Egyption Journal for Specialized Studies – Vol (1) – N (5) – October 2007
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Email: egyjournal@sedu.asu.edu.eg
ISBN: 1687-6164
ISNN: 2682-4353

Introduction

The quality of foods depends on the nutritive value i.e. various elements content such as vitamins. Vitamin C is considered as L-ascorbic acid, dehydroascorbic acid and other forms that are inactive. L-ascorbic acid is produced from glucose in the animal and vegetable cell by gulonolakton oxydase. Humans do not posses this enzyme so that they are not capable of producing L-ascorbic acid [(1)].

Fruit and vegetables, commonly considered good sources of vitamin C, are the foods most often left uneaten by children. Milk and milk products, however, is consumed by the majority of school children. Unfortunately, the milk contains small amount of vitamin C; 1.8 – 2.0 mg% in cow milk and goat milk [(2)]. Accordingly, the addition of ascorbic acid to milk could be one means of enhancing vitamin C consumption by children without adding greatly to the cost or bulk of school of meal [(3)].

L-ascorbic acid performs very important functions such as taking part in the hydroxylation process of the praline residues in collagen, participating in the synthesis of steryde hormones and in the hydroxylation of aroma compounds [(4)]. Moreover this vitamin preserve structural and function activity of DNA and lipoprotein and has some demonstrated as being an important antioxidant [(5)]. Regardless of its roles, ascorbic acid is known to be very unstable and easily destroyed during processing by temperature, pH, oxygen, UV light, *etc* [(6)].

Cow and Goat milk yoghurts are the products which combine the properties of fermented milk. They have a positive effect of intestinal flora; they blocked some kind of cancer and gave hypocholerosterolemic effects. Due to their properties they can be consumed by people allergic to casein alfa S1. Moreover, goat milk and goat yoghurts are often consumed by people who do not tolerate lactosis [(2)]. Cow and goat milk and its yogurts are very often consumed by children especially allergic children and elderly people. These groups of consumers need more vitamin C than others.

Cow's and goat's milk and its products are not rich in L-ascorbic acid. Therefore, enrichment those with L-ascorbic acid are becoming important. Our objective in this study was to identify the respective roles of the pasteurization process, fermentation and storage conditions for various duration periods and temperatures for non-fortified or fortified cows' or goat milk and its yoghurts on the stability of L-ascorbic acid content.

Material and Methods

Milk samples and fortification. Ten kilograms of pasteurized cow's and goat's milk were purchased from local market in Cairo, Egypt. Each of two kinds of milks was treated as follows:

- non-enrich milk
- enrich milk with 0.1 mg of analytically pure L-ascorbic acid dissolved in 50 ml distilled water
- enrich milk with 30 ml of lemon juice

This fortification has been done before and after pasteurazion of milk at 85 ^{o}C, for 5 minutes and then cooled. The Two kinds of milks were cooled in refrigerator to 5 ^{o}C. Then, each kind of milk was divided into two groups. First group was stored at chill temperature (approximately 5 ^{o}C) for 6 days. Second group was stored at ambient temperature (approximately 20 ^{o}C) for 6 hours. During the storage the content of L-ascorbic acid were daily estimated for the first group and every one hour for the second group.

Yoghurts. The examined yoghurts were produced by thermostatic method in the home economic microbiological laboratory, Faculty of Specific Education, Ain Shams University according to the standard method of making yoghurts [(2)]. The yoghurts were produce in enriched and non-enriched forms according to the required procedure. The milk used to make enrich yoghurts production were fortified by adding 0.1 mg of analytically pure L-ascorbic acid dissolved in 50 ml distilled water or 30 ml of lemon juice. The cow's and goat's milk were cooled to 10 ^{o}C and vaccinated with yoghurt culture (Biolacta-Texel). 150 ml of milk prepared in such a way in was poured into 200 ml cups of polyethylene and then lids were bringing back on tightly. The milk in cups was incubated at 37 ^{o}C for four hours. The yoghurts were cooled in refrigerator to 5 ^{o}C. Then, the yoghurts were divided into two groups. First group was stored at 5 ^{o}C for 12 days. Second group was stored at 20 ^{o}C for 6 days. During the storage the content of L-ascorbic acid were daily estimated by Tillmans methods applying 2, 6-dichlorophenolindophenol[(7)]. Three replicate series were tested.

Sensory evaluation. The sensory evaluation was conducted on fortified and non fortified cow's or goat's milks and its yoghurts at the end of storage periods. Ten panelists were recruited from the faculty members and graduate students in the Home Economics department, Faculty of Specific Education, Ain Shams University.

The intensity of off-flavor and sourness were scored on a 5-point scale (1= none, 2 = slight, 3 = moderate, 4 = strong and 5 = very strong), and overall preference was scored on a 5-point scale (1= like extremely, 2 = like moderately, 3 = neither like nor dislike, 4 = dislike moderate and 5 = dislike extremely). This balance of sensory evaluation was designed according to Lee et al., 2004 [(6)].

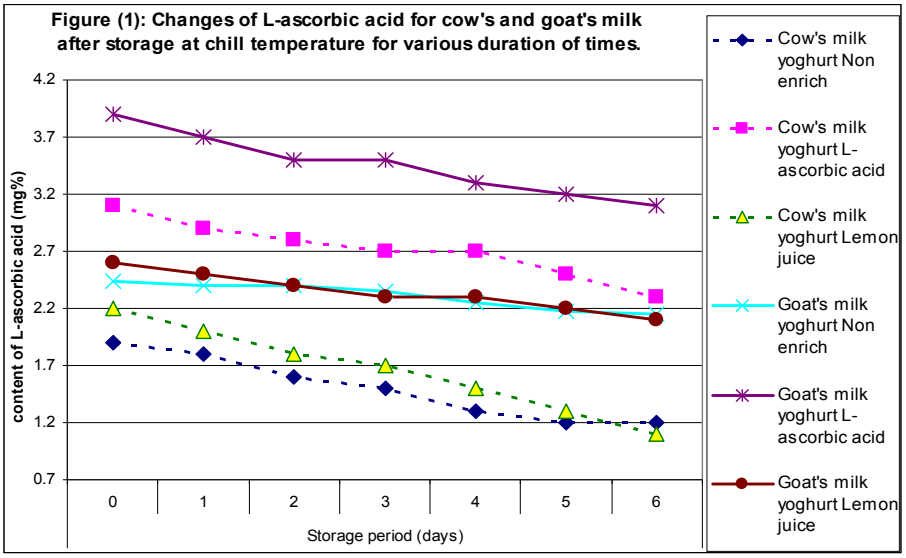
Result and discussion

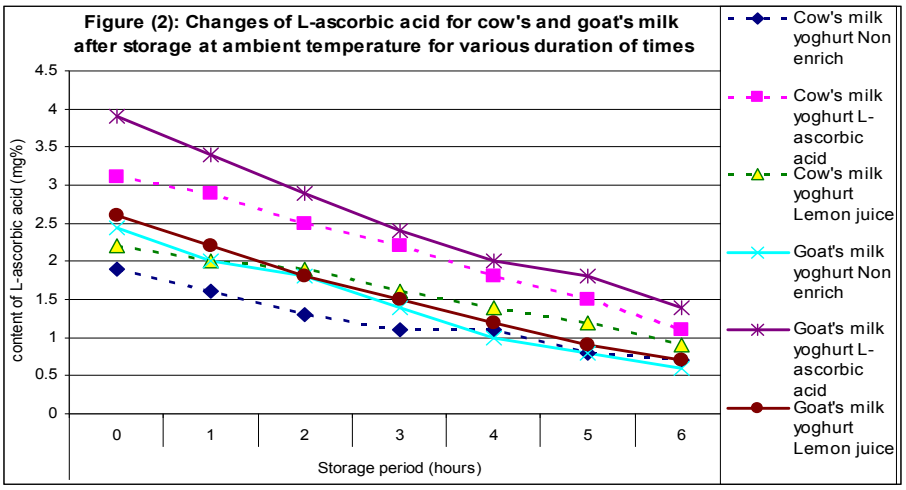
A - Degradation of L-ascorbic acid in non-fortified and fortified cow's and goat's milk during storage

Figure 1 presents the L-ascorbic acid degradation curve in fortified cow's and goat's milks as a function of storage time. The milk batch was fortified with 0.1 mg of analytically pure L-ascorbic acid or 30 ml of lemon juice/L before pasteurization treatment (value provided by the dairy plant).

It should be noted that fresh or fortified goat's milk with analytically L-ascorbic acid or lemon juice tended to have L-ascorbic acid higher than cow's milk counterparts. The current results observed a dramatic drop in the L-ascorbic acid content of samples stored at ambient temperature for 6 hours, and slow degradation for samples stored at chill temperature for 2 days. This result is completely within with the recent study observed that the

long storage time allowed by pasteurization technologies is associated with a significant physicochemical evolution of the food product. Among the reactions developing upon storage, oxidation of L-ascorbic acid is the best documented[8].





In both fortified milks, which were fortified with analytically pure of L-ascorbic acid, the mean decrease in L-ascorbic acid content after 6 days of storage at chill temperature was approximately 25% of the initial value (Figure1). This percentage has been increased to approximately 66% of the entail value after storage at ambient temperature for 6 hours only (Figure 2). It was observed that, the losses of L-ascorbic acid in both milks' enriched with lemon juice lower than its counterparts that enriched with analytically pure source. It may be due to the fact that L-ascorbic acid is particularly prone to degradation during processing because of its high susceptibility to oxidation in the presence of oxygen and metal ions [9].

Table (1). Effects of pasteurization milk treatment on L-ascorbic acid content

samples	Cow's milk								Goat's milk							
treatment	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G	H
L-ascorbic acid	2.1	1.91	2.4	2.1	1.9	3.1	2.7	2.5	2.4	2.0	2.6	2.2	2.0	3.9	3.3	3.1
% losses	-	9	-	12.5	20	-	13	19	-	16	-	15	23	-	15	20

A. Fresh samples (no treatment)
B. Pasteurized milk
C. Un-pasteurized milk enriched with lemon juice
D. Pasteurized milk enriched with lemon juice after pasteurization
E. Pasteurized milk enriched with lemon juice before pasteurization
F. Un-pasteurized milk enriched with pure L-ascorbic acid
G. Pasteurized milk enriched with pure L-ascorbic acid after pasteurization
H. Pasteurized milk enriched with pure L-ascorbic acid before pasteurization

Mean L-ascorbic acid in two batches of cow's milk and goat's milk was present in table 1. The first analysis of L-ascorbic acid was done immediately after and before pasteurization. After pasteurization, the L-ascorbic acid content was decreased to reach 12.5 to 20% of the initial concentration. There was a reduction in L-ascorbic acid due to pasteurization can be observed by comparing treatments A and B or C and D, even D and E. In table 1 the losses of

unfortified samples was similar to losses reported by Scott and Bish (1988)[10]. There was some evidence of interaction, i.e., less L-ascorbic acid loss due to pasteurization in the unfortified milk than the fortified counterparts. This result is agreed with those reported by Wojciechowska, (2002)[2]. In the fortified milk, L-ascorbic acid added after pasteurization deteriorated less rapidly than that added before pasteurization. The non-enriched cow's milk showed the least deterioration of L-ascorbic acid.

B- Degradation of L-ascorbic acid in non-fortified and fortified cow's milk yoghurt and goat's milk yoghurt during storage

To examine the stability of yoghurts L-ascorbic acid content during storage, the yoghurts after making stored for 12 days at chill temperature. The degradation of this acid content was then daily determined after 0 to 12 days as shown in table 2. The obtained results point out that yoghurts examined immediately after enriching with lemon juice resulted in a rise of L-ascorbic acid by 0.9 mg% and 1.1 mg% for cow's yoghurt and goat's yoghurt, in comparison to non-enrich yoghurts, respectively. Whereas, analytically L-ascorbic acid increased that amount by 1.9 mg% and 2.2 mg% for cow's yoghurt and goat's yoghurt, respectively. As seen from table 1, 2 and 3 the L-ascorbic acid content if fresh cow's and goat's yoghurts were higher than those in pasteurized milk, even higher than fresh milk. The observed increase in L-ascorbic acid in non-enriched fermented milk could be attributed to synthesis of this vitamin by microorganisms that were use in fermentation [1].

In the end of storage period at chill temperature, the losses of L-ascorbic acid were 35.7 % and 61 % for cow's milk yoghurt enriched with lemon juice and pure analytically L-ascorbic acid, respectively. While, the losses of L-ascorbic acid were 43.3 % and 57.14 % for goat's milk yoghurt enrich with lemon juice and pure analytically L-ascorbic acid, respectively. This result approximately agreed well with similar study which stated that the retention of L-ascorbic acid was ranged from 48 : 64% in examined enriched yoghurts from cow's milk after storage for 10 days at cold temperature [(11)]. In another study stated by Noh and Coop (1994) [(12)] claimed that the level of vitamin C in fortified cow's milk yoghurt decreased after 15 days of storage to 49.2% of the initial level. Moreover, Fiedlerove and Coop (1993) [(13)] and Wojciechowska et al., (2001) [(14)] observed that 50 : 70% of L-ascorbic acid were losses in fortified cow's milk yoghurts after 10 days of storage.

During storage of yoghurts the similar level of that acid was observed after 6 days at ambient temperature and after 11 days at chill temperature (Tables 2 and 3). Therefore, the minimum losses of L-ascorbic acid were in yoghurts stored at chill temperature. This observation in general agree with previous studies which reported that, The deleterious effect of variables such as oxygen and pH are influenced by temperature. Therefore, low temperature storage is imperative in order to regard L-ascorbic acid delay [(15 & 16)]. It may be due to considerable change of rhelogic properties of yoghurts (considerable stratification) during this kind of storage [(2)].

Table 2. Changes of L-ascorbic acid for cow's and goat's milk yoghurts after storage at chill temperature for various duration of times.

sample	treatment	Storage period (day)												
		0	1	2	3	4	5	6	7	8	9	10	11	12
Cow's milk yoghurt	Non enrich	2.3 ± 0.01[E]	2.0 ± 0.08[F]	1.7 ± 0.02[FG]	1.5 ± 0.04[G]	1.3 ± 0.1[GH]	1.1 ± 0.04[H]	1.0 ± 0.02[HI]	0.9 ± 0.04[HI]	0.8 ± 0.02[HI]	0.6 ± 0.01[I]	0.5 ± 0.04[J]	0.3 ± 0.01[K]	0.2 ± 0.02[K]
	L-ascorbic acid	3.9 ± 0.12[AB]	3.7 ± 0.06[B]	3.5 ± 0.11[C]	3.2 ± 0.14[CD]	3.0 ± 0.07[D]	2.8 ± 0.09[DE]	2.7 ± 0.11[DE]	2.4 ± 0.10[E]	2.2 ± 0.06[F]	2.1 ± 0.06[EF]	1.9 ± 0.08[F]	1.8 ± 0.14[F]	1.5 ± 0.03[G]
	Lemon juice	2.9 ± 0.08[D]	2.9 ± 0.06[D]	2.7 ±0.06[DE]	2.4 ± 0.07[E]	2.3 ± 0.15[E]	2.1 ± 0.06[EF]	2.0 ± 0.03[F]	1.9 ± 0.12[F]	1.8 ± 0.04[FG]	1.85±0.09[FG]	1.75 ±0.08[FG]	1.6 ± 0.08[FG]	1.5 ± 0.02[G]
Goat's milk yoghurt	Non enrich	2.4 ± 0.09[E]	2.1 ± 0.12[EF]	1.9 ± 0.03[F]	1.8 ± 0.01[FG]	1.7 ± 0.03[FG]	1.6 ± 0.12[FG]	1.5 ± 0.09[G]	1.5 ± 0.06[G]	1.4 ± 0.02[G]	1.2 ± 0.08[H]	1.1 ± 0.02[H]	1.1 ± 0.02[H]	1.0 ± 0.01[HI]
	L-ascorbic acid	4.2 ± 0.05[A]	4.1 ± 0.16[A]	3.9 ±0.16[AB]	3.7 ± 0.12[B]	3.3 ± 0.09[C]	3.1 ± 02.0[D]	3.0 ± 0.13[D]	2.8 ±0.12[DE]	2.6 ± 0.10[E]	2.3 ± 0.07[E]	2.1 ± 0.04[EF]	2.0 ± 0.05[F]	1.8 ± 0.09[FG]
	Lemon juice	3.0 ± 0.02[D]	2.9 ± 0.06[D]	2.7 ±0.07[DE]	2.5 ± 0.09[E]	2.3 ± 0.11[E]	2.2 ± 0.07[EF]	2.1 ± 0.07[EF]	2.0± 0.05[F]	2.0 ± 0.05[F]	1.95 ± 0.11[F]	1.8 ± 0.07[FG]	1.7 ± 0.12[FG]	1.7 ± 0.04[FG]

Means with the same letter are no differed significantly (0.05).

X ± S average ± standard error

Table 2. Changes of L-ascorbic acid for cow's and goat's milk yoghurts after storage at chill temperature for various duration of times.

sample	treatment	Storage period (day)												
		0	1	2	3	4	5	6	7	8	9	10	11	12
Cow's milk yoghurt	Non enrich	2.3 ± 0.01^{E}	2.0 ± 0.08^{F}	1.7 ± 0.02^{FG}	1.5 ± 0.04^{G}	1.3 ± 0.1^{GH}	1.1 ± 0.04^{H}	1.0 ± 0.02^{HI}	0.9 ± 0.04^{HI}	0.8 ± 0.02^{HI}	0.6 ± 0.01^{I}	0.5 ± 0.04^{J}	0.3 ± 0.01^{K}	0.2 ± 0.02^{K}
	L-ascorbic acid	3.9 ± 0.12^{AB}	3.7 ± 0.06^{B}	3.5 ± 0.11^{C}	3.2 ± 0.14^{CD}	3.0 ± 0.07^{D}	2.8 ± 0.09^{DE}	2.7 ± 0.11^{DE}	2.4 ± 0.10^{E}	2.2 ± 0.06^{E}	2.1 ± 0.06^{EF}	1.9 ± 0.08^{F}	1.8 ± 0.14^{F}	1.5 ± 0.03^{G}
	Lemon juice	2.9 ± 0.08^{D}	2.9 ± 0.06^{D}	2.7 ± 0.06^{DE}	2.4 ± 0.07^{E}	2.3 ± 0.15^{E}	2.1 ± 0.06^{EF}	2.0 ± 0.03^{F}	1.9 ± 0.12^{F}	1.8 ± 0.04^{FG}	1.85 ± 0.09^{FG}	1.75 ± 0.08^{FG}	1.6 ± 0.08^{FG}	1.5 ± 0.02^{G}
Goat's milk yoghurt	Non enrich	2.4 ± 0.09^{E}	2.1 ± 0.12^{EF}	1.9 ± 0.03^{F}	1.8 ± 0.01^{FG}	1.7 ± 0.03^{FG}	1.6 ± 0.12^{FG}	1.5 ± 0.09^{G}	1.5 ± 0.06^{G}	1.4 ± 0.02^{G}	1.2 ± 0.08^{H}	1.1 ± 0.02^{H}	1.1 ± 0.02^{H}	1.0 ± 0.01^{HI}
	L-ascorbic acid	4.2 ± 0.05^{A}	4.1 ± 0.16^{A}	3.9 ± 0.16^{AB}	3.7 ± 0.12^{B}	3.3 ± 0.09^{C}	3.1 ± 02.0^{D}	3.0 ± 0.13^{D}	2.8 ± 0.12^{DE}	2.6 ± 0.10^{E}	2.3 ± 0.07^{E}	2.1 ± 0.04^{EF}	2.0 ± 0.05^{F}	1.8 ± 0.09^{FG}
	Lemon juice	3.0 ± 0.02^{D}	2.9 ± 0.06^{D}	2.7 ± 0.07^{DE}	2.5 ± 0.09^{E}	2.3 ± 0.11^{E}	2.2 ± 0.07^{EF}	2.1 ± 0.07^{EF}	2.0 ± 0.05^{F}	2.0 ± 0.05^{F}	1.95 ± 0.11^{F}	1.8 ± 0.07^{FG}	1.7 ± 0.12^{FG}	1.7 ± 0.04^{FG}

Means with the same letter are no differed significantly (0.05).

$X \pm S$ average ± standard error

Fermented milk differed significantly with regards of L-ascorbic acid content in enriched and non-enriched yoghurts counterpart. No significant differences concerning L-ascorbic acid content in fresh cow's and goat's yoghurts were also found (table 2 and 3). After 7 days of storage at ambient temperature L-ascorbic acid content in all investigated samples was much lower than in fresh. Yoghurts from cow's or goat's milk which fortified with analytically pure L-ascorbic acid had the highest L-ascorbic acid values. On the other hand, the highest speed of L-ascorbic acid degradation in yoghurts fortified with analytically pure water solution of L-ascorbic acid occurs probably due to free form of acid used [14]. Furthermore, the acid was more subject to oxidants action in yoghurts than natural form (lemon juice) [1].

Table 4. Some sensory score for milk and yoghurt enriched with L-ascorbic acid for up to 12 days at chill temperature.

Sensory description	treatment	Storage period (day)										
		0		2		4		6		8	10	12
		milk	yoghurt	milk	yoghurt	milk	yoghurt	milk	yoghurt	yoghurt		
Sourness	Control	1.0 ^{A}	1.0 ^{A}	1.0 ^{A}	1.0 ^{A}	1.0 ^{A}	1.0 ^{A}	1.2 ^{A}	1.8 ^{AB}	1.9 ^{B}	1.9 ^{B}	2.2 ^{B}
	Ascorbic acid enrich	1.1 ^{A}	1 ^{A}	1.1 ^{A}	1.6 ^{AB}	1.2 ^{A}	1.6 ^{AB}	1.8 ^{AB}	3.3 ^{C}	4.0 ^{D}	4.0 ^{D}	4.3 ^{E}
	lemon juice enrich	1.2 ^{A}	1.0 ^{A}	1.0 ^{A}	1.2 ^{A}	1.1 ^{A}	1.6 ^{AB}	1.6 ^{AB}	2.2 ^{B}	2.8 ^{BC}	2.8 ^{BC}	3.1 ^{C}
Off-flavour	Control	1.0 ^{A}	1.0 ^{A}	1.2 ^{A}	1.2 ^{A}	1.2 ^{A}	1.2 ^{A}	1.2 ^{A}	1.4 ^{A}	1.4 ^{A}	1.8 ^{AB}	2.3 ^{B}
	Ascorbic acid enrich	1.3 ^{A}	1.1 ^{A}	1.3 ^{A}	1.3 ^{A}	1.3 ^{A}	1.6 ^{AB}	1.5 ^{AB}	2.6 ^{BC}	3.8 ^{D}	3.8 ^{D}	4.1 ^{D}
	Lemon juice enrich	1.1 ^{A}	1.0 ^{A}	1.0 ^{A}	1.2 ^{A}	1.3 ^{A}	1.2 ^{A}	1.2 ^{A}	2.4 ^{BC}	2.6 ^{BC}	2.6 ^{BC}	2.3 ^{BC}
Overall preference	Control	1.0 ^{A}	1.2 ^{A}	1.2 ^{A}	1.2 ^{A}	1.6 ^{AB}	1.8 ^{AB}	1.8 ^{AB}	2.0 ^{B}	2.0 ^{B}	2.3 ^{B}	2.3 ^{B}
	Ascorbic acid enrich	1.3 ^{A}	1.3 ^{A}	1.5 ^{AB}	2.6 ^{BC}	1.8 ^{B}	2.2 ^{B}	2.3 ^{B}	3.1 ^{C}	3.1 ^{C}	3.8 ^{D}	3.8 ^{D}
	Lemon juice enrich	1.0 ^{A}	1.1 ^{A}	1.1 ^{A}	1.3 ^{A}	1.4 ^{A}	1.9 ^{AB}	2.0 ^{B}	2.0 ^{B}	2.3 ^{B}	2.3 ^{B}	2.6 ^{BC}

Means in the column without the same letter are significantly different (P >0.05)

The sensory properties of milk and yoghurts fortified with pure analytically L-ascorbic acid and lemon juice were evaluated (table 4). When 0.1 mg of analytically pure L-ascorbic acid dissolved in 50 ml distilled water or 30 ml of lemon juice has been added to milk, the sourness score was not significant different from that of the control up to 6 days ($P > 0.05$), but increased thereafter up to 12 days for milk and yoghurts. In respect of the off-taste, both of L-ascorbic acid treated groups showed non-significant difference up to 3 days, but increase thereafter up to 6 days. When analytically pure L-ascorbic acid has been added sourness and off-taste increased and showed significant differences from that of the counterparts fortified with lemon juice after 8 days and longer. In respect of the overall preference the control and fortified milk and yoghurt with lemon juice showed high consumer preference up to 12 days of storage at chill temperature. While, samples fortified with analytically pure L-ascorbic acid impaired the consumer's milk and yoghurts preference after 4 days of storage. Therefore, the sensory quality of milk and yoghurt enriched with chemical forms of L-ascorbic acid was shown to be well sensory accepted up to 4 days at least. On the other hand, sensory quality of milk and yoghurt enriched with natural source (lemon juice) was well conventional up to 12 days of storage.

Conclusions

- Variation of L-ascorbic acid content in fermented milk during the storage period may give evidence of its synthesis by the micro-organism of the starter cultural.
- The L-ascorbic acid content for fortified and non-fortified has been sharply decreased after pasteurization.
- L-ascorbic acid degradation occurs in examined milk and yoghurt when they are stored at chill or elevated temperature. But, the chill temperature is more favorable form of storage.
- Cow's or goat's milk and yoghurt enriched with synthetic L-ascorbic acid tended to have L-ascorbic acid content higher than its counterparts enriched with natural source. However, synthetic L-ascorbic acid destabilization was higher than natural source. Subsequently, enrichment milk and yoghurt with natural source is the most favorable.
- All samples fortified with natural source were considered sensory properties acceptable up to 12 days. While, samples fortified with chemical source were considered harsh in taste and overall preference after 4 days of storage.

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