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EVALUATION YOGURT FORTIFIED WITH VEGETABLE AND FRUIT JUICE AS A NATURAL SOURCES OF ANTIOXIDANT

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ABSTRACT

The incorporation natural antioxidant compounds into yogurt provide a simple way to improve public health and/or reduce the risk of disease. Carrot and cantaloupe juice was used in forming structure of yogurt with different concentrations. Acid coagulation time of milk mixed with different concentrations of juices to reach the pH 5.2 and 4.6 was monitored. Apparent viscosity was also measured. Sensory characteristics, carotenoids content, β-carotene content and antioxidant activity (DPPH and FRAP) were investigated during refrigerated storage 5°C for 12 days. Initial stress of carrot yogurt was increased as carrot juice concentration was increased. Total carotenoid content in all yoghurt samples gradually decreased along the cold storage. There was great decline in the β-carotene of yoghurt after cold storage for 6 and 12 days whereas yogurt kept its activity as antioxidant through storage. Yogurt made with carrot juice with 8% was the best fermented dairy product form delivers the healthy benefits for carotenoids to consumer. Consumption of antioxidants is thought to provide protection against oxidative damage and contribute positive health benefits.

Key words: Yogurt, Carrot, Cantaloupe and antioxidant activity.

INTRODUCTION

There is strong evidence that reactive oxygen species (ROS) and free radicals play an important role in many degenerative diseases like cancer, atherosclerosis and diabetes (Beckman and Ames 1998). The recent abundant evidence suggesting the involvement of oxidative stress in the pathogenesis of various disorders and diseases has attracted much attention of the scientists and general public to the role of antioxidants in the maintenance of human health and prevention and treatment of diseases (Halliwell and Gutteridge, 2007; Papas, 1999).

Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Renaud et al. 1998; Temple, 2000). These natural antioxidants are a rich source of phytochemicals, such as carotenoids, flavonoids and other phenolic compounds.

Carotenoids are natural pigments which are synthesized by plants have antioxidant activity. Studies have reported that a diet high in carotenoids may reduce the risk of heart attack and candidates for cancer prevention (Steinmetz and Potter, 1996; Federmann and Federmann, 2000). Studies also showed that intake diet has high flavonoid predicted lower mortality from coronary heart disease and lower incidence of myocardial infarction in older men (Hertog, et al. 1993) and reduced the risk of coronary heart disease by 38% in postmenopausal women (Yochum, 1999). This fortification is one of the best ways used to deliver the antioxidant health benefits to human that may be useful for the prevention of these diseases.

This study depends on fortification yogurt with natural antioxidant through supplemented milk with vegetable and fruit juices. Carrot (*Dascus carota L.*) is one of the more commonly used vegetables of human nutrition. Carrot juice increases total antioxidant status and decreases lipid peroxidation in adults (Potter et al. 2011). It is rich in beta carotene, ascorbic acid, tocopherol and classified as vitaminized food (Alasalvar *et al.* 2001; Hashimoto and Nagayama, 2004). Cantaloupe, Cucumis melo, is a melon belonging to the gourd family, Cucurbitaceae. They are an excellent source of the antioxidants beta-carotene and vitamin C (Ensminger, et al. 1995). Incorporation natural sources of antioxidants into yogurt give a new of the potentially significant product trend for the dairy industry as healthy or functional dairy products. Functional dairy products became a key factor for increasing consumer demanded driving value sales growth in developed markets (Anonymous, 2005). The objective of this study after production new yogurt is examine its characteristics

(Formation gel at specific pH and its sensory), determination its carotenoids content besides β-carotene and monitor the antioxidant activity through product storage.

MATERIALS AND METHODS

MATERIALS

Skim milk powder (low heat, origin USA) was reconstituted in distilled water and left overnight at 4°C to allow full hydration. Full milk cream used as substrate to dissolve carotenoids, was add to skim milk through reconstituted. Carrot roots and cantaloupe from local market. Starter : freeze dried culture of Lactobacillus delbrueckii supsp bulgaricus and Streptococcus salivarious subsp.thermophilus (1:1) were obtained from Chr. Hansens laboratories Denmark. Folin–Ciocalteu reagent and 2,4,6-Tris [2- pyridyl]-s-triazine (TPTZ) were obtained from Fluka Chem. Co (Buchs, Swizerland), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical and the reagents, (S)-(-)-6-Hydroxy- 2,5,7,8- tetramethylchroman -2-carboxylic acid (TROLOX) from Sigma, St. Louis, Mo. USA) and gallic acid from (MP Biomedicals,. Inc. (Eschwege, Germany).

METHODS

PREPARATION OF CARROT AND CANTALOUPE JUICE

Carrot roots (vegetables) were washed thoroughly; the juice was obtained by blending in blender with sieves. Cantaloupe were washed thoroughly in cold running tap water were cut off and the seed cavity was gently scraped with a spoon to remove the seeds, each half was placed cut-side down on a cutting board and roughly cut into 2.5 cm thick slices using a thin 12.7 cm food knife. The cantaloupe juice was obtained by blending in blender with sieves.

PREPARATION FULL FAT MILK 3% INCLUDING NATURAL SOURCES OF CAROENOIDS

Reconstituted skim milk was mixed with different levels of natural sources of carotenoids, carrot and cantaloupe juice that mixed with milk fat to give (2%, 4%, 6%, 8%, and 10% v/v) and they homogenized to obtain full fat milk 3% with total solids 14 % that prepared of processing yogurt.

YOGHURT MANUFACTURE

Reconstitute full fat milk (3%) including different concentrate of juices were blended, heat treated at 90 °C for 10 min, and cooled to 43 °C, then homogenized at two-stags (200, 50 Kg/cm2) (Rannie homogenizer). Starter was added at the rate of 3% and incubated at the same temperature, incubated at 43°C for 4h until coagulation occur. After incubation and acidifying samples reached to pH 4.5, yoghurt then refrigerated at 5°C for 12 d and subsequent analysis. Plain yogurt was produced and the productions were duplicated. The yogurt was refrigerated at 5 C for 24 hours and apparent viscosity was measured.

CARROT AND CANTALOUPE YOGURT ANALYSIS

STUDY THE EFFECT OF FOUNDATION CAROTENOIDS SOURCE ON THE MILK ACIDIFICATION RATE

Reconstitute full fat milk (3%) with different levels of mixed juices were acidified with 3% (wt/vol) yogurt culture at 40°C. The pH of the samples was regularly measured using Knic Digital pH meter 646 at 40 ± 1°C and recorded the acid coagulation time to reach the pH 5.2 (start coagulum formation-casein micelles aggregation) and at the pH 4.6 (casein micelles stopped its aggregation). These pH measures are the critical pH in processing fermented milk.

PHYSICAL PROPERTIES OF THE YOGURT

APPARENT VISCOSITY OF YOGURT (η)

Apparent viscosity was based on measuring resistance to a rotating spindle (Brookfield Model DV III , Programmable rheometer).The instrument was equipped with an 18 measuring head. Rheological behavior depends on time of shearing. Test samples were subjected to constant shear rate a spindle speed of 50 rpm and spindle rotating velocities, at constant temperature (25°C) for 5 min. Samples were allowed to relax (more than 10 min) prior to measure their viscosity. All apparent viscosity measurements were expressed in cintipoise seconds (cP.s), performed in triplicate, and the apparent viscosity was measured as a function of shear time.

SENSORY EVALUATION (ORGANOLEPTIC PROPERTIES) OF YOGURT

Panelists: Ten trained panelists from the staff members of the Dairy Science Department, National Research Center, Egypt. They evaluated 20 g portions of each yoghurt sample and used a quality rating score card for evaluation of flavor (60 points) and body and texture (30 points) and appearance (10 points) as described by Nelson and Trout (1981).

DETERMINATION OF THE TOTAL CAROTENOID CONTENT (TC) OF YOGURT BY ABSORPTION UV-VIS SPECTROPHOTOMETRY

Carotenoids are expected to be dissolved in fat phase was evaluated by determination the total carotenoid content (TC) by absorption SP-2000UV UV-VIS spectrophotometry according to Lachman et al. (2003). Absorbance of organic extracts was then measured in 1 cm cuvettes at λ = 444 nm and total carotenoid content in mg/kg fw of sample was expressed as lutein equivalent from the equation:

(K + X)L = A444. 25. 15 (mg/kg fw) 0.259 m

Where:

(K + X)L is total carotenoid content (carotenes and xanthophylls)

A444 is absorbance of acetone extract at λ = 444 nm

m is sample weight (g)

MONITOR THE QUANTITY OF Β-CAROTENE CONTENT IN TOTAL CAROTENOID CONTENT (TC) OF YOGURT BY HPLC ANALYSIS

A sample yogurt with the highest juice concentrate (10%) was analyzed by reversed-phase HPLC as described by Lyan et al. (2001) at fresh day, 6 days and 12 days storage. Solvents (HPLC grade) was added to yogurt samples (12–20 g), which were homogenized in a Waring blender with 150 mL tetrahydrofuran containing 5 mg ethyl ß-apo-8'-carotenoate (Fluka, Milwaukee) as an internal standard and 0.01% butylated hydroxytoluene. The tetrahydrofuran extract was filtered through no. 1 Whatman filter paper. The total tetrahydrofuran extract was concentrated to 80 - 100 mL on a rotary evaporator at 30°C and then partitioned between petroleum ether and saturated sodium chloride solution. The petroleum ether phase was dried over magnesium sulfate and filtered through no. 2V Whatman filter paper. The sample was then analyzed by reversed-phase HPLC. A standard curve with a correlation of 0.9971 was prepared for quantification of β-carotene. The β-carotene standard used in this study was separated into 1 major β-carotene peak.

MONITOR THE ANTIOXIDANT ACTIVITY IN THE YOGURT

Two methods commonly used in antioxidant activity assays were the DPPH and FRAP procedures respectively.

SCAVENGING ACTIVITY OF DPPH RADICAL ASSAY

The stable free radical DPPH is a rapid test can provide information on the ability of a food compound contains antioxidants to act as free radical scavenger's ability or hydrogen donor's atom, (Xuewu et al., 2007). Briefly, 40µl of various concentrations of natural sources antioxidant was mixed with 2.9 ml of 0.1 mM DPPH methanol solution. After the solution was incubated for 30 min at 25 °C in dark, the decrease in the absorbance at 517 nm was measured. Control contained methanol instead of the antioxidant solution while blanks contained methanol instead of DPPH solution. The results are corrected for dilution and expressed as TE mg/100mL sample.

THE FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

The FRAP assay is quick and simple method to evaluate the antioxidant capacity (antioxidant power) of compounds materials in reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) than to scavenging free radicals. The FRAP assay was carried out by the method of Benzie and Strain (1996). Aliquots of 100 µL sample filtrate were mixed with 3 mL FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37 °C for 10 min. FRAP values were obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration [from The standard curve of [$FeSO_4 \cdot 7H_2O$].

STATISTICAL ANALYSIS

Data were analyzed statistically by running Student t test using Stat view 512+ software (1986). Chi square was performed to compare between the plain (control) and carrot yoghurt. Significant effects were declared at $P < 0.05$.

RESULTS AND DISCUSSION

STUDY THE EFFECT OF FOUNDATION CAROTENOIDS SOURCE ON THE MILK ACIDIFICATION RATE

As the milk mixed with different concentrates with carrot and cantaloupe juice was fermented with yoghurt starter, the pH was started to move down and acid coagulation time (min) needed to reach the pH 5.2 and 4.6 was recorded as shown in Fig 1. These results clearly indicated that that time was increased significantly with increasing the concentration of both carrot and cantaloupe juices. This finding presumably due to some inhibitory action of the added juices as carotenoid sources on yoghurt started activity, as reported by Salwa et al (2004). Upon acidification, the net negative charge continues to get lowered, resulting in the decrease in both electrostatic repulsion and steric stabilization. β-carotene has a positive charge (Ruth Edge, et al. 2007) and it may neutral these negative charge formed during acidification and therefore time need to reach the pH 5.2 and 4.6 was more than the plain yogurt.

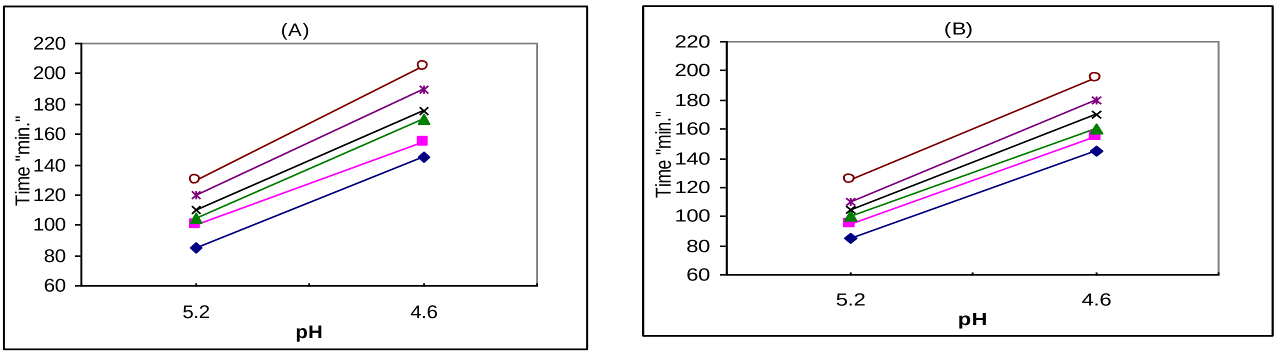


Fig1 Determination time (min) vs. pH 5.2 and 4.6 during acidification reconstitute full fat milk (3%) with different concentration of carrot (A) and cantaloupe (B) juice [(-♦-) 0%; (-■-) 2%; (-▲-) 4%; (-x-) 6%, (-*-) 8%; (-o-) 10%]. Data were analyzed by SAS (t) test, one-way ANOVA followed by LSD test (P≤0.05) with GraphPad Prism 3.0 (GraphPad Software) as appropriate.

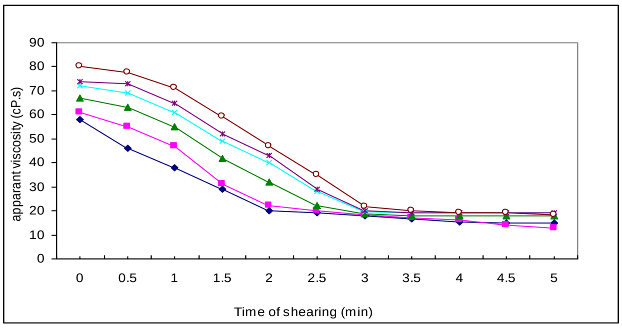
PHYSICAL PROPERTIES OF THE YOGURT

APPARENT VISCOSITY (CP.S) OF YOGHURT SUPPLEMENTED WITH CARROT AND CANTALOUPE

It was decided then to work with the average value of the parameters obtained for each formulation to estimate the effect of the variation of the composition on the rheological behavior of carrot and cantaloupe yogurt. At a constant shear rate, the apparent viscosity of both samples decreases rapidly with time within one minute of shearing. A decrease in apparent viscosity with time under constant shear rate exhibited thixotropic behavior. If sufficient force (shear stress) is exerted on a thixotropic solution, the structure can be broken and the apparent viscosity reduced.

Results showed apparent viscosity at initial stress of carrot and cantaloupe yogurt gel structure (14% TS, pH 4.5) with different concentrations of carrot and cantaloupe juices were decreased with the time under constant shearing, implying a progressive breakdown of structure within 1.0 - 2.5 minutes (Fig. 2.A, B). Initial stress of carrot yogurt was increased as carrot juice concentration was increased; it was 80.0 cP.s at 10% (Fig. 2.A) whereas in cantaloupe yogurt the initial stress was decreased with increasing cantaloupe juice concentration. It was 32.0 cP.s (Fig 2.B). The higher apparent viscosity of carrot yogurt due to the soluble dietary fibers in carrot which act as pectin had the ability to bind water and form highly viscous solutions tending to increase of the consistency of the yoghurt

(A)



(B)

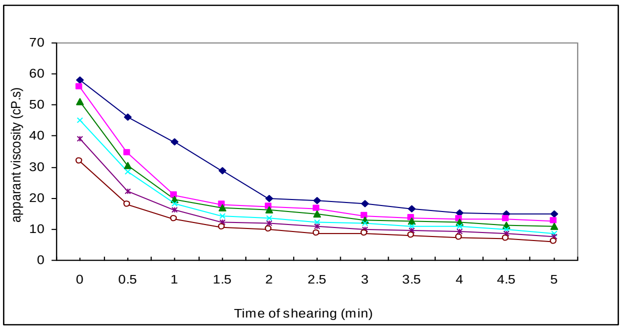


Fig 2 -The apparent viscosity of yoghurt supplemented with carrot juice (A) and cantaloupe juice (B). [(-♦-) without; (-■-) a2 %;(-▲-) 4%; (-x-) 6%; (-*-) 8% (-o-) 10%]. Data were analyzed by SAS (t) test, one-way ANOVA followed by LSD test (P≤0.05) with GraphPad Prism 3.0 (GraphPad Software) as appropriate

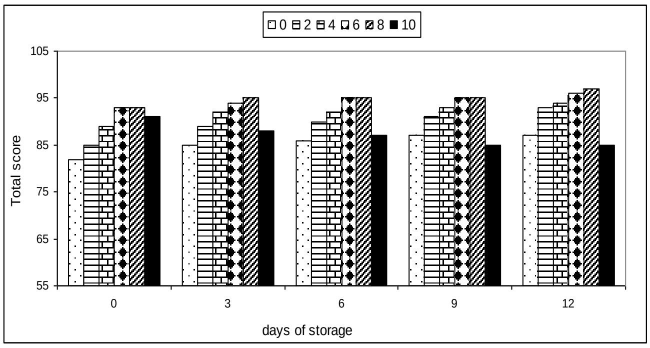
SENSORY EVALUATION (ORGANOLEPTIC PROPERTIES) OF YOGURT

Plain and functional yoghurt (supplemented with carrot and cantaloupe juices) were sensory evaluated for flavor, body texture and appearance when fresh and along cold storage at 50C for 12 days. The mean total scores for plain and functional yoghurt samples are shown in Fig. 3 A, B. The organoleptic properties showed that carrot treatments gained higher scores than control yoghurt Fig 3.A. The flavor and odor were pleasant and sweet, and appearance was normal. As addition of carrot evidently enhance not only the flavor, but also the consistency of yoghurt which led to higher scores of flavor as well as body & texture of the resultant yoghurt, nearly findings were reported by Al-shahib and Marshall (2003; Nazni and Gomathi, 2014). During cold storage the scores increased with increase in the percentage of added carrot juice.

On the other hand more addition of cantaloupe juice which has the high water content led to pronounced decrease in the body & texture scores and simultaneously ranked lower total scores. Addition of cantaloupe juice up to 4% improved the flavor and consequently raised the total score along the storage time than control yoghurt (without cantaloupe) whereas led to pronounced decrease in the body & texture scores and simultaneously ranked lower total scores (Fig 3 B).

The highest scores were recorded with increasing carrot juice percent up to 8% and 4% of cantaloupe juice.

(A)



(B)

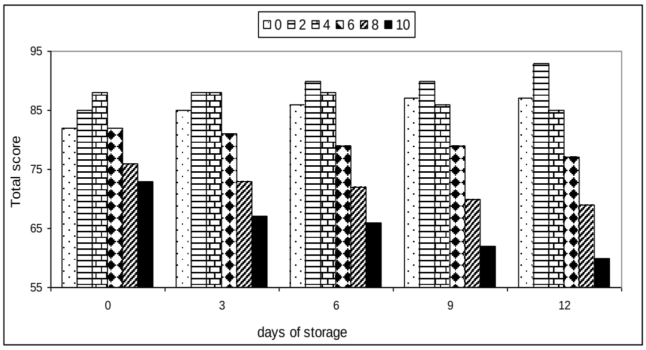
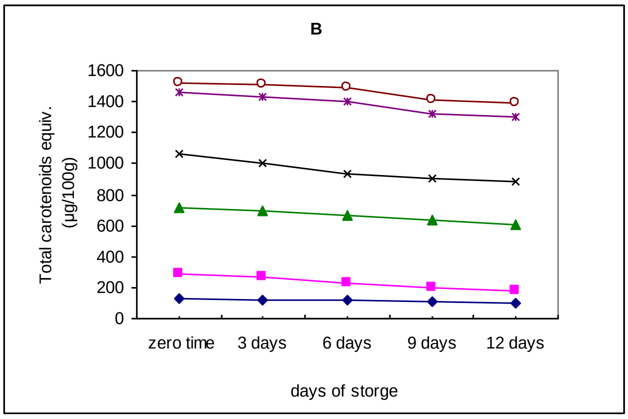


Fig. 3- Organoleptic properties of yoghurt supplemented with carrot juice (A) cantaloupe juice (B) during storage. Data were analyzed by SAS (t) test, one-way ANOVA followed by LSD test (P≤0.05) with GraphPad Prism 3.0 (GraphPad Software) as appropriate

TOTAL CAROTENOID CONTENT (TC) IN LIPOPHOFILIC FRACTION OF YOGURT SUPPLEMENTED WITH CARROT AND CANTALOUPE BY ABSORPTION UV-VIS SPECTROPHOTOMETRY DURING THE STORAGE

Results in Fig. 4 A, B show that total carotenoids of yoghurt supplemented with carrot juice had greatly higher content of carotenoids than yogurt with cantaloupe juice. Total carotenoids were increased significantly with increasing juices percentage in yogurt. On the other hand it was clear that the carotenoid content in all yoghurt samples gradually decreased along the cold storage, which may be owing to its degradation during storage. The results are given as µg carotenoid/g dry weight.

(A)



(B)

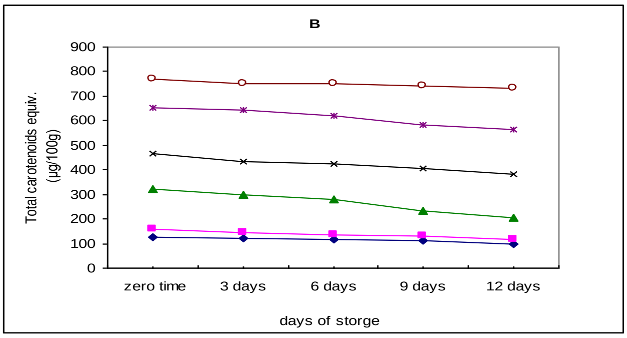


Fig. 4. Total carotenoids in yoghurt with different conc. of carrot (A) and cantaloupe (B) during storage [(-♦-) without; (-■-) 2 %;(-▲-) 4%; (-x-) 6%; (-*-) 8% (-o-) 10%.]. Data were analyzed by SAS (t) test, one-way ANOVA followed by LSD test (P≤0.05) with GraphPad Prism 3.0 (GraphPad Software) as appropriate.

MONITORING THE QUANTITY OF Β-CAROTENE CONTENT IN TOTAL CAROTENOID CONTENT (TC) OF YOGURT BY HPLC ANALYSIS DURING THE STORAGE

Beta-carotene is the most common carotenoid in fruits and vegetables can promote health when taken at dietary levels (Stahl and Sies, 1996). High performance liquid chromatography (HPLC) analysis was used to evaluate the stability of β-carotene and to quantify its amount in new product through storage. From the resulting acceptable chromatograms, the total peak areas of all detected β- carotene isomers as well as the peak area of all-trans β-carotene were determined and averaged for the duplicates. Separation was carried out for 30 min and β-carotene eluted after 5 min (Figure 5 a,b).

Fig.5. Monitor HPLC chromatogram of β-carotene extract of yogurt with 10% carrot juice (A) and 10% cantaloupe juice (B) during storage, milk mixed with juice (1), fresh yoghurt (2), yoghurt after 6 days (3) and yogurt after 12 days (4).

Quantitative LC analysis of the final test solution uses a reversed-phase C18 column relative to the β-carotene content. The determination of 13-cis-b- carotene was performed at 471 nm. The chromatograms and the content of β–carotene in standard solutions, in yogurt are shown in Figure 5 a & b. Results illustrated that β-carotene content (µg / ml) greatly affected with fermentation as well as storage time, as there was clear decline in β-carotene content of fresh yoghurt (8.69 µg / ml) to yogurt after 12 days storage (5.30) as compared with the milk from it was produced. There was great decline in the β-carotene of yoghurt after cold storage for 6 and 12 days. This decline may be because the β-carotene degradation increased during storage as stated by Vàsquez-Caicedo et al (2007). Although product was kept in cooled condition, β-carotene was exposed to degradation, and this may be consumed by starter bacteria. Therefore this bioactive component must be kept from the outer environments by encapsulation. β-carotene is believed to have a protective role against cancer (Handelman 2001; Nazni and Komathi, 2014). β-Carotene is a unique antioxidant that effectively scavenges certain reactive oxygen species (Burton and Ingold 1984) enhances endothelial cell growth (Melnykovych and Clowes 1981) and improves endothelial function in the setting of hypercholesterolemia (Keaney et al 1993).

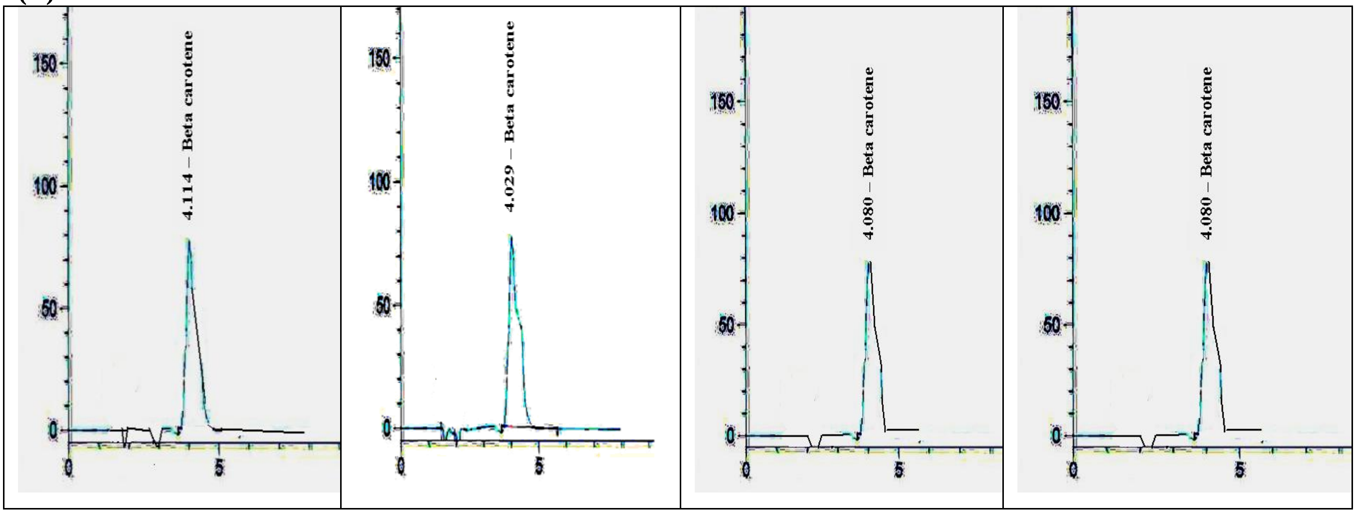
ANTIOXIDANT ACTIVITY

Antioxidant capacity assays used to monitor the antioxidant activity of phenols fortified to skim milk (used in processing yogurt) in the aqueous phase the major part of milk that able phenols are dissolving.

SCAVENGING ACTIVITY OF DPPH RADICAL

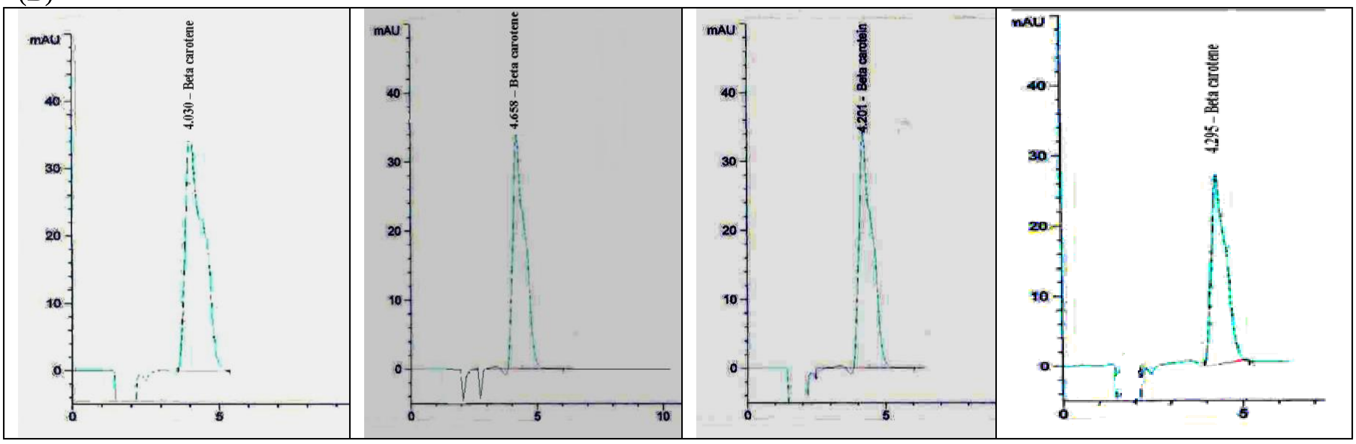
The stable radical DPPH (2, 2-Diphenyl-1-picrylhydrazyl hydrate) has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging with pure antioxidant compounds by hydrogen donation. DPPH radical-scavenging used to monitor antioxidant activity of yogurt supplemented with carrot juice and cantaloupe juice with different concentration at different days of storage (Fig.6 a, b). Results show that yogurt made from carrot juice had higher radical scavenging activity ($P < 0.05$) than yogurt with cantaloupe in all concentrations. The inhibition of DPPH radical by both yogurts was decreased with extent storage time. Plant extracts containing high levels of phenolic compound with strong H• - donating activity to free radical and inhibit the formation of reactive oxygen species (Lugasi et al 1995).

(A)



(1) (2) (3) (4)

(B)



(1) (2) (3) (4)

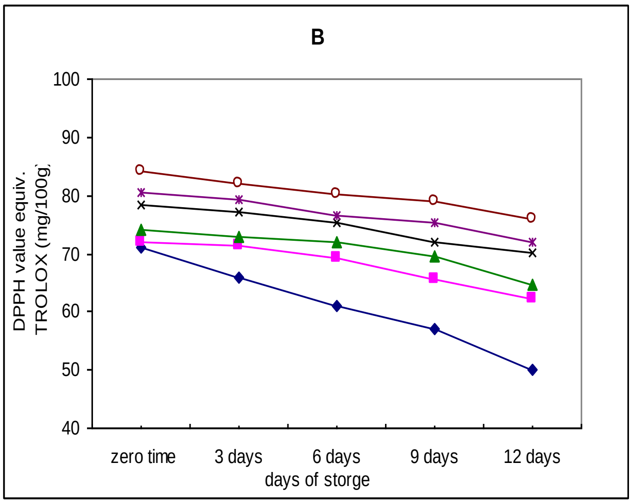
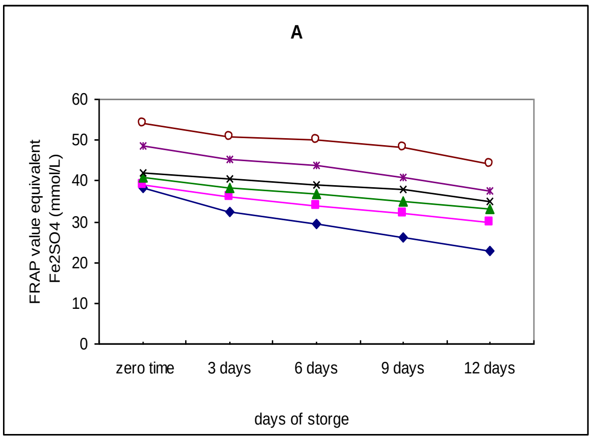


Figure 6- Antioxidant activity in yoghurt with different conc. of carrot (A) and cantaloupe (B). Using DPPH method [(-♦-) without; (-■-) 2 %;(-▲-) 4%; (-x-) 6%; (-*-) 8% (-o) 10%.]. Data were analyzed by SAS (t) test, one-way ANOVA followed by LSD test (P≤0.05) with GraphPad Prism 3.0 (GraphPad Software) as appropriate.

THE FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

Ferric Reducing Antioxidant Power (FRAP) is also quick and simple assay and was used to measure the antioxidant capacity of phenolic compounds of donating a single electron "donating activity to free radical". In this assay natural antioxidant can reduce ferric ions in a "ferric tripyridyltriazine reagent" to the ferrous form with blue colour which absorbs strongly at 593 nm.

FRAP reflects total antioxidant power involving the single electron transfer reaction was determined for both yogurt supplemented with carrot and cantaloupe juice. The ability of phenolic compounds in both yogurt with different concentrations to reduce a ferric $Fe3+$ to the ferrous form $Fe2+$ showing its ability in donating an electron, Fig.7.a.b. The reducing capacity of a yogurt may serve as a significant indicator of its potential antioxidant activity. The results revealed that the correlation coefficient (r2) between TC and FRAP values was 0.99.



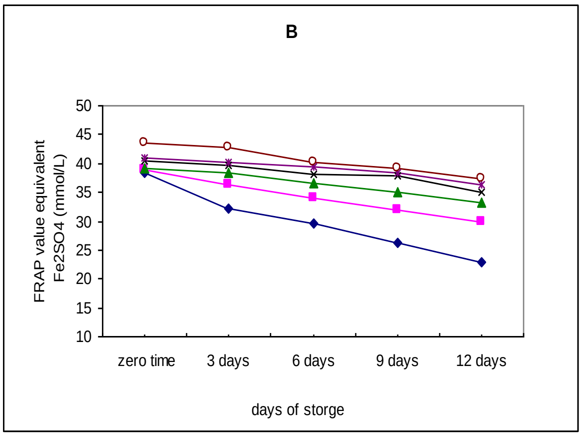


Figure 7 Antioxidant power of yoghurt with different conc. of carrot (A) and cantaloupe (B). Using FRAP method [(-♦-) without; (-■-) 2 %;(-▲-) 4%; (-x-) 6%; (-*-) 8% (-o-) 10%]. Data were analyzed by SAS (t) test, one-way ANOVA followed by LSD test ($P \leq 0.05$) with GraphPad Prism 3.0 (GraphPad Software) as appropriate.

Yogurt which supplemented with carotenoids acts as free radical scavengers or hydrogen donors can helps to reduce the risk of chronic diseases, such as cardiovascular disease, cancer, and age related neuronal degeneration (Ames et al 1993).

CONCLUSION

Recently, additional carotenoids have been the subjects of nutritional studies and are now also incorporated, both in combination products and in dietary supplements available to the general public. It is accepted that carotenoids in general and carotenes in particular provide significant antioxidant activity to the human food feed supply, and thus may be responsible for some of the significant correlations between increased intake of vegetables providing significant carotenoid content and improved health status. Functional yogurt rich with carotenoids have many functions such as acting as antioxidant, besides using for nutritional purposes as provitamine A agents. Yogurt kept its activity as antioxidant through storage. Degradation of β-carotene in the yogurt did not prevent its ability as scavenger of peroxyl radicals, especially at low oxygen tension. Yogurt made with carrot juice with 8% is the best fermented dairy product form delivers the healthy benefits for carotenoids to consumer. While the body has its defenses against oxidative stress by using this new product these defenses are thought to become less effective with aging as oxidative stress becomes greater.

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