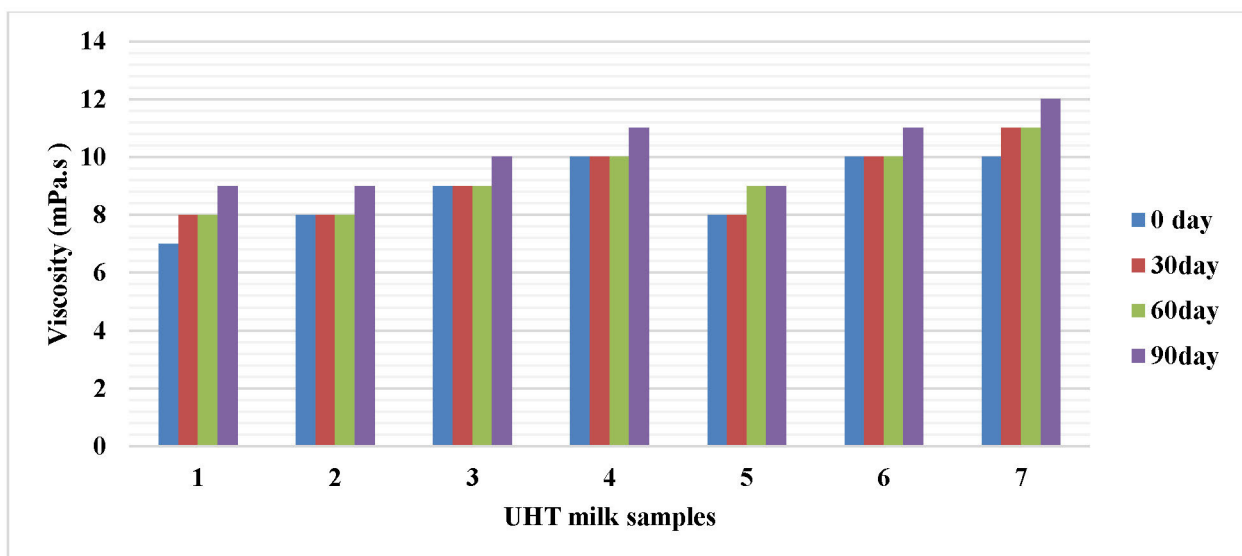


Fig.1: Change in viscosity of UHT milk samples during storage

1:UHTmilk made from grade A raw milk 100% by using Midagel stabilizer;2: UHTmilk made from grade B raw milk 100% by using Midagel stabilizer; 3: UHTmilk made from full fat milk powder(FFMP) 100% by using Midagel stabilizer ; 4: UHTmilk made from grade A raw milk 50% + 50% FFMP by using Midagel stabilizer; 5: UHTmilk made from grade B raw milk 50% + 50% FFMP by using Midagel stabilizer;6: UHTmilk made from grade A raw milk 50% + 50% FFMP by using Lacta stabilizer and 7: UHTmilk made from grade B raw milk 50% + 50% FFMP by using Lacta stabilizer

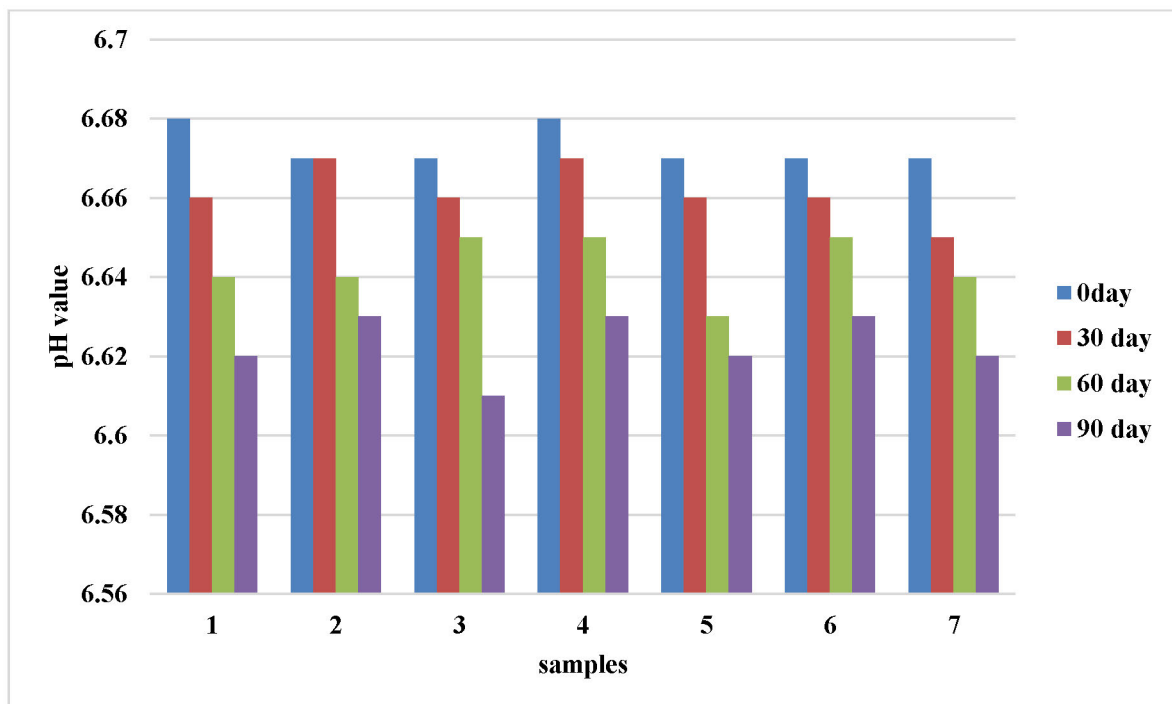


Fig. 2: Change in pH values for UHT milk during storage

1: UHT milk made from grade A raw milk 100% by using Midagel stabilizer; 2: UHT milk made from grade B raw milk 100% by using Midagel stabilizer; 3: UHT milk made from full fat milk powder (FFMP) 100% by using Midagel stabilizer; 4: UHT milk made from grade A raw milk 50% + 50% FFMP by using Midagel stabilizer; 5: UHT milk made from grade B raw milk 50% + 50% FFMP by using Midagel stabilizer; 6: UHT milk made from grade A raw milk 50% + 50% FFMP by using Lacta stabilizer and 7: UHT milk made from grade B raw milk 50% + 50% FFMP by using Lacta stabilizer

from GB milk after 90 days at 37°C. Regarding flavor, there were significant differences ($P \leq 0.05$) detected in the acceptability scores between panelists. The acceptability of flavour was higher in samples made from GA milk stored at 25°C comparing with that stored at 37°C. The flavour acceptability was lower after 90 days in samples made from GA milk. The result showed that milk stabilizers used have no effect on colour and flavour acceptability of final UHT milk's during storage at different temperature. The results showed that the storage temperature has a role in the gelation, flavour and colour of UHT milk. The sedimentation was not observed in samples stored at 25°C for 30 days, whereas, it was observed in samples made from GB milk storage at 25°C after 60 days. Notwithstanding, the sedimentation was observed in samples stored at 37°C after 30 days. The sedimentation was higher in samples made from GB milk when compared with that made using GA or milk powder. It could also be noticed that using Super Midagel stabilizer in the UHT process decreased sedimentation than Lacta 760R in samples made from GB milk (2M and 2L) after 30 days at 25°C. The obtained results in Table (7) showed that

the most effecting factors on the sedimentation in UHT milk were the quality of raw milk used in making UHT milk and storage temperature of final product, while stabilizer type has a little effect.

With increasing the storage period, there is a raise in acidity and viscosity with a decline in pH (Siddique *et al.*, 2016). In this study, there was increasing of viscosity during storage in all samples (Fig.3) especially at high storage temperature 37°C. There was a significant effect of stabilizer used on viscosity during storage at 37°C. Samples made using Super Midagel have low viscosity during storage at 37°C for 90 days when compared with samples made using Lacta stabilizer. Chavan, *et al.*, (2011) reported that the storage temperature significantly ($P \leq 0.05$) influences the gelation time of UHT milk. Gelation occurs more pronounced at room temperatures (20 to 25°C) than at low (4°C) or at high (35 to 40°C) temperatures. Structural of milk protein was changed during storage to form a gel in products stored at 22°C and heavy sedimentation could be detected when stored at 40°C (Malmgren *et al.* 2017). Samel *et al.* (1971) concluded that the gelation may be inhibited at 37°C due to blocking of

Table 7: Sensory evaluation of UHT milk during storage at different temperatures

UHT Milk samples	Time of storage(Days)	Temperature of storage	Sensory evaluation			
			Colour	Sedimentation	Flavour	Appearance
1M	30	25°C	4.9 ^j	0 ^a	5 ^c	5 ^c
		37°C	4.6 ^{ef}	1 ^b	4 ^b	4 ^d
	60	25°C	4.85 ^{ij}	0 ^a	5 ^c	5 ^e
		37°C	4.5 ^{de}	1 ^b	4 ^b	4 ^d
	90	25°C	4.85 ^{ij}	0 ^a	4 ^b	5 ^e
		37°C	4.25 ^b	2 ^c	3 ^a	3 ^c
Mean			4.65 ^A	0.66 ^C	4.33 ^A	4.33 ^A
2M	30	25°C	4.75 ^{ghi}	0 ^a	4 ^b	5 ^e
		37°C	4.25 ^b	2 ^c	3 ^a	3 ^c
	60	25°C	4.7 ^{fgh}	1 ^b	4 ^b	4 ^d
		37°C	4.5 ^{de}	3 ^d	3 ^a	2 ^b
	90	25°C	4.65 ^{fg}	2 ^c	4 ^b	3 ^c
		37°C	4 ^a	4 ^e	3 ^a	1.5 ^a
Mean			4.47 ^B	2 ^B	3.50 ^B	3.08 ^B
3M	30	25°C	4.75 ^{ghi}	0 ^a	4 ^b	5 ^e
		37°C	4.5 ^{de}	1 ^b	3 ^a	4 ^d
	60	25°C	4.6 ^{ef}	0 ^a	4 ^b	5 ^e
		37°C	4.5 ^{de}	1 ^b	3 ^a	4 ^d
	90	25°C	4.5 ^{de}	0 ^a	3 ^a	5 ^e
		37°C	4.25 ^b	2 ^c	3 ^a	3 ^c
Mean			4.51 ^B	0.66 ^C	3.33 ^C	4.33 ^A
1L	30	25°C	4.9 ^j	0 ^a	5 ^c	5 ^e
		37°C	4.65 ^{fg}	1 ^b	4 ^b	4 ^d
	60	25°C	4.9 ^j	0 ^a	5 ^c	5 ^e
		37°C	4.5 ^{de}	1 ^b	4 ^b	4 ^d
	90	25°C	4.85 ^{ij}	0 ^a	4 ^b	5 ^e
		37°C	4.2 ^b	3 ^d	3 ^a	2 ^b
Mean			4.66 ^A	0.83 ^C	4.16 ^{AB}	4.16 ^A
2L	30	25°C	4.8 ^{hij}	1 ^b	4 ^b	4 ^d
		37°C	4.3 ^{bc}	3 ^d	3 ^a	2 ^b
	60	25°C	4.8 ^{hij}	1 ^b	4 ^b	4 ^d
		37°C	4.5 ^{de}	3 ^d	3 ^a	2 ^b
	90	25°C	4.6 ^{ef}	2 ^c	4 ^b	3 ^c
		37°C	4 ^a	4 ^d	3 ^a	1.4 ^a
Mean			4.50 ^B	2.33 ^A	3.50 ^{BC}	2.75 ^B
3L	30	25°C	4.75 ^{ghi}	0 ^a	4 ^b	5 ^e
		37°C	4.5 ^{de}	1 ^b	4 ^b	4 ^d
	60	25°C	4.6 ^{ef}	0 ^a	4 ^b	5 ^e
		37°C	4.4 ^{cd}	1 ^b	3 ^a	4 ^d
	90	25°C	4.5 ^{de}	0 ^a	3 ^a	5 ^e
		37°C	4.25 ^b	2 ^c	3 ^a	3 ^c
Mean			4.50 ^B	0.66 ^C	3.50 ^{BC}	4.33 ^A

M: refer to Super Midagel stabilizer using in UHT process, L: Refer to Lacta stabilizer using in UHT process

1M: UHTmilk made from grade A raw milk 100% by using Midagel stabilizer; 2M: UHTmilk made from grade B raw milk 100% by using Midagel stabilizer; 3M: UHTmilk made from full fat milk powder (FFMP) 100% by using Midagel stabilizer, 1L: UHTmilk made from grade A raw milk 100% by using Lacta stabilizer; 2L: UHTmilk made from grade B raw milk 100% by using Lacta stabilizer; 3L: UHTmilk made from full fat milk powder (FFMP) 100% by using Lacta stabilizer

Means with the same letter(s) in the same column are not significant, but different letters are significant ($P < 0.05$)

Capital letters refer to differences among treatments,

Small letters: refer to differences among storage times at different temperature in the same column

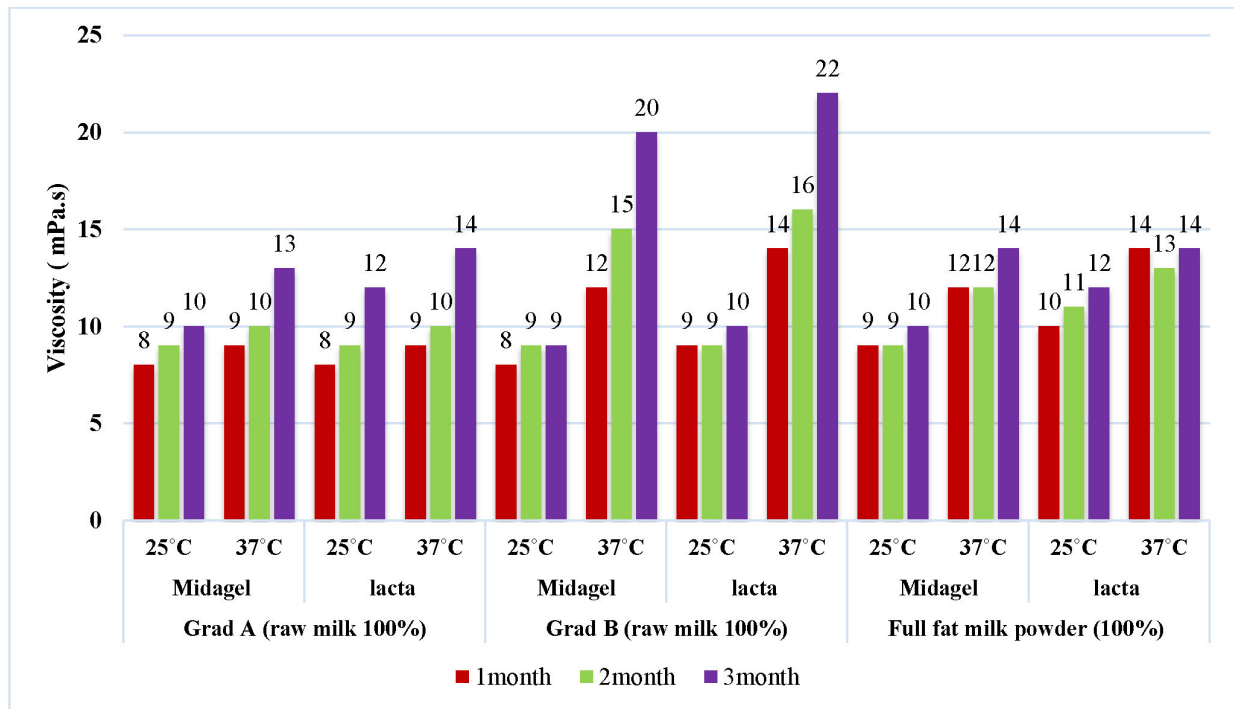


Fig. 3: Change in viscosity of UHT milk treatments during storage at different temperature

protein–protein interactions by casein–lactose interactions involving lysine residues. The browning observed in UHT milk stored at 37°C. The results of this study showed that the gelation or protein sedimentation was more pronounced in milk stored at 37°C when compared to that stored at 25°C. This was not in agreement with the hypothesis of Samel *et al.* (1971). On the other hand, the brown color of Maillard reaction and protein gelation are more pronounced in UHT milk made using hydrolyzed lactose than in that made using non-hydrolyzed lactose. Brownness of milk increased during storage of hydrolyzed-lactose UHT milk, which may be caused by Maillard-reaction. The formation of late-stage Millard products could also explain the increasing of the gel during storage time (Nielsen *et al.* 2017). So, the sedimentation in UHT GB milk is directly related to the low quality of raw milk, and storage temperature at higher than 25°C) in direct heating UHT milk.

SDS-PAGE of samples after storage for 90 days

SDS-PAGE was used to investigate the effects of storage (3 months) on protein profile of UHT milk treatments. It was studied the differences between total milk proteins and protein that precipitated on the wall of milk package. Results in (Fig.4) and the analysis of gel by Total Lab recommended no significant ($P \geq 0.05$) differences among all UHT treatments after storage period (3 months). As well as,

SDS-PAGE showed that no differences between soluble milk proteins and precipitated proteins on the wall of packages. Nielsen *et al.* (2017) studied the effect of indirect UHT process on milk proteins and they found that whey protein bands corresponding to the positions of α -lactalbumin and β -lactoglobulin were missing in the lower part of the gel, indicating that these two whey proteins become part of disulphide crosslinked aggregates during processing. In this study, no significant proteolysis was found in all stored samples for 90 days, compared to fresh sample. The proteolysis of UHT milk proteins is mainly of two origins: the plasmin (native milk alkaline proteinase) and heat-stable bacterial proteinases. These two groups of proteinases hydrolyze the milk proteins to reduce the stability of UHT milk during its shelf life (Datta & Deeth 2003). However, plasmin activity could be reduced by heat treatment at high temperatures for relatively long times such as in indirect UHT plants (Kelly & Foley 1997, Cauvin *et al.*, 1999). From these results it could be concluded that no proteolysis caused in UHT milk proteins during storage period and the sedimentation of proteins may be related to some change in physicochemical properties of milk proteins.

RP-HPLC of samples after storage for 90 days

The 12% TCA filtrates of samples were separated by RP-HPLC (Figs. 5-6). No significant differences among all treatments. This indicated that

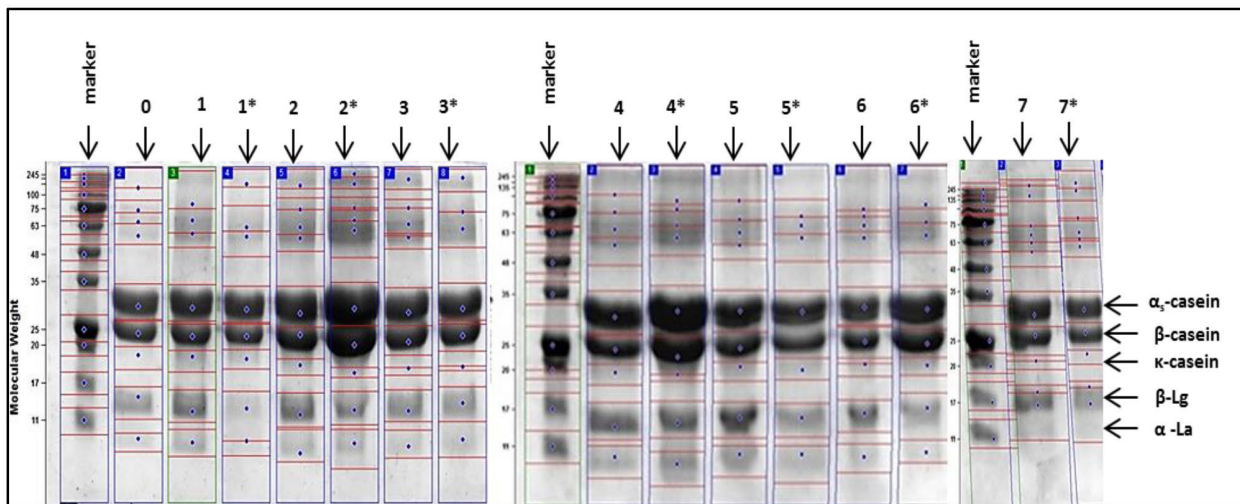


Fig. 4: SDS-PAGE (12.5%T) of UHT milk treatments

Sample No. 0 (Fresh UHT milk), 1:UHTmilk made from grade A raw milk 100% by using Midagel stabilizer;2: UHTmilk made from grade B raw milk 100% by using Midagel stabilizer; 3: UHTmilk made from full fat milk powder (FFMP) 100% by using Midagel stabilizer ; 4: UHTmilk made from grade A raw milk 50% + 50% FFMP by using Midagel stabilizer; 5: UHTmilk made from grade B raw milk 50% + 50% FFMP by using Midagel stabilizer;6: UHTmilk made from grade A raw milk 50% + 50% FFMP by using Lacta stabilizer and 7: UHTmilk made from grade B raw milk 50% + 50% FFMP by using Lacta stabilizer. Molecular weight= KD

(*) refer to the samples from precipitated protein in UHT milk packages

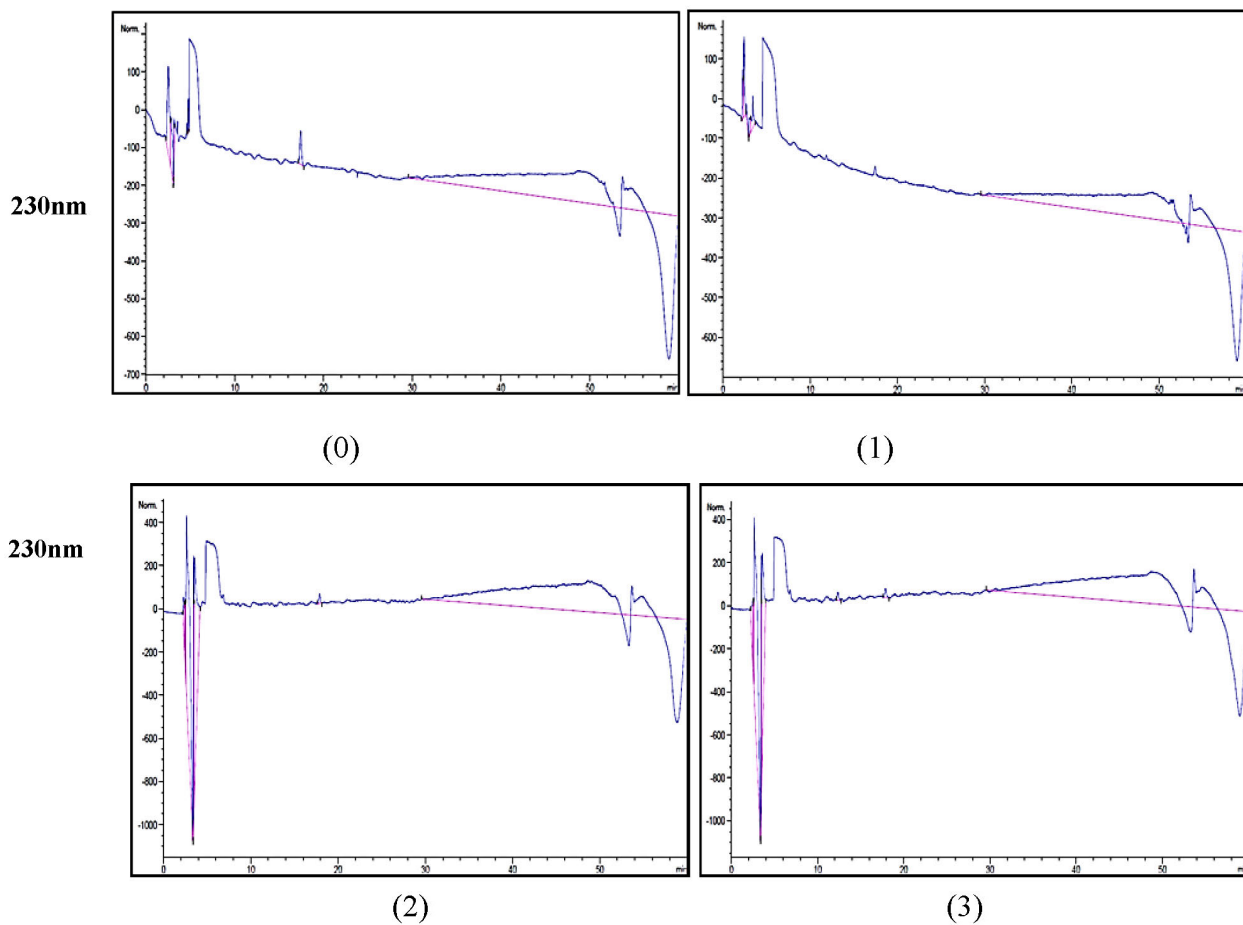


Fig.5: RP-HPLC for TCA filtrates of UHT milk samples

0: Fresh milk ; 1:UHTmilk made from grade A raw milk 100% by using Midagel stabilizer;2: UHTmilk made from grade B raw milk 100% by using Midagel stabilizer; 3: UHTmilk made from full fat milk powder (FFMP) 100% by using Midagel stabilizer after storage for 90 day.

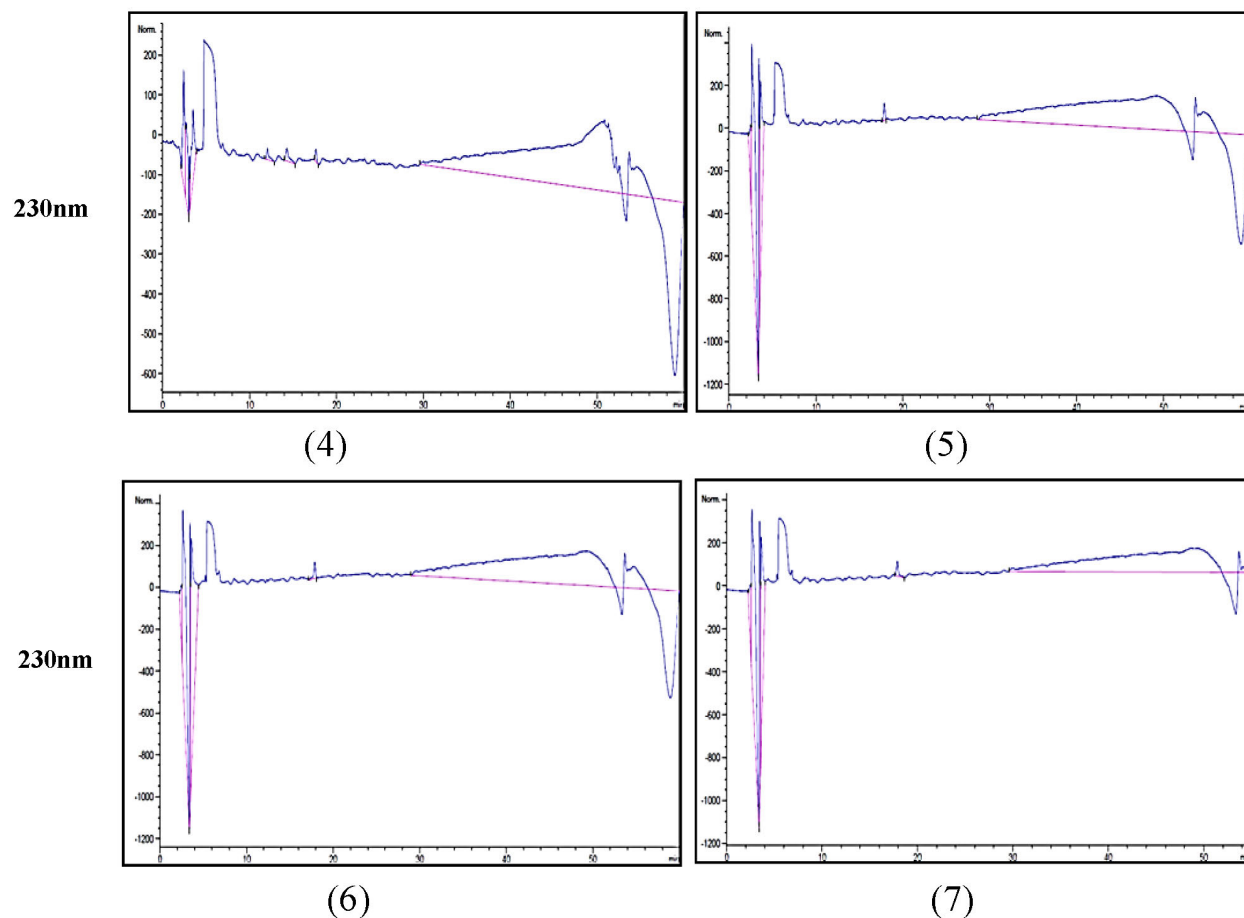


Fig. 6: RP-HPLC for TCA filtrates of UHT milk samples

4: UHTmilk made from grade A raw milk 50% + 50% FFMP by using Midagel stabilizer; 5: UHTmilk made from grade B raw milk 50% + 50% FFMP by using Midagel stabilizer; 6: UHTmilk made from grade A raw milk 50% + 50% FFMP by using Lacta stabilizer and 7: UHTmilk made from grade B raw milk 50% + 50% FFMP by using Lacta stabilizer after storage for 90 day.

there were no significant differences in proteolytic systems either milk enzymes or microbial enzymes. Since the total bacterial count and spore forming bacteria were significantly higher in GB milk than those of GA milk, the low molecular weight peptides may be further degraded and not appeared at RP-HPLC profiles. This finding may be recommended by the sedimentation of GB samples.

Low quality milk is more susceptible to gel formation than high quality milk. Microorganisms that produce heat-stable enzymes cause the most serious gelation problems (Chavan *et al.*, 2011). The results of SDS-PAGE and RP-HPLC did not show any proteolysis in UHT milk made using low quality milk. This finding suggested that the sedimentation of UHT milk is related to many other factors such as ionic strength, protein structure, storage temperature and direct or indirect heating system.

CONCLUSION

Consumption trends for UHT milk have increased especially in countries with shortage of raw milk. Milk with high microbial count and low quality is more susceptible to gel formation than milk with a low microbial count and high quality. The GB milk contains extremely high numbers of microorganisms due to poor hygienic practices during hand milking in the outdoors and milk handling practices. The sedimentation and fat separation that pronounced in GB UHT milk are not related to the proteolysis but are mostly related to stability of milk proteins and the storage temperature. The direct heating system used in processing UHT-milk in this study increased the sedimentation of GB milk.

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تأثير جودة اللبن والمثبتات على بعض الخواص الفيزيوكيماوية للبن المعامل بالحرارة الفائقة

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تم دراسة تأثير جودة اللبن والمثبتات على التغيرات غير المرغوبة في اللبن المعامل بالحرارة الفائقة (UHT) عند التخزين على درجة حرارة ٢٥ درجة مئوية أو ٣٧ درجة مئوية. أظهرت النتائج التي تم الحصول عليها أن العدد الكلي للميكروبات ، -psychophilic bacteria, mesophilic & thermophilic spore forming bac- ، كانت أعلى في اللبن منخفض الجودة (GB) عن اللبن عالي الجودة (GA). في هذا البحث تم إجراء سبعة معاملات باستخدام اللبن GA ، اللبن GB ، بودرة اللبن المجفف كامل الدسم (FCMP)، مزيج من GA مع FCMP ومزيج من GB مع FCMP، وفي الوقت نفسه، تم تقييم اثنين من المثبتات التجارية بشكل منفصل مع هذه المعاملات. أظهرت البكتيريا المكونة للجراثيم سواء mesophilic أو thermophilic مستوى عال في اللبن GB المعدل والمبستر مقارنة مع GA. من ناحية أخرى لم يكن لنوع المثبت أي تأثير على جودة اللبن المعامل بالحرارة الفائقة من لبن GB. في حين حصلت المعاملات المصنعة من لبن GB على مستويات منخفضة في الخواص الحسية (اللون والنكهة) ، كما يمكن ملاحظة فصل الدهن والترسيب أثناء التخزين بالمقارنة مع تلك المصنوعة من لبن GA أو FCMP. ظاهرة فصل الدهن والترسيب كانت أكثر وضوحاً في المعاملات المخزنة على درجة حرارة ٣٧ درجة مئوية مقارنة مع تلك المخزنة على ٢٥ درجة مئوية. لم تظهر نتائج الفصل الكهربائي للبروتينات (SDS-PAGE) وكروماتوجرافيا السائل عالي الكفاءة (RP-HPLC) أي اختلافات بين جميع المعاملات في التحلل البروتيني خلال فترة التخزين. وبناءً على ذلك ، فإن فصل الدهن والترسيب لا يرتبط فقط بالتحلل البروتيني ولكن أيضاً بجودة اللبن المستخدم في عملية التصنيع.