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Full Length Research Paper

A study of the physiochemical and microbiological analysis of the commercial milk market

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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 12 weeks. The parameters used for physicochemical analysis are; sedimentation, solids non fats (SNF), fat and protein %, 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. Colour, 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, % of fat contents, SNF (solids non fats) and proteins decreased during storage. The negative changes occurred in colour, aroma and flavor with reference to these physiochemical changes. Microbial counts for coliforms (e.g. *E. coli*), *B. cereus, B. subtilis* and heat resistant spores forming bacteria were zero. These all factors collectively limited the shelf life of UHT (ultra-high temperature) milk.

Key words: Physiochemical, microbiological, sensorial changes, heat resistant spores forming bacteria, UHT milk.

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 still can 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 (Singh, 1993). Pasteurization, sterilization (in bottle) and UHT (ultra-high-temperature) treatment integrated with aseptic packing. 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 micro-organisms 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 (250°F) for a fraction of a second. Milk simply labeled "pasteurization" is usually treated with the HTST method, whereas milk labeled "ultra-pasteurization" or simply "UHT" has been treated with the UHT method (Bylund, 1995).

Heating of milk accounts 2 main problems, age gelation and off flavor development, which limits shelf life of milk. UHT treatment of milk leads to a much larger production of small sized casein micelles compared to raw or pasteurized milk (Singh, 1993). Biochemical processes involve

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are heat resistance and reactivation of natural and bacterial proteases and survival of bacterial spores (Singh, 1993; Manji et al., 1988). Proteolysis of UHT milk during storage at room temperature is a major factor limiting the shelf life through changes in its flavor and texture (Datta et al., 2002) . The changes ultimately reduce the quality and limit the shelf life of UHT milk via development of off flavors, fat separation and sedimentation, which principally falls into 2 categories, liberation of volatile fatty acids such as butyric acid and oxidation of free or unsaturated fatty acids (Datta et al., 2002).

Above 135°C the protein deposited on the fat globule membrane form a network which makes the membrane denser and less permeable (Fink and Kessler, 1986). There is an increase in acidity and viscosity with a decrease in pH with the storage time increased both in UHT. Clare et al. (2005) 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 in container sterilized product is well known. The contamination can either through poor seal or through 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 down stream from the heat treatment stage of the process, which includes spores of *Bacillus cereus* (Davies, 1975 and Wilson et al*.,* 1960) and *Bacillus licheniformis* (Wilson et al*.,* 1960). Organisms of second type will probably have entered through poorly sealed container after aseptic filling.

The types of spores, which have been investigated as of particular relevance in the UHT, are those of *Bacillus stearothermophilus*, *Bacillus subtilis* and *Clostridium botulinum* has been studied. The high spore counts can occur at the dairy farm and that 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 (Westhoff and Dougerty, 1981). These problems had been reported internationally since long, hence the project was planned to observe the physicochemical, microbial and sensory changes in UHT processed milk till its maximum life. In this study the de-clared shelf life of different UHT milk available in market is studied.

MATERIALS AND METHODS

4 different UHT branded milk samples were taken from market. The samples were taken in sterilized syringes for microbiological analysis and in clean stainless steel containers of 1 liter for chemical and sensory analysis. The samples were analyzed at interval 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 similar conditions, as they are stored in market.

For microbiological analysis the samples were examined for aerobic plate counts (APC), *E. coli* counts, *B. cereus, B. subtilis* counts and for spore formers counts. The parameters examined for the chemical analysis were sedimentation, pH, and acidity as lactic acid %, fat % before and after shaking the milk, SNF % before and after shaking and protein % 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 following tests were carried out.

Sedimentation test

Sedimentation test was performed by following modified method as described by Ramsey and Swartzel (1984). According to this method, milk was drain from the cartons leaving the bottom 4 cm. The cartons were inverted for approximately 10 min, up righted and placed in the exhaust hood to dry. The cartons were allowed to dry for 48 h after the bottom flaps or wings of cartons had been opened to facilitate the drying of any sediment entrapped there. The cartons were weighted and then washed thoroughly to remove any sediment or residue adhering to the container. The washed cartons were again dried and weighted.

Solids non fats (SNF) %

Solids non fats (SNF) % was determined by lactometeric method as described by Ramsey and Swartzel (1984).

Total titratable acidity

Total titratable acidity determined according to the method of AOAC, (2005).

pH

The pH value of milk was determined by using a digital pH meter (AOAC, 2005). Prior to use, the pH meter was standardized with standard buffer solution of pH 4 and 7.

Fat

Milk fat % was determined by Gerber (1997) method as described by FAO (1997) by using the butyrometer.

Protein

The protein was estimated by formal titration method (Davide, 1977).

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 %			
	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

Microbial analysis

Microbial analysis was performed according to standard methods (AOAC, 2005).

Total viable counts

The plate count agar media (Bridson, 1995) was used for the total viable count in UHT milk samples (AOAC, 2005). Plates were incubated for 24 h at 37°C.

Determination of coliforms

Coliform counts were determined by pour plate method on violet red bile agar, prepared according to the manufacturer instructions. All plates were incubated at 37°C for 24 h.

Determination of *Bacillus* species

B. cereus selective agar base (Bridson, 1995) is used for isolation and enumeration of *B. cereus* and *B. subtilis.* All plates were incubated at 37°C for 24 h.

Determination of spore formers

Plate count agar media (Bridson, 1995) is used for the enumeration of spore formers. Sterile medium was poured into sterile petri plates and allowed the medium to solidify. Sample is heated at 80°C for 10 min using 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 h at 55°C.

***E. coli* Counts**

For *E. coli* count MacConkey's agar (Bridson, 1995) was used. Sample from lactose positive tubes in case of coliform counts were applied directly on the MacConkey's agar (Bridson, 1995) plates and incubated at 37°C for 24 h.

Sensory analysis

The st or ed milk s ampl es wer e evaluat ed sens orial for colour and fl avor by sc or ing met hod as described by Larmond (1977).

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 company of Pakistan shows shelf life of 12 weeks on the labels of milk packs, during this mentioned period. Milk must be in best condition for consumption. For storage time than a week or 2, these effects may be greater than those of the heat treatment. Changes in colour, flavor 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 1 type of process, the amount of sediments increases in the severity of the heat treatment (Vankatachalm and Macmahon, 1991; Sweetsur and White, 1975). The amount of sediment decreases with homogenization pressure (Robinson, 1994). Results obtained from sedimentation test in UHT milk during storage period of 3 months (12 weeks) shows that there is an effect of heat processing and subsequent storage on sedimentation in all 4 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 sediments after week 6. Sample III reaches up to 7.10/250 gml^{-1}, which is a considerable changes

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

and sample II showed formation of sediments after week 5. The alcohol test can be used to detect raw milk that is likely to give high level of the normal type of sediments and there are indications that it may be useful in predicting the abnormal type (Sweetsur and White, 1975). Processing operations influences acid base equilibrium in milk. UHT treatment results in a pH decrease, due to conversion of lactose into different organic acids (Fox and McSweeny, 1998). In milk, casein micelles are stable at natural pH, that is, 6.7. Lowering the pH facilitates aggregations of casein micelles and forms a gel. Results regarding effect of storage on pH of UHT processed milk during storage period of 90 days show that there is storage effect on pH level. Maximum pH value (6.81and 6.85 in samples 1 and 2, respectively and 6.75 in sam-ples III and IV) was recorded in 1^{st} week while minimum pH values obtained in 12^{th} 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 (1991) verified drop in pH and they associated it with browning reactions. Andrews et al. (1977) 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 subsequent 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 (Swartzel, 1983). Results obtained by the analysis for total titratable acidity (Table 1), show that there is storage effect on the total titratable acidity. The acidity value was 0.11% while during storage of UHT milk minimum acidity was recorded in 1^{st} week and maximum value (0.18%) at 90 days life in sample 1 while 0.15 in case of sample 1 and 0.13 in samples 2 and 4. 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 protein, antimicrobial proteins etc. These proteins coagulate after heating hence the texture of milk is dete-riorated during storage (Fox and McSweeny, 1998). Casein polymerization is greater at high storage tem-perature, 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 (Andrews, 1977). The results regarding protein % of stored UHT milk describes that there is effect of storage on protein contents of UHT processed milk. Results shown in Figure 2 give a glance on protein contents, that is, in week 1 protein contents were 3.30%, 3.70% for sample 1 and 2 while in week 12 of storage were 2.35 and 3.48 respectively (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 % 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 poly-acrylamide 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, like increased susceptibility of the fat to oxidation, since it is not protected by a mem-brane and release of free fatty acids by lipase activity. In short, fat % is reduced a little bit with storage (Fox and McSweeny, 1998). The results of fat % before shaking are shown in Figure 1, as in week 1, fat % is 3.55, 3.66, 3.88 and 3.50 and in last week, that is, week 12 it reaches to 2.70, 3.50, 1.85 and 3.00 for samples I, II, III and IV, respectively. In week 1, SNF % 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

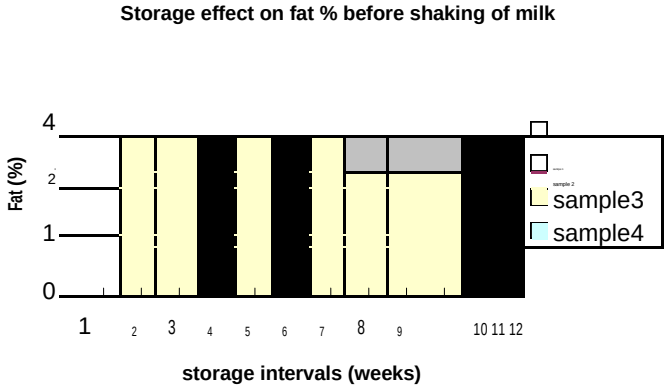

Figure 1. Effect of storage on fat % before shaking of milk.

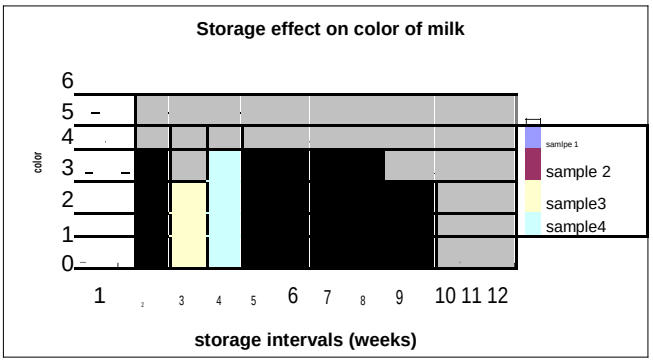

Figure 4. Effect of storage on colour of milk.

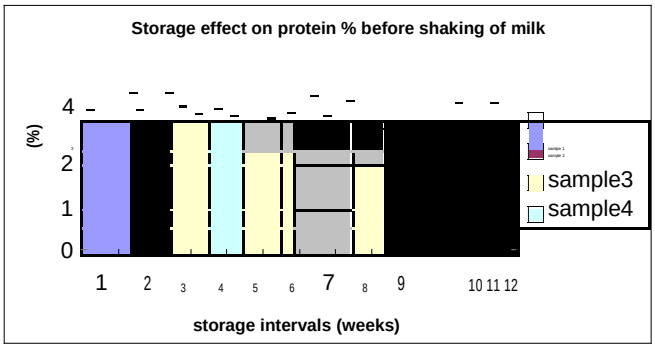

Figure 2. Effect of storage on protein % before shaking of milk.

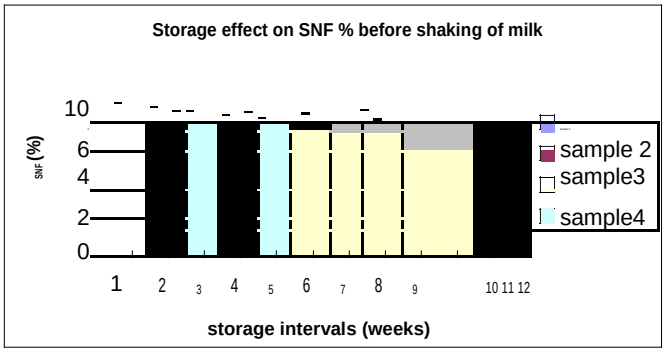

Figure 3. Effect of storage on SNF % before shaking of milk.

3); while there is no change in fats and SNF % in all samples after shaking of UHT milk (Table 2) .Not a single colony observed on aerobic plate count plates, *coliform* agar plates, *E. coli* plate, *B. cereus, B. subtilis*, and spore formers plates, in all the 4 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 organism is not observed in this study. The pasteurization or other treatment of milk re-moves the microorganisms, spore germination or recon-tamination can still cause quality deterioration. Further-more, heat resistant extracellular proteinases and lipases produced by psychrotrophic bacteria before processing represent a major spoilage factor of stored milk (Sorhaug and Stepaniak, 1997). Due to UHT process no micro-organism survived but the heat resistant enzymes may be present in UHT milk, which are responsible for spoi-lage of UHT milk during storage.

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 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. (2003), who observed that off white colour of UHT pro-cessed stored samples, was changed to light brown during storage. Maillard's reaction is one of the most important reasons of colour loss (Martel et al., 1987). Colour scores decreased with an increase in storage interval. In sample 1 during week 1, colour score was maximum that is, 3 and in week 12 and the lowest colour score was observed, that is, 1. In sample 2 during week 1, the colour score was i.e. 5 and in week 12, minimum score was observed, that is, 3. Score was 4, in sample 3 during week 1, and in week 12, 1 was colour score. In sample 4 during week 1, 4 were maximum score while it reaches to 3 with the end of 12 week. Data et al (2002) accounts various changes for flavor decrease, that is, maillard browning, sulphdryl flavor change as a result of lipid degradation, which principally falls into 2 categories, liberation of volatile fatty acids such as butyric acid fatty

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

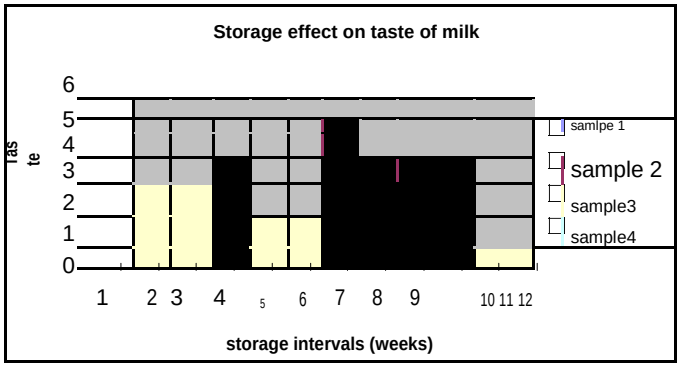

Figure 5. Effect of storage on taste of milk.

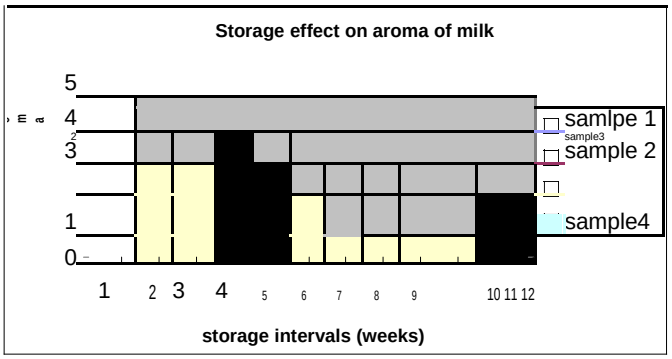

Figure 6. Effect of storage on aroma of milk.

acids with subsequent formation of volatile compounds. It was observed that 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 flavor score was 3 and in week 12 the lowest score was observed, that is, 1. In week 1, for sample II, the flavor score was 5 and in week 12 the lowest score was observed, that is, 3. In week 1 of sample III, the flavor score was 3 and in week 12 the lowest score was observed, that is, 1, while in sample IV during 1st week flavor score was 4 and it was changed to 2 in week 12. Results show (Figure 6) that there is effect of storage on the aroma attributes of the UHT milk samples. Aroma scores decrease with an increase in storage interval. The aroma score was maximum, that is, 3 and in week 12, the lowest score was observed, that is, 1, in sample I in week 1. In sample II during week 1, the aroma score was maximum that is, 5 and in week 12, the lowest score was observed, that is, 3. In sample III during week 1, the aroma score was maximum that is, 3 and in week 12, the lowest score was observed, that is, 1. The results showed that for sample IV, during week 1, the aroma score was 4 and in week 12 the score reached 2.

Conclusion

It is concluded from the whole study that there is an increase in sedimentation value, fat separation, titratable acidity during storage, while decrease was found in pH and protein % during storage of 12 weeks. The increase in sedimentation shows excessive protein denaturation during processing and subsequent storage. In UHT processed milks the fat separation was observed during storage. This high % of fat separation is attributed with less homogenizing efficiency during processing. On microbiological examination, not any colony found on TPC plates, *coliform* agar plates, *E. coli* plate*, B. cereus, B. subtilus*, and spore formers plates, in all the 4 samples of UHT milk during storage of 12 weeks. Sensory characteristics showed a significant decrease in scores during storage. These all are factors that limit the shelf life of UHT milk. The shelf life of milk mainly depend on the quality of raw milk and better quality of milk can be achieved in Pakistan, when the manufacturers have bet-ter milk collection system. The manufacturer of sample II has its own sophisticated type of milk collection system said to be VMCs (village milk collection centers). At these centers, milk is collected at small scale and in 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 better way before heat treatment or processing. While manufacturers of other dairy industries of Pakistan get milk from contractors and ice added milk is mostly supplied to these industries which disturb the mineral balance and natural emulsion and give higher water activity which leads to physicochemical, microbiological as well as sensory changes during shelf life of milk.

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