International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume:02, Issue:04

CHEMICAL ANALYSIS AND NUTRITIONAL VALUES OF WHEATGRASS COMPARED TO APPLE JUICE AND SOUR CHERRY/APPLE JUICE

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ABSTARCT

Changes in consumer's lifestyle and food consumption patterns provides a great opportunity in developing functional food sector. Nowadays food is not only used to satisfy hunger and provide necessary nutrients for human but it is also used to prevent disease and improve the physical and mental well-being of consumers. Such a functional food is considered also the Wheatgrass. Comprehensive data from number of studies has revealed the multitude effects of wheatgrass in thalassemia, hemolytic anemia, cancer, asthma, allergy, inflammatory bowel disease and detoxification. However, there is a very limited number of studies on chemical screening of the wheatgrass.

The aim of this research is to do a chemical screening of wheatgrass, apple juice and sour cherry/apple juice profiles, by conducting chemical, physical and physic-chemical analysis such as pH, Total Titrable Acidity (TTA), Total Soluble Solids (TSS) oBrix, Chlorophyll A mg/l, Chlorophyll B mg/l, Total Chlorophyll mg/l, Vit C g/l, Total polyphenolic index, antioxidant activity and minerals including P, Ca, Mg, Fe, Na, Zn for three juices subject of this research and then be able to compare nutritional values among them. Based on the findings of this research we can suggest that wheatgrass may be considered as a potential raw material for fruit processing industry as a nutritional value added superfood, as an excellent source of health promoting phytonutrients such as Chlorophyll, Vitamin C, Polyphenols, Minerals and high Antioxidant activity.

Keywords: wheatgrass, super-food, novel food, functional food, chemical analysis, nutritional values.

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1. INTRODUCTION

When nowadays, there is increased consumer demand for high-antioxidant foods. Drinking high-antioxidant beverages may help to protect against aging, Alzheimer's disease, and other chronic diseases [13].

Cereal grasses (young shoots of grain-bearing plants) including alfalfa, barley grass, wheatgrass are one such type of Green foods which are very beneficial for a healthy body [6].

The Wheat Grass refers to the young grass of the common wheat plant, Triticum aestivum that is freshly juiced or dried into powder for animal and human consumption. Both provide chlorophyll, amino acids, minerals, vitamins, and enzymes. Wheat grass is a humble weed that is a powerhouse of nutrients and vitamins for the human body. In the form of fresh juice, it has high concentrations of chlorophyll, active enzymes, vitamins and other nutrients [12]

Wheatgrass is proven to have high antioxidant activity. Antioxidant activity of wheatgrass juice was compared with the standard drug ascorbic acid. From the graph of percent inhibition and IC50, it shows that wheatgrass juice is having significant antioxidant activity that is comparable to the standard drug ascorbic acid [7].

Wheatgrass juice is an integral part of the macrobiotic diet under the complementary and alternative medicine (CAM) approach of anticancer therapy, due to its high antioxidant content [14].

Comprehensive data from number of studies has revealed the multitude effects of wheatgrass in thalassemia, hemolytic anemia, cancer, asthma, allergy, inflammatory bowel disease and detoxification. The structural homology of chlorophyll with hemoglobin indicates the role of chlorophyll as a blood builder in various clinical conditions involving hemoglobin deficiency – thus the name "green blood". To conclude, the wheatgrass seems to be a very promising herbal drug [15].

The pH (hydrogen molecules) value of both human blood and wheatgrass is about 7 (alkaline) and is therefore quickly absorbed in the blood and is highly beneficial [12].

There is growing evidence that dietary chlorophyll may have cancer preventing properties by limiting the bio-availability of carcinogens, and wheatgrass juice appears to be a good source of this phyto pigment.

Wheatgrass has almost all the vitamins, amino acids, antioxidants, minerals and useful trace elements, due to the occurrence of these components wheatgrass is highly nutritive and has shown advantageous effects in many diseases such as cancer, diabetes, ulcer, rheumatoid

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arthritis, hyperlipidemia, thalassemia, anemia, kidney stone, digestive problems skin diseases, asthma etc. Due to the high amount of chlorophyll it is highly oxygenated and improves the function of heart and lungs. It also helps with the appropriate function of organs. Wheatgrass contains high amount of both enzymatic and non-enzymatic antioxidants and show higher antioxidant activity than other fruits and vegetables, and there is increased levels of oxidative stress is noticed in chronic diseases, so wheat-grass can be used as an herbal antioxidant supplement to treat various chronic diseases in future [17].

The aim of this research is to do a chemical screening of wheatgrass, apple juice and sour cherry/apple juice profiles, by conducting chemical, physical and physic-chemical analysis such as pH, Total Titrable Acidity (TTA), Total Soluble Solids (TSS) oBrix, Chlorophyll A mg/l, Chlorophyll B mg/l, Total Chlorophyll mg/l, Vit C g/l, Total polyphenolic index, antioxidant activity and minerals including P, Ca, Mg, Fe, Na, Zn for three juices subject of this research and then be able to compare nutritional values among them.

2. MATERIAL AND METHODS

2.1. Materials

Green juice extracted from Wheatgrass and commercial pasteurized 100% apple, sour cherry/apple juice, (produced by a local producer in Gjilan Kosovo) were used in this research.

2.1.1 Juice extraction

Experimental land plots were planted with wheatgrass to be harvested on their so called jointing stage that means after the second leaf has grown as the half of the first leaf. Than the grasses were cleaned and immediately juiced and blast frozen; then kept at - 30˚C prior to dispatch.

The juicing has been conducted by Fruit, Vegetable and Wheatgrass juicer, Omega 8224 Nutrition Center Juicer, which is a masticating style juice extractor. Its ability to juice at low speeds – 80 RPM minimizes heat build-up and oxidation.

2.2 Methods

2.2.1 Determination of pH

- was done with a Uniscope pH meter previously standardized Referring to the AOAC Method 981.12 on pH of acidified food [2]

2.2.2 Total soluble solids (TSS) as oBrix (by using ISO Refractometer Method) [9]

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2.2.3 Titrable acidity, according to AOAC official Method 942.15 and A.O.A.C. official Method 920.149 [1] [3].

2.2.4 Chlorophyll analysis.

Chlorophyll content was determined using the procedure by Arnon (1949) [5]. 0.3ml of extract was removed from each beaker using a pipette and placed into labelled balloons, using digital scales to obtain an exact weight. 80% acetone was then added to make each balloon up to a volume of 10ml of solution. Than the material was transferred by individual pipettes from the balloons to individually labelled Fisherbrand ®15ml centrifuge tubes.

The tubes were then balanced and spun in an MSE Centrifuge, at 2000 rpm for 5 minutes. The supernatant was then carefully removed from each tube using a pipette and placed into a 1ml glass spectrophotometer cuvette. Zero was set on a Unicam UV/Vis Spectrophotometer using a blank cuvette containing 80% acetone and readings were taken for each sample at 645nm (Chlorophyll b) and 663nm (Chlorophyll a). Readings were recorded and using the equations from Arnon (1949), the fresh weight of total chlorophyll from each sample was determined as mg/100g. [20].

2.2.5 Determination of ascorbic acid (Vitamin C) content by titration - (Redox Titration Using Iodine Solution).

Determination of vitamin C. In order to determine the C vitamin content, it was used the adapted iodometric method. The method principle is color reaction between starch and KI + I solution. Determination of C vitamin is made with the aid of K2Cr2O7 in the KIstarch presence. Initially, C vitamin is oxidized and then the following reaction takes place:

Cr2O72- +6I- +14H+ = 3I2 +2Cr3+ + 7H2O

Thus liberated iodine stains starch blue. Was weighed a sample on analytical balance, then was brought quantitatively into a titration vessel, were added 10 ml of 2n hydrochloric acid, diluted to 50 ml with distilled water, triturated, added 1 ml of 1% starch solution (freshly prepared) and 1 ml 0.1 N potassium iodide, after which the solution was titrated with 0.1N potassium dichromate until persistent blue color. Quantification of vitamin C content was done according to the relation: 1 ml 0.1 N potassium dichromate is equivalent to 0.008806 g vitamin. All determinations were performed in triplicate, calculating their arithmetic mean [8].

2.2.6 Antioxidant activity of wheatgrass and fruit juices by DPPH free radical scavenging assay.

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The radical scavenging ability of fruit juices was tested on the basis of the radical scavenging effect on the DPPH free radical. The fruit juices (12.5 µL to 100 µL/mL) were prepared in methanol. In clean and labeled test tubes, 2 mL of DPPH solution (0.002% in methanol) was mixed with 2 mL of different concentrations of fruit juices separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517 nm using UV-Vis Spectrophotometer. The absorbance of the DPPH control was also noted. The scavenging activity of the juices was calculated using the formula:

Scavenging activity (%) = [(A – B) / A] x 100, where A is absorbance of DPPH and B is absorbance of DPPH and fruit juice combination [11].

2.2.7 Total phenol content

Was determined by Total Polyphenolics Index (TPI) method. This method uses the maximum of the absorbance in wave length 280 nm, as characteristic of benzoic cycle present in all polyphenols. The samples are diluted with distillated water with dilution scale 1:100. In spectrophotometer is registered specter between 200-350nm. Measurement of the optical density is done at 280nm. [17].

Calculation was done using the formula:

TPI (280) =Abs280 x dilution

2.2.8 Minerals

Wheatgrass and fruit juices was analyzed for Minerals including: Fe, Na, Zn, P, Ca. M.

P, Ca, M was analyzed in accredited laboratory of Institute of Public Health, Nis, Serbia. The methods used for this purpose was SRPS ISO 13730, 1999 for P and SRPS EN 1134 2005 for Ca and M. [18] [19]

Samples were analyzed also for Fe, Na, and Zn content. Those analyses took place in accredited laboratory at Institute of Public Health in Prishtina. The method used for this assay was A.O.A.C. official method 999.11 [4]

2.2.9 Statistical Analysis

For every single analyzed parameter were conducted triple tests and the mean of three results was presented as final result. Using Microsoft Excel was calculated Standard Deviation for every value of result. Correlation analyses for all parameters described above were conducted with

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SPPS. Some descriptive statistics are undertaken and Pearson Correlations in order to asses if there is a relationship among the considered parameters.

3. RESULTS AND DISCUSSION

3.1 pH, TTS, TTA, Acidity % and Sugar/acid ratio

Results for pH, TTS, TTA, Acidity % and Sugar/acid ratio for three samples involved in this research are shown in the following table. For each parameter there where done triple tests and the arithmetic average is taken as final result. Standard deviation was calculated by Microsoft excel 2010 and the SD values was not significant, therefore are not taken into consideration.

Table 1. Values of pH, TTS, TTA, Acidity % and Sugar/acid ratio for three samples. (a)

Sample code/ analysis	*Sample formulation*	*pH*	*Stdev*	*TSS oBrix*
1	*Wheatgrass juice 100%*	6.43	0.15	3.5
2	*Sour cherry 35%/apple juice 65%*	3.42	0.09	16.6
3	*Apple juice 100%*	3.60	0.1	13.53

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(b)

Stdev	TTA g/l malic acid	Stdev	Acidity %	Sugar/acid ratio (oBrix /%acid)
0.1	2.34	0.09	0.23	15.2
0.1	9.29	0.32	0.92	17.86
0.06	4.35	0.17	0.43	31.07

3.2 Chlorophyll analysis

Chlorophyll content was determined for first sample- wheatgrass, using the formula by Arnon (1949) as fresh weight in mg/l. Mean weights of three measurements at 645nm for Chlorophyll A and respectively at 663 nm for Chlorophyll B are presented in Table 2.

Table 2. The content of chlorophyll in wheatgrass

Sample	Chlorophyll A mg/l	Chlorophyll B mg/l	Total Chlorophyll mg/l
1 (Wheatgrass)	4.74545	2.596513333	7.33998

3.3 The results for Ascorbic acid (Vit C)

The results for Ascorbic acid (Vit C) content by redox iodine titration method, for three samples along with Anti-oxidation activity and Total Polyphenolic Index –TPI, are presented by the following chart:

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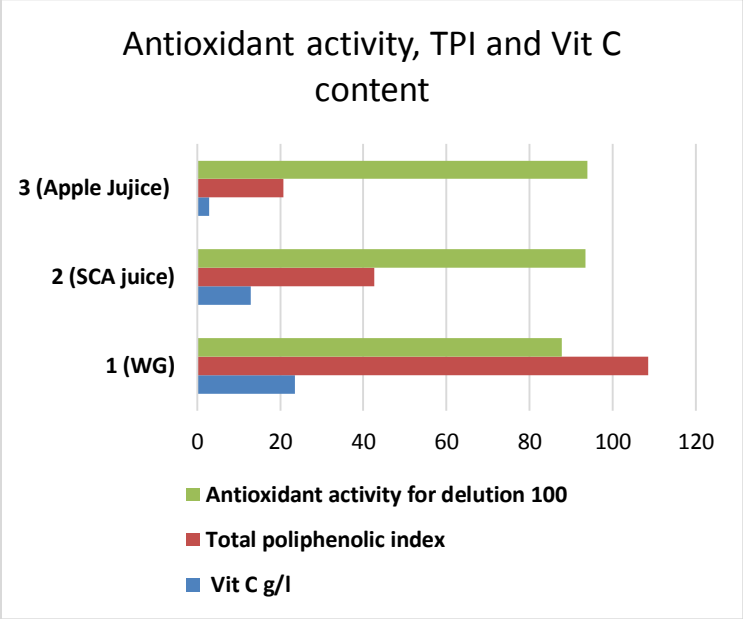

Figure 1. Concentration of Ascorbic Acid, Antioxidant activity and TPI

3.4 Minerals

Concentration of the minerals given in mg/l are presented in two charts, due to the huge differences on the results, and to enable to properly compare them.

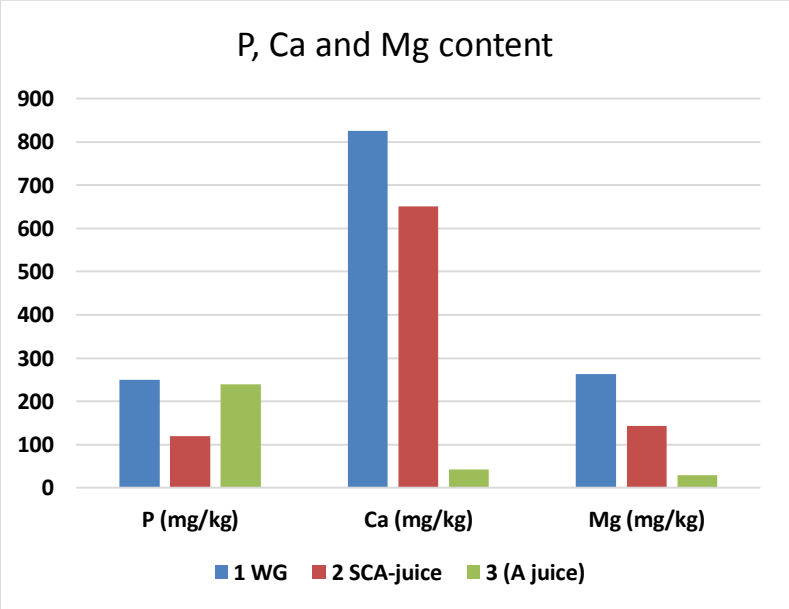

Figure 2. The content of P, Ca and Mg at sample 1 (WG), 2 (SCA) and 3 (A)

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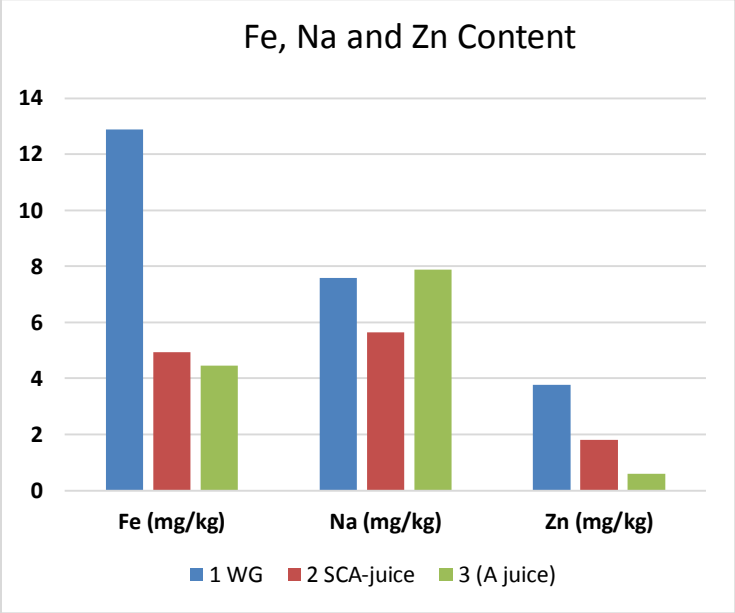

Figure 3. The content of Fe, Na and Zn at sample 1 (WG), 2 (SCA) and 3 (A)

3.5 Statistical Analysis

For every single analyzed parameter triple tests was conducted and the results of pair comparisons did not show any statistical difference ($p<0.05$). The mean of three results was presented as final result.

4. CONCLUSIONS

The present study showed that:

- Wheatgrass has a low acidity values and slightly acidic to neutral pH values.
- Wheatgrass is rich with chlorophyll with total chlorophyll of 7.34 mg/l, from which 4.74 mg/l as Chlorophyll A and 2.59 mg/l as Chlorophyll B. Therefore it is considered as an ingredient of interest when planning production of functional foods
- Wheatgrass is very rich in Ascorbic acid (vit C). Vitamin C concentration on wheatgrass is 23.55mg/l, which if compared to sour Chery/apple juice (sample 2) is 1.83 times higher in wheatgrass and to apple juice (sample 3) is 8.3 times higher in wheatgrass.
- The Index of Total Phenolic was the highest for wheatgrass, with the value of 108.54, while for the sour cherry/apple juice it was 42.69 and for the apple juice 20.80.

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- All sudden results were gained for antioxidant activity. Although with not significant differences, the higher antioxidant activity was found for sample no. 3 apple juice, with value of 93.97, while the second ranged is sample 2 sour cherry/apple juice with 93.45 and the last is wheatgrass with 87.74. It was unexpected considering the fact that wheatgrass was much richer with Vitamin C, Total phenolic compounds and chlorophyll. This conclusion is a sign for importance of fruit juices in human diet and their high content of antioxidants, what can be considered as a need for further detailed analyses in the future researches.

- Relating to minerals, Wheatgrass resulted to be the richest sample with all minerals, subject of this research. It is richer with Phosphor for 208% than Sour cherry/apple juice and 104% richer than apple juice. The Phosphor content is 250 mg/kg. Calcium content was 825.1 mg/kg and that is 126% higher at wheatgrass than at sour cherry/apple juice and 1955% than at apple juice. Magnesium was found to be present in wheatgrass 262.7 mg/kg, which is 183% higher than at sour Chery/apple juice and 884% higher than at apple juice. While iron is for 260 % higher in wheatgrass (with 12.89 mg/kg) than in Sour cherry/apple juice and for 290% higher than Iron concentration at apple juice. The value of 7.59 mg/kg of Natrium found in wheatgrass is 134% higher than the same values found at sample no 2 and 96% higher than at apple juice. Zinc was present in wheatgrass with 3.76 mg/kg and expressed in % it is 207% higher than at sour cherry/apple juice and 643 % higher than at apple juice. Thus can be concluded that Wheatgrass is an excellent source of P, Ca, Mg, Fe, Na, and Zn.

- Based on the abovementioned findings of this research we can conclude that wheatgrass may be considered as a potential raw material for fruit processing industry as a nutritional value added super food, as an excellent source of health promoting phytonutrients such as Chlorophyll, Vitamin C, Polyphenols, and Minerals.

5. ACKNOWLEDGEMENTS

1. National Institute of Public Health of Kosovo for analyses of Fe, Na and Zn.

2. Secondary school for Agribusiness and technology “Arberia” Gjilan, Kosovo for planting wheatgrass in their experimental parcel.

3. Center of Research Laboratory of Faculty of Biotechnology and Food, Agricultural University of Tirana for enabling to perform the chemical analyses on their laboratory premises.

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