Analysis of the Storage Stability of the Wheatgrass (*Triticum Aestivum* L.) Juice in Terms of Antioxidant Activity and Their Polyphenols Content

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A broad spectrum of of health benefits have been attributed to wheatgrass. The fresh juice has high chlorophyll content, essential vitamins (A, B, C, E and K), minerals (iron, calcium, and magnesium), enzymes, amino acids, phenolic compounds (ferulic acid and vanillic acid), and dietary fibers [1].The objective of this work was to evaluate the stability of the wheatgrass juice through the total polyphenol content (mg, gallic acid equivalents, GAE/g, determined according to the Folin-Ciocalteau method) and the antioxidant activity (mg, Trolox equivalent antioxidant capacity, TEAC/g determined by the 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay). The sample was divided into four subsamples of which one (E1) was analyzed immediately and the others were kept at -20^{0}C until analysis 11, 21 and 32 days, respectively. The stability assessment was carried out by the difference between the subsample averages, analyzed at the set time, and the E1 subsample average. Through statistical method for analysis of variance ANOVA, the standard deviation between subsamples and the repeatability standard deviation were determined on the basis of which was established the detection limit of the stability test. The limit of detection of instability for total phenolic content (TPC) was 0.08 mg GAE/g and of antioxidant activity (AA) was 0.11 mg TEAC/g. Initial properties of the sample are: TPC = 2.15 mg GAE /g and AA = 1.17 mg TEAC /g. The samples stored at -20 ^{0}C for 32 days shows instability towards initial properties.

Keywords: wheatgrass juice; total polyphenol content; Trolox equivalent antioxidant capacity, stability

Triticum aestivum L. is belonging to the family Poaceae, subfamily Pooideae and tribe Triticeae is one of the most important crops in the world and in Romania too. It has the recorded synonyms *Triticum vulgare, Triticum aestivum* subsp. *Aestivum* [2]. After germination the vegetative shoot apex initiates additional leaf primordia. The number of leaf primordia can vary from 7 to15 and is affected by genotype, temperature, light intensity and nutritional status of the plant. Temperature has a major influence on leaf appearance and extension. The minimum temperature for leaf extension is approximately 0^{0}C, the optimum 28^{0}C and the maximum greater then 38^{0}C [3]. Wheatgrass juice is a dietary supplement derived from common wheat which is produced by juicing the young shoots. The supplement is available commercially in liquid, powdered or concentrated forms, depending on the supplier and can be consumed on its own, or mixed with fruit juices [4].

Natural products are valuable source of bioactive compounds and a suitable origin for new drugs development. The name “green blood” of wheatgrass is attributable to its high chlorophyll content which accounts for 70% of its total chemical constituents [5]. Wheat grass juice is the richest source of vitamins A, B, C, E and K, calcium, potassium, iron, magnesium, sodium, sulphur and 17 forms of amino acids [6]. The various enzymes responsible for its pharmacological actions are protease, amylase, lipase, cytochrome oxidase, transhydrogenase, super oxide dismutase. The major clinical utility of wheatgrass juice is due to its antioxidant action which is derived from its high content of bioflavonoids like apigenin, quercitin and luteolin. Other compounds present, which make this grass therapeutically effective, is the indole compounds, apigenin and laetrile [7].

Different studies have shown that juicing wheatgrass is very beneficial for his biological activity: antioxidant activity [8, 9], detoxifying activity [10], anti-ulcer activity [11], apoptitic activity [12].

Despite the fact that comprehensive information on cultivation, breeding, and the physiology of the *Triticum aestivum* L. exist, the antioxidant properties have not been investigated to the same amplitude. Thus, the objective of this study was to evaluate the stability of the wheatgrass juice through the total polyphenol content (mg, gallic acid equivalents, GAE/g, determined according to the Folin-Ciocalteau method) and the antioxidant activity (mg, Trolox equivalent antioxidant capacity, TEAC/g determined by the 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay). Present study was conducted for storage temperature of -20 ^{0}C to provide information about the stability of wheatgrass juice during storage for a short period. The stability study consists of a series of measurements performed at different times. The results of our work can be useful for small manufacturers of wheatgrass juice since the literature’s studies about its stability are limited.

Experimental part

Instrument and Reagents

The Specord M40 spectrophotometer was used to measure the absorbance (DPPH, Folin-Ciocalteu

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assay). 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 6-Hydroxy-2,5,7,8- tetramethychroman-2- carboxylic (Trolox), Gallic acid and Folin-Ciocalteu, were purchased from Sigma-Aldrich. Stock solution of each standard was prepared in ethanol. All working standard solutions were freshly prepared prior to use. All reagents used in the experiments were of analytical grade.

Growing of the grass and preparation of grass juice

The seeds of wheat (*Triticum aestivum* L.) were procured and washed with tap water, followed by distilled water. The seeds were soaked in distilled water. Only wheatgrass of uniform size and shape was selected. The plants were washed and cut into small pieces. The wheatgrass juice was obtained by cold pressing procedure. The extracts were stored at -20 °C until further use.

Stability study

The stability of the wheatgrass juice was tested by measuring of the following properties: total phenolic contents and antioxidant activity. Samples were kept at -20^{0}C, over a period of 32 days. Tests were made at the beginning of the storage period and after 11, 21 and 32 days.

Determination of total phenolics

The Folin-Ciocalteu assay with some modification was applied to determine the TPC of the samples [13]. The linear regression equation was calculated and a good linear relationship was obtained between the standard concentrations (20-100 mg L^{-1}), and the absorbance at 750 nm of the reaction solution. The sample solution was tested following the procedure by the gallic acid solutions. Total phenolic contents of wheatgrass juice were expressed as the gallic acid equivalent from the calibration curve.

Determination of the antioxidant activity

The scavenging effect of the wheatgrass juice on a DPPH radical was determined as described [14] with some modification. Thus, the scavenging ratio of the sample and Trolox on DPPH was tested, and then a suitable concentration range of the Trolox and its scavenging percentage was found, a linear regression equation between the Trolox concentration and its scavenging percentage was find, and the Trolox equivalent antioxidant capacity (TEAC) was calculated through the equation, according to the scavenging percentage of the sample solution to the DPPH radical solution. Trolox solution of 1 mL was mixed with 2 mL of 70% ethanol solvent, 1mL Trolox solution was mixed with 2 mL DPPH, and 2 mL DPPH solution with 1mL of 70% ethanol solvent, to make three different reaction systems. The three different mixtures were put under 40°C for one hour without illumination, and then their absorbance at 517 nm were read by the Specord M40.

$$AA,\% = \frac{A_2 - (A_1 - A_0)}{A_2} \cdot 100 \quad (1)$$

For convenience, they were represented as A_0, A_1, A_2, so the scavenging effect of Trolox on the DPPH radical was calculated with the formula 1.

Results and discussions

Total phenolic content of the samples

The total phenolics content of wheatgrass juice are presented in figure 1. The amount of TP was calculated as the gallic acid equivalent from the calibration curve: y = 0.0046x - 0.0240, R^2 = 0.9987. It was found that the samples analyzed in term of short stability kept 83.4% TPC from initially determined values.

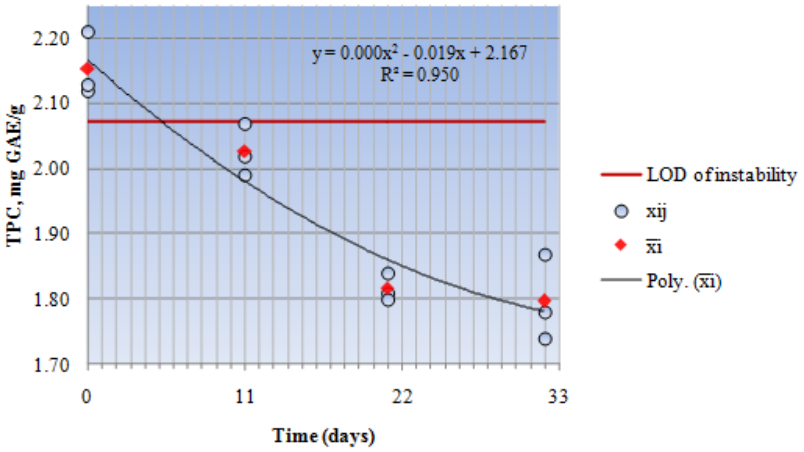

Fig. 1. Wheatgrass (*Triticum Aestivum L.*) juice – Total polyphenol content results of the short term stability at -20 ^{0}C (n=12; a=4)

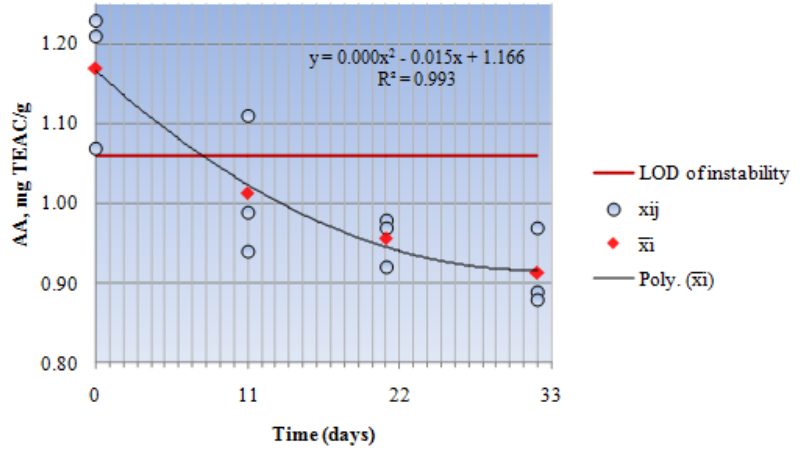

Fig. 2. Wheatgrass (*Triticum Aestivum L.*) juice – Antioxidant activity results of the short term stability at -20 ^{0}C (n=12; a=4)

Table 1

SUMMARY OF SUMS OF SQUARES AND DEGREES OF FREEDOM

Source of variation	Sum of squares	Degrees of freedom
Between-sample	$n\sum_i (\bar{x}_i - \bar{x})^2$	a-1
Within-sample	$\sum_i \sum_j (x_{ij} - \bar{x}_i)^2$	a(n-1)

n = the total number of observations, i vary from 1 to n; a = the total number of populations/samples, j varies from 1 to k; $\bar{x}_i$ = the sample mean for a group; grand mean $\bar{x}$ is the mean of all observations.

MSE = SSE/df; F = $MSE_{Between}/MSE_{Within}$

Table 2

STATISTICAL CRITERIA FOR PERFORMANCE EVALUATION OF THE STABILITY TEST (ANOVA) – TPC

Sums of Squares (mg GAE/g)		Degree of freedom		Mean Square Errors (mg GAE/g)		Test criterion	Critical value (95 %)
$SS_{Between}$	0.266	$df_{between}$	3	$MSE_{Between}$	0.0885	39.63	4.07
SS_{Within}	0.018	df_{within}	8	MSE_{Within}	0.0022		
$s_{meas.}$ (mg GAE/g)							0.04
LOD of instability (mg GAE/g), P = 95 %, k=2							0.08

Table 3

STATISTICAL CRITERIA FOR PERFORMANCE EVALUATION OF THE STABILITY TEST (ANOVA) - AA

Sums of Squares (mg TEAC/g)		Degree of freedom		Mean Square Errors (mg TEAC/g)		Test criterion	Critical value (95 %)
$SS_{Between}$	0.113	$df_{between}$	3	$MSE_{Between}$	0.0378	8.08	4.07
SS_{Within}	0.037	df_{within}	8	MSE_{Within}	0.0047		
$s_{meas.}$ (mg TEAC/g)							0.06
LOD of instability (mg TEAC/g) P = 95 %, k=2							0.11

Determination of the antioxidant activity

A linear regression equation was calculated with the Trolox solution concentrations as the independent variable (X) and the percentage of scavenging effect on the DPPH radical as the dependent variable (Y). A good liner relationship could be found between the Trolox concentrations (0.006-0.05 mg mL^{-1}) and the scavenging percentage on the DPPH radical. The AA was calculated as the TEAC equivalent from the calibration curve: y = 1709x + 10.84, R^2 = 0.992. It can be seen from figure 2 that the wheatgrass juice has maintained only 78.1% AA from initially determined values.

Statistical analysis

Data were statistically analyzed using Microsoft Excel software. Significant differences between groups were determined using one way ANOVA. The relationship between the sources of variation is presented in table 1, which outline the sums of squares and degrees of freedom. The repeatability standard deviations (s_r), mean squares between groups $MS_{between}$, mean squares within groups MS_{within} were calculated (table 2 and table 3). On the basis on MS_{within} [15, 16] were calculated $s_{measured}$ and limit of detection (LOD) of instability.

Conclusions

From our knowledge, this study represents the first report regarding short stability of the wheatgrass (Triticum Aestivum L.) juice in terms of TPC and AA. It has confirmed that wheatgrass represents an important source of polyphenols, thus the initial properties of the sample are: TPC = 2.15 mg GAE /g and AA = 1.17 mg TEAC /g. The results also indicate that the samples stored at -20 ^{0}C for 32 days shows instability towards initial properties. In addition, a significant correlation exists between the contents of TP and the DPPH radical scavenging ability. Through statistical method for analysis of variance ANOVA [15], the standard deviation between subsamples and the repeatability standard deviation were determined on the basis of which was established the detection limit of the stability test. The instability limit of detection of total phenolic content (TPC) was 0.08 mg GAE/g and of

antioxidant activity (AA) was 0.11 mg TEAC/g. This study indicates that wheatgrass (Triticum Aestivum L.) juice has a good short stability and high application potential based on its high TPC as well as a strong antioxidant activity.

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