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Mini-Review

Microbiological and physicochemical analysis of different UHT milk available in a local market

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Abstract

The aim of the study was to diagnose physiochemical, microbiological, sensorial changes and to differentiate the milk collection systems of the commercial milk market competitor during storage of twelve weeks. The parameters used for physicochemical analysis are; sedimentation, Solids Non Fats (SNF), fat and protein percentages, total titratable acidity and pH and for microbiological analysis; Total plate, coliform, *Bacillus cereus* and *Bacillus subtilus, Escherichia coli* and spore forming bacterial count were determined. Color, taste and aroma were observed during storage. The results strongly reflect an increase in sedimentation value with the ice mixing or dilution before processing which disturbed the salt balance, protein charges and natural emulsion. There was increase in acidity and sedimentation of milk but pH, percentages of fat contents, SNF (Solids Non Fats) and proteins decreased during storage. The negative changes occurred in color, aroma and flavor with reference to these physiochemical changes. Microbial counts for coliforms (e.g. *E.coli*), *Bacillus cereus, Bacillus subtilis* and heat resistant spores forming bacteria were zero. These all factors collectively limited the shelf life of UHT milk.

Keywords: physiochemical, microbiological, sensorial changes, heat resistant spores forming bacteria, UHT milk, Pakistan

Introduction

Raw milk is milk in its natural (unpasteurized) state. Contaminated raw milk can be a source of harmful bacteria, such as those that cause undulant fever, dysentery, salmonellosis and tuberculosis. "Certified" milk, obtained from cows certified as healthy, is unpasteurized milk with a bacteria count below a specified standard, but it can still contain significant numbers of disease-producing organisms.

Different heat and treatments are given to raw milk in order to remove pathogenic organisms, to increase the shelf life, to help subsequent processing, e.g. for warming before separation and homogenization or as an essential treatment before cheese making, yoghurt manufacture and production of evaporated and dried milk products [1]. Pasteurization, sterilization (in bottle) and UHT (ultra-high-temperature) treatment integrated with aseptic packing are two such treatments. Sterilization (in bottle) is the term applied to a heat treatment process which has a bactericidal effect greater than pasteurization. Although it does not result in sterility, it gives the processed milk a longer shelf life. As a result of the long holding time at this elevated temperature, the product has a cooked flavour and a pronounced brown colour. Unlike sterilization, pasteurization is not intended to kill all pathogenic microorganisms in the food or liquid. Instead, pasteurization aims to reduce the number of viable pathogens so they are unlikely to cause disease. Ultra-high temperature (UHT or ultra-heat treated) is also used for milk treatment. UHT processing holds the milk at a temperature of 138°C for a fraction of a second. Milk simply labeled "pasteurized" is usually treated with the HTST method, whereas milk labeled "ultra-pasteurized" or simply "UHT" has been treated with the UHT method [2].

Heating of milk accounts for two main problems, age- gelation and off- flavour development, which limits shelf life of milk. UHT treatments of milk leads to a much larger production of small sized casein micelles compared to raw or pasteurized milk [1, 3]. Biochemical processes involved are heat resistance and reactivation of natural and bacterial proteases and survival of bacterial spores [1, 4]. Proteolysis of UHT milk during storage at room temperature is a major factor limiting the shelf life through changes in its flavour and texture [5]. The changes ultimately reduce the quality and limit the shelf life of UHT milk via development of off-flavours, fat separation and sedimentation, which principally falls into two categories, liberation of volatile fatty acids such as butyric acid and oxidation of free or unsaturated fatty acids [5].

Above 135°C the protein deposited on the fat globule membrane forms a network which makes the membrane denser and less permeable [6]. There is an increase in acidity and viscosity with a decrease in pH with the storage time increase in UHT. Clare *et al* [7], determined that sweet aromatic flavor and sweet taste of UHT milk decreases during storage.

The microorganisms, which cause spoilage in milk, which is intended to be sterile (UHT treatment), are either resistant types that have survived the heat treatment, or organisms that have contaminated the product after the sterilization process. Contamination may either be by heat labile organism or heat resistant forms such as spores. Contaminating spores are, however, likely to be less heat resistant than those which might survive the heat treatment.

The problem of post treatment contamination of a product in a sterilized container is well known. The contamination can either be through poor seal or through a puncture or pinhole in the container. Post treatment contaminants in UHT milk may be either spores, which would not be expected to be heat resistant enough to survive the heat treatment, or non-heat resistant vegetative organisms. Organisms of first type will probably have entered from ineffectively sterilized plant downstream from the heat treatment stage of the process, which includes spores of *Bacillus cereus* [8, 9] and *B. licheniformis* [9]. Organisms of the second type will probably have entered through a poorly sealed container after aseptic filling.

The types of spores which have been investigated as being of particular relevance to UHT are those of *Bacillus stearothermophilus*, *B. subtilis* and *C. botulinum*. The high spore counts can occur at the dairy farm and feed and milking equipment can act as reservoirs or entry points for potentially highly heat-resistant spores into raw milk. Lowering this spore load by good hygienic measures could probably further reduce the contamination level of raw milk, in this way minimizing the aerobic spore-forming bacteria that could lead to spoilage of milk and dairy products [10].

These problems have been reported for quite some time, hence this research was planned to observe the physicochemical, microbial and sensory changes in UHT processed milk until it has reached its maximum life. In this study the declared shelf life of different UHT milk available in market is examined.

Materials and Methods

Four different UHT branded milk samples were purchased from a local market in Lahore. The samples were taken in sterilized syringes for microbiological analysis and in clean stainless steel containers of 1 litre for chemical and sensory analysis. The samples were analyzed at intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 weeks. During this period, samples were stored at room temperature (25°C) to provide them with conditions similar to storage in the market.

For microbiological analysis the samples were examined for aerobic plate counts (APC), *E. coli* counts, *B. cereus, B. subtilis* counts, and for spore former counts. The parameters examined for the chemical analysis were sedimentation, pH, acidity as lactic acid percentage, fat percentage before and after shaking the milk, SNF percentage before and after shaking and protein percentage before and after shaking. For sensory evaluation colour, aroma and taste were examined.

Physicochemical analysis of milk

To assess the physical and chemical changes in processed milk samples the following tests were carried out.

Sedimentation Test. Sedimentation test was performed by following a modified method as described by Ramsey and Swartzel [11]. According to this method, milk was drained from the cartons leaving the bottom 4 cm. The cartons were inverted for approximately 10 minutes, up righted and placed in the exhaust hood to dry. The cartons were allowed to dry for 48 hours after the bottom flaps or wings of cartons had been opened to facilitate the drying of any sediment entrapped there. The cartons were weighed and then washed thoroughly to remove any sediment or residue adhering to the container. The washed cartons were again dried and weighed.

Solids Non Fats (SNF) Percentage: Solids Non Fats (SNF) Percentage was determined by lactometeric method as described by Ramsey and Swartzel [11].

Total Titratable Acidity. TTA was determined according to the methods of AOAC [12].

pH: The pH value of milk was determined by using a digital pH meter [12]. Prior to use, the pH meter was standardized with standard buffer solution of pH 4 and 7.

Fat Percentage: Milk fat percentage was determined by the Gerber method as described by FAO [13] by using the butyrometer.

Protein Percentage: The protein was estimated by formal titration method [14].

Microbial analysis

Microbial analysis was performed according to the standard methods of AOAC [12].

Total viable counts: The Plate Count Agar media [15], was used for the total viable count in UHT milk samples [12]. Plates were incubated for 24 hours at 37^0C.

Determination of Coliforms: Coliform counts were determined by pour plate method on Violet Red Bile Agar, prepared according to the manufacturer's instructions. All plates were incubated at 37ºC for 24h.

Determination of *Bacillus* spp*:* *Bacillus cereus* selective agar base [15], was used for isolation and enumeration of *Bacillus cereus* and *Bacillus subtilis.* All plates were incubated at 37ºC for 24h.

Determination of spore formers: Plate count agar media [15], was used for the enumeration of spore formers. Sterile medium was poured into sterile petri plates and the medium allowed to solidify. The sample was heated at 80^0C for 10 min using a water bath. These plates were inoculated with 1 ml sample by using sterile pipette. After inoculation, the sample was well mixed in the petri plates by to and fro motion. All plates were incubated in an inverted position for 72 hours at 55^0C.

***Escherichia coli* Counts:** For *Escherichia coli* count MacConkey's agar [15], was used. Samples from lactose positive tubes in case of Coliform counts were applied directly on the MacConkey's agar plates and incubated at 37^oC for 24 hours.

Sensory analysis

The stored milk samples were evaluated by sensory analysis for colour and flavour by the scoring method as described by Larmond [16].

Results and Discussion

The changes that have taken place during storage depend on temperature of storage, extent of exposure of the milk to light and availability of oxygen. The dairy companies in Pakistan show a shelf-life of 12 weeks on the labels of milk packs. Milk must be in best condition for consumption. For storage time of more than a week or two, these effects may be greater than those of the heat treatment. Changes in colour, flavour and texture are readily detected by the consumer and may reduce the acceptability of the products. Other changes cannot be recognized by the consumer and are not necessarily correlated with organoleptic, recognizable changes, but are of potential nutritional importance. The quality of sediment depends on the raw milk and on the type and severity of the heat treatments. For any one type of process, the amount of sediment increases with the severity of the heat treatment [17, 18]. The amount of sediment decreases with homogenization pressure [19]. Results obtained from sedimentation tests in UHT milk during a storage period of 3 months (12 weeks) shows that there is an effect of heat processing and subsequent storage on sedimentation in all four samples of UHT milk (Table 1). The changes started in week 2 of shelf life for samples I and III and sample II showed formation of sediment after week 6; sample III reaches up to 7.10 g/250ml, which is a considerable change, and sample II showed formation of sediments after week 5.

Table 1. Effect of storage on sedimentation, pH and acidity of milk.

Analysis (Week)	Effect of storage on Sedimentation (gram)				Effect of storage on pH				Effect of storage on Acidity %age			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
1	0	0	0	0	6.81	6.85	6.75	6.75	0.12	0.11	0.11	0.11
2	0.37	0	0.5	0	6.75	6.85	6.70	6.75	0.12	0.11	0.11	0.11
3	0.60	0	1.37	0	6.73	6.85	6.67	6.74	0.12	0.12	0.12	0.12
4	0.72	0	1.85	0.20	6.70	6.80	6.63	6.70	0.12	0.12	0.13	0.12
5	0.85	0	2.35	0.40	6.65	6.80	6.60	6.65	0.13	0.12	0.14	0.12
6	1.25	0.22	3.12	0.82	6.65	6.80	6.52	6.65	0.13	0.12	0.15	0.12
7	1.60	0.22	4.21	1.00	6.65	6.76	6.45	6.62	0.14	0.12	0.15	0.12
8	2.25	0.37	4.32	1.70	6.60	6.75	6.32	6.60	0.14	0.13	0.16	0.13
9	5.50	0.37	5.37	2.37	6.58	6.72	6.27	6.60	0.14	0.13	0.16	0.13
10	2.72	0.47	5.70	2.62	6.40	6.70	6.20	6.60	0.14	0.13	0.16	0.13
11	3.30	0.47	6.90	2.87	6.30	6.68	6.19	6.57	0.14	0.13	0.17	0.13
12	3.61	0.47	7.10	3.00	6.20	6.65	6.17	6.55	0.15	0.13	0.18	0.13

The alcohol test can be used to detect raw milk that is likely to give a high level of the normal type of sediments, and there are indications that it may be useful in predicting the abnormal type [18]. Processing operations influence acid base equilibrium in milk. UHT treatment results in a pH decrease, due to conversion of lactose into different organic acids [20]. In milk, casein micelles are stable at natural pH i.e. 6.7. Lowering the pH facilitates aggregations of casein micelles and forms a gel. Results regarding the effect of storage on pH of UHT processed milk during a storage period of 90 days show that there is storage effect on pH level. Maximum pH value (6.81 and 6.85 in samples I and II, respectively and 6.75 in samples III and IV) was recorded in the 1st week, while minimum pH values were obtained in 12th week of shelf life (6.20, 6.65, 6.17 and 6.55 in sample 1, 2, 3 and 4 of UHT milk respectively) (Table 1).

Vankatachalm and McMahon [17], verified a drop in pH and they associated it with browning reactions. Andrews *et al* [21], confirmed similar effects and concluded that the level and extent of pH decrease was related to age-gelation. When milk is heated at a temperature above 100^{o}C and subsequently stored, lactose is degraded to acids. Formic acid is the principal acid produced due to which titratable acidity of milk rises. Increase in free fatty acids is also responsible for increasing the total titratable acidity of milk [22]. Results obtained by the analysis for total titratable acidity (Table 1) confirm that there is a storage effect on the total titrable acidity. The acidity value was 0.11%, while during storage of UHT milk minimum acidity was recorded during the first week and maximum value (0.18%) at 90 days life in sample I, while 0.15 in case of sample I and 0.13 in samples II and IV. The proteins of milk are the constituents most affected by heating and subsequent storage of milk. The principal changes in UHT milk during storage may be due to enzymes. Many proteins in milk are very heat labile e.g. whey proteins, vitamin binding proteins, antimicrobial proteins etc. [23]. These proteins coagulate after heating hence the texture of milk is deteriorated during storage [20]. Casein polymerization is greater at high storage temperature, but occurs significantly even under refrigeration: 50% of the protein may be in the polymer form after 6 months at 37°C and 21% after 6 months at 4°C [21]. The results regarding protein percentage of stored UHT milk demonstrates that there is an effect of storage on protein contents of UHT processed milk. Results shown in Figure 2 give an insight into protein contents i.e. in week 1 protein contents were 3.30%, 3.70% for samples I and II, while in week 12 of storage were 2.35 and 3.48 respectively (shown in Figure 2). In case of samples III and IV, protein contents were 3.40 in week 1 while it changes to 1.15 and 2.59, respectively, in week 12. There is no change in protein percentage in all samples after shaking of UHT milk (Table 2).

Chen *et al* (2005) showed almost a 90% loss and denaturation of ß-lactoglobulin (LG) of the UHT processed and dry milks by using polyacrylamide gel electrophoresis. The results of solid non fats are shown in Figure 6. Of the principle constituent, the fats are probably least affected by UHT treatment. However, significant changes do occur in milk during heat and subsequent storage, such as increased susceptibility of the fat to oxidation, since it is not protected by a membrane and release of free fatty acids by lipase activity. In short, fat percentage is reduced marginally with storage [20]. The results of fat percentage before shaking are shown in Figure 1, as in week 1, fat percentage is 3.55, 3.66, 3.88 and 3.50 and in the last week (12), it reaches 2.70, 3.50, 1.85 and 3.00 for samples I, II, III and IV, respectively. In week 1, SNF percentage is 8.60, 8.55, 8.85 and 8.55 and in week 12 it reaches up to 7.15, 8.35, 5.95 and 7.35 for samples I, II, III and IV, respectively (Figure 3), while there is no change in fats and SNF percentages in all samples after shaking of UHT milk (Table 2).

Table 2. Effect of storage on fat %, protein % and SNF % after shaking of milk.

Analysis (Week)	Effect of storage on fat % after shaking				Effect of storage on protein % after shaking				Effect of storage on SNF % after shaking			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
1	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55
2	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55
3	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55
4	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55
5	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55
6	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55
7	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55
8	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55
9	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55
10	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55
11	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55
12	3.55	3.60	3.80	3.50	3.30	3.70	3.40	3.40	8.60	8.55	8.85	8.55

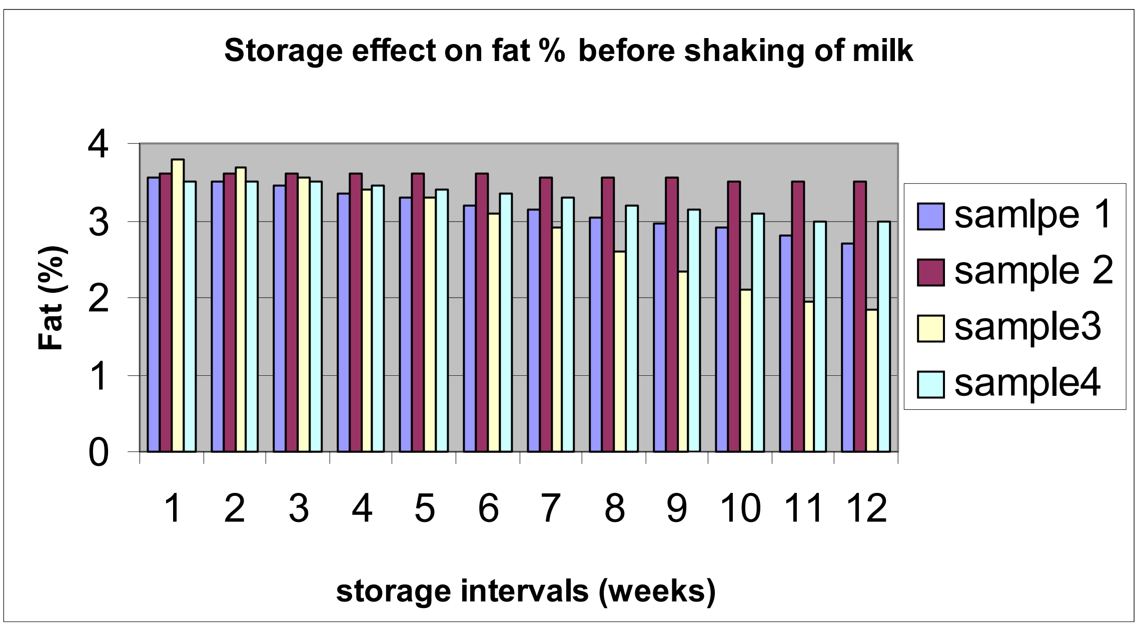

Figure 1. Effect of storage on fat % before shaking of milk.

Not a single colony was observed on aerobic plate count plates, *Coliform* agar plates, *E. coli* plate, *B. cereus, B. subtilis*, and spore formers plates, in all the four samples of UHT milk during storage, at room temperature, of 12 weeks. Results showed (Table 3) that there may be contamination of bacterial enzymes in raw milk which is being processed, which are causing lipolysis and proteolysis, but the presence of organisms is not observed in this study. The pasteurization or other treatment of milk removes the microorganisms, spore germination or recontamination can still cause quality deterioration; furthermore, heat-resistant extracellular proteinases and lipases produced by psychrotrophic bacteria before processing represent a major spoilage factor of stored milk [24]. Due to the UHT process no microorganism survived, but the heat resistant enzymes may be present in UHT milk which are responsible for spoilage during storage.

Table 3. Microbiological analysis of UHT milk during storage intervals (Week 1 to Week 12).

Treatments	Sample I	Sample II	Sample III	Sample IV
APC	Zero	Zero	Zero	Zero
Coliforms	Zero	Zero	Zero	Zero
E.coli	Zero	Zero	Zero	Zero
B. cereus	Zero	Zero	Zero	Zero
B. subtilus	Zero	Zero	Zero	Zero
Spore formers	Zero	Zero	Zero	Zero

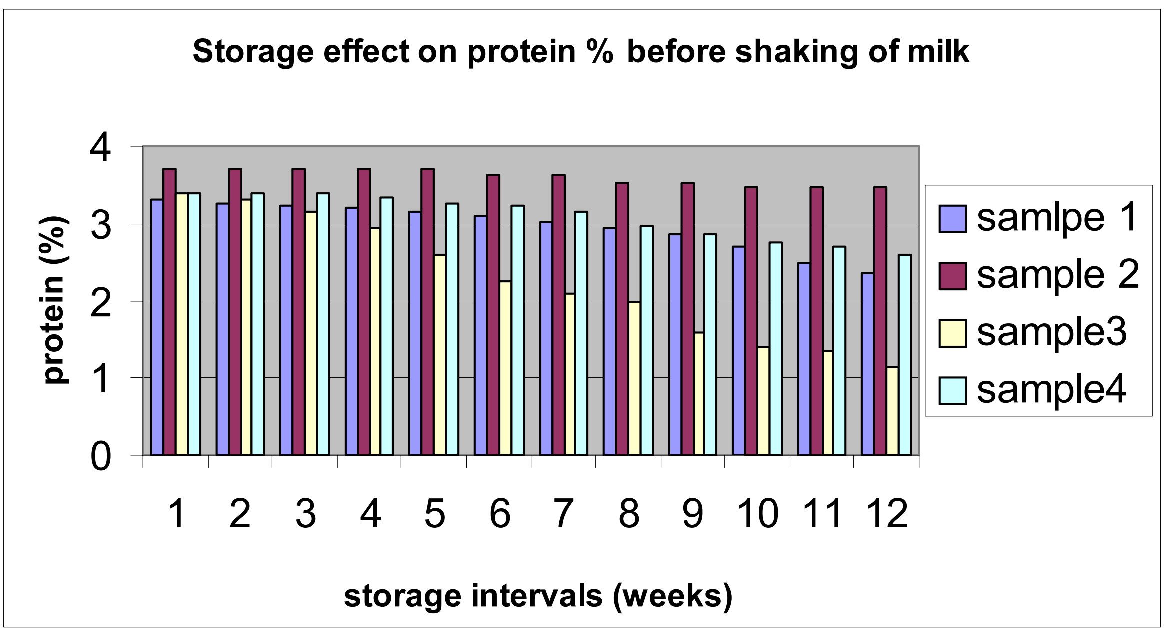

Figure 2. Effect of storage on protein % before shaking of milk.

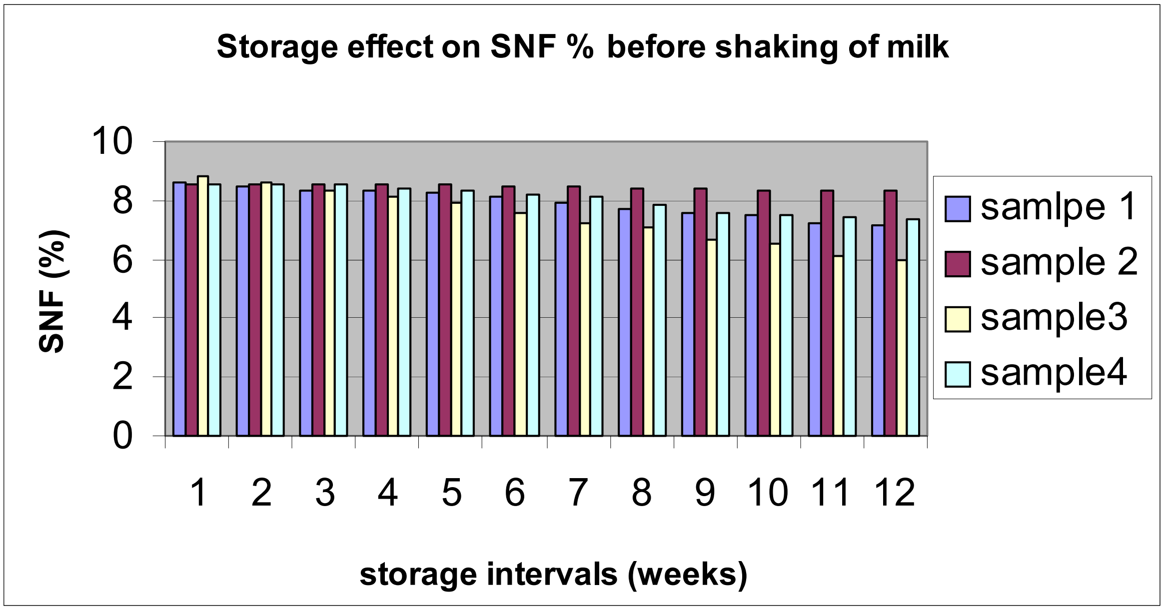

Figure 3. Effect of storage on SNF % before shaking of milk.

Colour is an important parameter that highly affects the consumer acceptability. After UHT treatment in milk, changes in casein size and denaturation of whey protein both increase the amount of light scatter (reflectance) and milk appears whiter. However, this improvement is balanced by browning, which lowers the degree of reflectance and gives a mild white colour. Results of the work presented here show that there is an effect of storage on the colour attributes of the UHT milk samples (Figure 4). In week 1 the colour score of samples I, II, III and IV was maximum, i.e. 3, 5, 4 and 4 respectively and in week 12, the lowest colour score was observed, i.e. 1, 3, 1 and 3 respectively. The change of colour in UHT processed milk during storage was also reported by Qamar, *et al* [25], who observed that off white colour of UHT processed stored samples was changed to light brown during storage. Maillard reaction is one of the most important reasons for colour loss [26]. Colour scores decreased with an increase in the storage period. In sample 1 during week 1, colour score was maximum, i.e. 3 and in week 12 the lowest colour score was observed, i.e. 1. In sample 2 during week 1, the color score was i.e. 5 and in week 12, minimum score was observed i.e. 3. The score was 4, in sample 3 during week 1, and in week 12, 1 was colour score. In sample 4 during week 1, 4 was the maximum score, while it reaches to 3 with the end of 12 week. Datta, *et al* [5], accounts various changes for flavour decrease, i.e maillard browning, sulphdryl compounds formation, formation of a range of carbonyl and other flavoursome compounds formation. They described flavour change as a result of lipid degradation, which principally falls into two categories, liberation of volatile fatty acids such as butyric acid and oxidation of free or unsaturated fatty acids with subsequent formation of volatile compounds.

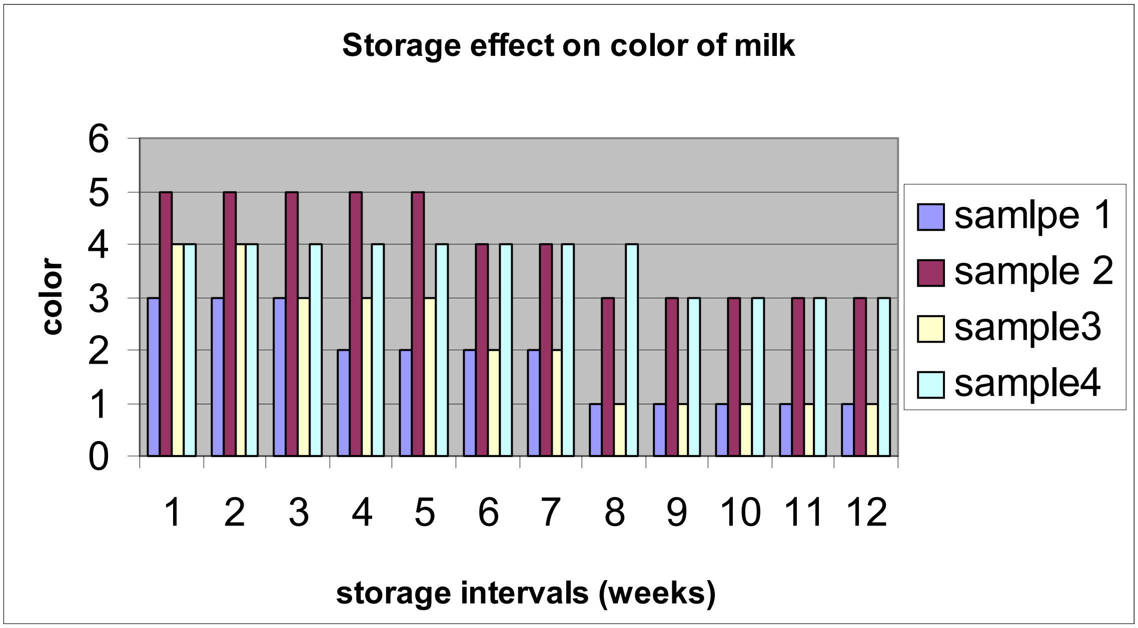

Figure 4. Effect of storage on colour of milk.

It was observed that the storage period affects the taste of the milk samples (Figure 5). There is a gradual decrease in taste score. During week 1, for sample I, the flavour score was 3 and in week 12 the lowest score was observed i.e. 1. In week 1, for sample II, the flavour score was 5 and in week 12 the lowest score was observed i.e. 3. In week 1 of sample III, the flavour score was 3 and in week 12 the lowest score was observed i.e. 1, while in sample IV during 1st week flavour score was 4 and it was changed to 2 in week 12.

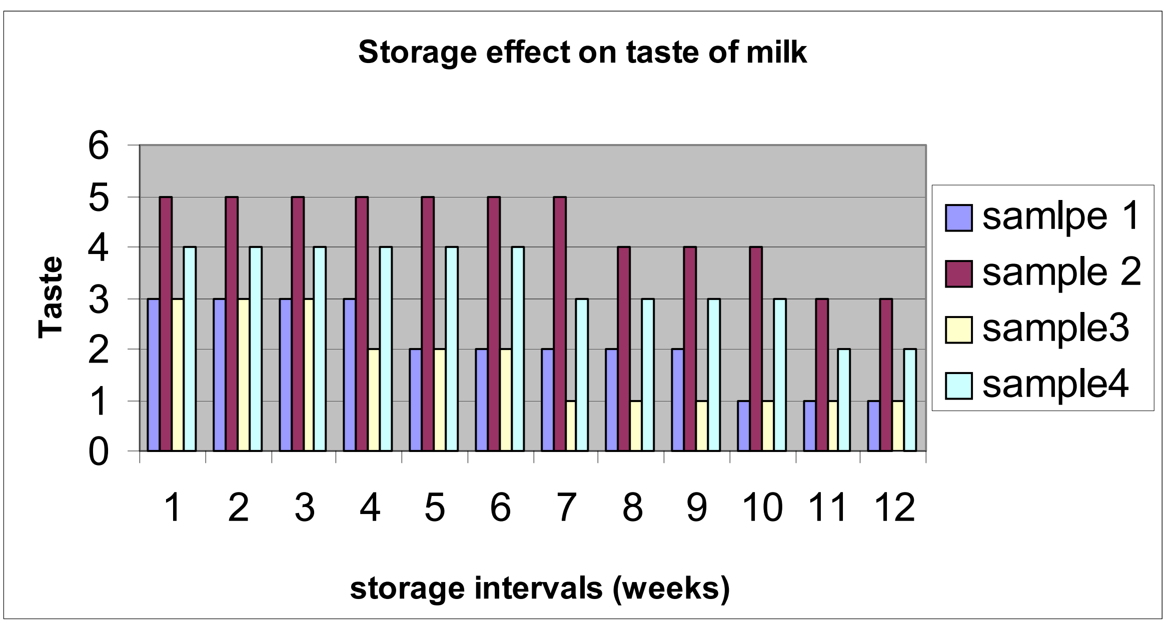

Figure 5. Effect of storage on taste of milk.

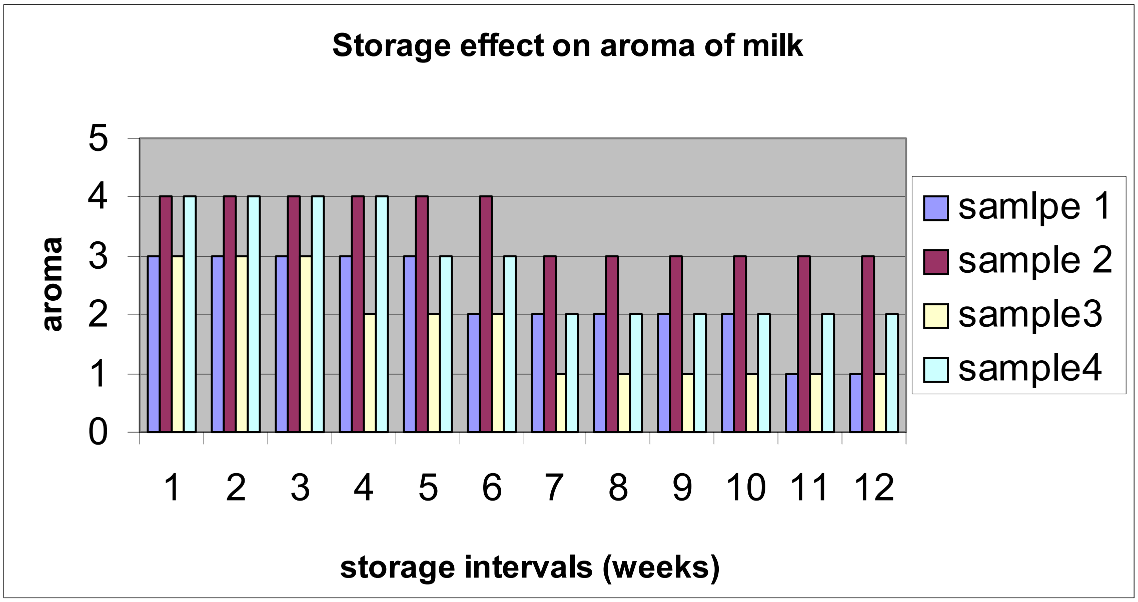

Figure 6. Effect of storage on aroma of milk.

Results show (Figure 6) that there is an effect of storage on the aroma attributes of the UHT milk samples. Aroma scores decrease with an increase in storage time. The aroma score was maximum i.e. 3 and in week 12, the lowest score was observed i.e. 1, in sample I in week 1. In sample II during week 1, the aroma score was maximum i.e. 5 and in week 12, the lowest score was observed i.e. 3. In sample III during week 1, the aroma score was maximum i.e. 3 and in week 12, the lowest score was observed i.e. 1. The results showed that for sample IV, during week 1, the aroma score was 4 and in week 12 the score reached 2.

Conclusions

It is concluded from the overall results of this study that there is an increase in sedimentation value, fat separation, titratable acidity during storage, while a decrease was found in pH and protein percentages during storage of 12 weeks. The increase in sedimentation shows excessive protein denaturation during processing and subsequent storage. In UHT processed milk, fat separation was observed during storage. This high percentage of fat separation is attributed to less homogenizing efficiency during processing. On microbiological examination, no colonies were found on TPC plates, *Coliform* Agar plates, *E. coli* plates*, B.cereus, B. subtilus*, and Spore formers plates, in all the four samples of UHT milk during storage of 12 weeks. Sensory characteristics showed a significant decrease in scores during storage. These are all factors that limit the shelf life of UHT milk. The shelf-life of milk mainly depends on the quality of raw milk and a better quality of milk can be achieved in Pakistan when the manufacturers have a better milk collection system. The manufacturer of sample II has its own sophisticated type of milk collection system known as VMCs (Village Milk Collection Centres). At these centres, milk is collected at small scale and in a short time it is transported at low temperature to the processing plant, avoiding contamination. Due to this practice the microbial as well as other contamination can be controlled in a better way before heat treatment or processing. The current general practice for manufacturers in other dairy industries of Pakistan is to get milk from contractors and ice added milk is mostly supplied to these industries which disturbs the mineral balance and natural emulsions and gives higher water activity, which in turn leads to physicochemical, microbiological as well as sensory changes during shelf-life of milk.

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