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Original Research Article

DETERMINATION OF THE LEVEL OF SOME VITAMIN IN WHEATGRASS AND GRASS

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Abstract: Various studies are performed by researchers using nutritional and physical techniques on the number of substances for health and long life, as known anti-aging or detox. One of the most striking detox or anti-aging materials are the sprouts or wheatgrass In this study it is aimed to emphasize the importance and the quantity of the certain vitamins in the green part of the wheatgrass obtained by germinating of wheat grains and grass. Washed over a clean container closed with a thin layer of cotton and wheat grains supplied water spilling allowed to remain moist to germinate. When the sprouted wheat length of 12-14 cm after germination was cut, were divided into one-gram pieces. These samples were crushed in porcelain pot and treated with in the appropriate solvent. Then, alpha and gamma tocopherol levels were measured by HPLC, and retinol and ascorbic acid were determined spectrophotometrically. The amounts of ascorbic acid, β-carotene, retinol, α-tocopherol, γ-tocopherol in analyzed samples of wheat grass; 341.63 mg/dL, 8424.81 mcg/dL, 60.45 mcg/dL, 2.15 mcg/dl, 4.00 mcg/dl, and in lawn 411.46 mg/dL, 9140.07 µg/dl, 142.86 µg/dl, 3.42 µg/dl, 5.04 µg/dl respectively. The average levels of vitamin C, α-tokoferol and γ-tokoferol was significantly important between 2 types of samples. B-carotene and retinol level differences were not significant. It was concluded that the daily requirement of antioxidant vitamins in humans the wheat grass juice has been concluded significant to meet.

Abbreviations: HPLC-_high performance liquid chromatography; WGJ-wheat grass juice; DNPH-_2,4-Dinitrophenylhydrazine.

Introduction: Wheat grasses (Poaceae) genus is common name for the annual herbaceous species of Triticum family in whole world. Wheat grass, young grass of common wheat plant, is freshly juiced or dried into powder for animal and human consumption- both the forms provide chlorophyll, amino acid, minerals, vitamins and enzymes. Wheat Grass Juice (WGJ) is an extract squeezed from the mature sprouts of wheat seeds (T. aestivum).Wheat; flour, is an essential nutrient used for production of livestock also as a feed ingredient for animal breeding. The sprouts or wheatgrass has become the new

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favorite of anti-aging research. In recent years wheatgrass had great interest as a food supplement and presented for consumption as herbal food capsules, tablets and frozen turf water [1,2].

Wheat grass has been shown to posses anti-cancer activity, anti-ulcer activity, antioxidant activity, anti-arthritic activity, and blood building activity in Thalassemia Major [2,3]. Some studies have proved the antioxidant activity of wheat grass juice, when regularly used by the cancer patients the lethal effect on cancer cells has been also found [4.5,6]. Its antioxidant potential which is derived from its high content of bioflavonoids such as apigenin, quercitin, luteoline [2]. It contains significant amount of iron, phosphorus, magnesium, manganese, copper and zinc. Wheat grass contains 20 kinds of amino acids and hundreds of different enzymes not found in other foods. Wheat sprouts grown in organic soil, it retains the natural minerals 90 of 102. Wheatgrass contains vitamins C, β- carotene and rich source of tocopherols with high vitamin E potency. Especially vitamin A content is suggested to be beneficial in the treatment of allergies and asthma [1]. The vanillic and ferulic acid contents increase during germination. It also contains chlorophyll that is responsible for the reduction of metabolic activity of carcinogens [7,8,9]. As the result of studies conducted in the United States; the content of chlorophyll, Mg and Fe in wheatgrass juice were determined useful in diseases such as anemia and cancer [10].

The importance of wheat grass is primarily due to the chlorophyll content of leaves that makes it appear green. Also the selenium and laetrile which have anti-cancer properties and strengthens the immune system are found on content of wheat grass. One of the most important features of chlorophyll is almost the same chemical structure with the oxygen-carrying red blood cells in human blood hemoglobin. When taken orally, it mixed immediately to red blood cells and cleanses the blood, improves the rate of healing of tissues. Wheat grass is consumed fresh; stimulates the metabolism, ensures healthy functioning of the thyroid gland, cleanses the lymph system, improves the heart's function, normalizes blood pressure, lowers cholesterol, internal organs especially the uterus, lung, liver, kidney and cleanses the intestines, eliminates bacteria that create bad odors in the digestive system [1,2].

Kulkarni *et al.,* [11] conducted a study to determine the mineral content of wheat grass. K, Na, Ca and concentration of minerals such as magnesium has been found to increase during the growth period. It made another study minerals that comprise the wheat grass juice; potassium, phosphorus, calcium, sulfur, magnesium, sodium, aluminum, zinc, copper, sulfur, aluminum, copper, iodine, magnesium, alkaline earth metal, selenium, Iron, boron and molybdenum [4]. especially vitamin content is suggested to be beneficial in the treatment of allergies and asthma [1].

In this study to determine and compare the amounts of ascorbic acid, β-carotene, retinol, α-tocopherol, γ-tocopherol in of wheat grass and lawn.

Materials and Methods: In the presented study about 50 g washed wheat were placed in a plastic box to obtain the wheat grass, placed on a very thin layer of sterile cotton soaked with water, then allowed at room temperature to germinate by watering intervals in an airy and bright atmosphere. The length of 10-12 cm when wheat sprouts were cut with scissors, was crushed in a porcelain mortar gram samples. Grass samples (Lolium perenne) were taken from the garden of Faculty of Veterinary Medicine. Same procedure was also performed for grass. By treatment with suitable solvents homogenizer was prepared for vitamin analysis. After centrifugation, the supernatant was removed and used for vitamin analysis.

Extraction and HPLC analysis

Vitamin E (α-tocopherol) levels of samples were determined using the extraction method stated by Miller and Yang [12]. For this purpose, 200 µl of extracted material was taken into a plastic

tube for each sample. It was added 200 µl ethanol to precipitate the protein and vortexed for 1 minute. Then 700 µl of hexane was added, homogenized again and vortexed for 1 minute. The homogenate was centrifuged at 2,000 RPM for 10 minutes and lipophilic phase was removed. Hexane application procedure was performed twice and the collected phases are dried under nitrogen gas flow. The residue was dissolved in 100 µl of ethanol and injected into the HPLC column. Vitamin E levels was determined in the HPLC separation with C18 column (25 cm x 4.6 mm) with a mobile phase in methanol-water (98: 2) and 1.5ml /min at flow rat. The diagnosis was carried out with 325 and 290 nm wavelength detector deidore [13]. Measuring the peak areas of stock solutions prepared in different concentrations of vitamin E a study graphics was drawn. the right equation of study graph was help to calculate the amounts of vitamins.

Determination of vitamin A and β-carotene: Vitamin A is a fat-soluble vitamin. Its determination is based on the dissolution in organic solvent and gives the absorbance at 325 nm. 1 ml wheatgrass extract was allowed plastic test tubes then 1 ml of ethanol and 3 ml of n-hexane were added. The tubes were mixed for 10 minutes. Afterwards the tubes were centrifuged for 10 minutes at 2000 rpm, the supernatant was removed and read against air in spectrophotometer at 325 and 453 nm. [14]. Calculations were made with the formula of Suzuki and Katoh [15].

Determination of Vitamin C: When the vitamin C extracted with perchloric acid then added DNPH, CuSO4 and thiourea mixture, a yellowish solution forms following sulfuric acid addition. It gives absorbance at 520 nm in a spectrophotometer [16].

Try building: 0.5 ml of wheatgrass extract was placed to test tube with plastic flap, Then 4.5 ml of 6%. perchloric acid solution was added. After standing for 15 minutes tubes were centrifuged for 10 minutes at 2500 rpm. Following centrifugation, 1 ml of supernatant were collected from each tube. 0.3 ml of a reaction solution (10 ml DNPH CuSO4 + 0.5 ml + 0.5 ml thiourea) was added and tubes were mixed. The tubes were incubated for 20 min at 90 °C, then allowed to cool in ice bath. After cooling 1.5 ml of 65% H_2SO_4 solution was added, tubes were vortexed and read at 520 nm against the blank.

Calculations were made with the formula of Omaye *et al.,* [17].

Vit.C (mg/dl) = (25.186 x O.D)- 0.023

Results: The results of vitamins analysis of germinated wheat germ in the laboratory and grass samples were shown in Table 1.

The amounts of vitamin C, α-Tocopherol, γ -tocopherol of wheatgrass and grass were significantly different as shown in Table 1. The average of β- carotene and retinol levels were also different but there is no statistical importance. The vitamin amounts of grass was higher than the wheatgrass extracts except retinol.

Table1: The levels of some vitamins of wheatgrass and grass

Parameters	**Wheatgrass**	**Grass**	**P**
Vitamin C (mg/dl)	341.67±35.79	411.46±38.88	0.022*
β-carotene (µg/dl)	8424.67±158.99	9140.07±183.24	0.36
Retinol (µg/dl)	238.95±10.68	142.86±21.67	0.37
α- tocopherol (µg/g)	2.15±0.24	3.42±0.31	0.034*
γ -tocopherol (µg/g)	4.00±0.16	5.04±0.23	0.007*

*P<0,05

Discussion: WGJ is commonly known as the "green blood" due to its high chlorophyll content which accounts for 70% of its chemical constituents. The presence of 70% chlorophyll, which is almost chemically identical to hemoglobin, in wheat grass makes it more useful in various clinical conditions involving hemoglobin deficiency and other chronic like

thalassemia, hemolytic anemia, cancer, asthma, allergy, inflammatory bowel disease and detoxification [18].

The content of the extraction water obtained from wheat grass is rich. In wheatgrass or sprouts a large amount of Vitamins A, B1, 2, 3, 5, 6, 8 and 12; C, E and K, ascorbic acid, dehydrated ascorbic acid, carotene, bioflavonoids, 17 amino acid, eight of which are essential, aspartic acid, threonine, asparagines, glutamine, proline, glycine, arginine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, tryptophan and serine, protease, amylase, lypase, cytochrome oxidase, transhydrogenase, superoxide dismutase enzymes are found [2].

The daily average of vitamin need is 75-90 mg and increasee during the period of rapid growth in childhood, pregnancy lactation and fever. The necessity of vitamins increase 5-10 times in the treatment of various wounds and burns. There is no storage of vitamin C so must taken daily with meals [1] reported the amount of vitamins in wheat grass as vitamin C 3.64 mg, vitamin A: 427 IU, vitamin E 15.2 IU.

The rich nutritional content of WGJ such as antioxidant vitamins (C and E) and bioflavonoids help to obtain beneficial effects on different diseases. WGJ prevents oxidative damage of deoxyribonucleic acid (DNA) and lipid peroxidation by its antioxidant activity [19].

The effects of the WGJ therapy may be due to the action of natural antioxidants on red blood cell (RBC) antioxidant function and corresponding effects on cellular enzyme function and membrane integrity. In addition to the stimulating and regenerative properties of chlorophyll, other constituents of WGJ like choline and its high mineral content are responsible for the therapeutic benefit.

Wheatgrass juice can be used for the anti-allergic actions because of its rich antioxidant and vitamin ingredients. antioxidants scavenge endogenous and/or environmental oxidant sources. Vitamin C may decrease the inflammatory effects of oxidizing compounds caused by phagocytic leucocytes vitamin C reacts with these oxidants. Antioxidant vitamins (pro-vitamins C, E and β-carotene) are considered to be potentially protective factors in the respiratory system, diabetes mellitus and some cancer types [20]. Dietary vitamin C and β -carotene have a protective effect on lung function but not on respiratory symptoms. It was shown that a higher intake of vitamin C was associated with a higher forced expiratory volume in one second [21] and vitamin C intake was not associated with most respiratory symptoms like cough, wheeze, shortness of breath. Subjects with a high intake of vitamin C had a higher forced expiratory volume in one second and higher forced vital capacity than those with a low vitamin C intake [22).

Intake of vitamin E was positively associated with lung function. Low levels of vitamin E have been associated with asthma and wheezing illness but higher intake of vitamin E was significantly associated with a lower incidence of asthma [23]. The gamma-tocopherols protect against inflammatory cell during an antigen challenge. Allergy treatment with these tocopherols also appears to block certain inflammatory immune cells from entering the airspace and tissue spaces of the lungs, nose and sinuses. WGJ reduces the IgE responses to allergic stimuli and reduces the frequency sensitization [24].

In the presented study, the vitamin levels of wheatgrass and grass was determined and obtained promising results. Wheatgrass juice is safe and the incidence of side effects is very low so during respiratory other chronic disease it can be taken safely. As conclusion wheatgrass seems to be very promising herbal drug and extensive research work is needed in order to explore its therapeutic application in various diseases. The obtained results will give confidence to clinicians for practical application.

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