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# USE OF GREEN POWDERS AS INGREDIENTS IN FUNCTIONAL BEVERAGES

– Research paper –

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**Abstract:** The aim of this study was to compare the quality of soft drinks with the addition of powderd chlorella, barley grass, wheatgrass, kale, matcha, *Moringa oleifera* leaves. The levels of ash, pH, total polyphenols (TPC), total flavonoids (TFC), individual phenolic compounds, chlorophylls, carotenoids, and antioxidant activity (ABTS, DPPH) were determined before and after pasteurization. Turbidity and colour parameters *(L*, a*, b*, C*, h°)* were analysed. The drinks averaged 48.9 mg of TPC, 16.37 mg of chlorophylls, and 4.33 mg of carotenoids, per 100 ml of fresh weight. The antioxidant activity per ml averaged 16.72 µM Trolox (DPPH) and 103.41 µM Trolox (ABTS). Gallic acid was present in the highest amount (103-228.11 mg/L) in beverages containing matcha before pasteurization. The matcha - containing drinks also had the highest antioxidant activity, and their turbidity was the highest. The drinks were characterized by values of parameter *a** in the range -1.67÷1.78, and parameter *b** in the range 3.21÷27.78. Pasteurization significantly reduced the level of analysed components and deteriorated the parameters of colour and turbidity. In conclusion, green powders can be used as an ingredient in beverages to create new products with antioxidant properties.

**Keywords:** green powders; non-alcoholic beverages; antioxidant properties; colour; turbidity

## INTRODUCTION

A wide range of soft drinks are available on the world market. The global soft drink industry revenue is estimated to exceed $950 billion in 2025, with a volume of more than 330 billion liters (Statista, 2024). These products influence on the body's water balance, provide energy, and exhibit refreshing and stimulating effects (Ramirez et al., 2022; Soufi et al., 2021). Depending on the type of product, they may also contain nutrients such as vitamins, minerals and often sugars added in the processing (Arora et al., 2020). Since the main component of soft drinks is water, increasing soft drink intake can improve fluid intake and water levels in the body if it is regarded as an "essential nutrient" (Redondo et al., 2014). However, sucrose-sweetened beverages (SSBs) are a major source of added sugars in the diet (Malik & Hu, 2022).
It should be noted that carbohydrates have become a health concern, especially when consumed insignificant amounts (Rippe & Angelopoulos, 2016; Wee & Henry, 2020). Excessive consumption of SSBs is one of the causes of modern chronic non-communicable diseases, which include diabetes, cardiovascular disease (metabolic hypertension syndrome), and even cancer (Ardeshirlarijani et al., 2021). Studies indicate a correlation between the consumption of SSBs and type 2 diabetes (Drouin-Chartier et al., 2019; Malik & Hu, 2022). The condition is the most common metabolic disorder contributing to significant mortality in humans (Sokołowska et al., 2022). Therefore, whenever possible, efforts should be made to reduce the intake of sugars, particularly in children's diets (Farhangi et al., 2020; Luger et al., 2017; Zhu et al., 2020).
Currently, there is a trend of green consumerism, i.e., consumer interest in and orientation towards natural and organic products, in which the use of synthetic additives is reduced (Corbo et al., 2014). The nutritional opportunities provided by such products can be particularly helpful in preventing and treating diet-related chronic diseases, such as obesity or diabetes. Consumers look for innovative foods that could significantly improve their health and provide valuable nutrients. They treat health

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and nutrition as a priority. Functional beverages may be the answer to these expectations (Nazir et al., 2019; Pamela et al., 2021).
The European Union has had a definition of functional foods since 1999, defined by FUFOSE (Functional Food Science in Europe) as foods that have a beneficial effect on one or more bodily functions over and above the nutritional effect. The goal is to improve health, well-being and/or reduce the risk of disease. Such foods must resemble conventional foods in form ("Scientific Concepts of Functional Foods in Europe Consensus Document," 1999). The production and consumption of functional foods are becoming increasingly important because they provide health benefits beyond basic nutritional function and well-being (Badejo et al., 2020; Corbo et al., 2014). For example, functional beverage consumption improves insulin resistance and decreases the degree of non-alcoholic fatty liver disease (Rubio-Rodríguez et al., 2021).
The variety of ingredients used in the production of functional beverages makes it possible to obtain products aimed at strengthening the immune system, relieving stress, or improving body weight (Lee et al., 2021). In addition to nutrients, such products also contain biologically active and medicinal compounds found naturally in, for example, herbs (Dilrukshi & Senarath, 2021). Valuable plant raw materials for functional beverages can be green powders from plants such as matcha, moringa, kale, chlorella, wheatgrass, and barley grass. They are a source of many components with health-promoting properties, including valuable chlorophylls (Farooq & Sehgal, 2018; González-Romero et al., 2020; Salanta et al., 2016; Satheesh & Workneh Fanta, 2020; Schüler et al., 2020; Y. Zeng et al., 2018).
Consuming foods or beverages that have functional properties is an element of healthy living, as the bioactive compounds in these products can fight free radicals, both those produced in the body and those coming from outside (Pamela et al., 2021; Vichaibun & Kanchanaphu, 2019). For example, in Japan, green juices made with ingredients such as young barley grass juice are known as "aojiru," and are widely consumed to enhance health (Sasaki et al., 2019). According to Tireki (2021), consumers will increasingly look for mood-enhancing and health-boosting functional products in the future. Sucrose-free, low-calorie, or calorie-reduced beverages are also becoming increasingly popular (Lohner et al., 2017; Malik & Hu, 2022). The aim of this study was to investigate the effect of the addition of green powders (chlorella, barley grass, wheatgrass, kale, matcha, *Moringa oleifera* leaves) on the quality of soft drinks sweetened with apple concentrate. The developed drinks were evaluated for antioxidant content and potential.

## MATERIALS AND METHODS

### Materials

The research materials were soft drinks with 1% and 3% green powders from chlorella (Look Food SA, Poland), barley grass, wheatgrass (Bio Planet S.A., Poland), kale (Bio Planet S.A., Poland), matcha (Taheebo sp. z o.o, Poland) and *Moringa oleifera* leaves (Intenson Europe sp. z o.o, Poland). All drinks included:

- pasteurized lime juice (Bio Planet S.A., Poland),
- apple concentrate, including natural apple flavour (GOMAR sp. z o.o., Pińczów, Poland),
- natural spring water, mineral content 259.72 mg/l, obtained from Kryształ, Kęty, Poland.

The following beverage variants were prepared:

C1/C1P - with 1% addition of chlorella before/after pasteurization,
C3/C3P - with 3% addition of chlorella before/after pasteurization,
Gb1/Gb1P - with 1% addition of barley grass before/after pasteurization,
Gb3/Gb3P - with 3% addition of barley grass before/after pasteurization
Gw1/Gw1P - with 1% addition of wheatgrass before/after pasteurization,
Gw3/Gw3P - with 3% addition of wheatgrass before/after pasteurization,
K1/K1P - with 1% addition of kale before/after pasteurization,
K3/K3P - with 1% addition of kale before/after pasteurization,
M1/M1P - with 1% addition of matcha before/after pasteurization,
M3/M3P - with 1% addition of matcha before/after pasteurization,
Mo1/Mo1P - with 1% addition of moringa before/after pasteurization,
Mo3/Mo3P - with 1% addition of moringa before/after pasteurization.

### Production of beverage

The production of beverages included the following technological operations: mixing the ingredients according to the recipe for the beverage variant, heating the beverage to temp. 70ºC, bottling (133 ml glass jars), sealing, pasteurization (temp. 82-85ºC, 13 min.), cooling. The beverages were evaluated before pasteurization and immediately after.

Beverage recipes were predetermined based on organoleptic analysis. The total soluble solids of the beverages was assumed to be 8 ºBx, the total acidity was assumed to be 1.2%, per malic acid, the proportion of each vegetable powder in the weight of the beverage was assumed to be 1% and 3%. On this basis, the amounts of ingredients of each variant of the beverage were calculated per 1030 g (1000 ml) of beverage such as:

- plant powder of 10.3 g and 31 g, respectively, for the beverage with 1% and 3% powder,
- apple concentrate with flavour condensate (E=71%, total acidity 3.02 g/100 g) at 116 g, as a sweetener and partial acidity regulator,
- lime juice (E=6.9%, total acidity 4.5 g/100 g) in an amount of 20 g, as an acidity regulator,
- spring water in the amount of 884 g (variant with 1% addition of powders) and 863 g (variant with 3% addition of powders), to dilute the ingredients.

**Chemical analysis**

The basic composition of the products (ash, pH) were determined by AOAC (AOAC International, 2006) methods. Ash content was determined by incineration of a sample (460 °C) in L9/S 27 furnace oven (Nabertherm GmbH, Lilienthal, Germany), and active acidity (pH) was measured using a potentiometric method (pH-/Ion Meter 692, Switzerland).

To determine total polyphenols (TPC), total flavonoids (TFC) and antioxidant activity, sample extracts were prepared using 80% methanol. Polyphenols were determined by spectrophotometric method with Folin-Ciocalteau reagent (Heimler et al., 2005). TFC content was detected spectrophotometrically with flavonoid-aluminum chloride ($AlCl_3$) complexation (Csepregi et al., 2013). The content of polyphenols and flavonoids was read from the standard curve prepared for (+)-catechin, and the results are expressed as milligrams catechin equivalents per 100 ml of plant extract (mg CE/100 ml).

Separation and identification of polyphenols was performed by high performance liquid chromatography (HPLC), according to the method described by Skoczylas et al. (2018), with our modifications. To 2 ml of the beverage, 2 ml of NaOH (2 mol/L) was added, mixed on a Labnet vortex mixer (Edison, USA) for 5 minutes and to settle for 4 hours in a dark place at room temperature. Then, using HCl (2 mol/L), the pH was set at 2.1-2.6 using a Metrohm pH meter (Herisau, Switzerland) and supplemented to 10 ml with 1% ascorbic acid in methanol (HPLC grade). The sample prepared in this way was centrifuged using an MPW - 260R centrifuge (Warsaw, Poland) (18,000 rpm; 20 min.; 4°C) and analysed by HPLC. Quantitative and qualitative chromotographic analysis was performed using HPLC-UV/Vis LaChrom ELITE (Merck, Germany). The column used was a Lichrospher® 100, RP-18 end-capped, LiChroCART® 4 mm, ID x 250 mm, 10 µm, Merc KGaA, (Darmstadt Germany). The mobile phase consisted of two eluents: phase A - 2.5% acetic acid in water, phase B – 100% acetonitrile, thermostat temperature 30ºC, wavelength (λ) = 220-400 nm, mobile phase flow rate - 1 ml/min. Chromatographic analysis lasted 97 min, using the following gradient: Eluent A-13 min. 92%; 23 min. 85%; 38 min. 80%; 53 min. 60%; 87 min. 97% by the end of the analysis. The results are presented in mg/L of beverage.

Total chlorophylls (a+b) and carotenoids were determined according to the methods of Lichtenthaler and Buschmann (Lichtenthaler & Buschmann, 2001a, 2001b). The measurement was carried out spectrophotometrically after prior extraction of the pigments with 80% acetone. The absorbance was read at 661.6 nm and 644.8 nm (the absorption maxima of chlorophyll a and chlorophyll b, respectively), as well as at 470 nm, the characteristic absorption of carotenoids. Pigment concentration (mg/100 ml) was calculated on the basis of absorption coefficients for the above wavelengths.

Antioxidant activity was determined by two spectrophotometric methods as the scavenging activity of the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) (Pekkarinen et al., 1999) and using the cation ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) (Re et al., 1999). The results were expressed as Trolox equivalent antioxidant activity in µmol Tx/ml. Trolox was prepared in phosphate buffer saline PBS (2.5 mM/L), pH 7.4.

A Hitachi U-2900 dual beam spectrophotometer (Hitachi Europe Ltd) was used to analyse TPC, TFC, chlorophylls, carotenoids, and antioxidant activity.

**Instrumental colour analysis**

The colour measurement was performed using the CIE ($L^*a^*b^*$) system (CIE 2014). A Konica MINOLTA CM-3500d equipment (Konica Minolta Inc., Tokyo, Japan) was used. The measurement was made with respect to the D65 illuminant and a visual angle of 10°. The following parameters were determined from the measurement: $L^*$ - lightness

(L*=0 blackness, *L**=100 whiteness), *a** - proportion of green (*a** <0) or red (*a** >0), *b** - proportion of blue (*b** <0) or yellow (*b** >0), and *C** - chroma, $h^o$ - hue angle.

**Turbidity and turbidity stability**

Turbidity of the beverages was measured with a HANNA HI 98703 turbidimeter (Hanna Instruments, Inc., Woonsocket USA), using 2.5-cm round cuvettes. Turbidity (T) was expressed in nephelometric turbidity units (NTU). Turbidity stability (T%) was calculated from the equation 1:

$$T\ (\%) = (Tc/To) \times 100 \quad (1)$$

where To and Tc are the beverages turbidities before and after centrifugation at 4200 x *g* for 15 min at 20 °C (Teleszko et al., 2016).

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**Statistical analysis**

Results were statistically analysed using Statistica 13.3 (Statsoft, Inc., Tulsa, OK., USA) and expressed as mean ± standard error. One-way analysis of variance was used. Significance of differences between mean values was determined by Fisher's test at $p < 0.05$. All measurements were performed in at least four duplicates. Principal Components Analysis (PCA) was performed to analyse differences between samples and interdependencies between analysed parameters

## RESULTS AND DISCUSSION

Soft drinks are a diverse group of products. They play an important role in the diet, and their health-promoting properties depend on the composition of such a product. In addition to spring water, they often contain fruit juice, vitamins, minerals, or other enriching riching ingredients. They can be a rich source of nutrients and phytonutrients, especially phenolic acids and flavonoids (Ferruzzi et al., 2020). In addition to hydrating the body, many soft drinks have been developed to provide specific health benefits, such as improving immunity and digestion (Kregiel, 2015).

**Ash and active acidity (pH)**

The beverages evaluated differed in the level of ash. It was in the range of 0.22-0.46 g/100 g, with pH ranging from 3.27-3.78 (Table 1). After pasteurization, the ash content did not change significantly, and the pH value increased slightly. Both ash levels and pH were always higher in beverages with a higher addition of plant powder. The highest levels of ash were observed in beverages with 3% wheatgrass, barley grass, and moringa.

Table 1. Ash and pH of beverages before and after pasteurization

| Type of beverage | Ash (g/100 g) | | pH | |
|---|---|---|---|---|
| | before pasteurization | after pasteurization | before pasteurization | after pasteurization |
| C1 | 0.26 ± 0.01 | 0.23 ± 0.02 | 3.34 ± 0.01 | 3.43 ± 0.02 |
| C3 | 0.38 ± 0.02 | 0.36 ± 0.01 | 3.71 ± 0.02 | 3.77 ± 0.01 |
| Gb1 | 0.27 ± 0.02 | 0.26 ± 0.02 | 3.31 ± 0.02 | 3.33 ± 0.01 |
| Gb3 | 0.40 ± 0.02 | 0.40 ± 0.03 | 3.54 ± 0.01 | 3.57 ± 0.02 |
| Gw1 | 0.22 ± 0.01 | 0.21 ± 0.02 | 3.36 ± 0.01 | 3.41 ± 0.01 |
| Gw3 | 0.46 ± 0.03 | 0.44 ± 0.03 | 3.42 ± 0.02 | 3.52 ± 0.01 |
| K1 | 0.24 ± 0.02 | 0.23 ± 0.01 | 3.37 ± 0.01 | 3.44 ± 0.02 |
| K3 | 0.33 ± 0.03 | 0.32 ± 0.01 | 3.78 ± 0.02 | 3.85 ± 0.02 |
| M1 | 0.26 ± 0.02 | 0.25 ± 0.01 | 3.27 ± 0.02 | 3.28 ± 0.02 |
| M3 | 0.34 ± 0.02 | 0.33 ± 0.02 | 3.48 ± 0.01 | 3.49 ± 0.01 |
| Mo1 | 0.29 ± 0.01 | 0.28 ± 0.02 | 3.39 ± 0.01 | 3.41 ± 0.02 |
| Mo3 | 0.46 ± 0.02 | 0.46 ± 0.02 | 3.66 ± 0.01 | 3.69 ± 0.02 |
| LSD p<0.05 | 0.044 | 0.025 | 0.022 | 0.066 |
| Total LSD | 0.035 | | 0.048 | |

Values are presented as mean value ± SD (n=3), in fresh matter

Beverages with green plant powders: C - chlorella; Gb - barley grass; Gw - wheat grass, K - kale, M - matcha, Mo - moringa; 1, 3 - percentage addition of plant powder

Wheatgrass is considered a very good source of nutrients, including minerals (Thakur et al., 2019). Therefore, its juice has become a popular beverage in many countries as a "health food" (Cores Rodríguez et al., 2022). Also, barley grass powder is a rich source of minerals (Cores Rodríguez et al., 2022). Y.-W. Zeng et al. (2009) report that compared to brown rice, this powder contains more than 10 times more K, Ca, and Fe, similar to moringa leaves. *Moringa oleifera* is a deciduous tree that has a remarkable range of beneficial effects used in nutrition, medicine, and other industrial purposes (Mouchili et al., 2019). It is one of the plants richest in nutrients (Olvera-Aguirre et al., 2022).

**Bioactive compounds content and antioxidant activity**

The levels of bioactive components and total antioxidant activity in the evaluated beverages are shown in Tables 2 and 3. The drinks had different levels of TPC in the range of 19.7-175.4 mg/100 ml, of which flavonoids accounted for 59-81%. Drinks with 3% moringa and matcha had the most TPC (58.4-175.4 mg/100 ml), while the others had significantly less (19.7-49.3 mg/100 ml). Noteworthy were drinks with matcha, which had the highest levels of polyphenols. In addition, the most phenolic acids (gallic acid, protocatechuic acid, cinnamic acid) were recorded in these drinks, especially in the drink with 3% matcha (Table 3)

Table 2. Bioactive compounds content and antioxidant activity of beverages before and after pasteurization

| Type of beverage | Total polyphenols mg CE/100 ml | Total flavonoids mg CE/100 ml | Total chlorophylls mg/100 ml | Total carotenoids mg/100 ml | ABTS µmol Tx/ml | DPPH µmol Tx/ml |
|---|---|---|---|---|---|---|
| before pasteurization | | | | | | |
| C1 | 19.8 ± 0.9 | 11.8 ± 0.4 | 18.51 ± 0.30 | 3.92 ± 0.08 | 18.2 ± 0.7 | 4.7 ± 0.1 |
| C3 | 29.6 ± 1.0 | 20.5 ± 1.1 | 30.46 ± 0.70 | 6.99 ± 0.19 | 26.2 ± 0.6 | 5.7 ± 0.1 |
| Gb1 | 19.7 ± 1.0 | 14.1 ± 0.1 | 10.26 ± 0.15 | 3.16 ± 0.08 | 36.1 ± 1.1 | 4.6 ± 0.2 |
| Gb3 | 32.3 ± 2.7 | 25.3 ± 1.9 | 18.24 ± 0.21 | 7.79 ± 0.13 | 49.9 ± 0.4 | 6.9 ± 0.2 |
| Gw1 | 28.3 ± 1.7 | 20.9 ± 0.1 | 10.13 ± 0.18 | 1.58 ± 0.12 | 30.0 ± 0.5 | 5.2 ± 0.1 |
| Gw3 | 49.3 ± 3.8 | 37.5 ± 0.5 | 15.93 ± 0.13 | 2.98 ± 0.12 | 62.5 ± 0.9 | 7.3 ± 0.2 |
| K1 | 23.0 ± 1.8 | 14.1 ± 0.5 | 14.76 ± 0.28 | 4.20 ± 0.07 | 24.2 ± 0.7 | 5.8 ± 0.1 |
| K3 | 41.0 ± 0.6 | 26.5 ± 0.3 | 22.95 ± 0.19 | 6.23 ± 0.06 | 37.6 ± 0.6 | 7.3 ± 0.2 |
| M1 | 70.8 ± 6.2 | 57.5 ± 2.2 | 13.21 ± 0.20 | 3.54 ± 0.07 | 239.5 ± 14.1 | 38.9 ± 1.1 |
| M3 | 175.4 ± 6.6 | 121.9 ± 5.3 | 27.81 ± 0.08 | 5.78 ± 0.06 | 548.0 ± 18.1 | 85.4 ± 1.2 |
| Mo1 | 39.0 ± 3.4 | 25.6 ± 0.6 | 4.09 ± 0.04 | 1.66 ± 0.02 | 62.5 ± 1.7 | 7.2 ± 0.8 |
| Mo3 | 58.4 ± 6.6 | 37.3 ± 1.3 | 10.08 ± 0.06 | 4.09 ± 0.02 | 106.1 ± 4.4 | 13.6 ± 0.2 |
| LSD p<0.05 | 6.34 | 3.28 | 1.13 | 0.33 | 20.58 | 0.78 |
| after pasteurization | | | | | | |
| C1P | 11.7 ± 0.5 | 8.4 ± 0.1 | 7.86 ± 0.18 | 3.86 ± 0.04 | 15.6 ± 1.0 | 4.2 ± 0.2 |
| C3P | 15.4 ± 0.9 | 14.1 ± 0.5 | 17.39 ± 0.77 | 6.79 ± 0.07 | 21.0 ± 1.3 | 5.0 ± 0.1 |
| Gb1P | 15.6 ± 0.3 | 10.6 ± 0.2 | 5.57 ± 0.29 | 2.18 ± 0.05 | 25.0 ± 0.6 | 3.7 ± 0.1 |
| Gb3P | 25.2 ± 0.5 | 18.1 ± 4.4 | 13.16 ± 0.33 | 5.63 ± 0.08 | 30.0 ± 0.6 | 5.5 ± 0.2 |
| Gw1P | 15.6 ± 0.7 | 11.5 ± 0.2 | 5.04 ± 0.08 | 1.09 ± 0.01 | 26.2 ± 0.8 | 4.8 ± 0.2 |
| Gw3P | 23.8 ± 2.9 | 19.8 ± 1.3 | 6.39 ± 0.12 | 1.94 ± 0.02 | 34.9 ± 1.4 | 6.3 ± 0.3 |
| K1P | 11.6 ± 1.0 | 10.8 ± 1.2 | 8.88 ± 0.29 | 3.84 ± 0.09 | 20.4 ± 0.4 | 5.2 ± 0.1 |
| K3P | 14.7 ± 1.0 | 13.5 ± 0.2 | 16.50 ± 0.17 | 5.76 ± 0.10 | 30.7 ± 0.4 | 6.7 ± 0.1 |
| M1P | 52.2 ± 3.5 | 34.4 ± 0.5 | 5.49 ± 0.16 | 2.38 ± 0.05 | 155.7 ± 3.1 | 31.5 ± 0.1 |
| M3P | 120.3 ± 4.1 | 62.8 ± 3.5 | 19.57 ± 0.21 | 3.84 ± 0.09 | 321.8 ± 2.3 | 62.6 ± 0.2 |
| Mo1P | 29.8 ± 2.7 | 15.2 ± 0.6 | 1.99 ± 0.08 | 1.06 ± 0.07 | 55.2 ± 1.0 | 6.9 ± 0.1 |
| Mo3P | 38.2 ± 1.6 | 22.9 ± 1.6 | 8.80 ± 0.22 | 2.62 ± 0.08 | 89.0 ± 1.1 | 12.4 ± 0.2 |
| LSD p<0.05 | 3.49 | 3.02 | 1.24 | 0.39 | 5.94 | 0.27 |
| Total LSD p<0.05 | 4.99 | 3.07 | 1.15 | 0.35 | 14.75 | 0.57 |

Values are presented as mean value±SD (n=3), in fresh matter

Beverages with green plant powders: C - chlorella; Gb - barley grass; Gw - wheat grass, K - kale, M - matcha, Mo - moringa; 1, 3 - percentage addition of plant powder; P - pasteurization

Also, the drink with 3% moringa powder stood out for its high content of TPC (58.4 mg/100 ml), with the highest level of coffee acid (113.66 mg/L). Matcha contains significant amounts of antioxidant and anti-inflammatory substances. In recent years, there has been an increased consumption of matcha as a drink and as a component in various beverages, snacks, and other food products (Devkota et al., 2021). Compared to other teas, it has ten times more bioactive components and polyphenols (Sivanesan et al., 2021). When consumed regularly, it can promote health and prevent disease (Kochman et al., 2020). Matcha is effective in suppressing reactive oxygen species, inhibiting blood glucose accumulation, and stimulating lipid metabolism (Xu et al., 2016). Moringa leaves, on the other hand, due to their significant content of bioactive compounds, have pharmacological properties, including anti-diabetic, anti-inflammatory, anti-cancer, antioxidant, cardioprotective, and antimicrobial. Due to their nutritional properties, powdered moringa leaves have been used for supplementation as an additive to cookies, cakes, pastries, meats, juices, and sandwiches (Milla et al., 2021).

Drinks with kale and barley grass are also noteworthy. Drinks with kale had the most chlorogenic acid (39.17 mg/L) and synapic acid (83.92 mg/L), with barley grass coumaric acid (104.12 mg/L) and ferulic acid (154.82 mg/L). The level of polyphenolic compounds was always higher in products with more plant powders.

An important group of bioactive compounds found in plant raw materials are carotenoids. In the beverages evaluated, they were present in the highest amount in the drink, with 3% barley grass (7.79 mg/100 ml). Barley grass is rich in bioactive components and can be one of the best raw materials for the modern diet (Y. Zeng et al., 2018). High levels of these compounds were also observed in drinks with 3% chlorella (6.99 mg/100 ml) and kale (6.23 mg/100 ml). Microalgae are a natural source of nutrients and bioactive compounds, including carotenoids used in the food and pharmaceutical industries (Diprat et al., 2020). Microalgae have been attributed to antioxidant, immunomodulatory, and anti-cancer properties (Verni et al., 2023). Consumption of microalgae can help physiological needs for vitamin A. Currently, the food industry uses whole microalgae biomass or their extracted purified compounds as new ingredients to formulate food products such as baked goods, pasta, noodles, and plant milk (Ampofo & Abbey, 2022). Also, as a superfood, kale is recognized as an important source of carotenoids, whose colour is masked by green chlorophylls (Korus et al., 2023; Schmidt et al., 2021).

Chlorophyll has a similar chemical composition and structure to hemoglobin, participating in the exchange of oxygen and $CO_2$, thus aiding the healing process through good oxygenation (Ru et al., 2020). Beverages with 3% added vegetable powders had significantly more chlorophylls compared to products with 1% added. In 100 ml of beverage, high levels of chlorophylls were recorded in the drink with chlorella (30.46 mg), matcha (27.81 mg), kale (22.95 mg), and barley grass (18.24 mg), and wheatgrass (15.95 mg). Microalgae are well known for their production of high value pigments and bioactive compounds such as carotenoids and chlorophylls. Chlorella vulgaris can potentially mass-produce chlorophyll, reaching up to 1-2% of its dry weight (Safi et al., 2014). Due to its unique composition of bioactive compounds, matcha green tea offers a wide range of potential health benefits. It contains high concentrations of phenolic acids and chlorophyll, surpassing those in other green tea varieties. Thanks to its powdered form, it is an easy-to-use food ingredient (Kochman et al., 2020). Cereal grasses, including wheatgrass and barley grass, are also interesting ingredients in functional beverages. Young green barley powder is one of the best functional foods for providing nutrition and eliminating toxins from cells in human beings (El-Dreny & El-Hadidy, 2018). Barley grass is rich in chlorophyll and antioxidants and contains vitamins, and minerals (Y. Zeng et al., 2018). Also, wheatgrass juice is known as "green blood" due to its high content of chlorophylls, which make up 70% of its chemical components (Chauhan, 2014).

Green plant raw materials are characterized by a significant content of polyphenols and chlorophylls, which results into high antioxidant activity. In the evaluated beverages before pasteurization, the total antioxidant activity against the ABTS radical was in the range of 18.2 (C1) - 548.0 (M3) µmol Tx/ml. Drinks with matcha stood out as having the highest level of antioxidant activity, followed by those with moringa > wheatgrass > kale > barley grass> chlorella. Noteworthy is the significant antioxidant activity in beverages with matcha. Macha infusions have high antioxidant potential, the highest among all types of tea (Jakubczyk et al., 2020). Beverages with matcha also stood out for their high activity in the range of 62.1-106.1 µmol Tx/ml. Olvera-Aguirre et al. (2022) believe that the high antioxidant activity of moringa leaf extracts (260-343 mM Trolox/100 ml) represents the potential to use this raw material as a human or animal supplement. According to Yang et al. (2007), moringa leaves have a much higher antioxidant content than strawberry fruit, considered a rich source.

Table 3. Polyphenolic compounds (mg/L) of beverages before and after pasteurization—

| Type of beverage | G | P | Ch | Ca | Co | F | Si | Ci | TP |
|---|---|---|---|---|---|---|---|---|---|
| before pasteurization | | | | | | | | | |
| C1 | nd | nd | nd | 10.27 ± 0.08 | 4.56 ± 0.08 | 0.59 ± 0.11 | nd | nd | 15.42 |
| C3 | nd | nd | nd | 16.91 ± 0.19 | 15.37 ± 0.05 | 1.45 ± 0.11 | nd | nd | 33.73 |
| Gb1 | 4.02 ± 0.02 | nd | 0.92 ± 0.15 | 5.47 ± 0.08 | 26.65 ± 0.18 | 43.09 ± 0.22 | 0.91 ± 0.15 | nd | 82.06 |
| Gb3 | 8.54 ± 0.12 | nd | 1.76 ± 0.21 | 14.82 ± 0.13 | 104.12 ± 3.09 | 154.82 ± 0.2 | 6.49 ± 0.19 | nd | 290.55 |
| Gw1 | nd | nd | nd | 20.67 ± 0.12 | 19.66 ± 0.07 | 41.15 ± 0.10 | nd | nd | 81.48 |
| Gw3 | nd | nd | nd | 37.03 ± 0.12 | 55.60 ± 0.56 | 124.44 ± 0.21 | nd | nd | 217.04 |
| K1 | 2.31 ± 0.08 | nd | 20.81 ± 0.16 | 30.20 ± 0.07 | 5.39 ± 0.06 | 8.81 ± 0.11 | 31.73 ± 1.25 | nd | 99.25 |
| K3 | 6.48 ± 0.13 | nd | 39.17 ± 1.87 | 44.19 ± 0.06 | 15.46 ± 0.71 | 18.68 ± 0.22 | 83.92 ± 1.96 | nd | 207.90 |
| M1 | 103.05 ± 0.65 | 4.00 ± 0.03 | 0.91 ± 0.04 | 8.57 ± 0.07 | 18.53 ± 0.82 | 3.34 ± 1.12 | 2.56 ± 0.06 | 0.81 ± 0.06 | 141.77 |
| M3 | 228.11 ± 3.08 | 9.42 ± 0.44 | 2.06 ± 0.01 | 19.83 ± 0.06 | 34.12 ± 1.27 | 8.12 ± 1.23 | 6.46 ± 0.11 | 2.74 ± 0.06 | 310.89 |
| Mo1 | 5.20 ± 0.27 | nd | 1.63 ± 0.04 | 48.78 ± 0.02 | 9.35 ± 0.75 | 2.56 ± 0.81 | 0.21 ± 0.01 | nd | 67.73 |
| Mo2 | 8.18 ± 0.13 | nd | 4.06 ± 0.27 | 113.66 ± 0.02 | 20.26 ± 1.40 | 6.94 ± 0.20 | 0.46 ± 0.05 | nd | 153.56 |
| LSD p<0.05 | 2.580 | 1.337 | 1.551 | 1.896 | 2.315 | 5.246 | 1.908 | 0.244 | |
| after pasteurization | | | | | | | | | |
| C1P | nd | nd | nd | 5.39 ± 0.04 | 3.49 ± 0.05 | 0.49 ± 0.03 | nd | nd | 9.37 |
| C3P | nd | nd | nd | 14.07 ± 0.07 | 12.05 ± 0.06 | 1.11 ± 0.06 | nd | nd | 27.23 |
| Gb1P | 2.24 ± 0.06 | nd | 0.72 ± 0.05 | 3.69 ± 0.05 | 18.19 ± 0.87 | 33.17 ± 0.25 | 1.45 ± 0.07 | nd | 59.46 |
| Gb3P | 6.23 ± 0.12 | nd | 1.33 ± 0.17 | 10.12 ± 0.08 | 68.24 ± 0.37 | 113.50 ± 6.25 | 4.77 ± 0.14 | nd | 204.19 |
| Gw1P | nd | nd | nd | 12.53 ± 0.01 | 14.13 ± 0.81 | 34.39 ± 1.44 | nd | nd | 61.05 |
| Gw3P | nd | nd | nd | 26.92 ± 0.02 | 40.32 ± 1.12 | 103.38 ± 2.64 | nd | nd | 170.62 |
| K1P | 1.99 ± 0.04 | nd | 16.12 ± 0.84 | 19.88 ± 0.09 | 3.50 ± 0.07 | 7.04 ± 0.07 | 20.43 ± 1.48 | nd | 68.96 |
| K3P | 2.43 ± 0.04 | nd | 29.24 ± 0.41 | 36.70 ± 0.10 | 9.57 ± 0.07 | 15.13 ± 1.92 | 63.11 ± 2.72 | nd | 156.18 |
| M1P | 60.03 ± 2.91 | 1.62 ± 0.03 | 0.68 ± 0.04 | 6.89 ± 0.05 | 14.81 ± 0.63 | 3.08 ± 0.18 | 1.92 ± 0.13 | 0.51 ± 0.02 | 89.54 |
| M3P | 124.79 ± 5.19 | 4.35 ± 0.06 | 1.73 ± 0.06 | 17.55 ± 0.09 | 31.01 ± 0.57 | 7.74 ± 0.08 | 5.24 ± 0.10 | 1.64 ± 0.10 | 194.03 |
| Mo1P | 4.61 ± 0.08 | nd | 1.29 ± 0.11 | 38.67 ± 0.07 | 7.76 ± 0.03 | 2.21 ± 0.06 | 0.15 ± 0.02 | nd | 54.69 |
| Mo3P | 6.26 ± 1.19 | nd | 3.12 ± 0.05 | 101.83 ± 0.08 | 18.47 ± 0.47 | 5.57 ± 0.11 | 0.39 ± 0.02 | nd | 135.64 |
| LSD p<0.05 | 4.857 | 0.212 | 0.785 | 1.541 | 1.219 | 4.532 | 2.530 | 0.305 | |
| Total LSD | 3.575 | 0.617 | 1.130 | 1.636 | 1.752 | 4.644 | 2.060 | 0.178 | |

Values are presented as mean value ± SD (n=2), in fresh matter. Type of beverage: G - gallic acid, P - protocatechuic acid, Ch - chlorogenic acid, Ca - caffeic acid, Co - coumaric acid, F - ferulic acid, Si - sinapic acid, Ci - cinnamic acid, TP - sum of the determined phenolics
nd - not detected

In contrast to the ABTS test results, green raw materials containing chlorophyll have low antioxidant capacity according to the DPPH test. In beverages, antioxidant activity against the DPPH was significantly lower, compared to the ABTS test, ranging from 4.6 (Gb1) - 85.4 (M3) µmol Tx/ml. According to Pękal and Pyrzynska (Pękal & Pyrzynska, 2015), the presence of $Mg^{2+}$ ions in the structure of chlorophyll may impair the reaction between antioxidant compounds and the DPPH radical, leading to a lower antioxidant capacity than their actual potential.

Pasteurization is a traditional method of preserving food. It destroys microorganisms and enzymes and, at the same time, affects changes in physical parameters and antioxidant properties. For example, pasteurization has been shown to reduce polyphenols and vitamin C content (DeBenedictis et al., 2023). In the tested beverages after pasteurization, the level of TPC decreased by 21-64%, TFC by 23-49%, chlorophylls by 13-60%, and carotenoids by 2-36%. Losses of phenolic acids were also high, ferulic acid being the smallest (5-27%) and protocatechuic acid the largest (54-60%). The antioxidant capacity also decreased by 12-44% (ABTS) and by 5-27% (DPPH). After the heat treatment, matcha and moringa drinks still had the highest TPC and antioxidant activity.

**Colour and turbidity**

In recent years, the availability of different types of beverages has increased. Colour is an important factor influencing their acceptance (Mielby et al., 2018). Colour, as one of the most important senses of sight, can be an indicator of food quality/defects. It affects the perception of taste and flavour, so loss or change of colour may be viewed by consumers as a reduction in product quality (Gérard et al., 2022). Research suggests that there may be a correlation between the colours of plant raw materials and their effects on the body. Mirmiran et al. (2015) found that higher consumption of red/purple fruits and vegetables was associated with lower weight gain and abdominal fat, while yellow, green, and white were associated with lipid parameters. It is also believed that colour is also related to antioxidant capacity (Cömert et al., 2020).

The results of instrumental colour measurement in the CIE system ($L^*a^*b^*$) are shown in Table 4. The beverages were characterized by different colour parameters, which was due to the plant powders used with different intensities of green colour. Before pasteurization, beverages with chlorella were the darkest, with an average of $L^*$=15.12, with the highest proportion of green colour ($a^*$=-1.03) and the lowest yellow colour (b*=3.65). In contrast, beverages witch matcha were the brightest ($L^*$=30.77-36.36). The beverages C1, C3, K1, K3, Gw3 were dominated by green colour ($a^*$<0), Gb1, Gb3, Gw1, M1, M3, Mo1, Mo3 by red colour (a*>0) and all beverages by yellow colour (b*>0).

After pasteurization, the drinks darkened and became less green, which was related to the demonstrated degradation of chlorophylls. The drinks were also slightly less yellow, and the proportion of red increased. A decrease in colour saturation ($C^*$) and hue angle ($h^o$) was also observed, indicating a shift in colour toward red-purple and correlating with higher values of the $a^*$ parameter. The exceptions were beverages with chlorella (C1P and C3P), which became more yellow than those before pasteurization and had a more intense, saturated colour. The hue angle corresponds to the relative amounts of redness and yellowness where 0$^o$ /360$^o$ is defined for magenta colour, 90$^o$ for yellow, 180$^o$ for green, and 270$^o$ for blue. Chroma indicates the saturation or intensity of the colour (Wojdyło et al., 2019).

In addition to the colour, an important determinant of the quality of cloudy beverages is the turbidity stability. The high stability of the product's turbidity is a guarantee of non-sedimentation of suspended raw material particles during storage (Teleszko et al., 2016). Studies carried out by a number of authors deal with the evaluation of turbidity and its stability only in juices. Dietrich et al. (1996) indicate that turbidity stability of at least 50% with a turbidity of at least 250 NTU is necessary to classify juices as turbid. The plant powder beverages evaluated in the study were characterized by a turbidity level of 810 (K1) -13280 (M3) NTU, with a slight turbidity stability of 0.43% (M3) - 7.75% (K1) (Table 5). Higher stability was observed in beverages with lower turbidity. The reason for the low turbidity stability may have been the turbid beverage production technology used. The centrifugation process, designed to remove easily sedimented particles from the beverage, was not carried out. Yusuf et al. (2023) also emphasize the impact of raw material (variety, species), and Teleszko et al. (2016) fruit maturity on turbidity stability in juices. After pasteurization, the beverages became more turbid, the level of this parameter increased significantly by 13-39%, compared to non-heat-treated beverages. In addition, turbidity stability decreased by 17-37%.

Table 4. Changes in colour parameters of beverages before and after pasteurization

| Type of beverage | | | *a** | *b** | *C** | *h°* |
|---|---|---|---|---|---|---|
| before pasteurization | C1 | 17.11 ± 0.25 | -0.83 ± 0.03 | 3.21 ± 0.08 | 3.32 ± 0.07 | 104.5 ± 0.75 |
| | C3 | 13.12 ± 0.11 | -1.22 ± 0.01 | 4.09 ± 0.09 | 4.27 ± 0.09 | 106.7 ± 0.33 |
| | Gb1 | 25.28 ± 0.24 | 0.66 ± 0.09 | 19.02 ± 0.10 | 19.03 ± 0.10 | 88.0 ± 0.29 |
| | Gb3 | 28.55 ± 0.65 | 0.55 ± 0.12 | 20.84 ± 0.18 | 19.86 ± 0.08 | 87.3 ± 0.36 |
| | Gw1 | 27.69 ± 0.23 | 0.26 ± 0.02 | 18.44 ± 0.12 | 18.44 ± 0.21 | 89.2 ± 0.08 |
| | Gw3 | 29.22 ± 0.81 | -0.18 ± 0.02 | 22.23 ± 0.13 | 22.23 ± 0.34 | 90.5 ± 0.04 |
| | K1 | 23.40 ± 0.10 | -1.67 ± 0.03 | 12.99 ± 0.04 | 13.09 ± 0.28 | 90.9 ± 0.78 |
| | K3 | 31.43 ± 0.12 | -0.35 ± 0.02 | 23.13 ± 0.13 | 23.13 ± 0.27 | 97.3 ± 0.26 |
| | M1 | 30.77 ± 0.21 | 1.88 ± 0.10 | 23.10 ± 0.10 | 23.18 ± 0.08 | 85.3 ± 0.03 |
| | M3 | 36.36 ± 0.71 | 1.78 ± 0.12 | 25.54 ± 0.08 | 23.61 ± 0.13 | 85.7 ± 0.25 |
| | Mo1 | 26.56 ± 0.72 | 0.78 ± 0.21 | 25.92 ± 0.04 | 25.93 ± 0.08 | 88.3 ± 0.06 |
| | Mo3 | 29.55 ± 0.31 | 0.51 ± 0.27 | 27.78 ± 0.13 | 27.78 ± 0.14 | 88.9 ± 0.06 |
| | LSD p<0.05 | 0.636 | 0.149 | 0.314 | 0.320 | 0.621 |
| after pasteurization | C1P | 11.15 ± 1.09 | 0.25 ± 0.15 | 6.02 ± 0.93 | 6.03 ± 0.93 | 87.8 ± 1.24 |
| | C3P | 10.26 ± 0.38 | 0.09 ± 0.09 | 5.73 ± 0.41 | 5.73 ± 0.41 | 89.5 ± 0.96 |
| | Gb1P | 22.86 ± 0.37 | 1.75 ± 0.04 | 14.83 ± 0.30 | 14.94 ± 0.30 | 83.3 ± 0.13 |
| | Gb3P | 27.35 ± 0.80 | 2.41 ± 0.19 | 17.63 ± 0.31 | 17.80 ± 0.30 | 82.2 ± 0.65 |
| | Gw1P | 25.69 ± 0.21 | 1.75 ± 0.06 | 15.77 ± 0.21 | 15.87 ± 0.21 | 83.7 ± 0.16 |
| | Gw3P | 28.04 ± 0.15 | 1.98 ± 0.08 | 18.57 ± 0.15 | 18.68 ± 0.46 | 83.9 ± 0.34 |
| | K1P | 21.60 ± 0.13 | 0.83 ± 0.04 | 13.43 ± 0.87 | 13.50 ± 0.17 | 87.4 ± 0.28 |
| | K3P | 26.50 ± 0.09 | 0.19 ± 0.03 | 18.43 ± 0.22 | 18.49 ± 0.08 | 89.2 ± 0.26 |
| | M1P | 30.46 ± 0.37 | 2.81 ± 0.04 | 20.38 ± 0.30 | 20.58 ± 0.09 | 82.2 ± 0.09 |
| | M3P | 34.31 ± 0.80 | 2.58 ± 0.19 | 21.98 ± 0.31 | 22.13 ± 0.12 | 83.3 ± 0.23 |
| | Mo1P | 24.50 ± 0.13 | 3.72 ± 0.04 | 21.87 ± 0.37 | 22.18 ± 0.37 | 80.3 ± 0.09 |
| | Mo3P | 26.52 ± 0.09 | 3.53 ± 0.03 | 22.66 ± 0.22 | 22.94 ± 0.21 | 81.1 ± 0.15 |
| | LSD p<0.05 | 0.759 | 0.156 | 0.638 | 0.636 | 0.879 |
| | Total LSD p<0.05 | 0.682 | 0.149 | 0.490 | 0.491 | 0.742 |

Values are presented as mean value±SD (n=4), in fresh matter. Beverages with green plant powders: C - chlorella; Gb - barley grass; Gw - wheat grass, K - kale, M - matcha, Mo - moringa; 1, 3 - percentage addition of plant powder; P - pasteurization

Table 5. Turbidity and the stability of turbidity of the beverages before and after pasteurization

| Type of beverage | Turbidity, NTU | | Stability of turbidity, % | |
|---|---|---|---|---|
| | before pasteurization | after pasteurization | before pasteurization | after pasteurization |
| C1 | 4960 ± 308 | 5770 ± 265 | 1.43 ± 0.20 | 1.19 ± 0.10 |
| C3 | 9333 ± 404 | 11100 ± 111 | 0.76 ± 0.10 | 0.61 ± 0.07 |
| Gb1 | 2073 ± 93 | 2532 ± 118 | 3.12 ± 0.16 | 2.53 ± 0.20 |
| Gb3 | 5453 ± 314 | 6583 ± 176 | 2.62 ± 0.32 | 1.80 ± 0.19 |
| Gw1 | 2300 ± 140 | 3100 ± 140 | 4.13 ± 0.15 | 3.53 ± 0.32 |
| Gw3 | 5873 ± 280 | 7067 ± 167 | 2.32 ± 0.10 | 1.47 ± 0.21 |
| K1 | 810 ± 77 | 1082 ± 30 | 7.75 ± 0.76 | 5.86 ± 0.28 |
| K3 | 2597 ± 65 | 3649 ± 44 | 5.12 ± 0.55 | 3.57 ± 0.16 |
| M1 | 8673 ± 241 | 9817 ± 225 | 0.82 ± 0.13 | 0.64 ± 0.03 |
| M3 | 13280 ± 193 | 16160 ± 228 | 0.43 ± 0.08 | 0.31 ± 0.02 |
| Mo1 | 3217 ± 76 | 4487 ± 167 | 2.27 ± 0.07 | 1.43 ± 0.10 |
| Mo3 | 7747 ± 240 | 9233 ± 130 | 1.08 ± 0.03 | 0.79 ± 0.05 |
| LSD p<0.05 | 387.0 | 290.3 | 0.442 | 0.273 |
| Total LSD p<0.05 | 333.3 | | 0.358 | |

Values are presented as mean value±SD (n=4), in fresh matter. Beverages with green plant powders: C - chlorella; Gb - barley grass; Gw - wheat grass, K - kale, M - matcha, Mo - moringa; 1, 3 - percentage addition of plant powder; P - pasteurization

**Principal component analysis (PCA)**

The results of the principal component analysis of the tested samples are shown in Figure 1. Two factors were selected for analysis, which together accounted for 89.17% of the variability (factor 1: 65.34%, factor 2: 23.83%). The first factor shows a positive correlation with all analysed parameters of colour and turbidity, content of antioxidant compounds, and antioxidant activity (0.085-0.976). The second factor is negatively correlated with the content of polyphenols, flavonoids, antioxidant activity, and brightness of the samples (ranging from -0.094 to 0.562), while positively correlated with the content of chlorophylls and carotenoids and turbidity (0.268-0.958).

The size of their vectors determines the significance of variables, and the angles between them represent correlations between variables. Figure 1 shows that all the analysed variables have similar significance. Closely correlated are the content of TPC, TFC and antioxidant activity (DPPH and ABTS), and the content of chlorophylls and carotenoids to a lesser extent.

The samples can be divided into three groups. The first (Gb1, Gw1, Gw3, Mo1) is negatively correlated with both the first and second factors. The second (C1, C3, Gb3, K1, K3) is positively correlated with the second factor and negatively correlated with the first. The third group (M1, M3, Mo3) is positively correlated with the first factor and divided in terms of the second factor (M3 is weakly positively correlated, the other samples negatively). Clearly distinguished are sample C3, which contained the most carotenoids after pasteurization, and sample M3, richest in TPC and TFC and with the highest antioxidant activity of ABTS and DPPH, both before and after pasteurization (Figure 1, Table 2).

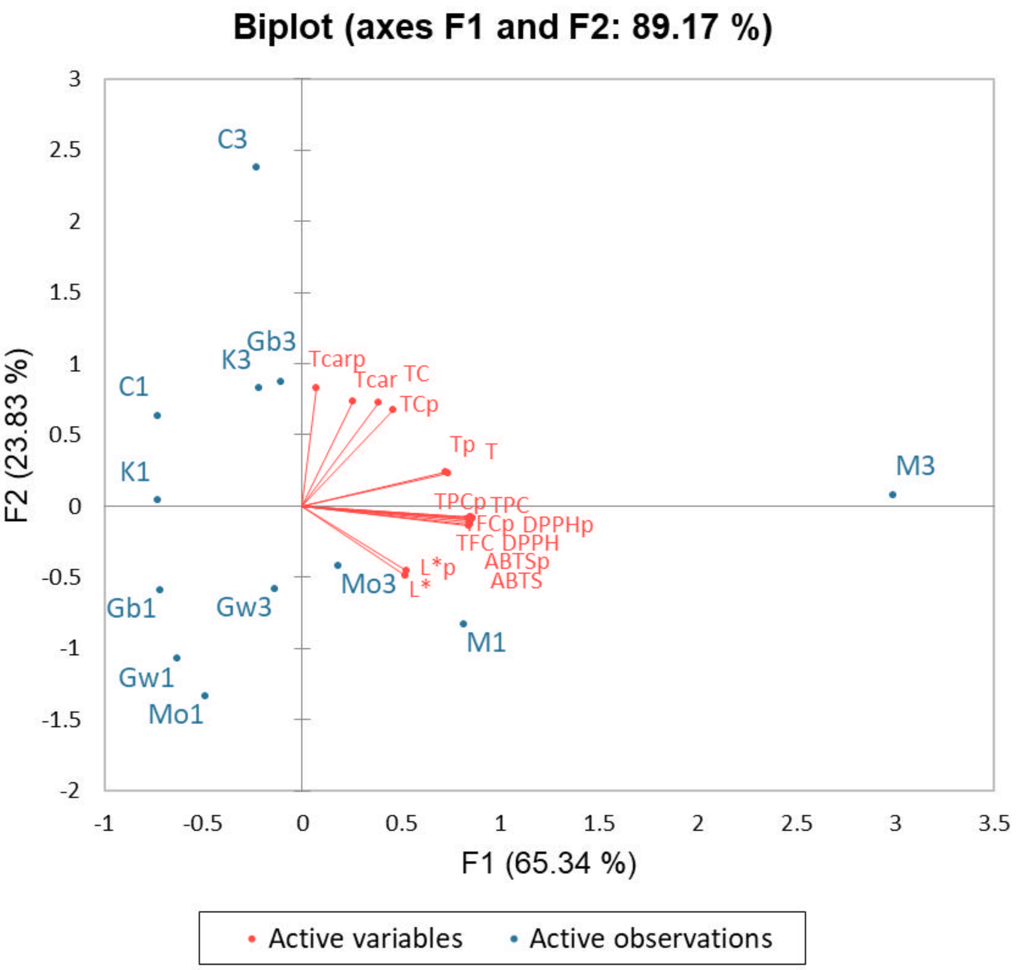


Figure 1. Principal Component Analysis (PCA) biplot for beverages with green plant powders: C - chlorella; Gb - barley grass; Gw - wheat grass, K - kale, M - matcha, Mo - moringa; 1, 3 - percentage addition of plant powder. TPC - total polyphenols content, TFC - total flavonoids content, TC - total chlorophylls content, Tcar - total carotenoids content, ABTS, DPPH - antioxidant activity against ABTS or DPPH free radicals, L* - lightness, T - turbidity. The index "p" indicates values after pasteurization

## CONCLUSIONS

Increased consumer awareness of health and proper nutrition has led to a strong demand for healthy foods, including beverages with functional and therapeutic properties. Consumers expect such products not only to hydrate but also to have a beneficial effect on the body. The right choice of beverages rich in health-promoting ingredients can be considered part of a healthy dietary pattern. Their consumption can, therefore, be a healthy alternative to other beverages available on the market.

Green powders can be a raw material for supplementing or enriching beverages with bioactive compounds. Drinks with green powders are a source of antioxidants, studies have shown. Among the green drinks evaluated, the most noteworthy are those with matcha and moringa with high antioxidant activity, and with chlorella and barley grass are a source of chlorophylls and carotenoids. It was shown that beverages before pasteurization had the highest quality.

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