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Full Length Research Paper

Use of natural plant antioxidant and probiotic in the production of novel yogurt

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Low fat fermented milk (1% fat) was supplemented with green bell pepper juice as a source of antioxidant compounds, and also carotenoids was produced and the impact of this ingredient on LAB was investigated. Pepper juice, in a ratio of 1:2 and 3%, was added to the low heat reconstituted milk powder. *Lactobacillus rhamnosus* (2%) was used only or with yoghurt starter (2%), and the growth rate of all probiotic strains and the viable count of lactic acid bacteria (LAB) during storage in refrigerator were determined. Moreover RSA, TPC and TCC of fermented milk samples were evaluated. Pepper juice was effective in enhancing the growth rate of *L. rhamnosus* when compared to the control, while the others of LAB count in the fermented milk, which contain pepper juice, was decreased. Furthermore, it was obvious that increasing the concentration of pepper juice increased RSA (%), TPC and carotenoids of the resultant low fat fermented milk, inoculated with *L. rhamnosus* for fresh and stored samples. During cold storage, RSA (%) slightly increased until 7 days and then decreased especially for samples supplemented with low concentration of pepper juice (1%), while TPC was gradually decreased by a prolongation of the storage period (15 days). The TCC was highly increased during the storage until 15 days. Moreover, adding yoghurt starter with *L. rhamnosus* strain in preparing fermented milk fortified with pepper juice also increased the RSA (%), TPC and TCC of the media. During storage, the TPC gradually decreased, whereas TCC was inversely increased. The percentage of RSA also increased during storage until 7 days and then decreased, in which case, it could be recommended that the addition of pepper juice has no effect on viability of probiotic strain.

Key words: Lactic acid bacteria, probiotic, carotenoids.

INTRODUCTION

Nutritional studies are now concentrating on examining food for their protective and disease preventing potential.

Recently, probiotic fermented dairy products provide a healthy functional food for health properties. Many previous researches mentioned that, milk fermented by selected culture of lactic acid bacteria (LAB) has high biochemical activity and antioxidant activity (Kullisaar et al., 2003; Sinyavskiy, 2005; Villani et al., 2005). Several early studies demonstrated the ability of supplementing yogurt with fruits (Lurton and Ouattrin, 2003; Skrede et al., 2004; Apostolidis et al., 2006), but was less supplemented with vegetables.

Recently, phytochemicals in vegetables have attracted a great deal of attention, which mainly concentrated on their role in preventing disease caused as a result of oxidation stress.

Sweet peppers are one of the popular vegetables, especially green bell pepper. They contain a wide array of phytochemicals that are important antioxidant components of a plant-based diet that reduce the risk of degenerative diseases (Zhang and Hamauzu, 2003). Green bell pepper is a good source of ascorbic, tocopherols and carotenoids (Materska and Perucka, 2005). However, peppers also contain various phenolics and flavonoids (Sun et al., 2007; Chatterjee et al., 2007; Marin et al., 2004). These compounds are antioxidants and can reduce harmful oxidation reactions in human body (Sun et al., 2007). Green peppers are also a good source of carotenoids (Deepa et al., 2007; O'Connell et al., 2007), including lutein, B-carotene, capsanthin and violaxanthin, which could contribute to antioxidant activity. Carotenoids are required for human epithelial cellular differentiation. O'Connell et al. (2007) reported that numerous potential health benefits have been

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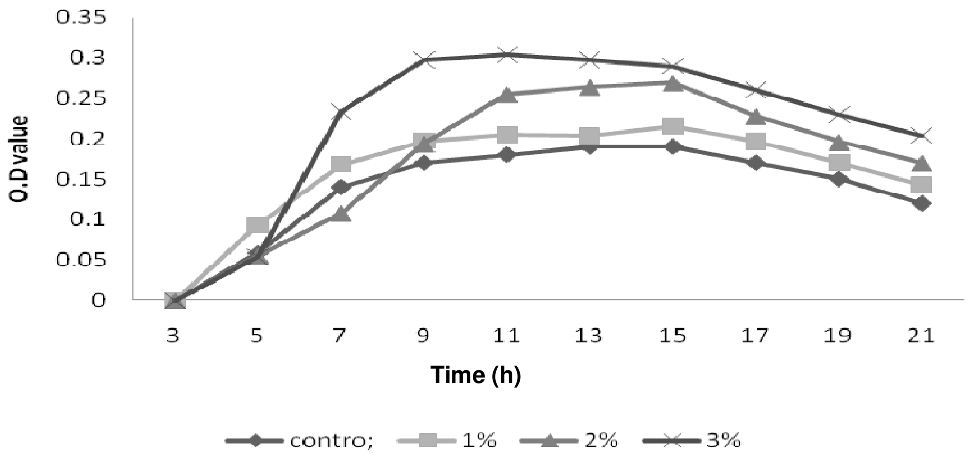

Figure 1. The growth curve (O.D at 600 nm) of *L. rhamnosus* at 37°C for 21 h.

associated with increased carotenoids consumption.

The use of microbial fermentation as a method for the production of natural antioxidant is promising.

MATERIALS AND METHODS

Preparation of green bell pepper juice

Fresh green bell pepper were washed and the seeds inside were removed, cut into smaller pieces and ground to mash using a kitchen blender with 2% olive oil and 0.5% emulsion to improve carotenoids bioavailability (Reddy et al., 1995). The mixture was incubated at 90°C in a water bath for 5 min, and then filtered with cloth to obtain clear filtrate which was transferred to a clean dark bottle, sealed and stored at 4°C until it was used.

The interaction between the vegetable matrix and the lipid fraction became more evident when the mixture was heated (Kaur and Kapoor, 2001).

Cultures

Streptococcus thermophilus, Lactobacillus delbreuckii subsp. *bulgaricus* and *Lactobacillus acidophilus* were obtained from Chr. Hansen's Lab., Denmark, while *Lactobacillus rhamnosus* B-445 was provided by Northein Regienal Research Lab., Illinois, USA, and NRRL.

Manufacturing of fermented milk samples

Low heat skim milk powder was reconstituted to 12% TS, while the milk fat was added and homogenized. Then, the green bell pepper juice was added in 1, 2 and 3% concentration. Fermented milk was prepared in similarity to the traditional method using 2% *L. rhamnosus* only, and also 2% yogurt starter plus 2% *L rhamnosus,* to ferment the milk. Afterwards, the samples were stored in the refrigerator at 4 ± 2°C for 15 days.

Methods

Total phenolics content, using Folin-Ciocalleae reagent (Zheng and Wang, 2001) and gallic acid as standard; and radical scavenging activity using 2,2,-di-phenyl-2-picryl-hydrazyl (DPPH) (Brand-Williams et al., 1995) and total carotenoids (Alberto, 1985) were evaluated.

Growth curve

The growth curve was estimated at OD 600.

Microbial analysis

Fermented milk was analyzed from the initial time to 15 days after the time. Lactic acid bacteria count was enumerated according to Elliker et al. (1956) and the plates were incubated at 37°C for 48 h. LC agar (1978) was used to enumerate *L. rhamnosus* anaerobically, at 27°C for 72 h, using "gen kits" in oxide jar, while yeasts and mould were counted on malt extract agar (Difco) at 25°C for 48 to 72 h.

RESULTS AND DISCUSSION

Figure 1 shows the effect of pepper juice on specific growth of all probiotic strains. This effect was dependent on concentration of pepper in the growth medium. Pepper juice was effective in enhancing growth rate of the strain, and when compared with the control, this may be due to the composition of juice which contains complex carbohydrate and dietary fiber. The growth rate of *L. rhamnosus* with 3% pepper juice was the highest rate and it gradually increased during the first 11 h, and then gradually decreased to the end of the experiment time (21 h). As shown in Figure 2, lactic acid bacteria count of all treatment decreased gradually during 7 days of storage, and then slightly increased on the 15th day, except the control which decreased after 5 days. This could be due to the fact that during the manufacturing process, bacterial starter increase in number and continue to multiply for approximately five days, whilst lactose is available in fermented milk. The results coincide with those stated by Mehanna et al. (2002) and

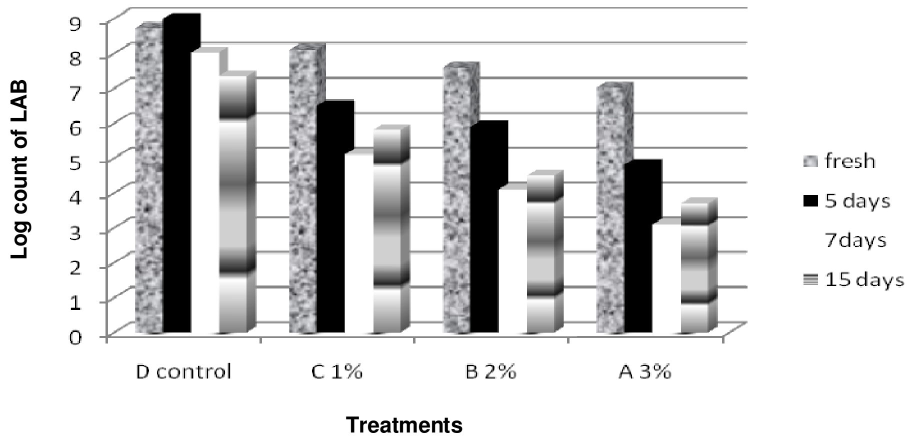

Figure 2. Change in the viable count of lactic acid bacteria during storage period of fermented milk supplemented with juice pepper.

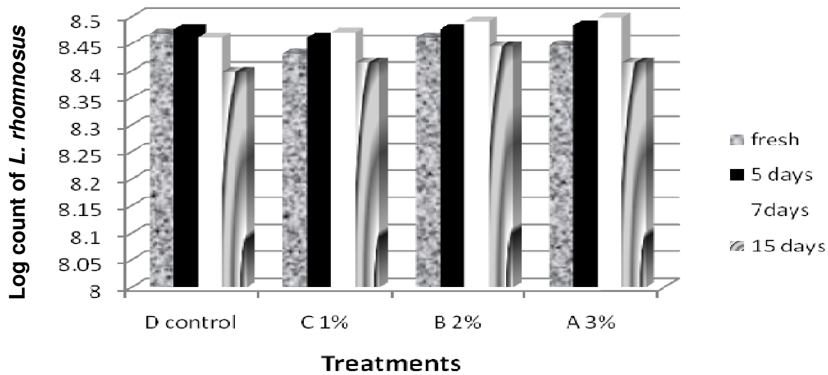

Figure 3. Change in the viable count of *L. rhamnosus* during storage period of fermented milk supplemented with juice pepper.

Mehanna and Hosney (2003). In addition, it may be noticed that the lactic acid bacteria count in the fermented milk which contain pepper juice decreased; this result may be due to the effect of some juice pepper components like phenols. Rodriguez et al. (2009) found that most of the phenolic compounds studied exert an inhibitory effect on LAB growth. This inhibition activity seems to be related to alterations in cytoplasmic membranes and in the cell wall. Also, we can notice that the differences between all treatments are significant.

The viability of *L. rhamnosus* increased in number till the 7th day of storage, except the control which increased for 5 days only (Figure 3). The count slightly decreased after that till the end of the storage period without insignificant difference between all treatments. From the fore going results, it could be recommended that the addition of pepper juice has no hazard or inhibition effect on viability of this strain.

Mould and yeasts were also examined in all treatments and were not detected until the end of the storage period.

This could be attributed to an inhibition effect of *L. rhamnosus* against yeasts and moulds.

Radical scavenging activity (RSA)

DPPH radical has odd electron (deep violet color), and when it accepts an electron or hydrogen radical, the absorbance is vanished. Nishino et al. (2000) reported that DPPH radical is known to be stoichiometrically decolorized by potent reducing substance and antioxidants such as cysteine, glutathione, ascorbic acid and tocopherols. Also, they mentioned that lactic acid fermentation by *L. casei* strain raised the RSA of the Millard skim milk samples, thereby heating low fat (1%), fat (12% TS) (control), low fat milk and pepper juice mixture (contain carbohydrates) in preparing fermented milk samples at 90°C per released products which had antioxidative and radical scavenging activity. It is obvious from Figure 4 that the fresh fermented milk samples inoculated with *L. rhamnosus* has RSA, and increasing the concentration of pepper juice slightly, increased RSA

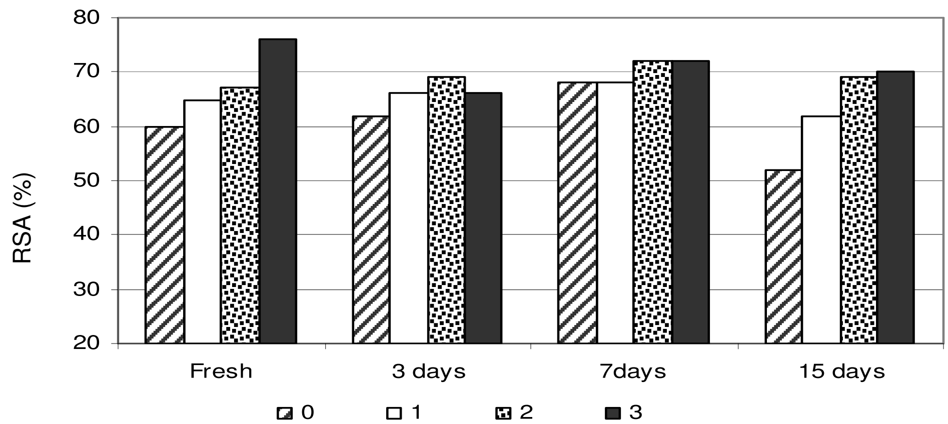

Figure 4. RSA (%) of fermented milk supplemented with bell green pepper juice and inoculated with *L. rhamnosus* during cold storage.

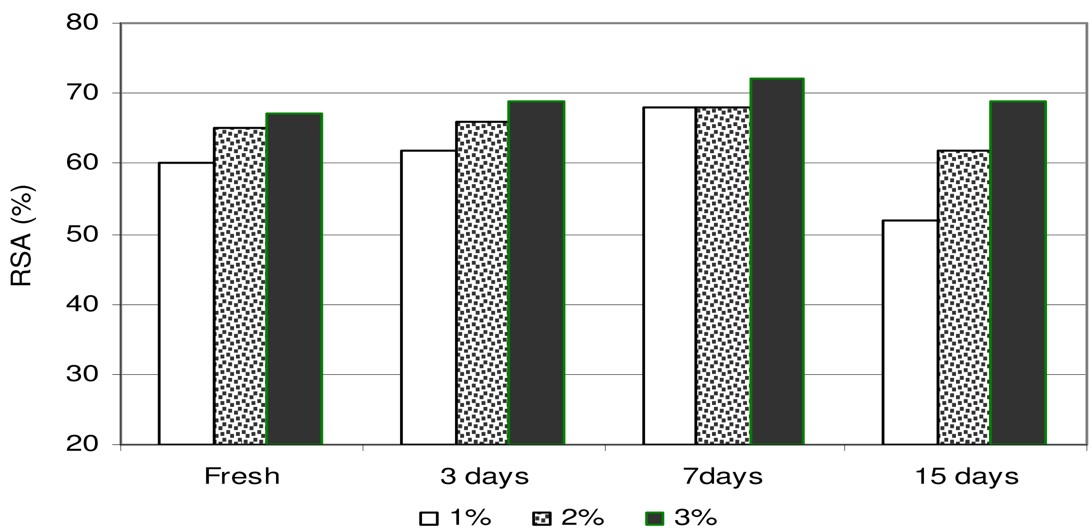

Figure 5. RSA (%) of fermented milk supplemented with bell green pepper juice and inoculated with *L. rhamnosus* and yoghurt starter during cold storage**.**

percentage. After 7 days of storage, the RSA percentage of all samples declined; and this was greatly noticed for samples without pepper juice (control).

The development of radical scavengers was more connected to the simultaneous development of proteolysis (Virtanen et al., 2008). They illustrated that the ferments with high scavenging activity possessed a high proportion of peptides in the molecular mass range of 4-20 KDa, and also the amount of hydrophobic amino acids was higher in these ferments. Consequently, the development of the antioxidant activity was strain-specific characteristic (thus, *L. rhamnosus* presumably does not have enzyme inhibition -superoxidedismutase- activity). Lin and Yen (1999) found that, this enzyme was not in the 19 strain of LAB. Moreover, the decrease in RSA percent, during 7 days, may be ascribed to the hydrolysis of the reducing compounds in the media during its growth until 7 days. After 15 days of storage, the RSA% which is nearly stable, may be attributed to the presence of the intercellular cell free extract as a result of the death of the strain which have antioxidant activity (Lin and Yen, 1999). This result may be due to the effect of one or more enzymes, related with *L. rhamnosus* as b-glucosidas, which may be activated in the first time (first 12 h) of fermentation and inhibited after that. Also, this enzyme may be affected by some component in milk or pepper juice at the first 12 h of fermentation.

Figure 5 illustrated the effect of supplementing milk with green pepper juice on the RSA percent of the resultant fermented milk using yoghurt starter with *L. rhamnosus* strain. It was clear that increasing the concentration of green pepper juice, increased the RSA percent in all fermented milk samples when fresh milk samples along the storage periods decreased on the 7th day. Kudoh et

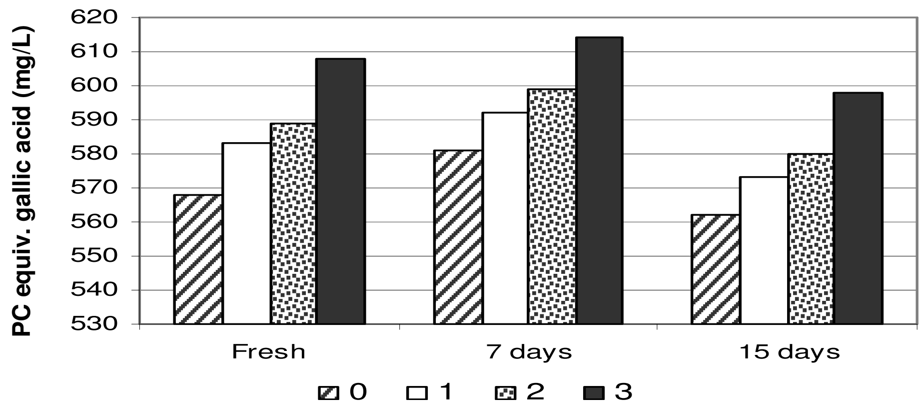

Figure 6. TPC of fermented milk supplemented with bell green pepper juice and inoculated with *L. rhamnosus* during cold storage.

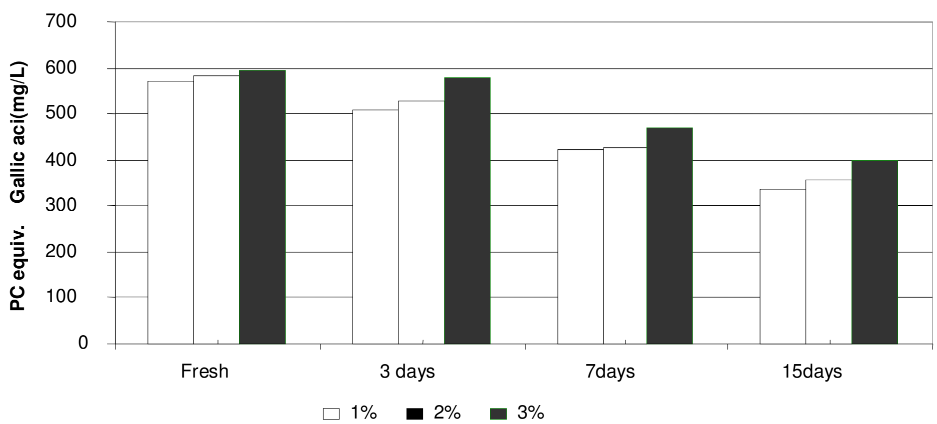

Figure 7. TPC of fermented milk supplemented with bell green pepper juice and inoculated with *L. rhamnosus* and yoghurt starter during cold storage.

al. (2001) and Fitzgerald et al. (2006) found potent antioxidant peptides during lactic acid fermentation. The presence of LAB with *L rhamnosus* inhibits the release of antioxidant compounds, since LAB has proteoltic and peptidolytic activity (Mattews et al., 2004). Said and Gilliland (2005) mentioned that most of the LAB has systems to cope with oxygen radicals. The most common systems are superoxide dismutase; and some species of lactobacilli produced a heme-dependent catalase for blocking the formation of peroxyl radicals. They also found that the greatest degree of antioxidant capacity was associated with the cell-free extracts of the cultures. This result agrees with Figure 2, which illustrates that the LAB was dead during the first 7 days in the fermented milk samples which contain pepper juice.

Total phenolic content (TPC)

The effect of supplementing low fat milk with green bell pepper juice on the phenolic content of fermented milk using *L. rhamnosus* strain is shown in Figure 6.

It is obvious that increasing the concentration of pepper juice increased the total phenolic content (TPC) of fresh and stored samples, and this attributed to the high level of PC in green bell pepper juice (7). Storage until 7 days increased the PC in the media, and this may be formed by the bacterial deamination of some aromatic amino acids derived from milk proteins by *LAC rhamnosus* (Abd El-Salam et al., 2004). Prolonging the storage to 15 days, decreased the phenolic content in the media for control and experimental samples as a result of the strain death, or as a result of the transformation of PC which is highly unstable to various reaction products (Cheynier, 2005). Es-Safi et al. (2007) supported the previous information, and they reported that PCs are highly unstable compounds that undergo numerous enzymatic and chemical reactions during food storage.

Preparation of low fat fermented milk containing pepper juice and mixture of yoghurt starter and *LAC rhamnosus* strain was investigated. Figure 7 illustrated that the PC content of all samples decreased during the storage

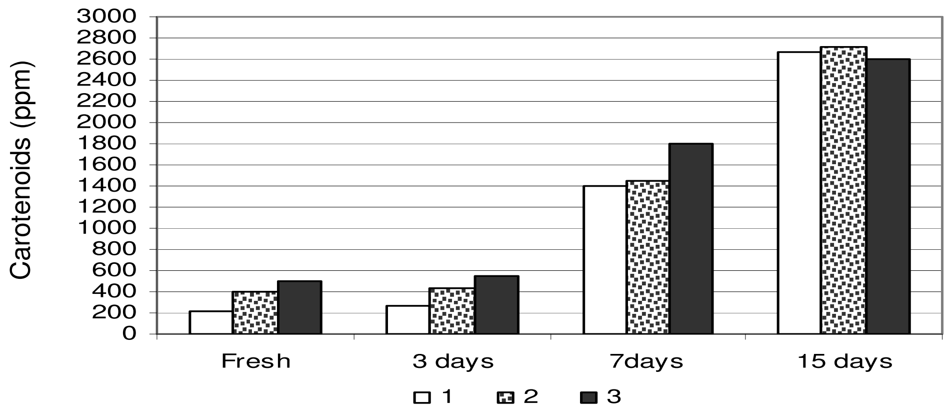

Figure 8. TCC of fermented milk supplemented with bell green pepper juice and inoculated with *L. rhamnosus* during cold storage.

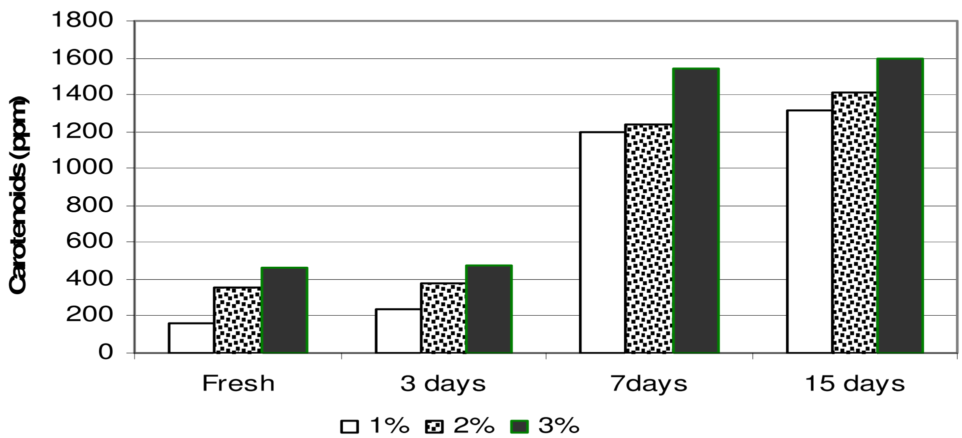

Figure 9. TCC of fermented milk supplemented with bell green pepper juice and inoculated with *L. rhamnosus* and yoghurt starter during cold storage.

period for all pepper juice concentration used. This is ascribed to the effect of pH of the media (as a result of the fermentation by yoghurt culture), and as such, the acidity enhances the hydrogen-donating activity of some PCs. Consequently, Keto compounds were formed (Satue-Gracia et al., 1999). Also, the effect of the yoghurt starter on the protein content of the media producing peptides interact with the PC of the juice during storage periods, and this is obvious with the addition of 1% pepper juice rather than 3% for the presence of extra PC with increasing the concentration of the juice.

Total carotenoids content (TCC)

Although carotenoids are fat soluble and show a weaker radical scavenge in the test, using DPPH method (7), they can potentially reduce the toxic effect of reactive oxygen species (J. Nut, 2004); thus, they act as antioxidant (Henry and Heppelle, 1998). Johnson and Schroeder (1996) reported that, several bacteria, fungi and yeasts are good carotenoid producers. Moreover, Buzzini et al. (2001) elucidated that the studies on carotenogenasis revealed a growing interest in using natural substrate with carbohydrate sources for microbial metabolite, as a carbon source. Green bell pepper, besides the fact that it contains carotenoids as mentioned before, still contains carbohydrates.

Consequently, it was obvious from Figure 8 that using *LAC rhamnosus* strain in preparing fermented milk increased the TCC in the media during the storage period until 7 days, and this was evident upon increasing the concentration of pepper juice to 3%. The TCC was nearly stable with prolonging the storage to 15 days. This may be attributed to the death of the strain, thereby stopping the carotenogenasis. Frengava et al. (2003) pointed out that these carotenoids were almost parallel with the cell growth.

Addition of yoghurt starter with *L. rhamnosus* strain in preparing fermented milk fortified with pepper juice (Figure 9) slightly increased the TCC in the media, upon increasing the concentration of the juice. Concerning the storage, the TCC was highly increased with elongating the storage period. Frengava et al. (2003) found 5 strains from 40 strains of *Lactobacilli* which have the basis of carotenoids-synthesing activity and can be carried out under conditions for lactose transformation into carbon sources (glucose, galactose and lactic acid). Moreover, the increase in the media's TCC in the end of the storage may be presumably caused by yeast contaminant. Emilina et al. (2004) demonstrated that the carotenoid yield was approximately two-fold higher in association with a mixed yoghurt culture with yeast (*Rhodorula rabra*).

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