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## Effect of *Spinacia oleracea* extract on physicochemical, phenolic content, antioxidant activity and microbial properties of yogurt

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### ABSTRACT

Yogurt flavored with kiwi flavor (1, 2 and 4%) and colored with spinach extract (1.25, 2.5 and 4%) and a control yogurt (no kiwi flavor or spinach extract) were evaluated for some chemical characteristics, viscosity, syneresis, L\*, a\* and b\* color values and microbial properties at seven-day intervals for 21 days. Significant differences were found between the control and kiwi-spinach yogurt with respect to syneresis and viscosity characteristics (P<0.05). Increasing the amount of spinach extract in yogurt resulted in a decrease in L\*, a\* values of yogurt, and increase in b\* color, syneresis, total phenolic content, antioxidant activity, titratable acidity and viscosity parameters (P<0.05). During the storage period, pH decreased but titratable acidity, syneresis and viscosity values of yogurt samples increased continuously (P<0.05). Storage affected LAB count significantly (P<0.05). © 2013 Trade Science Inc. - INDIA

### KEYWORDS

Kiwi-spinach yogurt;  
Total phenolic content;  
Antioxidant activity;  
*Spinacia oleracea*.

### INTRODUCTION

Spinach (*Spinacia oleracea* L.) is a leafy vegetable of the Chenopodiaceae family<sup>[1]</sup>. It is native to central Asia and widely thought to have originated in Iran<sup>[2-5]</sup>. It is commonly used as a salad, a cooked vegetable or as a component of many other cooked meat and vegetable dishes. The juice of leaves is cooling and very nutritive<sup>[6]</sup>. It is high in ascorbate,  $\beta$ -carotene, lutein, flavonoids, magnesium, folate, iron, potassium and unsaturated fatty acids<sup>[7,8]</sup>. Water-soluble spinach extracts have been shown to have antimutagenic<sup>[9]</sup>, antioxidative<sup>[10]</sup>, antitumor<sup>[11]</sup> and anti-inflammatory properties<sup>[12]</sup> in biological systems, but have no potential adverse estrogenic activity<sup>[13]</sup> or toxic effects in ani-

mals<sup>[13]</sup>. Fresh leaf juice of spinach increases breast milk and used in anemia, jaundice, cirrhosis of the liver and in conditions of general weakness<sup>[6]</sup>. These studies suggest that spinach extracts may exert beneficial effects such as chemo and central nervous system protection, and anticancer and anti aging functions<sup>[14]</sup>. Recently, there has been an increasing interest in the use of natural food additives and incorporation of health-promoting substances into the diet<sup>[15]</sup>. Yogurt is one of the most unique dairy products. The uniqueness of yogurt is attributable to the symbiotic fermentation involved in its manufacturing. Yogurt in different forms with appropriate local names is made throughout the world. In principle, worldwide, there are not any differences between manufacturing of homemade and factory-made yogurt<sup>[16,17]</sup>. Yo-

gurt is being enjoyed everywhere in the world for its beneficial properties. It is easily digestible, has high nutritional value<sup>[18-20]</sup> and has also therapeutic properties<sup>[21,22]</sup>. The production and consumption of fruit yogurt is low in Iran compared to plain yogurt, but Buraniy (or Spinachy yogurt) is widely made and consumed in Iranian homes. There is no commercial production of either Spinach yogurt in Iran. Therefore, The use of different vegetable-flavor in yogurt manufacture has been attempted increasingly. The aim of this study was to utilize spinach extract (with high iron content and good nutritional value) and kiwi flavors in developing a yogurt of high acceptability. Another objective of this study was to evaluate the effect of spinach additives on microbial, and physicochemical properties of yogurt.

## MATERIAL AND METHODS

### Material

The experiment conducted in the laboratory of the department of food technology & rural industries, Islamic Azad University, branch of Shahrekord. Fresh milk collected from dairy farm of Shimbar factory. Spinach for extract preparation, Kiwi flavor (Farmand Co, Iran) and starter culture (Chr. Hansen Co. Horsholm, Denmark) purchased from local market. Materials used in these experiments were Sodium hydroxide, methanol, sodium carbonate, methylene blue reagent, Folin-ciocalteu) 64171 Dansta dtj Germany), 1DPPH) Alrich D913-2, Germany) and devices used including pasteurizator and homogenizer (Made in Iran), juicer (JC-17E, Japan), centrifuge 3K30 (Germany), high speed agitator (mixer 12405, Germany), Brookfield viscometer (Stoughton, USA), Hunter Lab Color Quest (Memmert, Germany), pH meter (D-82362, Germany), refractometer (RX-500, Belgium).

### Preparation of spinach extract

Spinach was washed with clean water and the black spots removed from spinach with the help of knife. Spinach blended and then extracted by juicer. After blending, the extract filtered with clean cloth (hot water washed) and kept in plastic containers at freezing temperature (-20 °C) until preparation of spinach yogurt<sup>[23]</sup>.

### Yogurt manufacture

Raw milk pasteurized at 85 °C for 10 minutes and subsequently cooled to 39±1 °C. Inoculation was done

with desirable proportion of starter culture (2.5%). Once the starter, was completely mixed, the spinach juice incorporated into yoghurt at 1.25%, 2.5% and 4% level and was flavored with kiwi flavor at 1, 2 and 4% levels except in control sample<sup>[24]</sup>. The plastic cups were pre-washed with boiled water before using. The samples were incubated at 41-43 °C until formation/coagulation of yoghurt (8-12 hrs). The yoghurt samples were stored at about 4 °C at refrigeration until used.

### Chemical analysis of fresh milk

Moisture, total solid (TS) and ash content were determined according to AOAC<sup>[25]</sup>. Fat content determined by Babcock method using the procedure described by Aggarwala and Sharma<sup>[26]</sup>. Acidity determined by titration with 0.1 N sodium hydroxide solution using the procedure by Aggarwala and Sharma<sup>[26]</sup>. Crude protein determined by Kjeldahl described by Ranganna (1976) procedure<sup>[27]</sup>. Total carbohydrate content of the sample determined by subtracting the measured protein, fat, ash and moisture from 100<sup>[28]</sup>. pH measured with the help of a pH meter (D-82362; Germany).

### Lactic acid bacteria (LAB) and yeast and mold counts

Total LAB count determined for the starter culture and yogurt samples. Aliquots of 10 g diluted with 90-mL sterile peptone water (0.1% w/v) and serial dilutions were prepared. Mann, Rogosa, Sharpe agar (Oxoid, Basingstoke, U.K.) used for assaying total lactic acid bacteria by the double layer plating technique. Plates incubated at 32 °C for 48–72 h<sup>[29,30]</sup>. Yeast and mold counts also conducted on the yogurt samples. The serial dilutions were placed on potato dextrose agar (Oxoid) acidified with 1% lactic acid and the plates were incubated at 30 °C for 5 days<sup>[31]</sup>. All yogurt samples were duplicates plated at days 1, 7, 14 and 21.

### pH measurement

Yogurt pH measured in duplicate using a pH model (D-82362; Germany). Titratable acidity of milk and the yogurts, expressed as % lactic acid, was determined on triplicate samples following method 947.05 of the AOAC<sup>[32]</sup>. Yogurt pH and titratable acidity measured at days 1, 7, 14, and 21.

### Total titratable acidity measurement

Yogurt sample (1 ml) was mixed thoroughly with 9

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ml of distilled water Phenolphthalein solution (0.1%, 3 drops) was added and the yogurt suspension was titrated using 0.1 M NaOH. The mixture was stirred continuously and titrated was continued until the indicator changed to a definite pink color lasting for 30 seconds. The volume of NaOH required to neutralize the yogurt acid recorded and used to calculate the content of titratable acids (lactic acid percentage equivalent) using the following formula:

$$LA\% = \frac{10 \times V_{NaOH} \times 0.009 \times 0.1}{W} \times 100\%$$

Where 10 = Dilution factor; W = Weight of sample for titration; VNaOH = Volume of NaOH used to neutralize the lactic acid; 0.1 = Normality of NaOH.

### Syneresis measurement

One hundred grams of yoghurt sample placed on a filter paper resting on a top of a funnel. After 2 h of drainage at 7 °C, the quantity of whey collected in a 50 ml graduated cylinder was used as an index of syneresis<sup>[33]</sup>. Syneresis (%) was based on the volume of clear supernatant per 100-mL yogurt.

### Viscosity measurement

Apparent viscosity was determined by using a RV Brookfield viscometer (Stoughton, USA) on 100 mL yogurt samples at room temperature. Samples stirred for 40 sec before measurement. Readings converted to centipoises units. All viscosity values measured at 10 rpm with spindle #5<sup>[34]</sup>.

### Total phenolic content

Total phenolic content determined according to Apostolidis, et al<sup>[35]</sup>. The absorbance read at 725 nm and the values were converted to total phenolics, expressed in micrograms equivalents of gallic acid per gram (GAE/g) sample. Gallic acid used as standard.

### Color measurement

Color measured by using a Hunter Lab Color Quest (Memmert, Germany). In the CIELAB system, L\* in-

dicates degree of lightness or darkness (L\* = 0 indicating perfect black and L\* = 100 indicating perfect white); a\* and b\* indicate degree of redness or greenness and yellowness or blueness, respectively.

### Antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay

Antioxidant activity of yogurt samples by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition was determined by an assay modified from Apostolidis, et al<sup>[35]</sup>. The decrease in absorbance monitored at 517 nm until a constant reading was obtained. The readings compared with the control which contained distilled water (250 il) instead of yogurt water extract. The inhibition percentage calculated as follows:

$$\% \text{ inhibition} = \frac{(A_{\text{control}} - A_{\text{extract}})}{A_{\text{control}}} \times 100\%$$

### Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 1995)<sup>[36]</sup>. The means separated by using the least significant difference (LSD) test. Significant differences were determined at  $\alpha=0.05$ <sup>[37]</sup>.

## RESULTS

### Chemical analysis of milk

Quality of milk used for yoghurt production analyzed before using. Moisture, total solid, fat, protein, ash, lactose, acidity, pH, and solid non-fat (SNF) were determined. Results of chemical analysis of milk are shown in (TABLE 1). The results are more or less similar to other researcher. Protein percentage of raw milk samples was 3.32, which is within the normal range of 2.3 to 4.4. Mean acidity of the experimental samples was 0.17 percent (TABLE 1) which is within the normal range.

TABLE 1 : Composition of cow milk, used for yogurt making

| Composition | pH  | Acidity (%) | Moisture (%) | Fat (%) | Protein (%) | Ash (%) | Carbohydrate/Lactose (%) | SNF (%) |
|-------------|-----|-------------|--------------|---------|-------------|---------|--------------------------|---------|
| Raw Milk    | 6.7 | 0.17        | 87.52        | 4.16    | 3.32        | 0.74    | 4.26                     | 8.37    |

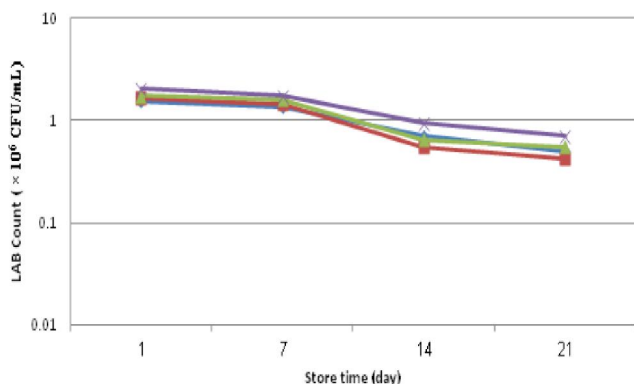
### Microbial properties of spinach- flavor yogurts

#### LAB and yeast and mold counts

The starter culture used in yogurt preparation had a LAB count of  $2.8 \pm 0.4 \times 10^6$  cfu/mL. LAB counts of

yogurt containing 1.25, 2.5 and 4% spinach extract shown in Figure 1, LAB counts of yogurts, except yogurt containing 4% spinach extract, reported  $3 \pm 0.1 \times 10^6$  cfu/mL at first day, which was suitable. During 21 days of storage, LAB counts decreased significantly

( $P < 0.05$ ). Addition of spinach extract affected LAB counts. Cueva and Aryana (2007)<sup>[38]</sup> reported that LAB counts of fruit-flavored yogurts were 8.9 log cfu/g and 8.4 log cfu/g at days 1 and 13, respectively, and Akin and Konar (2001)<sup>[39]</sup> found LAB counts for yogurts made from cows' milk varied between 8.3–8.7 log cfu/g at day 1 and 8.5–8.6 log cfu/g at day 15. In comparison to these reports, LAB counts in this study were generally less and decreased significantly over the 21-days period. The decrease was possibly related to the slightly higher storage temperature and larger pH reduction that was observed.



**Figure 1 :** Total lactic acid bacteria counts in control and spinach yogurts during 21 days of refrigerated storage at 5–7 °C: ♦CY: control, ■S1: 1.25%, ▲S2: 2.5%, and × S3: 4% Spinach extract respectively.

The result of viable bacteria count during storage at 4 °C for three weeks is shown in Figure 1. According to this Figure, the number of lactic acid bacteria in yogurt containing 4% spinach extract is more than the control at day 14, which may be due to stimulation by glucose, lactic bacteria, nitrogenous compounds and vitamins available in spinach extract. But the growth of bacteria significantly reduced until day 21. Guven and Gulmez (2006)<sup>[40]</sup> and Kucukoner and Tarakci., (2004)<sup>[41]</sup> observed a decrease in LAB count of LAB during storage period because of the reduction in pH and increasing the acidity. Land and Shepher (1988) stated that a minimum of 10<sup>7</sup>cfu/mL LAB is necessary for positively influence on the intestinal microbial flora<sup>[42]</sup>. Yeast and mold count in all the samples during 15 days of storage was below 5cfu/g and acceptable according Codex standard.

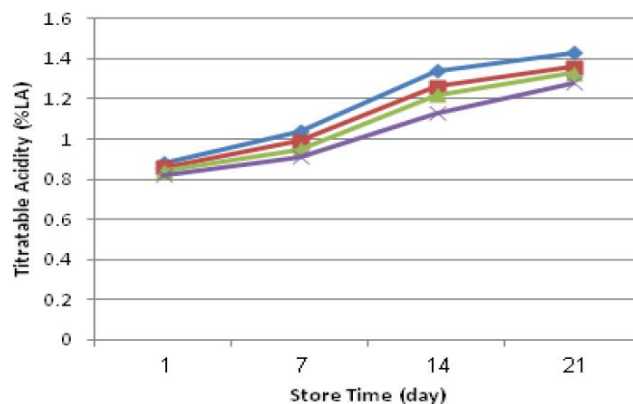
### Physical and chemical properties of yogurt samples

Plain yoghurt (no Flavor and extract added) was compared with yoghurts incorporating different concentrations (1.25%, 2.5% & 4%) of spinach extract

(S1, S2 & S3), and different dosages (1%, 2% & 4%) of Kiwi flavor (K1, K2 & K3).

### Titratable acidity properties of yogurt samples

The lowest mean value of titratable acidity found at the 1st day of storage (for S3), while the highest value found at 21st day of storage (control yogurt). Some authors reported similar results (Tarakci and Kucukoner, 2004)<sup>[40]</sup>. This might be due to the acid production in the experimental yogurts during storage as a result of the fermentation of lactose by the action of the starter cultures<sup>[43]</sup>. Laye et al., (1993)<sup>[44]</sup> reported lower titratable acidity values than our results and similar results were reported by Isleten and Karagul-Yuceer (2008)<sup>[45]</sup> for non-fat yogurt. Titratable acidity of the control and kiwi-spinach yogurts increased significantly during the storage period at 4 °C ( $P < 0.05$ ). Some researchers reported that the titratable acidity of fruit-flavored yogurts increased along with storage<sup>[39,46]</sup>.



**Figure 2 :** Effect of spinach extracts on titratable acidity of yogurts during storage at 4°C for 21 days. ♦: CY control and, ■S1: 1.25%, ▲ S2: 2.5% and × S3: 4% Spinach extract respectively.

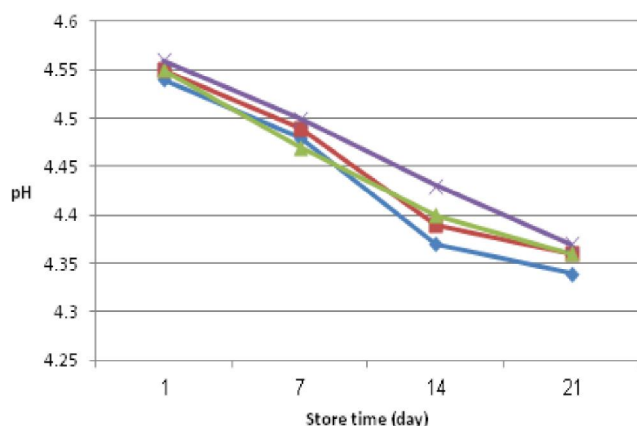
In yogurt sample containing 4% spinach extract, the rapid increase in titratable acidity continued up to the end of storage (Figure 2). The titratable acidity of kiwi-spinach yogurt containing 2.5% spinach extract found to be more than the yogurt with 1.25% spinach extract and control, while the acidity of the yogurt with 1.25% spinach extract was lower than the control (Figure 2).

### pH properties of the experimental yogurts

pH measures free H<sup>+</sup> ion whereas the total titratable acidity measure total organic acid that present in yogurt. Both measurements are important because acidification is the key mechanism during yogurt fermentation<sup>[47]</sup>. The declining of pH during fermentation

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was due to the proto cooperative action of two strain of bacteria *Str. thermophilus* and *Lb. bulgaricus*<sup>[47]</sup>. Presence of milk sugar (carbon source) and milk protein (nitrogen source) in the rich medium of milk and optimum incubation environment (pH 7 and 41 °C) encourage bacteria (*Str. thermophilus*) to grow rapidly. They transform lactose into lactic acid, acetaldehyde, diacetyl, and formic acid. The accumulation of all these fermentation products corresponds to the increasing of acid production during fermentation. The liberation of lactic acids reflects the high metabolic activity of the lactic acid bacteria<sup>[48]</sup>.



**Figure 3 : Effect of spinach extracts on pH of control and spinach yogurts during storage at 4°C for 21 days: ♦ CY: control and, ■ S1: 1.25%, ▲ S2: 2.5% and × S3: 4% Spinach extract respectively.**

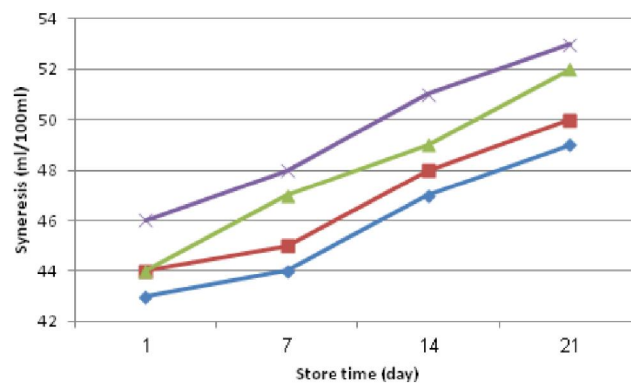
In general, the pH values of all samples decreased during storage and these differences were found to be significant ( $P < 0.05$ ). This can be explained by further metabolic activities of starter cultures during storage<sup>[49]</sup>.

pH of control and kiwi-spinach yogurts continued to decrease during the storage. Lower concentration of spinach extract in the yogurt resulted in a faster decrease in the pH, while higher spinach extract concentration caused to increase in the pH.

### Syneresis properties of the yogurts

The syneresis values of yogurts were affected significantly ( $P < 0.05$ ) by both spinach extracts concentration and storage time and the changes were shown in (Figure 4). The highest mean value (46.06 mL/100 g) of syneresis was recorded in sample S3 and the lowest mean value (42.62 mL/100 g) in sample CY (control). As seen in Figure 3, the addition of spinach extract caused an increase of syneresis value in all samples of kiwi-spinach yogurts and the differences between the

control and these samples were statistically significant ( $P < 0.05$ ). All yogurt samples with spinach extracts showed a higher syneresis percentage compared to plain yogurt. Yogurt with 4 % w/w spinach extract showed the highest syneresis (52.8%). This increasing in syneresis is probably due to decreasing in water holding capacity that led to more releases of whey<sup>[50]</sup>.



**Figure 4 : Syneresis of control and spinach yogurts during storage at 4°C for 21 days: ♦ CY: control and, ■ S1: 1.25%, ▲ S2: 2.5% and × S3: 4% Spinach extract respectively.**

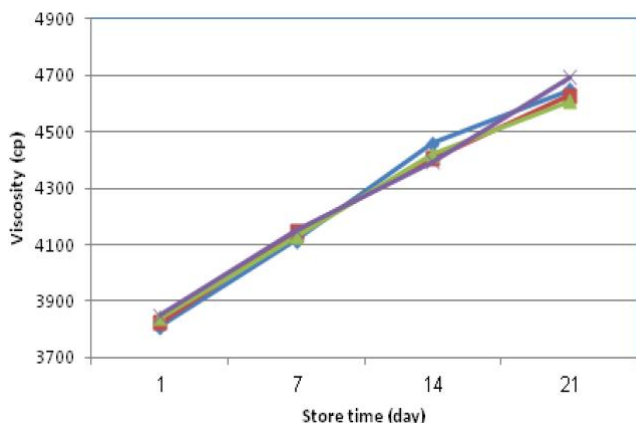
The introduction of spinach extracts did not increase the fiber contents in yogurt, which otherwise would hold the water and thus increase the syneresis. The watery structure of the extracts themselves may lead to more releases of whey in the spinach-flavor yogurts. The higher syneresis shown in spinach-flavor yogurt was most probably caused by higher active water content contributed by the added extracts.

### Viscosity properties of the yogurts

The viscosities of the control and kiwi-spinach yogurt increased rapidly up to day 7, and continued to increase slowly up to day 14 of storage and afterwards decreased slowly. Similar viscosity pattern of yogurt during gelation process was reported by Jumah et al (2001)<sup>[51]</sup>. On the other hand, the viscosity of the kiwi-spinach yogurt was influenced by the rate of the extracts addition (Figure 5). The addition of the spinach extract increased the mean viscosity values of all yogurts, and it was also found to be concentration-dependent ( $P < 0.05$ ). All yogurts formulated with spinach extracts showed no significantly higher viscosity values compared to the plain yogurt. Probably, the addition of spinach extracts reinforced the yogurt micelle matrix and the spinach fiber fragments did not interfere with the fine structure of the yogurt.

Yogurt, with the highest spinach extract content

(4%), showed the highest viscosity values. Lower levels of spinach extracts probably altered the casein micelle matrix structure of the yogurts in such a way as to have contributed to the low viscosity values. Similarly, Otero et al., (2000) observed higher viscosity values when the fiber content was increased in yogurt preparation<sup>[52]</sup>.

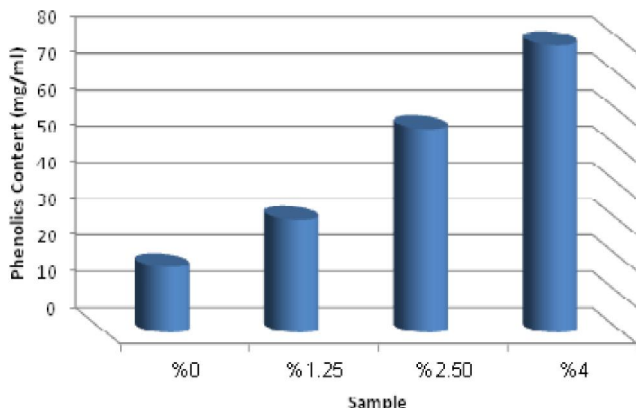


**Figure 5 :** Viscosity of control and spinach yogurts during storage at 4 °C for 21 days: ♦ CY: control and, ■ S1: 1.25%, ▲ S2: 2.5% and × S3: 4% Spinach extract respectively.

**Total phenolic content assay**

All spinach enriched yogurts showed an increment in total phenolic content compared to plain yogurt. Green spinach enriched yogurts showed higher increment in total phenolic content than plain yogurt.

Results showed significantly difference in phenolics content of spinach extracts enriched yogurt and plain yogurt and suggested that the addition of green spinach extract may change the phenolics content of yogurt. Besides, there is also significant difference in phenolic content of yogurt containing 4%, 1.25 and 2.5% spinach extracts (P<0.05) which showed that 4% spinach extract yogurt gave higher increment in phenolic con-



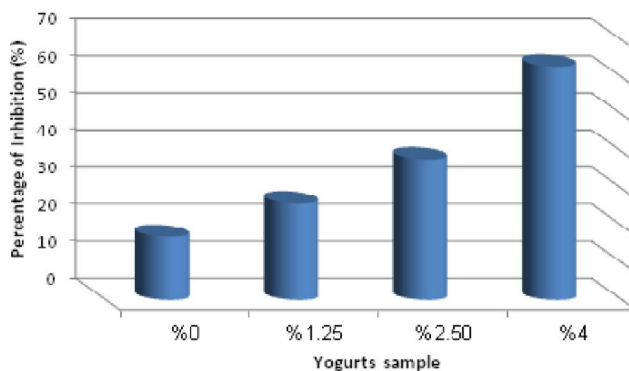
**Figure 6 :** Total phenolic content in yogurt with Added different percentage of spinach extract

tent compared to others. Chlorophyll, the pigment found in Spinach, also contributed to the total phenolics, due to a phenol structure in the molecule<sup>[14]</sup>. The existence of several flavonol glycosides in a methanolic extract of spinach leaves is reported<sup>[53,54]</sup>. The occurrence of at least 10 flavonoid glycosides is reported to be in spinach. These are glucuronides and acylated di- and triglycosides of methylated and methylene dioxide derivatives of 6-oxygenated flavonols<sup>[53,55]</sup>.

**Antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay**

The antioxidant capacity of spinach flavonoids has been determined by the free-radical scavenging assay using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical (7). Using the DPPH radical scavenging method, it was shown that all spinach enriched yogurts showed an increment in percentage of inhibition compared to plain yogurt.

It also shown that all spinach enriched yogurt showed a significant different in the percentage of inhibition compared to plain yogurt and it is suggested that addition of spinach extract into yogurt may change or



**Figure 7 :** Percentage of antioxidant inhibition in yogurt Added different percentage of spinach extract.

enhance the percentage of inhibition. However, the difference in the percentage of inhibition between adding 1.25% spinach extract to yogurt and plain yogurt is not significant (P>0.05). The higher antioxidant activity of both 2.5 and 4% spinach extract yogurts are a desirable characteristic that may enhance the therapeutic values of yogurt. This might be due to the addition of Spinach extract that contained vitamins, phytoalbumins, and lycopene which are highly valued for their antioxidant properties<sup>[56]</sup>. Furthermore, the increment in antioxidant activity in spinach enriched yogurts might be due to the increment in total phenolics content that shown in Figure 7, as we know that phenolic and polyphenolic compounds constitute the main class of natural antioxi-

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dants present in plants<sup>[56]</sup>. Flavonoids and other phenolic constituents act as antioxidants by the free-radical scavenging properties of their hydroxyl groups. Extensive conjugation across the flavonoid structure and numerous hydroxyl groups enhance their antioxidative properties, allowing them to act as reducing agents, hydrogen- or electron-donating agents, or singlet-oxygen scavengers<sup>[57,58]</sup>. Results from the in vitro oxygen radical absorbance capacity (ORAC) assay have shown that, among various fruit and vegetable extracts, foods with the highest ORAC activity include spinach, straw-

berries<sup>[59,60]</sup>, and blueberries<sup>[61]</sup>.

### The color characteristics of the yogurts

The color characteristics of the yogurt samples are shown in TABLE 2. Although the L\* (whiteness) and a\*(greenness) color values of yogurts decreased by spinach addition, b\* (yellowness/blueness) values significantly increased (P<0.05). The S3 yogurt showed the lowest values in L\* and a\* color. Increasing spinach extract ratios in yogurts had a darker green color with a higher degree of luminosity than that of the control yogurt.

TABLE 2 : Effect of spinach extract concentrations on color values of yogurt

| Properties Of Color | Treatment         | Storage time (day) |                    |                     |                    |                     |
|---------------------|-------------------|--------------------|--------------------|---------------------|--------------------|---------------------|
|                     |                   | 1                  | 7                  | 14                  | 21                 | $\bar{Y}_X$         |
| L value             | CP                | 87±0.58            | 89±1.08            | 90±1.89             | 91±0.92            | 89.25 <sup>a</sup>  |
|                     | S1                | 84±0.65            | 86±1.23            | 87±0.12             | 89±2.09            | 86.5 <sup>ab</sup>  |
|                     | S2                | 81±2.42            | 82±0.65            | 82±0.34             | 84±0.98            | 82.25 <sup>b</sup>  |
|                     | S3                | 78±0.14            | 78±0.21            | 79±0.45             | 80±1.13            | 78.75 <sup>c</sup>  |
|                     | $\overline{ST}_X$ | 82.5 <sup>B</sup>  | 83.75 <sup>B</sup> | 84.5 <sup>A</sup>   | 86 <sup>A</sup>    |                     |
| a value             | CP                | -3.71±0.56         | -3.68±1.64         | -2.35±1.33          | -1.44±0.53         | -2.795 <sup>c</sup> |
|                     | S1                | -7.05±1.22         | -6.68±0.43         | -6.34±0.65          | -6.12±0.95         | -6.55 <sup>b</sup>  |
|                     | S2                | -11.37±0.4         | -11.29±0.8         | -11.06±1.3          | -10.75±1.7         | -11.12 <sup>a</sup> |
|                     | S3                | -12.35±0.7         | -12.09±2.1         | -11.94±0.1          | -11.78±2.3         | -12.04 <sup>a</sup> |
|                     | $\overline{ST}_X$ | -8.62 <sup>B</sup> | -8.44 <sup>B</sup> | -7.92 <sup>AB</sup> | -7.52 <sup>A</sup> |                     |
| b value             | CP                | 8.12±0.64          | 9.23±1.76          | 11.86±2.71          | 13.17±1.16         | 10.56 <sup>b</sup>  |
|                     | S1                | 11.45±1.24         | 11.96±0.34         | 12.33±0.87          | 12.97±1.39         | 12.18 <sup>b</sup>  |
|                     | S2                | 23.11±0.89         | 24.03±1.78         | 24.98±2.29          | 25.26±0.56         | 24.35 <sup>ab</sup> |
|                     | S3                | 28.43±0.16         | 28.98±0.93         | 29.23±1.53          | 29.74±0.91         | 29.1 <sup>a</sup>   |
|                     | $\overline{ST}_X$ | 17.78 <sup>A</sup> | 18.55 <sup>A</sup> | 19.6 <sup>A</sup>   | 20.29 <sup>A</sup> |                     |

CY, Control yogurt, S1, S2 and S3 yogurts added spinach extract at ratios of 1.25, 2.5 and 4 %, respectively abcd Letters indicate significant differences among yogurts ( $\bar{Y}_X$ ), P<0.05, ABC Letters indicate significant differences among storage times ( $\overline{ST}_X$ ), P<0.05

## CONCLUSION

Various amounts of spinach extract and kiwi flavors were used in production of kiwi-spinach yogurt and the effects of these additives on physical, chemical and organoleptic properties of the product were examined. Results shows that 4% spinach extract and 4% kiwi flavor led to a product preferred over another samples. The flavor, body and texture of the spinach flavored yogurt tended to decrease during storage. Storage had a marked effect on viable LAB counts. All the panelists recommended that kiwi addition level could increase the flavor scores of the spinach-yogurt samples.

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