

## Production of a pasteurized functional drink containing wheatgrass and apple juice

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### Abstract

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The novelty in this investigation is the development of a new product, production of a pasteurized functional drink containing wheatgrass. The aim of this research was to develop a pasteurized functional drink containing wheatgrass and apple juice and to give some concrete data on the changes of nutritional values of the fruit juice as well as after adding wheatgrass in its recipes. It also aims to determine the most appropriate pasteurization scheme that ensures the food safety of the product and that minimally denaturizes the valuable health promoting phytochemicals. The raw materials and the produced juices have undergone quantitative chemical analyses for Chlorophyll, Vitamin C, total polyphenols, antioxidant activity, and minerals (P, Ca, Mg, Fe, Na, and Zn). Microbiological analyses for the presence of pathogens: *E. coli*, *Salmonella* and *L. monocytogenes* were conducted as well. Also, through this research, the identification of functional ingredients of wheatgrass is done; which is added to fruit juice thereby producing a stable functional beverage, which is safe in terms of food safety and that enriches the nutritional value of fruit juice.

*Keywords*: functional beverage; novel food; nutritional; wheatgrass

### Introduction

Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans, but also to prevent nutrition-related diseases and improve the physical and mental well-being of the consumers (Siró et al., 2008b). Consumers more and more believe that foods contributes directly to their health (Mollet & Rowland, 2002). Changes in consumers' lifestyles and food consumption patterns provide

a great opportunity in developing the functional food sector. While there is still no universal accepted definition for functional food, the industry is growing steadily worldwide (Rezai et al., 2012). The increasing demand on such foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for improved quality of their later years (Kotilainen et al., 2006). The term “functional food” itself was first used in Japan in the 1980s, for food products fortified with special

constituents that possess advantageous physiological effects (Hardy, 2000; Kwak & Jukes, 2001; Siró et al., 2008a; Stanton et al., 2005). The concept of functional food was first promoted in 1984 by Japanese scientists who studied the relationships between nutrition, sensory satisfaction, fortification and modulation of physiological systems (Burdock et al., 2006; Roberfroid, 2000). Typically, a food marketed as functional contains added technologically developed ingredients with a specific health benefit (Niva, 2007). Although the term “functional food“ has already been defined several times (Roberfroid, 2000), so far there is no unitary accepted definition for this group of food (Alzamora et al., 2005).

The European Commission’s Concerted Action on Functional Food Science in Europe (FuFoSE), coordinated by International Life Science Institute (ILSI) Europe defined functional food as follows: “a food product can only be considered functional if together with the basic nutritional impact it has beneficial effects on one or more functions of the human organism, thus either improving the general and physical conditions or/and decreasing the risk of the evolution of diseases. The amount of intake and form of the functional food should be as it is normally expected for dietary purposes. Therefore, it could not be in the form of a pill or capsule, just as normal food form” (“Scientific Concepts of Functional Foods in Europe Consensus Document,” 1999). On the contrary to this latter statement, since 2001 FOSHU products in Japan can also take the form of capsules and tablets, although a great majority of products are still in more conventional forms (Ohama et al., 2006). Novel Food is defined as food that has not been consumed to a significant degree by humans in the EU prior to 1997, when the first Regulation on novel food came into force. ‘Novel Food’ can be newly developed, innovative food, or food produced using new technologies and production processes as well as food traditionally eaten outside of the EU (European Union, 2015). Such a new technology, is also using nanotechnology in novel food /functional beverages production (Wang et al., 2016).

Most early developments of functional foods were those fortified with vitamins and/or minerals such as vitamin C, vitamin E, folic acid, zinc, iron, and calcium (Sloan A.E., 2018). Subsequently, the focus shifted to foods fortified with various micronutrients such as omega-3 fatty acid, phytosterol, and soluble fiber to promote good health or to prevent diseases such as cancers (Sloan A.E., 2004); production of non-dairy fermented probiotic functional beverages (Chavan et al., 2018) etc. More recently, food companies have taken further steps to develop food products that offer multiple health benefits in a single food (Sloan A.E., 2004). Functional food products are not homogeneously scattered over

all segments of the food and drink market and consumer’s health concerns and product preferences may vary between markets. These products have been mainly launched in the dairy, confectionery, soft-drinks, bakery and baby-food market (Kotilainen et al., 2006; Menrad, 2003; Siró et al., 2008a).

One of the important product categories within the functional food segment is non-alcoholic beverages fortified with vitamins A, C and E or other functional ingredients. Other types of functional drinks are those of cholesterol lowering drinks (with combination of omega-3 and soy), “eye health“ drinks (with lutein) or “bone health“ drinks (with calcium and inulin) (Siró et al., 2008a). Several mid- and long-term developments in society, as well as socio-demographic trends are in favor of functional food, so that it can be assumed that functional food represents a sustainable category in the food market (Bech-Larsen & Scholderer, 2007). This increasing consumer’s awareness in combination with advances in various scientific domains, provides companies with unique opportunities to develop an almost infinite array of new functional food concepts (Biström & Nordström, 2002).

It should also be considered, that functional foods are sold at higher prices, thus containing larger profit margins than conventional foods, which obviously make the sector attractive for the players in the supply chain (Kotilainen et al., 2006). Against the above-mentioned advantages, the development and commerce of these products is rather complex, expensive and risky as special requirements should be answered (Kleef et al., 2005). This development and marketing requires significant research efforts. This involves identifying functional compounds and assessing their physiological effects; developing a suitable food matrix, taking into account bio-availability and potential changes while processing and food preparation, consumer education, and clinical trials on product efficacy in order to gain approval for health-enhancing marketing claims (Kotilainen et al., 2006). It is a multistage process that requires input from commercial, academic and regulatory interests with a critical need to achieve acceptance by the consumers (Jones & Jew, 2007). Wheatgrass is considered to be such a functional food.

Comprehensive data from a number of studies have revealed the multitude effects of wheatgrass in thalassemia, hemolytic anemia, cancer, asthma, allergy, inflammatory bowel disease and detoxification (Padalia et al., 2010). However, there are a very limited number of studies on the chemical screening of wheatgrass. 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 (Mujoriya & Bodla, 2011). 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 (Padalia et al., 2010).

Tandon and others in their research on Antioxidant Profiling of wheatgrass and its Antiproliferative Activity in MCF-7 Breast Cancer Cell provides preliminary data about the anticancer activity of wheatgrass extract and its potential to be considered as an anticancer agent (Tandon, 2011). Antioxidants are chemical compounds that can bind to free oxygen radicals thus preventing these radicals from damaging healthy cells, which could lead to cancer. The young grass of the common wheat plant, *Triticum aestivum* is known as wheatgrass, family *Poaceae*. Wheatgrass is known to be a rich source of vitamins, antioxidants and minerals. It also contains Vitamin A, B1, C and E, many minerals and trace elements including calcium, iodine selenium and zinc. Wheatgrass is known to contain antioxidant enzymes Superoxide dismutase (SOD) and cytochrome oxidase that have the potential to convert Reactive oxygen species (ROS) to a hydrogen peroxide and an oxygen molecule (Padalia et al., 2010). Chlorophyll, one of the primary components in the wheatgrass extract, was found to augment blood formation and strengthen the immune system through inhibition of metabolic activation of carcinogens (C. Lai et al., 1978; C.-N. Lai, 1979).

It also possesses the ability to inhibit oxidative DNA damage (Falcioni et al., 2002). 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 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 the heart and lungs. It also helps with the appropriate function of organs. Wheatgrass contains a high amount of both enzymatic and non-enzymatic antioxidants. It shows higher antioxidant activity than other fruits and vegetables, and the increased levels of oxidative stress are noticed in chronic diseases, so wheat-grass can be used as an herbal antioxidant supplement to treat various chronic diseases in the future (N. Singh et al., 2012).

On the other hand epidemiological studies link the consumption of apples with a reduced risk of developing some chronic diseases (Boyer & Liu, 2004a; Wolfe et al., 2003) such as cancers (Feskanich et al., 2000; Gerhauser, 2008; Le Marchand et al., 2000), cardiovascular disease (P. Knekt et al., 1996; Sesso et al., 2003), asthma (Shaheen et al., 2001; Woods et al., 2003) and diabetes (Paul Knekt et al., 2002). Most of these health benefits of apples are associated with

their high content in phenolic compounds (especially flavonoids), vitamin C, antioxidants and dietary fiber (Boyer & Liu, 2004b). The concentration of these diverse phytochemicals may vary depending on several factors such as the apple cultivar, climate, agronomic, harvest and storage conditions, and of course processing practices (Boyer & Liu, 2004a; Leja et al., 2003a; Matthes & Schmitz-Eiberger, 2009; van der Sluis et al., 2001).

According to sensory characteristics and consumer's acceptance of wheatgrass in formulation with 6 different 100% fruit juices (apple juice, wild apple (*Malus sylvestris*) juice, Strawberry and Apple (30:70) Juice, Sour cherry and apple juice (30:70), peach and apple juice (30:70), apricot and apple juice (30:70), among the most preferable combinations was the formulation wheatgrass and apple juice (30:70) (Hasani et al., 2018). There is no similar study encountered for the enrichment of nutritional values of pasteurized fruit juices by adding wheatgrass in the formulation and making it a life-long functional drink easily available and more convenient for the consumers.

The aim of this research was to: 1) study the development of a potential new product, a functional drink with a base of apple juice, enriched with wheatgrass to give some concrete data on the changes of nutritional values of the fruit juice after the introduction of the wheatgrass in its recipes and 2) to determine which is the most appropriate pasteurization scheme that ensures the food safety of the product and that minimally denaturizes the valuable health promoting phytochemicals. Also, through this research the identification of the functional ingredients of wheatgrass is done, which is added to fruit juice thereby producing a stable functional beverage, which is safe in terms of food safety and that enriches the nutritional value of fruit juice.

## Material and Methods

### *Plant material and experiment design*

Green juice extracted from wheatgrass (sample 1), 100% apple (*Malus domestica*) juice (sample 2) and their formulations of 30% wheatgrass and 70% apple juice pasteurized in 85 °C for 15 seconds (sample 3), and 30% wheatgrass and 70% apple juice pasteurized in 71.7°C for 15 seconds (sample 4) were used in this research study. 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. Then the grasses were cleaned and immediately juiced and then kept at 7°C prior to dispatch. The juicing process was conducted by Fruit, Vegetable and wheatgrass juicer, Omega 8224 Nutrition Center Juicer, which is a masticatingstyle juice extractor. Its ability

to juice at low speeds – 80 RPM minimizes heat build-up and oxidation. The same juicer also was used for the extraction of apple juice.

### **Chemical and Microbiological analyses**

Representative samples, coded 1, 2, 3 and 4 as described above underwent chemical and microbiological analyses of the following parameters and with their respective international standard methods:

Determination of ascorbic acid (Vitamin C) content was conducted by titration – (Redox Titration Using Iodine Solution) (Rekha et al., 2012). Antioxidant activity of wheatgrass and fruit juices was determined using DPPH (2, 2-difenil-1-pikrilhidrazil) method of free radical scavenging assay (Rekha et al., 2012).

Chlorophyll content was determined using the procedure by Arnon (Arnon, 1949; Rajalakshmi & Banu, 2015). Total phenol content was determined by Total Polyphenols Index (TPI) spectrophotometric method. All samples involved in this research were analyzed for determination of mineral content such as P, Ca, Mg, Fe, Na and Zn. The concentration of Fe, Na, Mg, Ca and Zn were determined by flame atomic absorption spectrometry (FAAS), whereas P was detected spectrophotometrically (at 430 nm) as yellow colored complex which was obtained with vanadate molybdate reagent (SRPS ISO 13730:1999, 1999). Microbiological analyses for the presence of pathogens: *E. coli* (ISO 16649-2:2001, 2001) *Salmonella* (ISO 6579-1:2017, 2017) and *L. monocytogenes* (ISO 11290-1:2017, 2017) was conducted by using their respective standardized methods as cited.

### **Statistical analyses**

Descriptive statistical analyses were performed for all obtained results. All measurements were performed over three repetitions. Pearson's correlation between assays, the analysis of variance (ANOVA) and Principal Component Analysis (PCA) of the obtained results were performed using StatSoftStatistica 10.0 software. Significant differences were calculated according to post – hoc Tukey's (HSD) test at  $p < 0.05$  significant level, 95% confidence limit. Further, principal component analysis (PCA) was applied successfully to classify and discriminate between the different juice

samples. PCA was applied within the results' descriptors in order to characterize and differentiate between all samples.

## **Results and Discussion**

### **Chemical parameters**

The name "green blood" of wheatgrass is attributable to its high chlorophyll content, which accounts for 70% of its total chemical constituents. However, the most remarkable feature of the wheatgrass juice is its high chlorophyll content. Chlorophyll bears structural similarity to hemoglobin and has been found to regenerate or act as a substitute of hemoglobin in hemoglobin deficiency conditions. This might be the reason behind the utility of wheatgrass in clinical conditions like thalassemia and hemolytic anemia (Padalia et al., 2010).

Chlorophyll content was determined for all samples, using the formula by Arnon (1949) as fresh weight in mg/100 ml. Mean weights of three measurements at 645 nm for Chlorophyll A and respectively at 663 nm for Chlorophyll B was used to calculate the Total chlorophyll. Those values along with standard deviation (SD) are presented in Table 1.

The chlorophyll content of wheatgrass was 73.40 mg/100 ml. This value is comparable with results of other studies (Patrick Wakeham, 2014). The total chlorophyll content was much higher in wheatgrass (WG) +Apple juice when using the pasteurization scheme 85°C for 2 s. Some studies have confirmed the presence of Chlorophyll which is believed to be a pharmacologically active component in wheatgrass as an anti-diabetic agent (Ashok, 2011b). The benefits of wheatgrass have been attributed to its high chlorophyll content in much of the anecdotal literature. Scientific studies regarding the health benefits of chlorophyll have shown anti-cancer effects in animal models, and studies have been extended to human subjects. Two studies published in 2005 by de Vogel et al. (Ashok, 2011b) found that chlorophyll inhibited haeme-induced cytotoxicity and reduced epithelial cell turnover (hyper proliferation) in rat colons; the first (de Vogel, Jonker-Termont, Katan, et al., 2005) found this effect to be specific to natural chlorophyll and cannot be mimicked by sodium copper chlorophyllin. The second study (de Vogel, Jonker-Termont, van Lieshout, et al., 2005) found spinach and chlorophyll caused cytotoxic inhibition and concluded that green vegetables in the diet

**Table 1. The content of chlorophyll in four samples**

Sample	Chlorophyll A, mg/l	SD	Chlorophyll B, mg/l	SD	Total chlorophyll, mg/100 ml	SD
Wheatgrass	4.75	0.01	2.60	0.01	73.49	0.02
Apple juice	4.33	0.00	2.73	0.02	7.06	0.00
WG+Apple J 85°C/2 s	3.11	0.01	0.69	0.01	37.99	0.03
WG+Apple J 71.7°C/15s	1.35	0.01	0.82	0.01	14.32	0.02

**Table 2. Ascorbic acid, total polyphenols and antioxidant activity of four samples**

Sample	Ascorbic Acid, g/l	SD	Total polyphenols, mg/l	SD	Antioxidant Activity, 100/ g/l	SD
Wheatgrass	235.62	0.23	54.54	0.01	140.35	0.02
Apple juice	20.47	0.10	17.81	0.02	31.33	0.00
WG+ Apple J 85 °C/2 s	50.63	0.02	4.47	0.00	54.72	0.02
WG+ Apple J 71.7°C/15s	40.34	0.01	4.63	0.01	50.05	0.01

may decrease colon cancer risk, as chlorophyll prevents the detrimental cytotoxic and hyper proliferative effects of dietary heme (P. Wakeham, 2013)

The role of ascorbic acid in health promotion is really well known and wheatgrass is considered an important source of vitamin C. The results for Ascorbic acid (Vitamin C) content by redox iodine titration method, for four samples are presented in Table 2.

The content of ascorbic acid in sample 1 (wheatgrass) it was 2.355 g/l, which is in compliance with literature review and other researches where it is claimed that wheatgrass contains 2 g/l ascorbic acid (*Wheat Grass Nutrition Facts & Calories*, 2020). Apple juice, canned, without added sugar or ascorbic acid contains 0.009 g/l or 1% of daily recommended intake of ascorbic acid. The concentration of vitamin C (g/l) in fresh apple juice is 0.204 g/l, red grapefruit 0.816 g/l, beet 0.680 g/l, red cabbage 0.604 g/l, fresh tomato juice 0.233 g/l (*Office of Dietary Supplements – Vitamin C*, 2019).

Based on the results for ascorbic acid in wheatgrass, if it is taken the average of RDA of ascorbic acid for a mature human 0.08 g/day, the content of ascorbic acid of wheatgrass represents 294.4% of RDA per 0.1 l wheatgrass. The fulfillment of 100% of RDA for ascorbic acid requires consumption of only 0.027 l of wheatgrass daily. Among newly produced juices, formulation of the wheatgrass with apple juice was 0.651 g/l for sample WG + Apple J 85°C/2s and 0.526 g/l for sample WG+ Apple J 71.7°C/15s. From the above demonstrated results, it is evident that ascorbic acid concentration was higher at juice pasteurized at 85°C/2s, in comparison to the one pasteurized at 71.7°C/15s.

Wheatgrass juice is an integral part of the macrobiot-

ic diet under the complementary and alternative medicine (CAM) approach of anticancer therapy, due to its high antioxidant content (Padalia et al., 2010). The results of the analyses of DPPH testing, for determination of Antioxidant Activity for dilution with 100 for all samples involved in this research are shown in the Table 2.

Since antioxidant activity is dedicated to synergistic action of some polyphenolic compounds, this activity depends directly on the effect of the temperature on these components (Kanner et al., 1994). Different literature sources refer to the wheatgrass as a rich source of polyphenols. Therefore, samples involved in this research were screened for their polyphenolic profile.

The results of total polyphenols among with antioxidant activity and ascorbic acid are presented in Table 2. The obtained results for Ascorbic acid show that the Vitamin C concentration in wheatgrass is 11.5 times higher than its concentration in apple juice. A similar situation appeared also for polyphenols, total polyphenols for wheatgrass ( $54.54 \pm 0.01$ ) compared with apple ( $17.81 \pm 0.02$ ). Among pasteurized juices WG+ Apple J 85°C/2 s has the highest value of total polyphenols. Antioxidant activity of wheatgrass ( $140.35 \pm 0.02$  g/l) is obviously higher than of the apple juice ( $31.33 \pm 0.00$ ). Among pasteurized juices WG+ Apple J 85°C/2s was richer with ascorbic acid, total polyphenols and has higher antioxidant activity than WG+ Apple J 71.7°C/15 s.

#### Minerals content

Concentration of the minerals given in g/kg for phosphorus and mg/kg for Ca, Mg, Fe, Na, and Zn are presented in the Table 3.

**Table 3. The content of minerals in all analyzed samples**

Element	Wheatgrass	SD	Apple juice	SD	WG + Apple juice 85 °C/2 s	SD	WG+ Apple juice 71.7°C/15 s	SD
P, g/kg	0.25	0.00	0.24	0.01	0.24	0.02	0.22	0.02
Ca, mg/kg	825.0	0.10	42.17	0.15	624.67	0.58	216.10	0.10
Mg, mg/kg	262.63	0.06	29.67	0.06	137.40	0.20	120.03	0.06
Fe, mg/kg	12.89	0.01	4.44	0.01	8.65	0.01	8.97	0.01
Na, mg/kg	7.58	0.01	7.87	0.01	16.66	0.01	17.21	0.01
Zn, mg/kg	3.75	0.01	0.57	0.01	1.02	0.01	1.56	0.01

**Table 4. ANOVA calculation of chemical and mineral content for all analyzed samples**

Element	df Effect	SS Effect	df Error	SS Error	F	p
TotChlor	3	8060	8	0.00	6961047	0.000
Vitamin C	3	90044	8	0.13	1891460	0.000
ITPoly	3	5026	8	0.00	15916292	0.000
AO	3	21215	8	0.00	40029127	0.000
P	3	0.0	8	0.00	2.0	0.186
Ca	3	1170155	8	0.75	4142141	0.000
Mg	3	82774	8	0.10	2207316	0.000
Fe	3	107	8	0.00	428618	0.000
Na	3	255	8	0.00	1699733	0.000
Zn	3	18.0	8	0.00	102352	0.000

As the wheatgrass is claimed to be a very rich source of minerals as well as fruit juices are widely consumed through the world, the evaluation of those for the mineral content is an important factor for health and safety issues related to consumers. The concentration of phosphorus does not change significantly between the samples; it ranges from 0.22 g/kg (WG+ apple juice, 71.7°C/15s) to 0.25 g/kg (wheatgrass).

Recommended daily allowance (RDA) for phosphorus is based on the amount needed to maintain adequate blood concentration and depends on age and gender. Tolerable upper intake levels (UL) have been set by the Institute of Medicine of the National Academies. In the United States, adults have an average intake of about 1300 mg/day for men and 1000 mg/day for women (National Institutes of Health (NIH), 2018).

The content of Ca in samples ranged from 42.17 mg/kg (apple juice) to 825 mg/kg (wheatgrass). Based on the results presented in Table 3, the important role of wheatgrass addition to the fortification of apple juice with Ca is very clear. The Ca content in wheatgrass + apple juice pasteurized in 85°C/2s is 14.8 times higher than the content of Ca in apple juice. RDA for minerals in humans varies based on different factors such as the age, gender and special circumstances (like pregnancy, lactation etc.). However the average of the RDA for Ca for an adult can be taken 1000-1300 mg/day ("Calcium and Vitamin D: Important at Every Age," 2018). Regarding to the Fe content, wheatgrass may be considered as an important source for fortifying apple juice with Fe by increasing Fe concentration from 4.44 mg/kg at apple juice to 8.97 mg/kg at sample 4 (WG+apple juice, 71.7°C/15 s), respectively 8.65mg/kg at sample 3 (WG+apple juice, 85°C/2 s). The average daily recommended intake for iron from foods and supplements is 13.7–15.1 mg/day in children aged 2–11 years, 16.3 mg/day in children and teens aged 12–19 years, and 19.3–20.5 mg/day in men and 17.0–18.9 mg/

day in women older than 19. The median dietary iron intake in pregnant women is 14.7 mg/day (National Institutes of Health (NIH), 2018).

Similar results are revealed for Na as well as Zn where the formulation of apple juice containing wheatgrass (samples 3 and 4) are richer in Na and Zn than the apple juice (Sample 2). The FDA recommends that individuals consume no more than 2.3 g of sodium per day, and that certain groups limit intake to 1.5 g per day (*The FDA Recommended Sodium Intake*, 2018). Zinc is involved in numerous aspects of cellular metabolism such as involvement in catalytic activity of approximately 100 enzymes, its role in the immune system, protein synthesis, and cell division. A daily intake of Zinc is required to maintain a steady state because the body has no specialized zinc storage system. RDA of zinc varies from 2 mg at infants 0–6 months to 11 mg to adults 19+years (National Institutes of Health (NIH), 2018). Although with slight differences compared to apple juice pasteurized on 85°C/2s, composition, wheatgrass and apple juice pasteurized on 71.7°C/15 s has the highest content of Fe, Na and Zn. All analyzed minerals in all samples were within given limits of their respective RDA values.

The one-way ANOVA test was performed to evaluate the effectiveness of the wheatgrass to the chemical parameter-sand minerals content when it was mixed with apple juice. This analysis is presented in Table 4.

The Anova analysis revealed that the content of all measured parameters (except the P content) are affected by the sample and by the way of pasteurization, statistically significant at  $p < 0.01$  level. Antioxidant activity (AO) showed a significant positive linear correlation with ascorbic acid (Vitamin C) and total chlorophyll (totChlor), ( $r = 0.996$ ,  $p < 0.01$  and  $r = 0.95$ ,  $p < 0.05$ , respectively). Also, vitamin C showed a significant positive linear correlation with TotChlor and total polyphenols index (ITPoly), ( $r = 0.939$ ,  $p < 0.10$

and  $r = 0.926$ ,  $p < 0.10$ , respectively). Pearson correlation matrix for TotChlor, Vitamin C, ITPoly and AO are shown in Table 5a, while Pearson correlation matrix for minerals content is presented in Table 5b.

**Table 5a. Pearson correlation matrix for TotChlor, Vitamin C, ITPoly and AO**

Element	Vitamin C	ITPoly	AO
TotChlor	0.939**	0.785	0.952*
Vitamin C		0.926**	0.996 <sup>+</sup>
ITPoly			0.889

Correlation statistically significant at  $p < 0.01$

\*Correlation statistically significant at  $p < 0.05$

\*\*Correlation statistically significant at  $p < 0.10$

**Table 5b. Pearson correlation matrix for minerals content**

Element	Ca	Mg	Fe	Na	Zn
P	0.753	0.551	0.407	-0.533	0.472
Ca		0.915**	0.867	-0.060	0.751
Mg			0.985*	-0.130	0.939**
Fe				0.005	0.916**
Na					-0.372

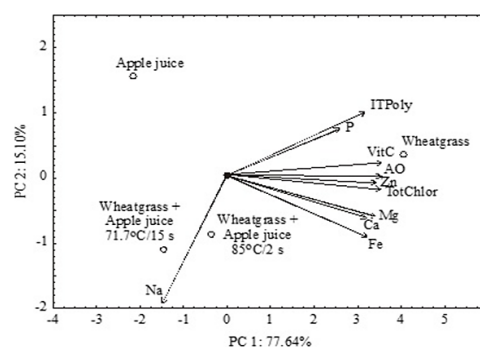
Correlation statistically significant at  $p < 0.01$

\*Correlation statistically significant at  $p < 0.05$

\*\*Correlation statistically significant at  $p < 0.10$

The PCA allows a considerable reduction in the number of variables and the detection of structure in the relationship between measuring parameters and different samples that give complimentary information. The number of factors retained in the model for proper classification of experimental data, in the original matrix regarding loading and scores were determined by applying Kaiser and Rice's rule. This criterion retains only principal components with Eigenvalues  $> 1$ . The full auto-scaled data matrix consisting of ten variables were submitted to PCA. For visualizing the data trends and the discriminating efficiencies of the used descriptors, a scatter plot of samples using the first two principal components (PCs) issued from PCA of the data matrix were obtained (Figure 1).

As could be seen, there was a neat separation of the juice samples, according to the applied assays. Quality results showed that the first two principal components, accounting for 92.74% of the total variability could be considered sufficient for data representation. PC1 correlated positively with: TotChlor (the variable contributed 12.4% for PC1 calculation), Vitamin C (12.6%), ITPoly (9.9%), AO (12.7%), Ca (10.1%), Mg (11.7%), Fe (10.2%) and Zn (11.6%), while the second component PC2, correlated positively with: ITPoly (13.3%), and negatively with Fe (12.2%) and Na (54.3%).



**Fig. 1. Biplot for the different juice samples**

### Microbiological analyses

Microbiological tests for the presence of pathogens such as *E. coli*, *Salmonella* and *L. monocytogenes* were conducted only for formulations of wheatgrass with apple juice (WG+ Apple J 85°C/2 s -sample 3 and WG+ Apple J 71.7°C/15 s -sample 4), pasteurized under the two schemes of pasteurization. Both samples resulted negative for the presence of *E. coli*, *Salmonella* and *L. monocytogenes*.

The results of microbiological analyses prove that the both schemes applied for pasteurization of juices produced in this study are safe in terms of food safety.

### Conclusion

The work done in this study indicates that wheatgrass may be considered a potential raw material for the fruit processing industry as a nutritional value adding superfood, as an excellent source of health promoting phytonutrients such as Chlorophyll, Vitamin C, Polyphenols, and Minerals. If used in its fresh form, nutritional values of wheatgrass are much higher. This study also indicated the importance of wheatgrass in the nutritional values increase of apple juice when added prior to pasteurization. From the results obtained in this work, composition of 70% apple juice + 30% wheatgrass, shows high content of ascorbic acid, total polyphenols, total chlorophyll, antioxidant activity and minerals content. Moreover, we studied the two pasteurization schemes (85°C/2s; 71.7°C/15s).

Both of the schemes applied for pasteurization of juices produced in this study were safe in terms of food safety. While among pasteurized juices WG+ Apple J 85°C /2 s was richer with ascorbic acid, total polyphenols and has higher antioxidant activity than WG+ Apple J 71.7°C/15 s. Regarding minerals, the concentration of phosphorus does not change significantly between the samples; it ranges from 0.22 g/kg (WG+apple juice, 71.7°C/15 s) to 0.25 g/kg

kg (wheatgrass). The content of Ca in samples ranged from 42.17 mg/kg (apple juice) to 825 mg/kg (wheatgrass). The higher content of Ca is in wheatgrass + apple juice pasteurized in 85°C/2 s. Composition, wheatgrass and apple juice pasteurized on 71.7°C/15 s has the highest content of Fe, Na and Zn. All analyzed minerals in all samples were within the limits of their respective RDA values. ANOVA test showed that the content of all measured parameters (except the P content) is affected by the sample and by the way of pasteurization, statistically significant at  $p < 0.01$  level. In addition, the results also give a practical conclusion for the consumers: 70% apple juice + 30% wheatgrass provide a variety of bioactive components that are vital in health promotion and disease prevention.

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