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The effect of addition of β-carotene on casein, lactose & acidity of whole milk

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Abstract: *The present study utilised the antioxidant property of β-carotene to arrest or lessen the degradation of some principal constituents of cow milk. Application of β-carotene in different concentrations towards retardation of deteriorating stages of protein, sugars and acidity of milk during the experimental period of 8 days were observed. Each of milk samples fortified with β-carotene has shown to retain a better nutritional quality on storage in comparison to the non-fortified sample and 100 ppm β-carotene fortification resulted in the maximum retention of protein (loss of only5.14%) during the study period. According to the study, the best quencher and acidity blocker (acidity decreased by 58.40%) as well as the highest retention and availability of milk sugars (milk sugars increased by 3.35%) has been depicted by the maximum (2000ppm) fortification of β-carotene.*
Keywords – *antioxidant property, β-carotene, casein, lactose, milk acidity, milk quality Introduction*

I. Introduction

Milk is considered to be the most satisfactory single food substance elaborated by nature. Milk is the lacteal secretion, particularly free from colostrums, obtained by complete milking of one or more healthy cows, which contains not less than 8.25 percent milk solid non fat and not less than 3.25 percent milk fat [1].

1.1 Composition of milk: Milk is a complex mixture of lipids, carbohydrate, proteins and many other organic compounds and inorganic salts dissolved or dispersed in water. The most variable component of milk is fat followed by protein. Milk also contains trace amounts of other substances such as pigments, enzymes, vitamins, phospholipids (substances with fatlike properties), and gases. The residue left when water and gases are removed is called the dry matter (DM) or total solids content of the milk.

Nutritive value of cow milk (per 100 gm): [2]

Moisture (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
87.5	67	3.2	4.1	4.4

1.2 Milk fat: One of the most important constituents of milk is the milk fat that is the triglycerides and phospholipid comprising of several medium and long chain fatty acids like lauric & linoleic acid, α-linolenic acid etc. On storage at ambient temperature, the fat, especially, the free fatty acids undergo oxidation and several low molecular weighted compounds like aldehydes, ketones are formed which makes the fat rancid and give off foul smell [3]. Cow's milk contains about 4.1 % fat, which is of great economical and nutritive value. The flavour of milk is due to the presence of milk fat. Milk is a true emulsion of oil-in-water. The dispersed fat globules are surrounded by a thin layer which is composed of a lipid-protein complex and a small amount of carbohydrate. The lipid portion includes both phospholipid and triglycerides [4]. Fatty acid present in milk also possess potent antimicrobial antiviral as well as antifungal activity [5].

Fats in foods are subject to two types of deterioration that affect the flavour of food products.

Hydrolytic rancidity: Fatty acids are broken off from the glycerol molecule by lipase enzymes produced by milk bacteria. The resulting free fatty acids are volatile and contribute significantly to the flavour of the product.

Oxidative rancidity: Fatty acids are oxidised. In milk products it causes tallowy flavours. Oxidative rancidity of dry butterfat causes off-flavours in recombined milk. The oxidation of milk fat not only contributes to off flavour and off odour development but also the milk spoilage. The free fatty acids are believed to have an anti microbial property thus the presence of free fatty acid may retard or delay the microbial infestation in milk during storage.

1.3Milk protein: Milk proteins represent one of the greatest contributions of milk to human nutrition.

Casein: Casein is a group name for the dominant class of proteins in milk. The caseins easily form polymers containing several identical or different types of molecules. Due to the abundance of ionisable groups and hydrophobic and hydrophilic sites in the casein molecule, the molecular polymers formed by the caseins are very special. The polymers are built up of hundreds and thousands of individual molecules and form a colloidal solution, which is what gives skim milk its whitish-blue tinge. These molecular complexes are known as casein micelles.

1.4 Milk carbohydrates

Lactose is the major carbohydrate fraction in milk. It is made up of two sugars, glucose and galactose. The average lactose content of milk varies between 4.7 and 4.9%, though milk from individual cows may vary more. It can be broken down to glucose and galactose by bacteria that have the enzyme galactosidase. The glucose and galactose can then be fermented to lactic acid. This occurs when milk goes sour. Under controlled conditions they can also be fermented to other acids to give a desired flavour, such as propionic acid fermentation in Swiss-cheese manufacture. Lactose is present in milk in molecular solution. In addition to lactose, milk contains traces of glucose and galactose. Carbohydrates are also present in association with protein. K-casein, which stabilises the casein system, is a carbohydrate-containing protein**.**

1.5 Minor milk constituents

In addition to the major constituents discussed above, milk also contains a number of organic and inorganic compounds in small or trace amounts, like salts, vitamins, some of which affect both the processing and nutritional properties of milk.

1.6 Spoilage of milk

Milk is an ideal medium for the growth of many organisms, having high water content and abundant nutrients, and being nearly neutral pH (6.4–6.8). A plentiful supply of food for energy is available in the form of milk sugar (lactose), milk fat, citrate, and nitrogenous compounds (proteins, amino acids, ammonia, urea and other non-protein nitrogenous compounds). In addition, the Eh (oxidation-reduction potential) of milk is above 0.3 volts, thus enabling aerobes to grow readily. Microorganisms present in milk can be classified into two main groups: **pathogenic** and **spoilage** organisms, although some may play a dual role (e.g. **Bacillus cereus**). By virtue of their elaborated enzymes (e.g. protease, peptidase, lipase, esterase, oxidase, polymerase, β-galactosidase), spoilage organisms are capable of hydrolyzing milk components such as protein, fat and lactose in order to yield compounds suitable for their growth. Such reactions can lead to spoilage of milk, manifested as off-flavours and odours, and changes in texture and appearance. Microflora of raw milk, the types of organisms present in raw milk is influenced by temperatures and time of storage as well as methods of handling during and after milking.

Due to high water activity, milk is a highly perishable food item and is very much prone to microbial infestation that leads to spoilage and deterioration of the nutrients especially, the milk sugar lactose, casein and many else. Along with it, the acidity of milk usually increases over time as lactose fermenting bacteria attack the lactose and produces lactic acid which lowers the p^H to about 4.6 from an average of 6.75 that leads to firstly souring of milk and then ultimately curdling.

1.7. Role of β-carotene

β-carotene, a well known natural antioxidant extracted from raw carrot using food grade ethanol as solvent [6], addition of which prevents the free fatty acid oxidation [7] that is on one hand beneficial for the retardation of off-flavour development [8] and analogously the intact fatty acid may contribute antimicrobial activity [9] which delays the spoilage of milk on storage. Optimisation of storing process to retain more bioavailable β-carotene in carrot was established in the previous work of the present authors [10]. In an oxidation process, organic systems produce free radicals which start off chain reaction. Antioxidants terminate these chain reactions and prevent or slowdown the process of oxidation reaction by means of removing free radical intermediates or by being oxidized themselves. Hence, most antioxidants are reducing agents. As the microbial load on milk is lower, the process of fermentation - thereby lowering of p^H and souring or curdling of milk is delayed and the shelf life of milk can be increased as well as the nutritional quality can be maintained at much superior level.

Thus, the present study has been focused to restrict or lessen the degradation of some milk constituents like protein, sugars and acidity – by impregnating with various proportions (100, 200, 400, 800, 1000 & 2000ppm) of the potent antioxidant β-carotene.

II. Materials And Methods

Roots of raw carrot were cut to slices, grinded with mixer grinder, were extracted in water bath at 50 degree centigrade using food grade Isopropanol (100%), shaken every 10 minutes. after eight hrs of extraction it was filtered, dried at 40 degree centigrade in water bath.

The carotene extract was added in milk at different proportion.

- Dry matter: 9.85%
- Yield : 0.262%(w.r.t. raw carrot)
- Milk :"Amul Tazza Homogenized Toned Milk"

The raw milk sample was analysed immediately after procurement w.r.t. milk proteins, milk sugars and milk acidity. The raw sample was the fortified with 6 different concentrations of β-carotene (extracted from carrot with food grade ethanol) like 100, 200, 400, 800, 1000 & 2000ppm. Above 2000 ppm, fortification was carried out but the precipitation occurred substantially hence the work was not performed beyond 2000 ppm. All the samples (blank and fortified) were then stored in refrigerator at about $4^{o}C$ and taken out on each day for the entire analysis and restored.

2.1 Reagents & Materials

1. Food Grade Isopropanol
2. Potassium Sulphate
3. Copper Sulphate
4. Sulphuric Acid
5. Zinc Dust
6. Sodium Hydroxide
7. Fehling I & II
8. Methylene Blue Indicator
9. Phenolphthalein Indicator
10. Distilled Water.

All reagents/chemicals are being purchased from Merck and raw carrot and milk being procured from local market.

2.2 Methods

Milk Protein : Estimated by formaldehyde titration [11].

Milk Sugars : Estimated by the Lane and Eynon constant titre method - mixed Fehling's solution is titrated with sample using methylene blue as indicator. [12].

Milk Acidity: Estimated by titrimetry using phenolphthalein as indicator.

III. Results and Discussion

Table 1 Effect of β-carotene addition w.r.t. milk protein, milk acidity and milk total sugars

Parameters	β-carotene added (ppm)	Day 1	2	3	4	5	6	7	8	% decrement/ increment
Milk Protein (%)	Blank	3.70	3.48	3.42	3.37	3.30	3.23	3.16	2.87	-22.43
	100		**3.57**	**3.57**	**3.57**	**3.57**	**3.57**	**3.55**	**3.51**	**-5.14**
	200		3.65	3.65	3.61	3.61	3.57	3.54	3.49	-5.68
	400		3.65	3.66	3.61	3.60	3.54	3.52	3.45	-6.78
	800		3.66	3.61	3.60	3.59	3.51	3.41	3.39	-8.38
	1000		3.74	3.69	3.63	3.56	3.48	3.43	3.37	-8.92
	2000		3.79	3.72	3.66	3.57	3.48	3.40	3.32	-10.27
Milk Acidity (as Acetic acid) (%)	Blank	0.125	0.1399	0.1400	0.1404	0.1407	0.1460	0.1676	0.1980	58.40
	100		0.1358	0.1331	0.1326	0.1303	0.1250	0.1469	0.1563	25.04
	200		0.1317	0.1328	0.1341	0.1355	0.1250	0.1355	0.1460	16.80
	400		0.1276	0.1239	0.1235	0.1335	0.1200	0.1312	0.1459	16.72
	800		0.1276	0.1242	0.1219	0.1200	0.1192	0.1245	0.1398	11.84
	1000		0.1276	0.1239	0.1224	0.1200	0.1043	0.1312	0.1459	16.72
	2000		**0.128**	**0.1242**	**0.1219**	**0.1200**	**0.1043**	**0.0781**	**0.052**	**-58.40**
Milk Total Sugars (as lactose) %	Blank	3.28	3.14	3.10	3.06	3.06	2.91	2.62	2.53	-22.87
	100		3.15	3.15	3.19	3.26	3.24	3.19	3.15	-3.96
	200		3.32	3.32	3.32	3.30	3.29	3.21	3.20	-2.44
	400		3.33	3.33	3.34	3.30	3.25	3.25	3.25	-0.91
	800		3.31	3.32	3.32	3.33	3.32	3.31	3.31	0.91
	1000		3.48	3.48	3.48	3.45	3.45	3.37	3.33	1.52
	2000		**3.65**	**3.64**	**3.53**	**3.45**	**3.45**	**3.45**	**3.39**	**3.35**

Note: Bold letters highlight the best effect w.r.t. a particular composition of β-carotene & milk

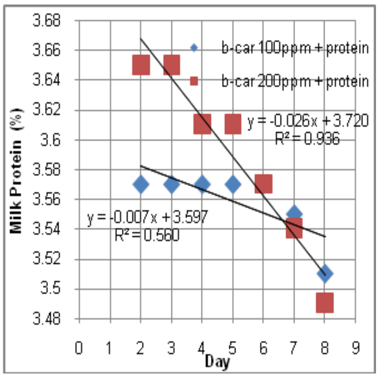

Figure 1a
β-carotene (100,200)ppm & milk protein

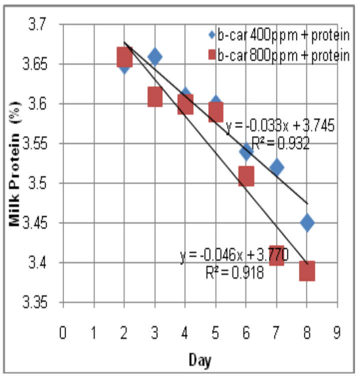

Figure 1b
β-carotene (400,800)ppm & milk protein

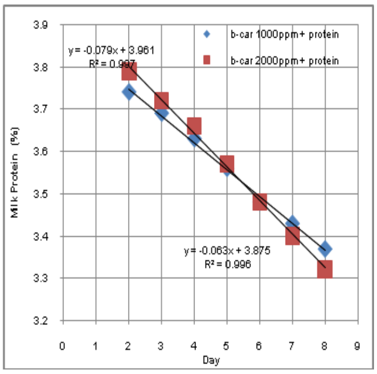

Figure 1c
β-carotene (1000,2000)ppm & milk protein

Figure 1 Correlation between added β-carotene & milk protein (%)

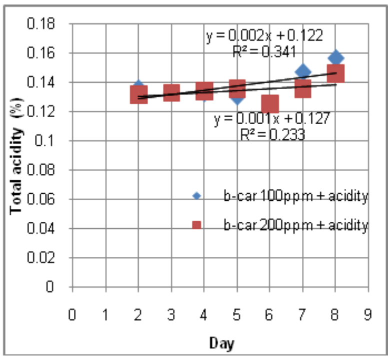

Figure 2a
β-carotene (100,200) ppm & milk acidity

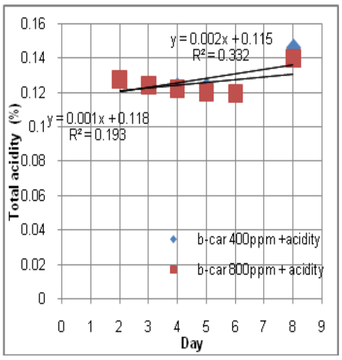

Figure 2b
β-carotene (400,800) ppm & milk acidity

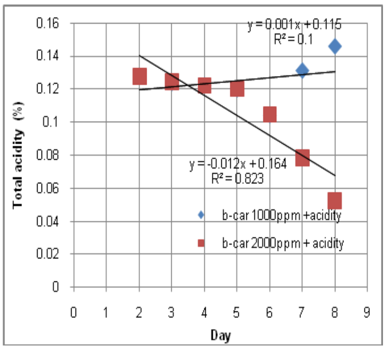

Figure 2c
β-carotene (1000,2000) ppm & milk acidity

Figure 2 Correlation between added β-carotene & milk Total acidity (%)

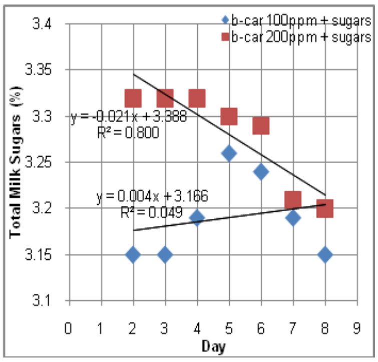

Figure 3a
β-carotene (100,200) ppm & milk sugars

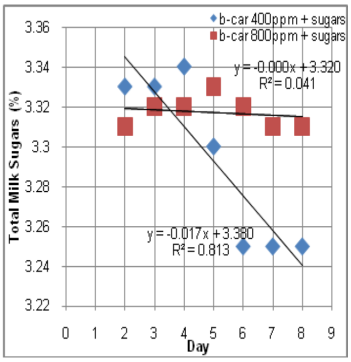

Figure 3b
β-carotene (400,800) ppm & milk sugars

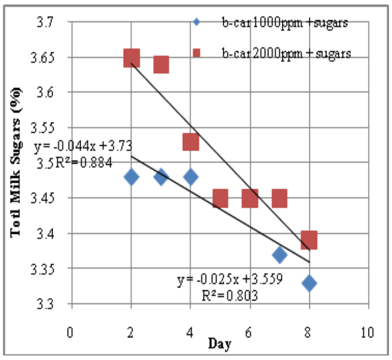

Figure 3c
β-carotene (1000,2000) ppm & milk sugars

Figure 3 Correlation between added β-carotene & milk sugars (%)

β-carotene, a well known natural antioxidant extracted from raw carrot, has been added to milk in different concentrations to study the effect of addition w.r.t. general milk spoilage based on casein, acidity and milk sugar, where a somewhat different picture has been noticed in terms of retention of more nutrient (milk protein, carbohydrates as lactose) and the buffering of acidity.

3.1 Milk Protein

The initial percentage of protein in raw and non-fortified milk was about 3.7%, which decreased day by day during refrigerated storage at 4°C and ended at 2.87% (after 8th day, loss of 22.43%), depicting loss of nitrogen in milk due to microbial infestation. The fortified milk showed variable results of protein on the 2nd day (from 3.57 to 3.79%). The 100ppm β-carotene fortification resulted in the highest retention of protein from 3.57 to 3.51% **(Table 1)**, and depicted a correlation coefficient, r=0.936 **(Fig. 1a).** On the other hand, the 200ppm β-carotene fortification resulted in the retention of protein from 3.65 to 3.49% **(Table 1)** and depicted the correlation coefficient r=0.560 **(Fig. 1a)**. The protein retention with 400, 800, 1000 & 2000 ppm β-carotene after the experimental period were 3.45, 3.39, 3.37 & 3.32% respectively **(Table 1)**, with r = 932, 918, 996 & 997 respectively **(Fig. 1b & 1c). The antioxidant property of** β-carotene has been the reason towards quenching the free radical generation during storage, as well as protecting and arresting any oxidative degradation of the compound responsible for spoilage. Further, β-carotene with its ability to guard the oxidation of free fatty acids of milk also helps to nullify the deteriorating action. **Thus, each of the fortified milk has shown to retain a better nutritional quality on storage in comparison to the non-fortified sample and 100 ppm β-carotene fortification resulted in the maximum retention of protein (loss of only5.14%) during the study period.**

3.2 Milk Acidity (as lactic acid)

Acidity of milk is the result of formation of lactic acid by the enzymatic action of lactase and the non-fortified milk represented an increase of about 58.40% acidity on storage for 8 days. Each of the β-carotene fortified milk resulted in lessening of acidity development (as lactic acid). The value steadily declined from 58.40% to 11.84%, as fortification increased from 100ppm to 800ppm. Thereafter, the acidity increased with 1000ppm and then declined sharply to give a positive value (-58.40%), when 2000 ppm fortification was done, where a lowering of acidity has been observed (from 0.125 to 0.052). The result may be explained on the antioxidant property of β-carotene, which exerts its buffering action towards nullifying the formation of hydrogen ions as well as arresting/hindering the action of lactase enzyme against microbiological action. The action of β-carotene against further breakdown of free fatty acids present in milk, thereby formation of more acids in the medium has also been noticed. The respective 'r' values may be cited from the **Fig. 2a, 2b & 2c**. **In this case, the maximum fortification considered in our study (2000ppm) has been found to be the best quencher and acidity blocker.**

3.3 Milk Total Sugars (as lactose)

The total sugars analysed by Lane & Eynon method expressed results in percentage and the non-fortified milk resulted in the 22.87% decrement (from 3.28 to 2.53%) on the completion of 8 day experimental period **(Table1)**. But, the varied concentrations of β-carotene fortified samples have been found to contain the loss of total sugars and resulted in remarkable retention during the period of analysis. From the loss of 22.87% in case of non-fortified milk, β-carotene fortified samples steadily lessened the degradation of total sugars upto 400ppm fortification (loss of -3.96 to -0.91%). The role of β-carotene as a potent antioxidant has come into play to lessen the degradation of lactose. But, in case of higher additions of β-carotene to milk, say 800, 1000 & 2000ppm, the final sugar contents have been found to increase from the initial value (3.28%) and resulted in 3.31, 3.33 & 3.39% respectively. This might be explained from the fact that β-carotene, being a lipophilic compound reacts with the milk fat and tend to free the bound lactose into the medium resulting in higher total sugar contents, which could not have been possible to that extent with the lower concentrations. From the **Table1** it appears that 2000ppm fortification resulted in the highest retention and availability of milk sugars. The **Fig. 3a, 3b & 3c** shows the correlation coefficient values of β-carotene & milk sugars.

IV. Conclusion

In this present study, milk is fortified with β-carotene in different concentrations like 100, 200, 400,800,1000 & 2000 ppm, where a somewhat different picture has been noticed in terms of retention of more nutrient (lactose, casein) and the buffering of acidity. The antioxidant property of β-carotene has been utilised by impregnating it in milk and the present study encompasses the effect of β-carotene towards controlling the deteriorating stages of protein, sugars and acidity of milk during the experimental period of 8 days. Each of the fortified milk has shown to retain a better nutritional quality on storage in comparison to the non-fortified sample and 100 ppm β-carotene fortification resulted in the maximum retention of protein (loss of only5.14%) during the study period. According to the study, the best quencher and acidity blocker (acidity decreased by 58.40%) as well as the highest retention and availability of milk sugars (milk sugars increased by 3.35%) has been represented by the maximum fortification considered in our study (2000ppm).

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