

## Levels of Fat-Soluble Vitamins in Some Foods

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The levels of vitamins in nine food of winter season which are grown in Turkey were determined. High performance liquid chromatography (HPLC) was used in this work. Eight vitamins were detected in the foods. The contents of vitamins were determined as  $\beta$ -carotene, 38.4-144.5;  $\delta$ -tocopherol, 0.63-6.15;  $\gamma$ -tocopherol, 0.7-936.2;  $\alpha$ -tocopherol, 0.7-72.8;  $\alpha$ -tocopherol acetate, 3.7-113.1; vitamin D<sub>2</sub>, 5.2-47.8; vitamin D<sub>3</sub>, 36.4-642.2 and vitamin K<sub>1</sub>, 12.6-67.4  $\mu\text{g g}^{-1}$ . For the simultaneous determination of all the vitamins, the mixture of acetonitrile/methanol (3/1, v/v) was used as the mobile phase. Detection was performed at 202 nm for vitamins. The results showed that vitamin D<sub>3</sub> level is high for corn and  $\gamma$ -tocopherol is rich in bulgur.

**Key Words:** HPLC, Vitamin, Food, Cereals.

## INTRODUCTION

Vitamins are biologically active controlling agents for an organism's health and growth. These agents are often accompanied by an excess of compounds with similar chemical properties. Human diet should contain appropriate amount of vitamin for its essential functions. Systematic absence of vitamins in human diet can results in deficient growing and development<sup>1,2</sup>. Vitamins generally are chemically unstable, especially in high temperature, light or oxygen. Thus not only quantification but also instantaneous identification is mandatory for the detection of vitamins in food. For separation and detection, different HPLC methods are used at room conditions in order to compensate the effect of such parameters on stability of molecules under investigation<sup>3</sup>.

Vitamins, studied in this work, are critical components of many antioxidant processes. Any deficiency in the level of these vitamins in living organism may impair the function of overall oxidant system because these compounds (*i.e.* vitamins) are essential in living systems<sup>4</sup>.

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The correlation between diet and health has led the consumers to ingest foods containing vitamins, included the fortifier ones and more recently, the pharmaceutical supplements. But the addition of these nutrients to industrial products can be utilized as only marketing strategy and several times, the commercial advantage has priority in damage of individual real necessity<sup>5</sup>.

There are a lot of analytical procedures including spectrophotometry<sup>6</sup>, spectrofluorimetry<sup>7</sup>, voltammetry and chromatography<sup>8,9</sup> to carry out the assay of vitamins in food.

In this study, eight fat soluble vitamins ( $\beta$ -carotene, vitamin D<sub>2</sub>, vitamin D<sub>3</sub>,  $\gamma$ -tocopherol,  $\delta$ -tocopherol,  $\alpha$ -tocopherol,  $\alpha$ -tocopherol acetate, vitamin K<sub>1</sub>) in nine winter season foods of Turkish origin were determined, using HPLC.

### EXPERIMENTAL

The vitamins standard stock solution, 100.0 mg L<sup>-1</sup>, were prepared by dissolving 10 mg of each reactive obtained from Sigma-Aldrich (St. Louis, USA) in 100 mL of methanol using dark brown volumetric flasks. These solutions were stable for at least 1 month when stored in the dark at 4 °C. Working solutions were prepared from the stock solutions by appropriate dilution with ethanol and shielded from light.

The chromatographic system was equipped with a Shimadzu LC-9A pump, SPD-M10AVP photodiode array detector. Supelcosil LC 18 DB column (250 mm  $\times$  4.6 mm, 5  $\mu$ m; Sigma, USA) for separation of vitamins.

Corn, pepper, sweet basil, bean, aubergine examples used in study were gathered from vegetable market in August 2007 taking into consideration to be grown in Elazig (Turkey). Each of these three numbers corn, pepper, aubergine and a hundred each gram packed green bean and sweet basil examples were obtained from various sales. After collection, the samples were washed and then dried under the sun for 20 days. Tarhana, Bulgur, Red lentil and chickpea were bought from cereal market in Elazig. Tarhana, a traditional fermented food made from a mixture of white wheat flour and yoghurt, is widely consumed in Turkey<sup>10</sup>.

**Determination of fat soluble vitamins in foods:** Dried vegetables (1 g) were homogenized in 6 mL acetonitrile/methanol/isopropanol (2/1/1, v/v/v)-containing tubes and the samples were vortexed for 30 s and centrifuged at 6000 $\times$ g for 10 min at 4 °C. Supernatant was dried under nitrogen in the 37 °C, it was redissolved in 1 mL mobile phase. Samples were transferred to auto sampler vials of the HPLC instrument. For vitamins, the mixture of acetonitrile/methanol (3/1, v/v) was used as the mobile phase and the elution was performed at a flow-rate of 1 mL/min. The temperature of column was kept at 40 °C. Detection was performed at 202 nm for vitamins. Identification of the individual vitamins was performed by frequent comparison with authentic external Standard mixtures analyzed under the same conditions. Quantification was carried out by external standardization using Class VP software<sup>11</sup>.

## RESULTS AND DISCUSSION

Some of the vitamins such as A, E, D<sub>3</sub> and K<sub>1</sub> are high hydrophobic whereas some are hydrophilic. Studies on hydrophobic vitamins are based on present high affinity of the selected C<sub>18</sub> group stationary phase, but in studies about other group there are difficulties thus, to promote the elution of fat-soluble vitamins from the column, even in conventional methods exploiting reversed phase chromatography, it is necessary the usage of high concentrations of methanol and acetonitrile<sup>12</sup> to increase the affinity of the vitamins by the mobile phase, becoming possible to elute them from the column. The levels of vitamin in some foods were shown in Table-1.

TABLE-1  
AMOUNT OF VITAMINS OF FOODS ( $\mu\text{g g}^{-1}$ )

Foods	$\beta$ -Carotene	Vitamin D <sub>2</sub>	Vitamin D <sub>3</sub>	$\gamma$ -Tocopherol	$\delta$ -Tocopherol	$\alpha$ -Tocopherol	$\alpha$ -Tocopherol acetate	Vitamin K <sub>1</sub>
Corn	38.4 $\pm$ 2.6	18.1 $\pm$ 1.5	642.2 $\pm$ 41.2	ND	6.15 $\pm$ 0.71	16.8 $\pm$ 1.2	25.8 $\pm$ 1.5	25.2 $\pm$ 2.6
Pepper	65.1 $\pm$ 7.2	5.2 $\pm$ 0.6	90.3 $\pm$ 5.4	ND	ND	72.8 $\pm$ 4.2	113.1 $\pm$ 13.4	23.3 $\pm$ 1.9
Sweet basil	93.4 $\pm$ 11.6	15.6 $\pm$ 1.2	129.3 $\pm$ 8.5	242.4 $\pm$ 18.4	3.25 $\pm$ 0.27	0.7 $\pm$ 0.1	9.7 $\pm$ 0.8	12.6 $\pm$ 1.3
Bean	79.2 $\pm$ 5.6	13.6 $\pm$ 1.4	42.1 $\pm$ 2.9	82.4 $\pm$ 7.6	ND	ND	3.7 $\pm$ 0.4	67.4 $\pm$ 8.4
Aubergine	144.5 $\pm$ 9.4	9.7 $\pm$ 0.8	36.4 $\pm$ 4.7	ND	ND	8.2 $\pm$ 0.7	48.7 $\pm$ 3.5	32.5 $\pm$ 2.1
Bulgur	81.9 $\pm$ 6.4	ND	38.1 $\pm$ 2.4	936.2 $\pm$ 72.4	2.62 $\pm$ 0.32	5.8 $\pm$ 0.6	42.2 $\pm$ 2.8	18.4 $\pm$ 1.6
Tarhana	116.1 $\pm$ 12.8	ND	59.1 $\pm$ 4.5	371.1 $\pm$ 26.3	1.34 $\pm$ 0.21	9.1 $\pm$ 1.4	20.8 $\pm$ 1.6	21.9 $\pm$ 1.4
Red lentil	55.7 $\pm$ 4.8	47.8 $\pm$ 3.8	100.8 $\pm$ 7.6	47.3 $\pm$ 3.6	ND	3.4 $\pm$ 0.5	10.8 $\pm$ 0.9	17.4 $\pm$ 1.5
Chickpea	46.3 $\pm$ 3.4	18.2 $\pm$ 1.4	97.2 $\pm$ 6.4	6.9 $\pm$ 0.8	0.63 $\pm$ 0.12	22.0 $\pm$ 1.3	17.6 $\pm$ 1.8	23.2 $\pm$ 3.2

ND: Not detectable, Data presented are in means  $\pm$  standard deviation (n = 3).

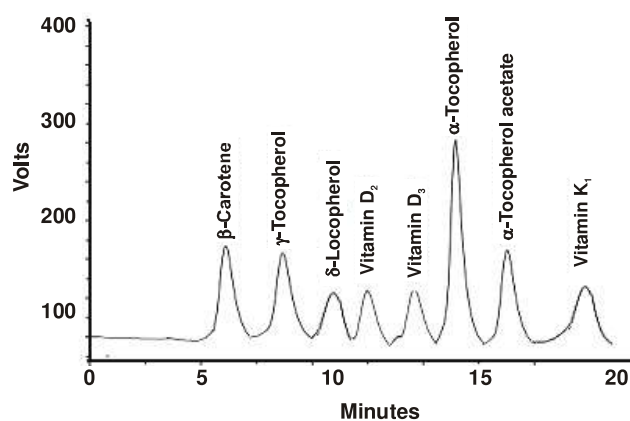


Fig. 1. Chromatography of standard vitamins

Vitamin A belongs to fat soluble group is nearly found in trace amount in nature, but its modified (*i.e.* a little structural change between) form, the substance  $\beta$ -carotene, is found naturally in plants extensively. If  $\beta$ -carotene is taken abundantly, necessity

of vitamin A has been met, too. Vitamin A is stored mostly in liver in the animal tissue<sup>13</sup>.

The mean  $\beta$ -carotene (vitamin A) content varied between 38.4 and 144.5  $\mu\text{g g}^{-1}$  dry weight. The highest and lowest levels of  $\beta$ -carotene were found in aubergine, tarhana and sweet basil. It is found in the study done in literature that level of the  $\beta$ -carotene in aubergine is 169 mg/100 g raw weights<sup>14</sup>.

All people need vitamin A to maintain strong bodies, mental sharpness, fight off disease and bear healthy children<sup>15</sup>.

Liver development also needs vitamin A because of its ability to turn off a particular liver promoting gene. The high dietary intake of vitamin A reduces the risk of some healthy problems such as cancer, cardiovascular disease and other diseases<sup>16</sup>. Vitamin A depletion is associated with low phosphoenolpyruvate carboxykinase mRNA levels during late fetal development and at birth in mice<sup>17</sup>.

Vitamin E, is mainly stored in adipose tissue, the liver and muscle, belongs to a fat-soluble class present in all cellular membranes. Vitamin E is an essential antioxidant in the living organism and protects the organism from polyunsaturated fatty acids in cell membranes from peroxidation. In fact, singlet oxygen can damage DNA and be mutagenic. Vitamin A is a singlet oxygen quencher, neutralizing these highly reactive and unstable singlet oxygen molecules. This vitamin also protects the double bonds of  $\beta$ -carotene from oxidation and thus exhibits a sparing effect. Due to the ability of vitamin E to work at higher oxygen existence, free radicals are scavenged and tissue injury is minimized. Besides its anti-aging properties, vitamin E is known to afford protection against cancer, ischemia and reperfusion injury, cataract, arthritis and certain neurological disorders<sup>18</sup>.

Vitamin E has eight different forms  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocopherols (Fig. 2) and  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  tocotrienols with varying activity and mechanism profiles relevant to cancer prevention<sup>19</sup>. The U.S. average daily intake of vitamin E is *ca.* 10 mg for men and 7 mg for women of naturally occurring or all-rac-tocopherol levels that are substantially lower than the 15 mg recommended by the Institute of Medicine Food and Nutrition Board for both men and women<sup>20</sup>. Although all forms of vitamin E are absorbed, only certain stereoisomers of  $\alpha$ -tocopherol are maintained in human plasma and tissues. Therefore, only  $\alpha$ -tocopherol currently is considered to contribute to the recommended daily allowance for vitamin E.

The contents of tocopherols in food samples were found in the ranges, 3.4-78.8, 6.9-936.2, 0.63- 6.15 and 3.7-113  $\mu\text{g g}^{-1}$  (dry matter) for  $\alpha$ ,  $\gamma$ ,  $\delta$ -tocopherols and  $\alpha$ -tocopherol acetate, respectively.  $\gamma$ -Tocopherols were found as 936.2 and 371.1  $\mu\text{g g}^{-1}$  for Bulgur and Tarhana, respectively.

The highest content of  $\delta$ -tocopherol was 6.15  $\mu\text{g g}^{-1}$  in corn, whereas the lowest  $\delta$ -tocopherol was 0.63  $\mu\text{g g}^{-1}$  in chickpea. The levels of  $\delta$ -tocopherol were not detectable in pepper, bean and aubergine.  $\alpha$ -Tocopherol levels were determined to be 72.8  $\mu\text{g g}^{-1}$  in pepper and 0.7  $\mu\text{g g}^{-1}$  in sweet basil. The higher  $\alpha$ -tocopherol acetate content was found in pepper as 113.1  $\mu\text{g g}^{-1}$ .

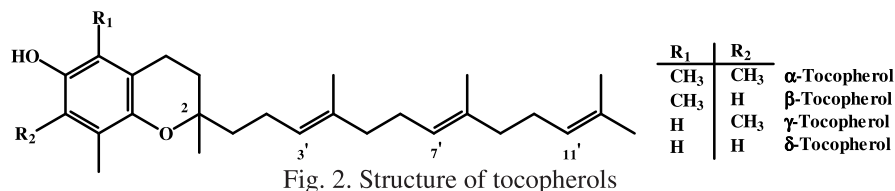


Fig. 2. Structure of tocopherols

Vitamin D belongs to a group of several related sterols, with the two most important ones being D<sub>2</sub> and D<sub>3</sub>. Vitamin D<sub>2</sub> is derived from plants and can substitute for D<sub>3</sub> in the human body. Vitamin D<sub>3</sub> is the natural form found in foods and made by the skin when exposed to sunlight. A dietary source of vitamin D is thought to be required only in animals not exposed to sunlight. Metabolism of vitamin D in ruminants has been reviewed<sup>21</sup>. The best known role of vitamin D is maintaining Ca homeostasis by increasing intestinal Ca absorption and is regulating Ca metabolism in bone. However, this view to function of vitamin D should be expanded to include regulation of numerous Ca binding proteins and Ca function in many types of living organisms and especially in much kind of cells<sup>22</sup>.

The mean vitamin D<sub>2</sub> content varied between 5.2 and 47.8 µg g<sup>-1</sup>. The highest and lowest levels of vitamin D<sub>2</sub> were found in Red lentil and pepper. It is not detectable in Bulgur and Tarhana. The contents of vitamin D<sub>3</sub> in food samples were found in the ranges 36.4-642.2 µg g<sup>-1</sup>. The higher vitamin D<sub>3</sub> content was found in corn.

Vitamin K is naturally occurring in all nine foods as naphthoquinone ring structure as menadione, but differ in the structure of the side chain at the position 3. They are traditionally classified into two groups according to whether they are synthesized by plants or bacteria. In plants the only major form of this vitamin is phyloquinone (vitamin K<sub>1</sub>), which has the same phytyl side chain as chlorophyll. Bacteria, on the other hand, synthesize a family of compounds called menaquinones (vitamin K<sub>2</sub>) with side chains based on a number of repeating prenyl units<sup>23</sup>.

The application to foods of vitamin K<sub>1</sub> is shown in the items of corn (25.2 µg g<sup>-1</sup>), pepper (23.3 µg g<sup>-1</sup>), sweet basil (12.6 µg g<sup>-1</sup>), bean (67.4 µg g<sup>-1</sup>), aubergine (32.5 µg g<sup>-1</sup>), bulgur (18.4 µg g<sup>-1</sup>), tarhana (21.9 µg g<sup>-1</sup>), red lentil (17.4 µg g<sup>-1</sup>) and chickpea (23.2 µg g<sup>-1</sup>). Bean is rich in point of vitamin K<sub>1</sub>. The reported vitamin K<sub>1</sub> values in the literature for plants were found 30, 20 and 190 µg g<sup>-1</sup> (dry matter) for corn, red pepper and bean, respectively<sup>23</sup>.

### Conclusion

The results showed that used winter season foods are rich in vitamin D<sub>3</sub>, α-tocopherol acetate and γ-tocopherol levels and include less level of vitamin D<sub>2</sub> and vitamin K<sub>1</sub>. Levels of fat soluble vitamins in sweet basil, tarhana and bulgur are high.

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