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N. H. M. Rubel Mozumder, Hajee Mohammad Danesh Science and Technology University, Bangladesh Saadia Ambreen, The University of Lahore, Pakistan Agnieszka Ryznar-Luty, Wroclaw University of Economics, Poland Maria Flores Cordova, Autonomous University of Chihuahua, Mexico

*CORRESPONDENCE

Thomas Dippong, □ dippong.thomas@yahoo.ro

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Quality of fruit juices in terms of physico-chemical, microbiological, thermal and antioxidant properties, evaluation of stability during storage

Thomas Dippong*, Flavia Pop, Zorica Voşgan and Cristina Mihali

Faculty of Science, Technical University of Cluj-Napoca, Baia Mare, Romania

Introduction: The storage stability and quality of seabuckthorn, aronia and black currant juices, unsweetened and with added honey, was analyzed in terms of sensory, physico-chemical, microbiological, thermal and antioxidant properties.

Methods: 6 juices assortments were obtained and analyzed using fresh seabuckthorn with honey and without honey, aronia with honey and without honey, black currant with honey and a juice prepared of 50% seabuckthorn and 50% aronia without honey. The thermal analysis of juices which evaluated the thermal behavior of juice assortments in air up to 600 °C. The physico-chemical were analyzed: total sugar content, acidity, dry matter content, electrical conductivity, ascorbic acid content, polyphenol content, pH and antioxidant capacity. Sensory attributes of juices such as appearance, color, smell, taste, foreign bodies were analysed. The microbiological analysis monitored the degree of preservability over time, evaluating the samples immediately after unpacking and subsequently at 7-day intervals up to 56 days of refrigeration.

Results and Discussion: The thermal analysis of juices has been less studied; however, it is highly interesting due to the specifics provided on juice composition and practical uses, particularly the potential to produce dry powder forms of juices. Thermal behavior of juices was assessed. Based on thermal analysis, details on the juice composition were obtained such as dry matter, water content, honey content of juices. The juices showed low pH values (2.78 - 3.75), higher sugar content in the case of honey sweetened juices (38.4 -42.9 Brix) compared to juices without honey (9.5 - 18.3 Brix), high ascorbic acid content (62.4 - 94.19 mg/100 g), high concentrations of polyphenols (2211.47 -4614.17 mg EAG/L) and high antioxidant capacity as scavenging capacity of 2,2diphenyl-1- picrylhydrazyl (DPPH) (87.05 - 94.19 %). The microbiological analysis monitored the degree of preservability over time, evaluating the samples immediately after unpacking and subsequently at 7-day intervals up to 56 days of refrigeration. The microbiological quality of the juices remained within acceptable limits up to the 14th day of refrigeration, after which a significant increase in yeasts and molds was observed, exceeding 5 log CFU/mL. The

prepared juices were high in compounds with health benefits such as vitamin C, polyphenols, that showed a high antioxidant capacity and can be consumed up to 14 days of storage in refrigerated conditions.

KEYWORDS

juice, buckthorn, aronia, black currant, thermal analysis, microbiology 1

1 Introduction

Juices are a valuable source of micronutrients (phytochemicals) and compounds benefic to human health such as vitamin C, polyphenols, beta-carotene, lycopene, antioxidants compound responsive to the scavenging of free radicals showing antioxidant activity which may contribute to microbiological safety by inhibiting the spoilage of microorganisms and pathogens (Evrendilek and Özkan, 2024; Meremäe et al., 2024; Paunovic et al., 2024). Fruit juice, which is a rich source of vitamins, minerals, bioactive compounds, that maintain a nutritional balance as well as a combination of fibers, sugars, and acids along with polyphenols, flavonoids, carotenoids, and other organic components, is now widely accessible and consumed worldwide (Bal et al., 2011; Evrendilek and Özkan, 2024). Juices quality is degraded by several factors, such as fruit's quality, the processing method, and the activity of enzymes (pectin esterase, lipoxygenase, polyphenol oxidase, and polygalacturonase) (Lan et al., 2023; Mounir et al., 2024).

The following fruits for juices were used in this work: sea buckthorn, aronia, and black currant. Sea buckthorn fruits contain vitamins (B1, B2, E, K), fatty acids, trace elements, essential amino acids (threonine, valine, methionine, leucine, lysine, tryptophan, isoleucine and phenylalanine), polyphenols, tocopherols, carotenoids, flavonoids, necessary for human health (Bal et al., 2011; Song et al., 2023). Aronia melanocarpa L. fruits are one of the richest sources of polyphenols, including anthocyanins, flavonols, proanthocyanidins, and phenolic acids, which exhibit antioxidant action, a rich source of natural food pigments, and are popular as nutritional supplements (Ćujić et al., 2018; Daskalova et al., 2019; Parzonko et al., 2015; Yi et al., 2022). Although black aronia is difficult to eat due to its harsh, sour, unpleasant astringent taste and smell of pungent bitter almonds, however, it is used in various food industries to produce a variety of juices, preserves, extracts, fruit teas and nutritional supplements (Gajic et al., 2020). Blackcurrants are rich in phytochemicals such as phenolic compounds, volatile aromatic compounds, polyunsaturated fatty acids, anthocyanins, proanthocyanidins, quercetin, myricetin, isorhamnetin and phenolic acids (Tian et al., 2023; Paunovic et al., 2024; Pott et al., 2023). Sea buckthorn juice quality is influenced by variety, origin, and processing. Despite being abundant in vitamins, antioxidants, and other beneficial substances, the juice may taste sour due to its high organic acid concentration. Blackcurrant berries have a high chemical content due to differences in the cultivar's profiles, amounts of sugars, organic acids, volatile compounds, tannins, and other phenolic compounds (Mattila et al., 2016; Tian et al., 2023). Xia et al. reported the nutritional composition of sea buckthorn juice, which includes the following values: total soluble solids at 2.63%, total acidity at 6.34%, total sugar at 1.87%, vitamin C at 356.90 mg/ 100 mL, total phenols at 382.23 mg GAE/100 mL, and total carotenoids at 0.36 mg/100 mL (Xia et al., 2023). Sea buckthorn berries possess these food chemical constituents, which enhance their unique tastes and flavors (Xia et al., 2023).

The spoilage of fruit juices can be influenced by several factors such as pH, nutrient availability, the presence of antimicrobial compounds, and microbiota. The consequences of microbial spoilage lead to the development of undesirable flavors, increased $\rm CO_2$ content, and changes in color, texture, and appearance (Lawlor et al., 2009; Sospedra et al., 2012). Food safety and quality are a top priority for all stakeholders in the food chain, and everyone shares the common responsibility to ensure that food is safe and suitable for consumption (Baranyi et al., 2024).

The antioxidant character of juices is mainly reflected in their vitamin C content, which also has a stabilizing role, vitamin C levels in fruits juice may be decreased by enzymatic activities (Kietzmann, 2023). The time of harvest, transportation, storage type, storage duration, and kitchen preparation all affect the vitamin C concentration (Kietzmann, 2023). The content of vitamin C in currant berries far exceeds the content of this antioxidant compared to other berries (Djordjević et al., 2014). In previous research evaluating the overall phenolic content and antioxidant capacity of various fruits, chokeberry has been found to exhibit substantially higher total antioxidant capacity when compared to other fruits like cranberries, blueberries, and blackcurrants. Black chokeberry boasts a wealth of polyphenols, vitamins C and E, and essential minerals like copper and zinc (Denev et al., 2018; Zhang et al., 2021). Aronia extract, juices, and pomace may be useful as functional ingredients, given their polyphenol content (Shakoori et al., 2024; Yi et al., 2022). Honey's antimicrobial properties arise from several factors, such as its elevated sugar levels, low acidity, hydrogen peroxide generation, and the presence of certain substances like methylglyoxal and defensins. These components function synergistically to inhibit the growth and viability of different microorganisms such as bacteria, fungi, and certain viruses (Almasaudi, 2021). The health benefits of honey are attributed to its biochemical composition, which includes a diverse range of antioxidants. Honey contains phenolic compounds that demonstrate antioxidant properties, reducing oxidative stress and inflammation while additionally promoting the healing process. These bioactive substances improve honey's antimicrobial characteristics and offer further health advantages, including anti-inflammatory and immunemodulating effects. This versatile approach makes honey a desirable choice in integrative medicine, where natural products are gaining recognition for their ability to enhance conventional treatments (Vîjan et al., 2023; Wang et al., 2022). Its rich chemical composition contributes to its flavor and



FIGURE 1
Types of Juice: SBH (sea buckthorn with honey), SB (sea buckthorn), AH (aronia with honey), A (aronia), BCH (black currant with honey), SBA (50% sea buckthorn and 50% aronia).

sweetness and underpins its health benefits and therapeutic potential (Ogwu and Izah, 2025).

Fruit juices contain a microbiota that is normally present on the surface of fruits during harvesting and processing (Tournas et al., 2006). Yeasts form the main flora of fruits before processing include *Candida, Dekkera, Hanseniaspora, Pichia, Saccharomyces*, and *Rhodotorula*, which are frequently responsible for juice spoilage. These microorganisms can grow exponentially in fruit and vegetable juices at room temperature, leading to food spoilage (Sezer et al., 2022; Xia et al., 2023).

The purpose of this research adopts an interdisciplinary approach, combining physical-chemical, sensory, microbiological and thermal methods (TG-DTA), to provide a complex and integrated understanding of the changes that occur in natural fruit juices during storage.

The thermal analysis (TG-DTA) which is less studied was performed to assess the thermal behavior and to elucidate the decomposition processes and transformations in the juices composition. The sensory analysis of the juices was performed using a scaling method, aronia juice had an astringent taste. The physico-chemical methods include: total sugar content, acidity, dry matter content, electrical conductivity, ascorbic acid content, pH, antioxidant capacity as DPPH radical scavenging capacity, and total polyphenols analysis. The microbiological analysis tested the degree of juices preservation, immediately after unwrapping and at 7-day intervals up to 56 days of refrigeration. Berry juices offer a way to increase nutrient intake, particularly for those who do not consume enough fresh fruit. Furthermore, the study of berry juices can lead to the development of healthier, appealing juice products and inform strategies for promoting healthier eating habits.

The novelty of this study consists in the following: (i) the literature is enriched with the extensive characterization of different juices with and without honey, (ii) identifying the temperature range where honey decomposes and estimating the honey content in honey juices, (iii) the relationship between the thermal behavior, antioxidant properties and microbiological contamination, (iv) comparison between sensory and physicochemical quality as a results of the use of sea buckthorn, aronia

and black currant fruits in juices, (v) the influence on thermal behavior, antioxidant activity and microbiological parameters and, (vi) implementation of thermal analysis which is of great interest due to the specifics provided on juice composition and practical uses, particularly the potential to produce dry powder forms of juices.

2 Materials and methods

2.1 Materials

Figure 1 shows the types of analyzed juices: SBH (sea buckthorn with honey), SB (sea buckthorn), AH (aronia with honey), A (aronia), BCH (black currant with honey), SBA (50% sea buckthorn and 50% aronia). The juices were not dried, in all analyses they were used in their natural liquid state. These juices are concentrated juices, without water addition, being made up only of the fruit juice and the rapeseed honey which has not changed the natural flavor of the fruit. Each of the juices was processed in an appropriate environment to preserve product quality and prevent contamination, and after packaging were stored at refrigeration temperatures until the analysis were performed. The fruits were picked at the end of summer and the beginning of autumn, when they are fully ripe, having a high content of vitamin C and nutrients. The fruits were washed under cold water to remove any impurities, dried and cleaned, removing the leaves and impurities. The fruits were crushed into a pulp using a commercial fruit pulper (Linxiao Juicer Model X20, Linxiao Machinery Co., Ltd., Taizhou, China) operating at 850 rpm rotational speed and equipped with a 0.5 mm stainless steel sieve to remove skins and seeds. The juice was filtered through a fine sieve, mixed with honey and homogenized until the honey completely dissolves. The obtained juices were packed in plastic containers and kept under refrigeration at 0-4 °C for analysis performing.

2.2 Thermal analysis

A quantity of 100 mg of juices from six different samples was measured and introduced into a special crucible in the derivatograph

SDTQ600 (TA Instruments, New Castle, DE, United States). The curves of thermogravimetry (TG) and differential thermal analysis (DTA) were recorded. The registration of these curves was carried out by a SDT Q600 (TA Instruments, New Castle, DE, United States) instrument in air up to 600 °C with a speed of 5 °C/min heating rate using alumina standards.

2.3 Sensory analysis

The sensory analysis of the juices was performed according to the scoring quality assessment method (Dippong, 2017). The analysis was carried out by a group of 10 taster volunteers (five women and five men) aged between 20 and 30 years. The tasters examined the characteristics of the six juices by comparison with the scoring scale of 20 cumulated points for six juices according to the sensory analysis standards (Dippong, 2017). The juices were evaluated for appearance (using scores ranging from 0 (very poor) to five (excellent)), color (0-3), smell (0-3), taste (0-7) and foreign bodies (0-2).

2.4 Determination of total sugar content, carbohydrates content, dry matter, titrable acidity

Total sugar content was determined by a digital refractometer (PAL-RI, Tokyo, Japan) with the following technical characteristics: range of use 1.3306–1.5284, resolution of 0.0001, accuracy of 0.0003, measuring temperature between 5°C–45°C (with 1°C resolution), measuring time of 3 s, in accordance with the requirements of the EMC directive 93/68/EEC. The refractometer is calibrated with distilled water, after which 2 drops of the sample are placed on the optical prism and the sugar content is displayed in Brix degrees.

Determination of carbohydrates from juices was analyzed by UHPLC (1,260 Infinity II), which contains a quaternary pump (Agilent Technologies, G7111B, 1,260 Infinity II, Santa Clara, CA, United States), an Agilent Autosampler with an injection valve fitted with a 10 μ L sample loop. The separation was performed on a 2.5 μ m Polaris NH $_2$ 250 μ m \times 4.6 mm (Agilent Technologies, Santa Clara, CA, United States). All the samples were filtered through a 0.45 μ M PTFE filter for LC analysis.

The dry matter (DM) content was determined by the air oven method after drying 2 g sample at 105°C in a Universal Oven ULE 500 (Memmert GmbH + Co. KG, Schwabach, Germany) to a constant weight. The titrable acidity (TA) was measured by titrating 10 g of juice that had been homogenized with 100 mL distilled water. The initial pH of the samples was recorded before titration with 0.1 N NaOH. The acidity was expressed as the percentage of citric acid equivalent to the quantity of NaOH used for the titration (Jakobek et al., 2007).

2.5 Determination of vitamin C content

Vitamin C content was determined by spectrophotometric method using a UV-VIS spectrophotometer Perkin-Elmer Lambda 25 (Perkin-Elmer Lambda 25, United States, 2015).

10 g of sample was homogenized with 50 mL of 5% metaphosphoric acid and 10% acetic acid solution, then was transferred into a 100 mL volumetric flask and gently stirred until homogenized. It was diluted to the mark with 10% acetic acid and 5% metaphosphoric acid solution. The solution was filtered, and the obtained filtrate was collected for the determination of vitamin C. Bromine water was added to the filtrate to oxidize ascorbic acid into dehydroascorbic acid. A few drops of thiourea were added to remove excess bromine, thus obtaining a clear solution. The standard solution of ascorbic acid was introduced, and then 1 mL of 2,4-dinitrophenylhydrazine solution. The obtained mixture was maintained for 3 h at a temperature of 30 °C in a water bath. After incubation, the mixture was cooled in an ice bath, treated with 5 mL H₂SO₄ 85% solution, and constantly stirred. The absorbance was recorded at 254 nm, and results were expressed as milligrams per 100 mL of juice based on a standard calibration curve of ascorbic acid (0-50 mg/L).

2.6 Determination of total polyphenols content, β -carotene, lycopene and of 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging capacity

2.6.1 Determination of the total content of polyphenols

The total content of polyphenols was determined by the Folin-Ciocâlteu method, which was based on the reduction of the Folin-Ciocâlteu reagent by the polyphenols from the juice samples that have reducing capacity. The used method was adapted from those presented in the literature (Singleton et al., 1999; Jakobek et al., 2007; Bontsidis et al., 2021) and consisted of obtaining a juice extract by stirring 1 g of juice with 20 mL of 40% methanol extractant with 1% HCl. 1 mL of the juice extract was placed in a 100 mL volumetric flask over which distilled water, 1 mL of Folin-Ciocâlteu reagent and 15 mL of 7.5% Na₂CO₃ alkaline solution were added. The sample was made up to the mark with distilled water and kept in the dark for 2 h. The absorbance of the sample was read at a wavelength of 750 nm against a blank consisting of 40% acidic methanol solution. Beforehand, a calibration curve was drawn with gallic acid solutions of 10-500 mg/L concentrations. The results were expressed in mg gallic acid equivalents (EAG)/L juice taking into account the dilution made by mixing with the extractant solution. A UV-VIS spectrophotometer with double beam was used for the analysis of polyphenols (Perkin-Elmer Lambda 25, United States, 2015). The UV-VIS spectrophotometer was checked metrological every year. The quality of spectrophotometric measurements was ensured by performing every analysis in triplicate. Tests of inter-day reproducibility of absorbance of the blue complex formed by Folin-Ciocâlteu reagent and a 100 mg/L gallic acid standard solution were performed. The 2-days reproducibility of six samples, for each day, n = 12 was less than 2%. Control samples were used for every 10 samples analyzed. The measured concentrations of the control samples were in the range of 95%-105% of their values. The reagents used for spectrophotometric analysis were of PA degree and the solutions were prepared with distilled water.

2.6.2 Determination of β -carotene and lycopene content

The β -carotene and lycopene concentrations in the juices samples were determined according to literature Ivanova et al., 2023 by compounds extraction in acetone at room temperature in dimmed light, followed by their transfer in petroleum ether. 1 g juice was mixed with 50 mL acetone for 20 min, followed by filtration. The extraction was repeated twice mixing the residue with another portion of 30 mL acetone. The acetonic extracts were collected in a separating funnel where 75 mL of pethroleum ether were added. The extracts of β -carotene and lycopene were washed three times with water, treated with anhydrous sodium sulphate to remove water traces, collected into a 100 mL flask and brought to volume with pethroleum ether.

The concentrations of β -carotene and lycopene in the extracts were analysed by measuring the absorbances at 450 nm and 503 nm using a UV-Vis Perkin Elmer–Lambda 25 spectrometer according to Equations 1, 2 (Tongnuanchan et al., 2012; Ivanova et al., 2023).

$$β$$
-carotene ($μg/mL$) = 4.624 × A_{450} -3.091 × A_{503} (1)

lycopene (
$$\mu g/mL$$
) = 3.956 × A₄₅₀ -0.091 × A₅₀₃ (2)

2.6.3 Determination of 2,2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging capacity

Antioxidant activity was determined spectrophotometrically, using a Perkin-Elmer Lambda 25 spectrometer (Perkin-Elmer Lambda 25, United States, 2015). The method was adapted from that reported by Lime et al., 1957; Tongnuanchan et al., 2012. A quantity of 0.5 g of juice sample was mixed with 10 mL of methanol and stirred for 2 h. The sample was filtered and a volume of 3 mL of methanolic extract was taken and treated with 3 mL of ethanolic DPPH solution of 0.15 mM concentration (DPPH in 95% ethanol) and kept in the dark for 30 min at room temperature. The mixture was stirred, and the absorbance was measured at 517 nm. In parallel, a control sample was prepared consisting of 3 mL of 0.15 mM ethanolic DPPH solution and 3 mL of methanol, which was kept in the dark for 30 min. Antioxidant activity was calculated based on Equation 3.

$$A_{activity} (\%) = \left(1 - \frac{A_{sample}}{A_{blank}}\right) \cdot 100 \tag{3}$$

2.7 Determination of pH, electrical conductivity and dissolved oxygen in juices

To perform the determination, the samples were diluted with water, in a proportion of 1:1, 50% water and 50% juice. The pH was determined with an HI98161 pH meter (Hanna Instruments, Bucharest, Romania). For electrical conductivity and dissolved oxygen, a HI6522 Modular Multiparameter was used (Hanna Instruments, Bucharest, Romania). The pH electrode used was thoroughly cleaned after the analysis and stored in a KCl solution. Buffer solutions were used to calibrate the pH-meter before each series of measurements.

2.8 Microbiological analysis

For microbiological analysis, the juice samples were evaluated immediately after opening and at 7-day intervals until day 56 during storage at 4 °C. The total number of viable aerobic mesophilic bacteria, yeasts and molds, as well as Enterobacteriaceae and Staphylococcus sp., was determined to monitor microbiological changes that occur during storage. Each sample was analyzed in triplicate (3 technical replicates). The samples were serially diluted with 0.85% NaCl solution and 1 mL of each dilution was inoculated into Petri dishes with the corresponding culture medium. To control for potential contamination, uninoculated plates were used as negative controls. The developed colonies were counted, and the results were expressed as colony-forming units per milliliter (log CFU/mL).

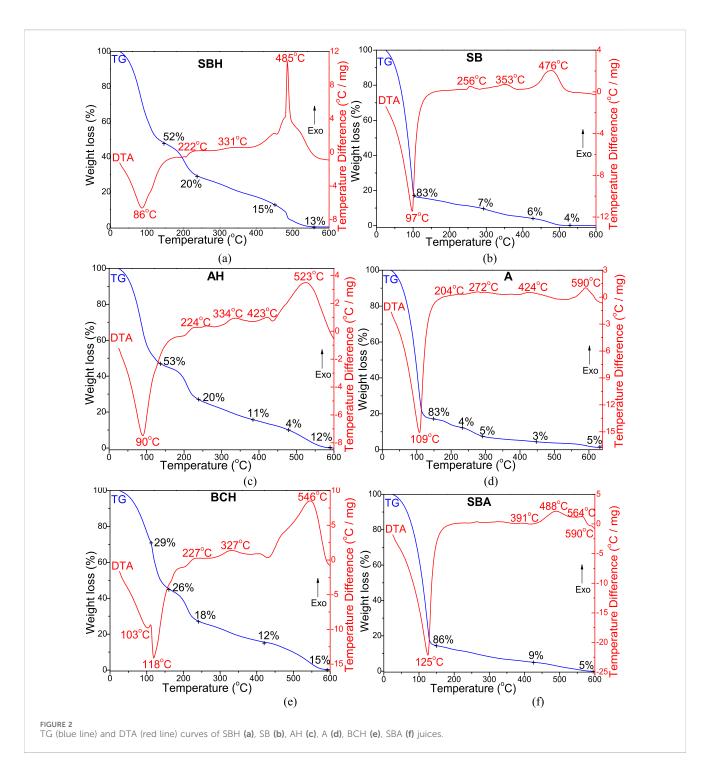
2.8.1 Mesophilic aerobic bacteria count

It represents a general sanitary microbiological indicator, which provides data on the state of contamination of the researched product. Plate count agar (Standard Methods Agar) was used to determine the total number of aerobic mesophilic colonies after incubation at 37 $^{\circ}$ C for 48 \pm 2 h (Hou et al., 2021).

The procedure consisted in making serial decimal dilutions of 10⁻¹, 10⁻² and 10⁻³. 1 mL of sample was added to 9 mL of sterile sodium chloride solution using the sterile pipette to obtain the first dilution (10⁻¹). This was followed by obtaining the other dilutions (i.e., to obtain the second dilution, 1 mL of the first dilution was added to 9 mL of sterile saline) and the inoculum was passed (1 mL of each diluted juice sample) in two Petri dishes for each dilution in part. Then, 12-15 mL of sterilized PCA culture medium cooled to 45 °C was poured onto each plate. The time elapsed between the end of the preparation of the initial suspension (dilutions) and the moment when the medium was poured into the plates should not exceed 45 min. The inoculum was carefully mixed with the medium by rotating the Petri dish and the mixture was allowed to solidify. After they had become solid, the prepared plates were turned face down and placed into the incubator at a temperature of $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. After the incubation period, the colonies on the plates were counted using Funke Gerber ColonyStar equipment, choosing the pair of boxes in which were developed between 30-300 colonies. The colonies in the two boxes were counted, the sum was multiplied by the dilution factor and divided by 2, being expressed as colonyforming units per milliliter (log CFU/mL).

2.8.2 Determination of the total number of yeasts and molds

The total number of yeasts and molds indicates the existence of improper hygienic conditions for obtaining and storing of the product. They can cause the alteration of products, modification of organoleptic characters, and their development can be accompanied by the release of some mycotoxins harmful to consumers. To count yeasts and molds, Sabouraud Dextrose Agar (SDA) with Chloramphenicol medium was used by incubation at 25 °C for 3–5 days (Ali et al., 2017). From the initial sample and the dilutions, 1 mL was passed with a sterile pipette in each prepared Petri dish, then were added 15 mL of SDA medium with Chloramphenicol, previously liquefied and maintained at 45 °C, in a water bath. The inoculum was carefully mixed, after which the



mixture was allowed to solidify. The boxes were placed with the lid down, in a place protected from light at 25 °C, for 4–5 days. After incubation, the developed colonies were counted from the pairs of boxes where the number falls between 10-100 colonies. The sum was multiplied by the dilution factor and divided by 2. The number of yeasts and molds in 1 mL of product was thus determined (log CFU/mL).

2.8.3 Determination of Enterobacteriaceae

Violet-red-beads-glucose agar (VRBG) medium was used for the detection of enterobacteria. The juice samples and their dilutions

were inoculated in two Petri dishes, over which the medium cooled to 45–50 °C was added, which had the appearance of a slightly opalescent or clear, red-purple gel. The plates were incubated at 37 °C for 24 h according to the ISO 21528-2:2017 method. Colonies that developed on the plates (dark purple surrounded by reddish halos) were counted and expressed as colony forming units per milliliter (log CFU/mL) for each sample.

2.8.4 Determination of Staphylococcus sp.

Detection of *Staphylococcus* sp. was achieved by cultivating on the selective and differential Baird-Parker agar supplemented with

egg yolk tellurite emulsion medium. Three Petri plates were inoculated from the juice samples and their dilutions for each juice variety, which were incubated at 37 °C for 36–48 h, in accordance with ISO 6888-1:2021.

2.9 Statistical analysis

Statgraphic XVIII programme was used to performe correlation analysis and Principal component analysis of the data aiming to find the similarities between the juice assortments and to reduce the dimensionality of the data set. All experiments were conducted in triplicate. The data were presented as the mean \pm standard deviation. Data were analyzed via factorial ANOVA using the General Linear Model in Minitab 19.1.1 (LEAD Technologies, Inc., Charlotte, NC, United States). Tukey's honest significance test was carried out at a 95% confidence level (p < 0.05).

3 Results and discussion

3.1 Thermal behavior of juices

The thermal behavior of SBH, SB, AH, A, BCH and SBA juices is presented through TG and DTA diagrams in Figure 2, where is shown the decomposition up to 600 °C. SBH (sea buckthorn with honey) and SB (sea buckthorn) juices, presented four stages: (i) the endothermic effect at 86-97 °C, with a mass loss of 52% (SBH had lower water loss, due to the honey content which did not decompose at this temperature) and 83% (SB had the highest weight loss due to higher water content) which corresponded to drying of juices and desorption of physically absorbed water molecules (at these temperature values, the heating of the shell can be related to moisture and water linked with the structure evaporation phenomenon (Melnikova et al., 2023)); (ii) the exothermic effect at 222-256 °C, with a mass loss of 7%-22% (SBH contained more volatile compounds and sugars than SB which was a watery juice) is attributed to the decomposition of flavor substances, polyphenols and sugars; (iii) the exothermic effect at 331-353 °C, with a mass loss of 6%-15% corresponded to the breakdown of fatty acids (saturated, monounsaturated, polyunsaturated), amino acids, proteins and carbohydrate (dextrose, sucrose, starch); (iv) the exothermic effect at 476-485 °C, with a mass loss of 4%-13%, corresponded to the decomposition of cellulose, hemicellulose and lignin (on the DTA diagram, a sharp peak was observed and on either side of this peak there were shoulders, the explanation lies in the separate decomposition of cellulose, hemicellulose and lignin in this type of juice) (Cai et al., 2019; Dippong et al., 2022; Dippong et al., 2023; Liu et al., 2024; Tian et al., 2016). Hemicellulose, a compound that possesses a lower degree of polymerization, is a mixture of various polymerized monosaccharides (mannose, xylose, galactose, glucose, and arabinose). At the same time, cellulose is a high-molecularweight compound consisting of a long linear chain of D-glucosyl groups (Cai et al., 2019).

AH (aronia with honey), A (aronia), BCH (black currant with honey) and SBA (50% sea buckthorn and 50% aronia) juices, showed five corresponding effects on the DTA curves: (i) the endothermic effect from 90-125 °C, with a mass loss of 53%, corresponded to

drying of juices and could be related to humidity and evaporation (SBA and A juices contained the highest amount with a mass loss of 83%-86%); (ii) the exothermic effect from 204-227 °C, with a mass loss of 4%-20% (the higher mass loss for AH and BC was due to the decomposition of honey in this temperature range) was attributed to the decomposition of flavor substances, polyphenols and the pectic polysaccharides; (iii) the exothermic effect at 227-391 °C, with a mass loss of 5%-12% corresponded to the decomposition of lipids (fatty acids) (saturated, monounsaturated, polyunsaturated), amino acids, proteins and carbohydrate breakdown; (iv) the broad exothermic effect at 423-488 °C (just in the case of AH, A and SBA), with a mass loss of 3%-4%, corresponded to the decomposition of cellulose; (v) the exothermic effect at 523-590 °C, with a mass loss of 5%-15%, corresponded to the decomposition of hemicellulose and lignin (in the case of AH, A and BCH, a broad exothermic effect was observed due to the decomposition of hemicellulose and lignin, while in SBA, the decomposition of hemicellulose and lignin was observed in two stages, clearly evident by the exothermic effects from 564 °C to 590 °C (Cai et al., 2019; Dippong et al., 2021; Dippong et al., 2022; Liu et al., 2024; Tian et al., 2016). The crystalline structure of cellulose renders its thermal degradation more difficult than that of hemicellulose. In addition, on account of the complex composition, the degradation of lignin and macromolecular substances was also more strenuous than that of hemicellulose (Cai et al., 2019).

In juices that do not contain honey, a highest water loss of 83%–86% was observed, up to 125 °C, while in honey juices in the temperature range of 120–227 °C, a mass loss of 18%–20% occurs. If we compare the results with juices without honey, a mass loss of 4%–7% was observed in the range of 120–227 °C, it can be assumed by the difference that the decomposition of bee honey was carried out in this temperature range and could be 13%–16%.

The total mass loss was 100% in all cases, this means that all organic compounds decomposed up to 600 °C without remaining carbonized residues and did not contain mineral compounds. The activation energy necessary for the thermal decomposition of a mixture of components depends on the energy barrier for the individual constituents and is influenced by the chemical composition of studied juices (Sunooj et al., 2011).

3.2 Sensory analysis

The sensory analysis was performed on juices: SBH (sea buckthorn with honey), SB (sea buckthorn with honey), AH (aronia with honey), A (aronia), BCH (blackcurrant with honey), SBA (50% sea buckthorn and 50% aronia). The juices had an intense orange color (those made from sea buckthorn or with sea buckthorn) or dark blue, a slightly viscous consistency, a sweet taste (those with added honey) or sour (those unsweetened), and a flavor specific to the fruits from which they were prepared. The appearance was analyzed in terms of uniformity, homogeneity, opacity, watery consistency or degree of concentration, by awarding a score from 5 (very good) to 0 points (altered). The color was analyzed according to intensity, degree of shine, uniformity, by awarding a score from 3-0 points. For the smell, between three and 0 points were awarded and was observed whether

TABLE 1 Results of sensory analysis for juice samples.

Sensory attribute	SBH	SB	АН	А	всн	SBA
Appearance	$4.7 \pm 0.5^{\rm b}$	4.2 ± 0.4^{ab}	4.8 ± 0.4 ^b	3.9 ± 0.3°	4.8 ± 0.4 ^b	4.0 ± 0.4°
Color	2.5 ± 0.3 ^b	2.7 ± 0.3 ^{bc}	2.8 ± 0.3 ^{bc}	2.6 ± 0.3 ^b	2.6 ± 0.3 ^b	2.0 ± 0.2 ^a
Smell	2.8 ± 0.2^{ab}	2.5 ± 0.3 ^a	2.9 ± 0.2 ^{ab}	2.3 ± 0.2°	2.9 ± 0.3 ^{ab}	2.4 ± 0.2°
Taste	6.6 ± 0.6^{ab}	6.3 ± 0.6^{ab}	6.8 ± 0.5 ^b	6.1 ± 0.5 ^a	6.8 ± 0.6 ^b	5.9 ± 0.5 ^a
Foreign bodies	1.9 ± 0.2 ^{ab}	2.0 ± 0.2 ^b	1.9 ± 0.1 ^b	1.8 ± 0.2 ^{ab}	1.9 ± 0.2 ^{ab}	1.5 ± 0.1 ^a
Total	18.5 ± 0.4 ^{bc}	17.7 ± 0.4 ^b	19.3 ± 0.3°	16.7 ± 0.3 ^{ab}	19.0 ± 0.4°	15.8 ± 0.3 ^a

SBH (sea buckthorn with honey), SB (sea buckthorn), AH (aronia with honey), A (aronia), BCH (black currant with honey), SBA (50% sea buckthorn and 50% aronia).

Values are expressed as mean ± standard deviation of three replicates for each parameter.

Different letters in the same line indicate statistically significant differences at p < 0.05 (Tukey's test).

TABLE 2 Results of physico-chemical analysis for juice samples.

Sample Parameter	SBH	SB	АН	А	ВСН	SBA
Total sugar content (Brix)	38.4 ± 3.6°	9.5 ± 0.9 ^a	42.9 ± 4.1 ^f	18.3 ± 1.8°	42.3 ± 4.3 ^f	14.5 ± 1.4 ^b
Titrable acidity (% citric acid)	3.86 ± 0.56°	2.68 ± 0.21 ^a	3.54 ± 0.48 ^b	3.12 ± 0.25 ^b	2.94 ± 0.31 ^{ab}	2.63 ± 0.16 ^a
Carbohydrate (%)	3.95 ± 0.35 ^d	0.93 ± 0.10 ^a	5.12 ± 0.50°	1.55 ± 0.14 ^{bc}	5.65 ± 0.55 ^{ef}	1.13 ± 0.10 ^b
Dry matter content (%)	19.3 ± 0.26°	17.4 ± 0.50 ^{bc}	16.3 ± 0.2 ^b	14.8 ± 0.4°	15.7 ± 0.21 ^{ab}	14.5 ± 0.32 ^a
рН	2.86 ± 0.25 ^a	3.57 ± 0.38 ^{bc}	3.75 ± 0.34°	2.78 ± 0.26 ^a	3.09 ± 0.29 ^b	3.38 ± 0.31 ^{bc}
Electrical conductivity (mS/cm)	1.54 ± 0.13 ^a	3.56 ± 0.28°	1.77 ± 0.15 ^{ab}	3.70 ± 0.33°	1.98 ± 0.17 ^b	3.62 ± 0.34°
Dissolved oxygen (mg/L)	6.32 ± 0.63 ^b	6.75 ± 0.68 ^{bc}	6.91 ± 0.69 ^{bc}	8.57 ± 0.85 ^d	4.81 ± 0.45 ^a	7.00 ± 0.70°

SBH (sea buckthorn with honey), SB (sea buckthorn), AH (aronia with honey), A (aronia), BCH (black currant with honey), SBA (50% sea buckthorn and 50% aronia).

Values are expressed as mean ± standard deviation of three replicates for each parameter.

Different letters in the same line indicate statistically significant differences at p < 0.05 (Tukey's test).

it was specific to the assortment, well specified, characteristic to the natural smell of the fruit, without foreign odors or other nuances and without astringent tints. The taste was analyzed from the perspective of the specificity of the fruit variety, well-pronounced, pleasant, specific to the natural fruit from which it comes, without the presence of other foreign taste nuances, awarded between 7-0 points. Foreign bodies were analyzed from the perspective of the absence of foreign bodies, without a gelatinous or watery appearance (2 points), the presence of foreign bodies (0 points) (Dippong, 2017). Juices with added honey had higher scores for all sensory characteristics. Honey improved the taste of the fruit juice, making it more pleasant to the taste. The highest total score was for aronia with honey juice (AH), and the lowest score was for SBA juice (50% sea buckthorn and 50% aronia), probably due to the lack of appropriate mixing from a sensory point of view. The preference series of the 10 tasters was: AH > BCH > SBH > SB > A > SBA (Table 1).

3.3 Physico-chemical analysis

According to Table 2, the samples with added honey showed a higher content of total sugar compared to the plain samples. The addition of honey in the case of sea buckthorn juice led to a 4 times higher sugar content compared to simple juice and for aronia juice

2.3 times higher. Honey brings to fruit juice an important supply of fructose and glucose easily assimilated by the body, while sugar contains sucrose that must be metabolized to be assimilated (Li et al., 2023; Feszterová et al., 2023). Sugars are polyhydroxyaldehydes or ketones and their derivatives or condensations. Depending on the number of molecules that form monosaccharides, they can be classified into three types: monosaccharides (such as glucose and fructose), oligosaccharides (such as sucrose and maltose), and polysaccharides (such as starch and cellulose) (Li et al., 2023). The dry matter content of the samples ranged from 14.5% to 19.3%. The highest value was determined for sea buckthorn with honey juice. Acidity is given by the content of organic acids in the product. A higher value of acidity was determined for the juice samples with the addition of honey. The highest titratable acidity was found for sea buckthorn with honey sample (3.86%), whereas the lowest was observed for the mixture 50% sea buckthorn and 50% aronia sample (2.63%) (Table 1). Denev et al., 2018, reported titratable acidity in the range of 0.89%-1.06% as citric acid in chokeberry juices from different growing seasons. Xia et al., 2023, have documented the nutritional profile of SB juice, which encompasses the following measurements: total soluble solids at $2.63\% \pm 0.06\%$, total acidity at $6.34\% \pm 0.07\%$, total sugar at $1.87\% \pm 0.06\%$ 0.00%, vitamin C at 356.90 \pm 8.13 mg/100 mL, total phenols at 382.23 ± 2.58 mg GAE/100 mL, and total carotenoids at 0.36 \pm 0.00 mg/100 mL.

Insoluble dietary fiber is a complex of polysaccharides, including cellulose, hemicellulose, and lignin. These components serve as the primary building blocks of cell microfibrils. The presence of insoluble dietary fiber in juices can enhance nutritional value and provide various health benefits, such as: digestive health, increased satiety, glycemic control, and cancer prevention (Liu et al., 2024). Honey juices had a higher carbohydrate content AH (5.12%), BCH (5.65%), found mainly in the form of sugar and fiber. The exact proportion of carbohydrates in juices varies depending on the processed fruit, processing methods, and added ingredients (Liu et al., 2024).

Previous research indicated that the concentration of soluble solids in black chokeberry juices varied between 18.15% and 25.61% across different growing seasons (Denev et al., 2018), while other studies reported levels of 13.30%–20.99% (Sidor and Gramza-Michałowska, 2019) or 12.50%–20.10% in commercially available juices sourced solely from black chokeberry (Veberic et al., 2015).

All the samples were acidic, the pH varied between 2.78 and 3.75. A high conductivity was found in all juices, which showed that these samples were dense, had a high viscosity and were rich in minerals and ionized organic acids. The pH values were low, being influenced of the degree of ripeness of the fruit. The pH values and fluctuations found during juice analyses may be impacted by the region's predominant soil type and the buildup of organic matter (Adesakin et al., 2020). In chokeberry juice (A) the electrical conductivity was the highest (3.62 ms/cm), and the lowest value of conductivity was found for chokeberry juice with honey (AH), with a value of 1.77 ms/cm. Dissolved oxygen values were low in most cases, except for chokeberry juice (8.57 mg/L), which had the greatest exposure to the attack of microorganisms. Blackcurrant juice with honey (BCH) had the lowest concentration of dissolved oxygen (4.81 mg/L), followed by SBH (6.32 mg/L) and SB (6.75 mg/ L), being the least exposed product to the action of microorganisms.

3.4 Antioxidant capacity

3.4.1 Ascorbic acid content

The juice enriched with honey showed a higher content of vitamin C compared to simple juice. It has been shown that the daily consumption of honey for a period of 2 weeks in doses of 1.2 g honey/kg body weight, increased the concentration of vitamin C in the blood by 47%, of β -carotene with 3% and other antioxidants with 19%, compared to the group that did not consume honey (Li et al., 2023). The highest vitamin C content was obtained in the case of sea buckthorn juice with honey (78.9 mg vitamin C/100 g sample), and the lowest concentration in the mixture of 50% sea buckthorn and 50% aronia juice (62.4 mg vitamin C/100 g sample). Feszterová et al., 2023 showed that to ensure the highest vitamin C content in fruit juices, it is best to store the juice in glass containers, at a temperature of 4 °C, and for as short a time as possible. Denev et al., 2018, reported that the ascorbic acid content varied from 37 to 92 mg/ 100 g in 23 Bulgarian aronia samples.

3.4.2 Polyphenol, β -carotene, lycopene content and DPPH radical scavenging capacity

It was observed that unsweetened aronia juice was the richest in polyphenols followed by sea buckthorn juice mixed with aronia (50% sea buckthorn and 50% aronia) and aronia juice sweetened with honey. The addition of honey, led to a decrease in the concentration of polyphenols by decreasing the proportion of fruit in the composition of the juice. This aspect was observed in both aronia and sea buckthorn juices. In sea buckthorn juice, the addition of honey led to a decrease in the concentration of polyphenols by 30.64%, and in the case of aronia juice by 31.09% (Table 2). Sea buckthorn and aronia juice had a polyphenol content equal to the average concentration of polyphenols in simple sea buckthorn and aronia juices. Aronia juice was the richest in polyphenols followed by sea buckthorn juice mixed with aronia juice, sea buckthorn juice and then blackcurrant juice sweetened with honey. Other studies have shown that aronia juice has a particularly high content of polyphenols. Jakobek et al., 2007, reported a higher content of polyphenols in fresh aronia juice, 7194.40 ± 78 mg/L and 5435.06 ± 31 mg/L in blackcurrant juice, higher than those determined in the present study. For sea buckthorn juice, Mendelová et al., 2016, indicated a content of polyphenols varying between 2000-2920 mg/L depending on the variety of sea buckthorn used to prepare the juice, the values were lower than those determined in the present study. In the study of Mendelová et al., 2016, the polyphenol content was not analyzed immediately after preparation, but after several months of storage. The fruit juices were prepared from fresh fruit and preserved with sodium benzoate and stored under refrigerated conditions.

A substantial amount of the phytonutrients in the human diet are polyphenols, which are phytochemicals present in meals originating from plants, such as fruits, vegetables, and grains (Rasouli et al., 2017). They are potent antioxidants in vitro and are thought to have many potential beneficial effects on health, for example, reducing the risk of cardiovascular disease, cancer, neurodegenerative diseases, diabetes and osteoporosis. However, more studies are needed to fully understand how these molecules interact with human physiological and pathological processes. In food, polyphenols may contribute to the bitterness, astringency, color, flavor, and oxidative stability of products (Mattila et al., 2016). Polyphenols include phenolic acids, flavonoids, stilbenes and lignans that are present in plants in a wide range of forms, including glycosides, esters and aglycones (without linked groups). Compounds are often divided into three groups according to structural characteristics such as phenolic acids, flavonoids and aggregated non-flavonoids or other polyphenols (Rasouli et al., 2017). Plant polyphenols are secondary types of plant metabolites with anti-inflammatory, antioxidant and other biological properties, can inhibit the reproduction of various viruses and harmful bacteria, and can prevent and treat a variety of cardiovascular diseases (Huang et al., 2023).

 β -carotene and lycopene were only found in juices containing sea buckthorn, respectively in sea buckthorn juice, sea buckthorn juice with honey, and in sea buckthorn and chokeberry juice. β -carotene concentrations were slightly higher than those of lycopene, by approximately 1.07–1.1 times (Table 3). Both compounds have antioxidant capacity and are beneficial to human health. Ingested through food, β -carotene and lycopene help manage and prevent a number of diseases, including cancer, metabolic, inflammatory, cardiovascular, hepatic, ophthalmic, skeletal, and infertility disorders (Tufail et al., 2024). In aronia and currant juices, β -carotene and lycopene were absent, but these juices contain flavonoids and anthocyanins.

TABLE 3 The vitamin C, total polyphenol, β -carotene and lycopene content, DPPH radical scavenging capacity of juice samples.

Sample	Vitamin C comtent (mg/100 mL)	Polyphenol content, mg EAG/1,000 mL	β -carotene, mg/ 1,000 mL	Lycopene, mg/ 1,000 mL	Scavenging capacity of DPPH %
SBH	78.9 ± 0.62^{d}	2211.468 ± 26.691 ^a	87.18 ± 0.67 ^b	80.76 ± 0.63 ^b	94.19 ± 0.88°
SB	66.8 ± 0.53 ^b	3188.604 ± 29.521°	114.82 ± 0.90°	104.28 ± 0.94°	93.23 ± 0.62 ^{bc}
AH	72.1 ± 0.59°	3179.429 ± 18.23°	-	-	91.68 ± 0.82 ^b
A	64.5 ± 0.50 ^{ab}	4614.17 ± 22.253°	-	-	89.37 ± 0.62 ^{ab}
ВСН	66.8 ± 0.54 ^b	2767.247 ± 14.153 ^b	-	-	87.05 ± 0.83 ^a
SBA	62.4 ± 0.49 ^a	3968.485 ± 22.267 ^d	58.31 ± 0.97 ^a	45.96 ± 0.54 ^a	91.82 ± 1.14 ^b

SBH (sea buckthorn with honey), SB (sea buckthorn), AH (aronia with honey), A (aronia), BCH (black currant with honey), SBA (50% sea buckthorn and 50% aronia).

Values are expressed as mean \pm standard deviation of three replicates for each parameter.

Different letters in the same column indicate statistically significant differences at p < 0.05 (Tukey's test).

TABLE 4 Correlation analysis matrix (Pearson) of the physico-chemical characteristics of juices.

Variable	TSC	TA	DMC	рН	Carb	EC	DO	Vitamin C	SC-DPPH	Lyc	β-car	T-polyph
TSC	1											
TA	0.71	1										
DMC	0.30	0.62	1									
рН	-0.07	-0.27	-0.07	1								
Carb	0.98*	0.57	0.24	0.01	1							
EC	-0.95*	-0.77	-0.57	-0.01	-0.91**	1						
DO	-0.55	-0.01	-0.30	-0.11	-0.65	0.61	1					
Vitamin C	0.64	0.91**	0.88**	-0.12	0.53	-0.82**	-0.26	1				
SC-DPPH	-0.25	0.31	0.65	0.26	-0.36	-0.03	0.23	0.51	1			
Lyc	-0.47	-0.09	0.65	0.12	-0.49	0.17	-0.06	0.26	0.79	1		
β-car	-0.50	-0.14	0.60	-0.26	-0.52	0.20	-0.06	0.22	0.79	0.99*	1	
T-polyph	-0.62	-0.50	-0.81**	-0.07	-0.62	0.81**	0.78	-0.78	-0.29	-0.37	-0.34	1

TSC, total sugar content; TA, titrable acidity; DMC, dry matter content; Carb, carbohydrate content; EC, electrical conductivity; DO, dissolved oxygen; SC-DPPH, scavenging capacity of DPPH; lyc, lycopene; β -carcotene; T-polyph, total polyphenol content.

**P < 0.05.

P-values below 0.01 indicate statistically significant non-zero correlations at the 99.0% confidence level while P-values below 0.05 indicate statistically significant non-zero correlations at the 95.0% confidence level. Correlation coefficients with high positive or negative values are written in bold.

All juices had high antioxidant activity, ranging between 87.05% and 94.19% (Table 3) compared to the control sample containing no juice but methanol mixed with the DPPH solution. The highest antioxidant activity was found in sea buckthorn juice sweetened with honey, followed by plain sea buckthorn juice and sea buckthorn juice mixed with chokeberry. In this case, the addition of honey had the effect of slightly increase of the antioxidant activity, probably due to the antioxidant contribution provided by honey, which is rich in antioxidants (Shakoori et al., 2024). The main classes of antioxidants present in honey as well as in fruits and in the fruit-derived products are phenolic compounds, carotenoids, tocopherols, ascorbic acid and its derivatives (Pena Junior et al., 2022).

3.5 Correlation analysis and principal component analysis

The physico-chemical characteristics of juices samples, namely, total sugar content (TSC), titrable acidity (TA), dry matter content (DMC), pH, carbohydrate content (Carb), electrical conductivity (EC), dissolved oxygen (DO), vitamin C content, scavenging capacity of DPPH (SC-DPPH), lycopene (Lyc), β -carotene (β -car) and total polyphenol content (T-polyph) that were shown in Tables 2,3 were statistically analyzed using correlation analysis and Principal component analysis to reduce the variability of data.

^{*}P < 0.01.

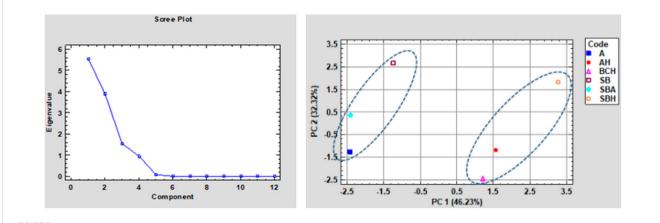


FIGURE 3
Principal component analysis of fruit juices based on their 12 physico-chemical characteristics (total sugar content, titrable acidity, dry matter content, pH, carbohydrate content, electrical conductivity, dissolved oxygen, vitamin C content, scavenging capacity of DPPH, lycopene, β -carotene and total polyphenol content) (left) Eigenvalues of components; (right) Diagram of juice samples based on the two principal components PC1 and PC two.

The correlation analysis of data (Table 4) showed high positive correlations between β -carotene and lycopene, titratable acidity and vitamin C, EC and vitamin C, carbohydrate and total sugar content, dry matter content and vitamin C, while other variables showed negative high correlations: EC and total sugar content, EC and vitamin C, carbohydrate and EC.

The results of principal component analysis are shown in Figures 3. Principal component analysis was conducted aiming to obtain a small number of liniar combinations of the variables considered (12 physico-chemical characteristics of the juices samples) which account for most of the variability in the data.

Figure 3 (left) depicted the eingenvalue of the components extracted by linear combination of the 12 variables. The principal component analysis showed three components extracted that present eigenvalues higher than or equal to 1.0: component one (eigenvalue of 5.55) account for 46.23% of the variability in the original data, component 2 (eigenvalue of 3.88) which account for 32.32% and component 3 (1.53) which account for 12.76% (Figure 3) (left), while component 4 with an eigenvalue of 0.96 account for 7.96% of the variability (Figure 3) (left). The first two principal components which account were choosen to represent the juices samples (Figure 3) (right). The total percentage of the first three principal components together accounted for 91.31% of the variability in the original data. The first two principal components PC1 and PC 2 were used to represent the juice samples (Figure 3) (right) with the aim of finding possible similarities.

The juice samples (observations) were represented in two dimensions diagram shown in Figure 3 (right) where the axes are component 1 and component 2. The honey juices formed a distinct group in the diagram, while the juices without honey are also grouped due to their similarity considering their lower dry matter, lower carbohydrate concentrations, higher titratable acidity and lower value of pH.

The principal component analysis showed that honey addition to juices modify the properties of juices by increasing their carbohydrate content, total sugar content and decreased their EC values and total polyphenol concentrations as well as their vitamin C concentrations.

3.6 Microbiological analysis

Natural juices are extremely vulnerable to spoilage by microorganisms (Xia et al., 2023), especially yeasts and molds could grow exponentially in fruit and vegetable juice at room temperature (McKay et al., 2011). The source of fresh fruit juices contamination comes from the microflora that is normally present on the surface of the fruit, from the air and soil. The results of the microbiological analysis on the variants of juices based on sea buckthorn, aronia and black currants can be seen in Table 5. This analysis was necessary to determine the validity period of the analyzed samples from the moment the sample was opened up to 56 days, during which the juices were kept under appropriate conditions, following the process of contamination and multiplication of microorganisms. With the extension of the storage period at 4 °C, the microbial load increases variably depending on the variety. Upon opening, no degree of contamination was observed, and with the passage of time, a progressive increase in CFU/mL was observed.

The limited number of juice variants (n = 6) is due to the fact that this study was designed as an initial exploratory investigation. Although the results provide valuable insights into microbial stability and the antimicrobial effect of honey in fruit juices, they should be regarded as a solid foundation for potential future research expansion. Future studies that involve biological replicates and a broader variety of formulations would help validate and expand these findings.

The microbiological evolution during storage revealed that, under refrigeration at $4\,^{\circ}$ C, the microbial load increased progressively over time, with variation depending on the juice composition.

In the case of the analyzed juice samples, the number of microorganisms (bacteria, yeasts, molds) registered a progressive increase, and reached a high quantitative level at 56 days of refrigerated storage (Table 5). This was also reported in other studies (Iqbal et al., 2015; Yi et al., 2022; Xia et al., 2023) whose results showed that unpasteurized fruit juices were highly contaminated, especially after 4 weeks of refrigerated storage. The monitoring of the general state of contamination for sea buckthorn,

TABLE 5 Microbial changes in the analyzed juices during storage.

Microbiological analysis	The number of microorganisms (log CFU/mL*)									
	Upon opening	7 days	14 days	21 days	28 days	56 days				
Sea buckthorn juice with honey - SBH										
Mesophilic aerobic bacteria count	n.d	1.19 ± 0.14 ^a	1.76 ± 0.11 ^{ab}	2.49 ± 0.09 ^b	3.05 ± 0.06°	4.87 ± 0.02 ^d				
Yeasts and molds	n.d	n.d	1.29 ± 0.20°	2.53 ± 0.05 ^b	3.16 ± 0.04°	5.05 ± 0.04 ^{de}				
Enterobacteriaceae	n.d	n.d	n.d	n.d	n.d	n.d				
Staphylococcus sp.	n.d	n.d	n.d	n.d	n.d	n.d				
Sea buckthorn juice without added honey - SB										
Mesophilic aerobic bacteria count		1.32 ± 0.21 ^a	1.99 ± 0.07 ^{ab}	2.51 ± 0.04 ^b	$3.09 \pm 0.04^{\circ}$	4.95 ± 0.02 ^d				
Yeasts and molds	n.d	n.d	1.31 ± 0.15 ^a	2.60 ± 0.02 ^b	3.18 ± 0.05°	5.09 ± 0.02 ^{de}				
Enterobacteriaceae	n.d	n.d	n.d	n.d	n.d	n.d				
Staphylococcus sp.	n.d	n.d	n.d	n.d	n.d	n.d				
	Aronia	juice with adde	d honey - AH							
Mesophilic aerobic bacteria count	n.d	1.36 ± 0.07 ^a	2.04 ± 0.12 ^{ab}	2.58 ± 0.08 ^b	3.22 ± 0.08°	5.12 ± 0.06 ^{de}				
Yeasts and molds	n.d	n.d	1.33 ± 0.15^{a} 2.63 ± 0.03^{b}		3.19 ± 0.09°	5.19 ± 0.10 ^{de}				
Enterobacteriaceae	n.d	n.d	n.d	n.d	n.d	n.d				
Staphylococcus sp.	n.d	n.d	n.d	n.d	n.d	n.d				
	Aronia	juice without ad	ded honey - A							
Mesophilic aerobic bacteria count	n.d	1.37 ± 0.14 ^a	2.19 ± 0.03^{ab}	2.59 ± 0.05 ^b	3.52 ± 0.06 ^{cd}	5.61 ± 0.09 ^{de}				
Yeasts and molds	n.d	n.d	1.61 ± 0.18 ^a	2.83 ± 0.11 ^b	3.25 ± 0.02°	5.85 ± 0.14°				
Enterobacteriaceae	n.d	n.d	n.d	n.d	n.d	1.41 ± 0.27 ^a				
Staphylococcus sp.	n.d	n.d	n.d	n.d	n.d	n.d				
	Black o	currants juice with	n honey - BCH							
Mesophilic aerobic bacteria count	n.d	1.35 ± 0.14 ^a	2.02 ± 0.12^{ab}	2.57 ± 0.06 ^b	3.11 ± 0.05°	5.02 ± 0.02 ^d				
Yeasts and molds	n.d	n.d	1.34 ± 0.14 ^a	2.65 ± 0.04 ^b	$3.21 \pm 0.06^{\circ}$	5.21 ± 0.08^{de}				
Enterobacteriaceae	n.d	n.d	n.d n.d		n.d	n.d				
Staphylococcus sp.	n.d	n.d	n.d	n.d	n.d	n.d				
Sea buckthorn juice and aronia juice 50% of each fruit - SBA										
Mesophilic aerobic bacteria count	n.d	1.36 ± 0.13 ^a	2.08 ± 0.10 ^{ab}	2.60 ± 0.02 ^b	3.30 ± 0.06°	5.33 ± 0.08 ^{de}				
Yeasts and molds	n.d	n.d	1.52 ± 0.28 ^a	2.77 ± 0.09 ^b	3.23 ± 0.04°	5.41 ± 0.15 ^{de}				
Enterobacteriaceae	n.d	n.d	n.d	n.d	n.d	0.99 ± 0.16 ^a				
Staphylococcus sp.	n.d	n.d	n.d	n.d	n.d	n.d				

n.d. - not detected (count ≤ 10 CFU/mL).

Values are expressed as mean ± standard deviation of three replicates.

Different letters in the same line indicate statistically significant differences at p < 0.05 (Tukey's test).

aronia and blackcurrant juices, plain and with the addition of honey, with aerobic mesophilic bacteria, yeasts and molds, enterobacteria, indicated differences depending on time and the variety of fruit.

The initial results, upon opening the samples, showed the absence of any microorganism in all analyzed samples, which suggested proper hygiene during the preparation and packaging

of the product. After the first 7 days of storage, the results showed a development of aerobic mesophilic bacteria to 1.19 ± 0.14 log CFU/mL in sea buckthorn juice, and to 1.37 ± 0.14 log CFU/mL in aronia juice; while yeasts, molds and enterobacteria were absent (Table 5). After 14 days, there was an increase in aerobic mesophilic bacteria in a proportion of 47.9% for sea buckthorn with honey, 49.63% for blackcurrant juice with honey, 50% for aronia juices with honey,

50.8% for sea buckthorn, 52.94% for sea buckthorn and aronia 1:1, and 59.85% for aronia juices without honey.

After 21, respectively 28 days, the values regarding aerobic mesophilic bacteria presented in fruit juices were maintained within the accepted limits <4 log CFU/mL (Gulf Standards. 2000; Codex Stan., 2005), but an increase was observed in sea buckthorn juice and aronia juice 50% of each fruit (3.30 ± 0.06 log CFU/mL) and aronia (3.52 \pm 0.06 log CFU/mL) juice without added honey. These data are in accordance with other findings (Tasnim et al., 2010; Rahman et al., 2011; Ndife et al., 2022) who reported the number of bacteria in fruit juices within the standard limits, but some situations were reported where the microbial load was above the standard consumption limit (Bagde and Tumane, 2011; Rashed et al., 2013; Yi et al., 2022; Xia et al., 2023). After 56 days of storage, a significant deterioration of the juices was observed, with microbial counts exceeding five log CFU/mL, thereby surpassing the maximum limits established by relevant food safety standards (Gulf Standards, 2000, Codex Stan. 2005), which generally recommend a threshold of <4 log CFU/mL. This was especially evident in samples containing aronia and a mixture of aronia and sea buckthorn without honey. Therefore, at the end of the storage period at 4°C, the degree of contamination of the analyzed juices is presented in the following increasing order: SBH →SB → BCH \rightarrow AH \rightarrow SBA \rightarrow A. The addition of honey to juices, through a possible synergistic effect with bioactive compounds in the fruits, such as vitamin C, polyphenols, and organic acids, can enhance the preservative action of the juices, thereby contributing to the slowing of microbial growth and the extension of the shelf life.

Yeasts and molds were counted in fruit juices at 14 daysof storage, with values between 1.29 \pm 0.20 and 1.61 \pm 0.18 log CFU/ mL, with a higher degree of contamination in aronia varieties without added honey (A). It follows an increase of yeasts and molds of over 50% on the 21st day of determinations for all samples, with values between 2.53 \pm 0.05 and 2.83 \pm 0.11 log CFU/mL. The results were above compliance, according to the legislation in force (Commission Regulation (EC) 2073/2005). According to de (Correa et al., 2024), juices should present yeasts and molds in a maximum number of 10² CFU/mL, i.e., < 2 log CFU/mL. After 28 days, were recorded values of 3.16 \pm 0.04 log CFU/mL for SBH, and of $3.25 \pm 0.02 \log CFU/mL$ for A, but after 56 days values were >5 log CFU/mL for all juice samples. The degree of contamination is presented in the following ascending order: SBH \rightarrow SB \rightarrow AH \rightarrow BCH \rightarrow SBA \rightarrow A. In terms of fungal contamination, the juices can be consumed within the first 14 days after opening by keeping them at refrigeration temperature (4 °C). Over this time interval (at 21, 28, respectively 56 days), the juices were no longer safe to drink, and the obtained results confirm their inappropriateness from a microbiological point of view.

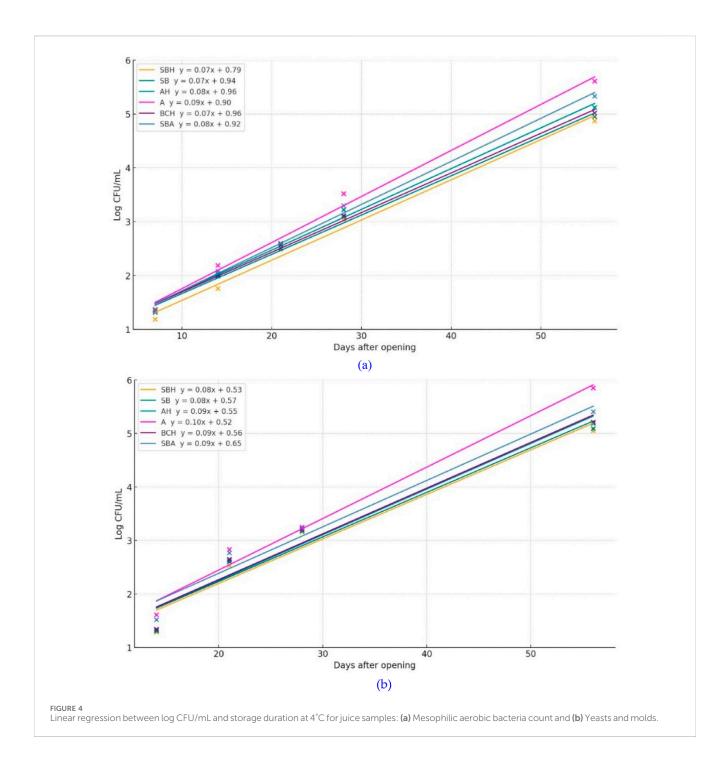
Enterobacteriaceae contamination of juice samples was not detected in the first 28 days after opening, only after 56 days in aronia samples A (1.41 \pm 0.27 log CFU/mL), respectively sea buckthorn juice and aronia juice 50% of each fruit (0.99 \pm 0.16 log CFU/mL) SBA sample. Enterobacteriaceae were absent in the samples with added honey. Usually, Enterobacteriaceae are sensitive in acidic environments (Sawada and Yamada, 2024), in the three varieties of juice where the acidity had the highest values were not detected throughout the monitored period.

In the case of *Staphylococcus* detection, the values reported as \leq 10 CFU/mL correspond to the detection limit of the method and do not reflect an exact number of microorganisms. It rather indicates a very low presence or absence, below the quantifiable threshold. In all analyzed samples, *Staphylococcus* sp. was either not detected or was present at levels \leq 10 CFU/mL, remaining within the permitted limits by food safety regulations (Commission Regulation (EC) No 2073/2005). These findings support the hypothesis of an antimicrobial effect exerted by the bioactive compounds naturally present in berry juices. The content of major bioactive components in sea buckthorn, chokeberry, and blackcurrant berries makes them true resources against infectious agents. Similar results regarding the antimicrobial properties of berries against potentially pathogenic bacteria such as *Staphylococcus sp* were also reported (Criste et al., 2020; Daoutidou et al., 2021; Ren et al., 2022; Trajković et al., 2023).

Although the Fisher LSD test allowed for the comparison of average microbial values between juice variants at each time point, it did not capture the overall dynamics of microbial growth during storage. To better understand the microbial evolution over time, linear regression models were applied to the microbial load data over the 56-day storage period, as presented in Figure 4.

The validity of fruit juices also varies depending on the assortment, because some fruits are used both for consumption and for the natural antimicrobial agents they contain. Their microbiological quality can be improved by honey addition, which exhibits antimicrobial activity and helps preserve the drinks (Gomashe et al., 2014). In general, sea buckthorn berries (Hippophae rhamnoides L.) retain their nutritional value and medicinal efficacy in juice (Abliz et al., 2021), maintaining the natural taste described as sour and astringent (Ma et al., 2017). The sugar content of sea buckthorn fruits was quite low, which makes the acidity more obvious, the source of the acidity is due to the high organic acid content (Liu et al., 2022). This is also evident in the case of the pH of the natural juice subjected to research, both plain (pH = 2.78) and with the addition of honey (pH = 2.86), which allows for a higher degree of preservation than for the other varieties of juice. The total number of aerobic mesophilic bacteria, yeasts and molds in sea buckthorn juice with honey compared to the other varieties of juice, Enterobacteriaceae and Staphylococcus sp. were not detected during the entire monitored period.

Black aronia fruits (Aronia melanocarpa L.) are notable for high levels of phenolic compounds, with strong antioxidant properties and other functional attributes beneficial to human health (Bontsidis et al., 2021; Bontsidis et al., 2024), but they may have different antimicrobial effects (Denev et al., 2019). This could also be observed in the case of the analyzed juice samples through an increase in the number of microorganisms, with higher values than in other types of juice for aerobic mesophilic bacteria, as well as for yeasts, molds and Enterobacteriaceae. The added honey showed a beneficial effect of inhibiting bacterial growth compared to the simple aronia juice, where microbial growth was recorded due to the high content of dissolved oxygen (8.57 mg/L). Blackcurrant berries (Ribes nigrum L.) are characterized by a high content of sugar, acids and pectin (Laaksonen et al., 2014), the juice with the added honey had a good storage capacity over time. The number of microorganisms falls within the intermediate values of buckthorn juice, respectively aronia, throughout the monitored



period. Honey added to fruit juice contributed to improving the antibacterial properties and helped to remove contaminants, a fact also proven in the research conducted by (Iqbal et al., 2015).

4 Conclusion

The study analyzed and evaluated the nutritional quality, physicochemical properties, microbiological contamination and thermal behavior of seabuckthorn, aronia and black currant juices, unsweetened and with added honey. In thermal

analysis, only in the case of BCH juice, there was observed a differentiated decomposition of water in juice, respectively honey, but in the case of SBA juice, the separate decomposition of cellulose was observed, compared to hemicellulose and lignin. In all TG thermal diagrams, water loss and decomposition of volatile aromatic compounds, fatty acids, amino acids, proteins, sugars, polyphenols, cellulose, hemicellulose and lignin were observed. In juices without honey, the mass loss due to water volatilization was 83%–86%, up to 125 °C, while in honey juices in the temperature range of 120–227 °C, the mass loss was 18%–20%. In juices without honey,

a mass loss of 4%-7% was found in the range of 120-227 °C, and the difference of 13%-16% in mass loss was given by the decomposition of bee honey. The total mass loss was 100% in all cases, this means that all organic compounds decompose up to 600 °C and do not contain mineral compounds. The highest sensory total score was for aronia with honey juice and the lowest score was for SBA juice (50% sea buckthorn and 50% aronia), probably due to the lack of appropriate mixing from a sensory point of view. The preference series of the 10 tasters was: AH > BCH > SBH > SB > A > SBA. The addition of honey significantly (p < 0.05) influenced the total sensory score in the case of sea buckthorn juice, respectively (p < 0.01) in the case of aronia juice. High contents of phenolic substances and high values of antioxidant properties were observed in the studied products. Black chokeberry juice is an excellent source of vitamin C and polyphenols and should be incorporated into the diet as a fruit juice with remarkable health benefits, although it is difficult to accept by consumers, mainly due to its high astringency. Total sugar content and carbohydrate content were significantly (p < 0.001) influenced by the addition of honey in the case of sea buckthorn and aronia juices. The assessment of the microbial contamination level of the juices enabled the determination of their shelf life, highlighting the significant impact of microorganisms on the microbiological stability and storage duration of the products. The results of the measurements confirmed that the studied juices were in accordance with the quality standards up to the 14th day of refrigeration when they can be recommended for public consumption. After this interval, the microbial counts progressively increase, particularly those of yeasts and molds, which exceed the threshold of five log CFU/mL by day 56, as well as those of mesophilic aerobic bacteria, reflecting the deterioration and decline in the microbiological quality of the products. Enterobacteriaceae were absent in most samples, except for low levels detected at day 56 in aronia juice and in the 50:50 mixtures of aronia and sea buckthorn juices. Staphylococcus sp. was not detected in any of the samples during the entire storage period. Juice varieties with added honey showed the lowest degree of microbial contamination. The addition of honey positively influenced the microbiological stability of the fruit juices due to its natural antimicrobial properties, acidic pH, and the osmotic effect exerted by the sugars present, which inhibit the growth of bacteria, yeasts, and molds. This study provides important information for consumers concerning the nutritional microbiological stability of seabuckthorn, aronia and black currant juices during refrigerated storage.

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Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

TD: Funding acquisition, Supervision, Writing – original draft, Software, Writing – review and editing, Formal Analysis, Investigation, Visualization, Resources, Data curation, Validation, Methodology. FP: Visualization, Validation, Formal Analysis, Resources, Writing – review and editing, Writing – original draft. ZV: Writing – original draft, Formal Analysis, Visualization, Resources, Validation, Writing – review and editing. CM: Resources, Writing – original draft, Formal Analysis, Validation, Writing – review and editing, Visualization.

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