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Chokeberry Pomace Utilization for Improving Selected Quality Parameters of Green Tea Leaves or Hibiscus Flower Infusions

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Abstract: The utilization of food industrial by-products, especially pomace after juice processing, could be a big challenge. Chokeberry pomace is a valuable source of phenolic compounds, including procyanidins and anthocyanins. These compounds have, e.g., antioxidant, anticancer, hypoglycemic, hypotensive, hepatoprotective and cardioprotective effects. In this work, dried chokeberry pomace (2.0 g, 2.5 g and 3.0 g) was added to green tea leaves or white or red hibiscus flowers (0.5 g of each) to obtain mixtures for preparing infusions. Such products could be a valuable source of bioactive compounds. After brewing and cooling, selected quality parameters of the obtained infusions were tested: pH, total phenolic content (TPC), anthocyanin content and the instrumentally and sensorily determined color. The composition of the infusion had an impact on the color. Those from a mixture of chokeberry by-products and hibiscus possessed lower L* and higher a* and b* values than those composed of chokeberry and green tea leaves. Infusions prepared from the mixture containing dried chokeberry pomace (3.0 g) and green tea leaves (0.5 g) had a higher content of anthocyanins (17%) and phenols (48%), respectively, than other samples with the same proportions of ingredients. According to PCA analysis, the highest content of the tested bioactive compounds was obtained in infusions of green tea leaves with the addition of chokeberry pomace.

Keywords: green tea; hibiscus; infusion; phenols; anthocyanins; color; by-products; utilization

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1. Introduction

Beverages obtained by brewing various dried parts of plants (e.g., leaves, flowers, fruits or roots) are very popular in many countries. They are valued by consumers for their attractive taste and aroma as well as their positive health effects. Plant-based infusions can also replace high-sugar beverages in the diet as a part of the recommended daily fluid intake for adults [1].

One of the most popular raw materials used for the industrial production of infusions are green tea leaves and hibiscus flowers [2–5]. Both raw materials are good sources of bioactive compounds. Up to 30% of the dry weight of green tea is made up of polyphenols such as flavanols, flavandiols and phenolic acids. Catechins are the most significant flavonoids and make up around 10% of the dry weight basis. The biologically active catechins in green tea are catechin, epicatechin, epigallocatechin, gallocatechin gallate, epigallocatechin gallate and epicatechin gallate [6]. Studies have indicated that catechins are important in weight control [7] and cancer prevention [8,9]. They also have significant anti-inflammatory, antioxidant and chemopreventive properties [10]. Hibiscus flowers are also a good source of bioactive compounds. They are important in the prevention of civilizational diseases. In water infusions from this raw material, the following phenolic compounds were identified: derivatives of chlorogenic acid, quercetin, kaempferol and myricetin [2,11]. Infusions obtained from red flowers also contain anthocyanins, mainly delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside [12]. Studies indicate the antioxidant, hepatoprotective, anti-cancer and anti-diabetic activities of hibiscus extracts [4].

Currently, the high content of compounds positively affecting human health in many food products is very desirable. It is possible to manipulate the composition of the product to achieve this effect. Literature data indicate the possibility of improving the quality of infusions as well. One way is through the addition of the by-product from the fruit industry [13]. For instance, peels after pre-treatment or pomace remaining after juice production are often sources of a higher content of bioactive compounds than the final products [14]. By-products can be not only an addition to infusions, but also their base. Studies on the use of chokeberry pomace to obtain infusions were conducted by Bober and Oszmiański [15]. They found that the pomace infusion contained more polyphenols and had a better flavor than the whole fruit infusion. Chokeberry is a raw material rich in phenolic compounds, especially anthocyanins and procyanidins [16]. Literature data indicate, e.g., the high antioxidant, anti-inflammatory, antidiabetic, hepatoprotective and hypotensive potential of these compounds [17,18]. Some authors have also suggested the cardioprotective and anticancer effects of extracts from black chokeberry fruits [19,20]. Chokeberry is often processed into nectar because its unfavorable flavor limits its consumption in its fresh form. The addition of chokeberry pomace to infusion can not only contribute to increasing the content of health-beneficial compounds, but also give the desired color from the consumer's point of view. A study by Vagiri and Jensen [21] indicates that the content of anthocyanins in pomace from chokeberries may range from 239 to 384 mg/100 g. In juices, this content is significantly lower and within the range of 130 to 210 mg/100 g.

The studies on the use of by-products in the preparation of infusions have, so far, mainly focused on ingredients such as banana peel [22], avocado peel [23], onion peel [24], dragon peel [25] and a mixture of the by-product from different sources [13]. The aspect of using dried chokeberry pomace as components of infusions has not been extensively studied.

The aim of this study was to determine the effect of the addition of dried chokeberry pomace to green tea leaves or white or red hibiscus flowers on the selected quality parameters of the obtained infusions. The pH, total phenolic content (TPC), anthocyanin content and instrumentally and sensorily determined color of the obtained infusions were checked. An additional aspect not yet investigated was the determination of the influence of the other compounds (green tea leaves, hibiscus flowers) on the extractability of active compounds from the chokeberry by-product to infusions.

2. Materials and Methods

2.1. Materials

Dried green tea leaves (GTL) originated from China, dried white hibiscus flowers (WHF) originated from Nigeria and dried red hibiscus flowers (RHF) originated from Egypt were purchased from internet stores. Dried chokeberry pomace (CP) after juice pressing was obtained from GreenField Sp. z o.o. Sp. k. (Warsaw, Poland). Food grade citric acid was purchased from a local market. Ingredients were weighted in the amounts presented in Table 1 and mixed and crushed for 10 s at 4400 rpm in a Thermomix TM31 (Vorwerk & Co. KG, Wuppertal, Germany).

2.2. Infusion Preparation

Each mixture for infusions was put into a ceramic cup, followed by pouring 200 mL of boiling tap water (pH of water: 7.0) and mixing with a teaspoon. Then, the cup was covered with a ceramic cover and left for 3 min. After this time, the infusion was filtered through a sieve lined with a cheesecloth and left to cool to room temperature. In the literature, we found brewing time ranging from 3 min to even 1200 min [26,27]. In our study, the shortest time of 3 min was chosen, which is often recommended by manufacturers. A long brewing time can lead to undesirable changes, such as the degradation of anthocyanins [28]. Each sample was prepared in three replicates. All analyses were performed in infusions at room temperature.

Table 1. Composition of tea infusions.

Sample Code	Dried Green Tea Leaves [g]	Dried White Hibiscus Flowers [g]	Dried Red Hibiscus Flowers [g]	Dried Chokeberry Pomace [g]	Citric Acid [g]
GTL	0.5	0.0	0.0	0.0	0.05
GTL + CP2.0	0.5	0.0	0.0	2.0	0.05
GTL + CP2.5	0.5	0.0	0.0	2.5	0.05
GTL + CP3.0	0.5	0.0	0.0	3.0	0.05
WHF	0.0	0.5	0.0	0.0	0.05
WHF + CP2.0	0.0	0.5	0.0	2.0	0.05
WHF + CP2.5	0.0	0.5	0.0	2.5	0.05
WHF + CP3.0	0.0	0.5	0.0	3.0	0.05
RHF	0.0	0.0	0.5	0.0	0.05
RHF + CP2.0	0.0	0.0	0.5	2.0	0.05
RHF + CP2.5	0.0	0.0	0.5	2.5	0.05
RHF + CP3.0	0.0	0.0	0.5	3.0	0.05

GTL—dried green tea leaves (0.5 g); WHF—dried white hibiscus flowers (0.5 g); RHF—dried red hibiscus flowers (0.5 g); CP—dried chokeberry pomace; 2.0, 2.5 and 3.0—indicate pomace addition in g.

2.3. Analysis of pH

The pH analysis of infusions was conducted using an electronic pH-meter HI 2223 (Hanna-Instruments, Smithfield, VA, USA). The instrument probe was directly immersed in the infusion, and the result was read from a pH-meter display.

2.4. Analysis of Color

The color of infusions was evaluated by instrumental analysis as well as by a sensory panel. Instrumental color analysis was performed by using a Konica Minolta 3600d spectrophotometer (Konica-Minolta, Osaka, Japan). The color parameter of the CIE L*a*b* system was assessed using D65 illuminant and a 2° observation angle. Light was transmitted through a sample held in a 10 mm thick glass cuvette. L* (lightness), a* (green-red component) and b* (yellow-blue component) were recorded. Then, the total color differences (ΔE) between infusions with and without the addition of chokeberry pomace were calculated according to the following equation [29]:

$$\Delta E = \sqrt{\left(L_0^* - L_{CP}^*\right)^2 + \left(a_0^* - a_{CP}^*\right)^2 + \left(b_0^* - b_{CP}^*\right)^2} \quad (1)$$

where L_0^*, a_0^*, b_0^* are the color parameters of infusions without pomace addition, and L_{CP}^*, a_{CP}^*, b_{CP}^* are the color parameters of infusions with pomace addition.

Sensory color assessment was performed by ten panelists, in the range of 20 to 45 years old, the same number of males and females, with formal classroom training in sensory evaluation. Samples were evaluated on a 5-point scale: 1—brown, cloudy, non-accepted; 2—weakly brown, detectable clouds, weakly desirable; 3—pink, brown shadow, clear, average desirable; 4—pink towards red, clear, intensive, desirable; 5—intensive red, clear, very desirable. About 200 mL of samples were presented in transparent plastic cups (top diameter 70 mm, bottom diameter 44 mm, height 95 mm). Coded samples, about 1 h after preparation, were tested by panelist in the room intended for sensory analysis under white light [30].

2.5. HPLC Determination of Anthocyanins

The HPLC methodology developed by Oszmiański and Sapis [31] was applied for anthocyanin analysis. An Agilent 1260 Infinity system (Agilent, Santa Clara, CA, USA) equipped with a Zorbax C18 column (150 × 4.6 mm, 5 µm) was used. Detection was performed by the diode array detector (DAD) set to scan mode between 400 and 700 nm. Anthocyanin content in samples was calculated as milligrams of cyanidin-3-*O*-glucoside per 100 mL using a five-point standard curve (1–5 mg/100 mL).

2.6. Total Phenolic Content (TPC)

The Folin–Ciocalteu reagent was used for TPC analysis. TPC content was calculated as gallic acid equivalent (GAE) mg/100 mL of a sample using a ten-point standard curve (0–50 mg/100 mL). The detection wavelength was set at 765 nm using a spectrophotometer Helios α (Thermo Separation Product, Waltham, MA, USA) against the blank [32].

2.7. Statistical Analyses

ANOVA (two-way analysis) and the Tukey test were performed at $p < 0.05$. The correlation between the variables was tested using the Pearson correlation. Principal component analysis (PCA) was performed for the following variables: content of phenolic compounds, anthocyanins, pH value and L*a*b* color parameters. Statistica 13.1 software (TIBCO Software Inc., Palo Alto, CA, USA) was used for statistical analyses.

3. Results and Discussion

3.1. pH Values of Infusions

Figure 1 shows the effect of the addition of chokeberry pomace to green tea leaves and hibiscus flowers on the pH value of the obtained infusions.

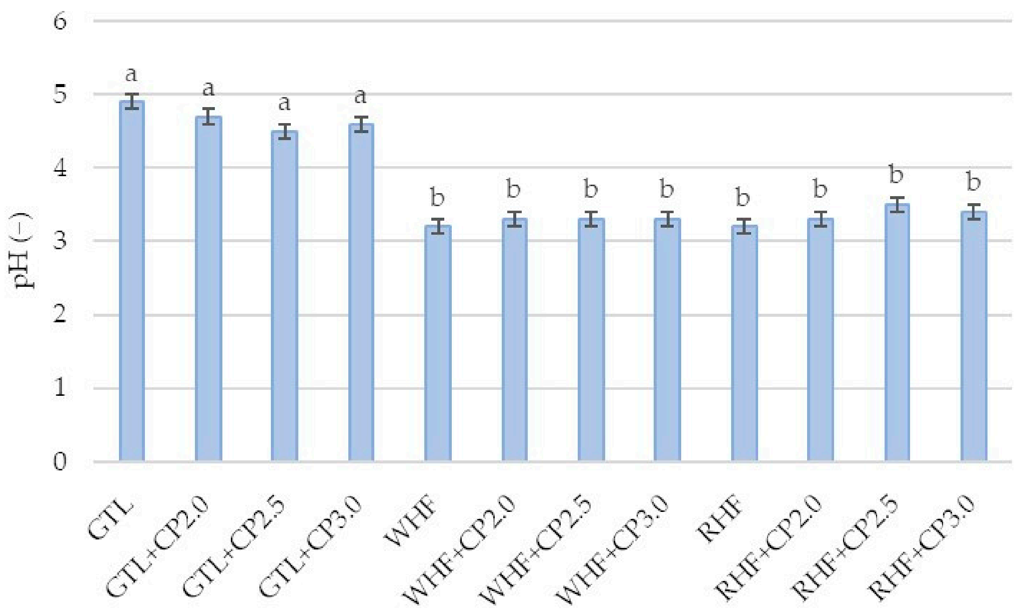

Figure 1. Influence of dried pomace addition to green tea leaves or hibiscus flowers on the pH value of infusions. The different letters above the bars indicate a significant difference at $p < 0.05$ for the two−way ANOVA and Tukey post hoc test; GTL−dried green tea leaves (0.5 g); WHF−dried white hibiscus flowers (0.5 g); RHF−dried red hibiscus flowers (0.5 g); CP−dried chokeberry pomace; 2.0, 2.5 and 3.0—indicate pomace addition in g.

The pH values of GTL, WHF and RHF infusions were 4.9, 3.2 and 3.2, respectively. The addition of dried chokeberry pomaces did not cause statistical differences in pH values. However, the pH value of infusions for dried green tea leaves (both with and without the addition of CP) was about 40% higher than that of hibiscus flower infusions. The values of the tested parameters of infusions from green tea leaves and hibiscus flowers in commercial mixtures were at a similar level, 4.9 and 2.7, respectively [33]. Tan et al. [34] also found that the pH values of green tea leaf infusions ranged from 5.03 to 6.10 and depended on the initial pH and mineral content of the water. The research of Tolić et al. [35] shows that the pH value of infusions obtained from chokeberry pomace ranges from 4.01 to 4.13. In our case, a slight decrease in the pH value with increasing content in the mixture of dried chokeberry pomace was observed for the green tea infusion. However, these changes were not statistically significant. Initially, the low pH of hibiscus flower infusions remained at a similar level of 3.3 after the addition of dried chokeberry pomace.

3.2. Color of Infusions—Instrumental and Sensory Analysis and Anthocyanin Pigment Content

An attractive color for infusions is an important requirement from a consumer's point of view. The addition of colored compounds such as dried chokeberry pomace could increase the intensity and improve the tone of color. On the other hand, drying may cause a browning reaction that results in an increase in undesirable browning compounds, which could affect the color of infusions. The results of the instrumental and sensory color analysis of the obtained infusions are presented in Table 2 and Figure 2.

Table 2. Influence of dried pomace addition to green tea leaves or hibiscus flowers on the color parameters of infusions.

Infusions	L*	a*	b*	ΔE
GTL	94.4 ± 0.1 [ab]	−0.7 ± 0.4 [c]	6.6 ± 0.7 [b]	-
GTL + CP2.0	68 ± 5 [c]	27 ± 6 [bd]	9.8 ± 0.8 [b]	38 ± 8 [e]
GTL + CP2.5	64 ± 4 [cd]	33 ± 3 [b]	10 ± 1 [b]	52 ± 6 [cde]
GTL + CP3.0	59 ± 4 [cde]	35 ± 4 [b]	11 ± 1 [b]	58 ± 7 [bcde]
WHF	95.3 ± 0.1 [a]	−0.6 ± 0.1 [c]	4.5 ± 0.1 [b]	-
WHF + CP2.0	59 ± 6 [cde]	55 ± 7 [a]	31 ± 9 [a]	71 ± 12 [abc]
WHF + CP2.5	53 ± 4 [e]	57 ± 3 [a]	33 ± 6 [a]	75 ± 7 [ab]
WHF + CP3.0	50 ± 4 [e]	61 ± 2 [a]	42 ± 7 [a]	84 ± 6 [a]
RHF	84.6 ± 0.1 [b]	22 ± 0.3 [d]	4.2 ± 0.1 [b]	-
RHF + CP2.0	59 ± 3 [cde]	56 ± 3 [a]	28 ± 4 [a]	49 ± 5 [de]
RHF + CP2.5	57 ± 4 [de]	59 ± 4 [a]	33 ± 9 [a]	56 ± 9 [bcde]
RHF + CP3.0	51 ± 1 [e]	61.7 ± 0.4 [a]	40 ± 2 [a]	63 ± 1 [bcd]

Mean ± standard deviation; the different letters indicate a significant difference at $p < 0.05$ for the two-way ANOVA and Tukey post hoc test. GTL—dried green tea leaves (0.5 g); WHF—dried white hibiscus flowers (0.5 g); RHF—dried red hibiscus flowers (0.5 g); CP—dried chokeberry pomace; 2.0, 2.5 and 3.0—indicate pomace addition in g.

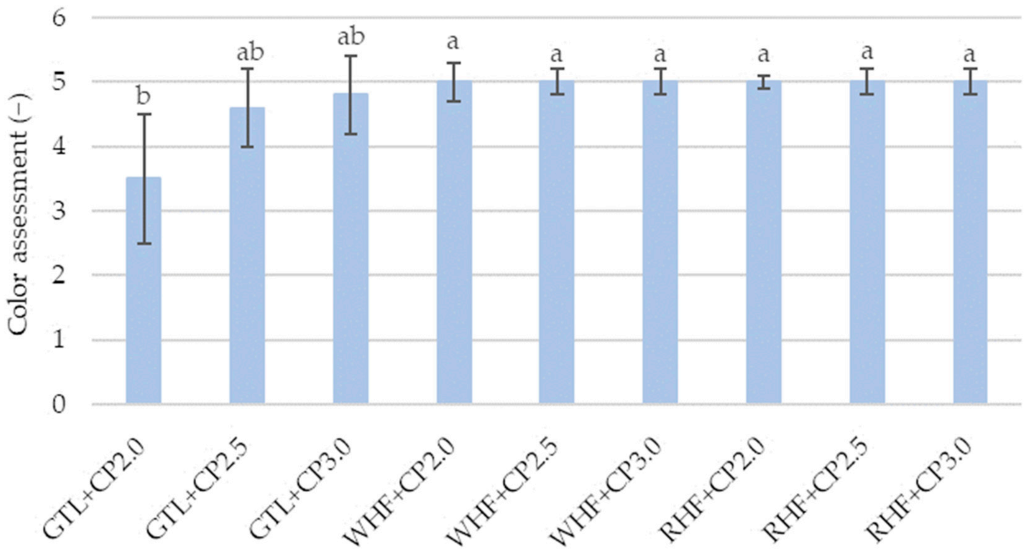

Figure 2. Influence of dried pomace addition to green tea leaves or hibiscus flowers on the sensory assessment of color in infusions (the different letters above the bars indicate a significant difference at $p < 0.05$ for the two-way ANOVA and Tukey post hoc test). GTL—dried green tea leaves (0.5 g); WHF—dried white hibiscus flowers (0.5 g); RHF—dried red hibiscus flowers (0.5 g); CP—dried chokeberry pomace; 2.0, 2.5 and 3.0—indicate pomace addition in g.

The initial color of the GTL and WHF samples was rather low-saturated and pale. The values of the L* parameter for infusions of dried GTL and WHF were at a similar level and ranged from 94.4 to 95.3. Values of the L* parameter ranging from 84.3 to 93.0 were also found for green tea infusions by Tan et al. [34]. In the case of the RHF infusion, this parameter was about 10 units lower, so this infusion was darker. The addition of chokeberry pomace caused a significant decrease in lightness in all tested samples (Table 2). The reduction of the L* value after the addition of 2.0 g of dried chokeberry pomace reached 28%, 38% and 30% for GTL + CP2.0, WHF + CP2.0 and RHF + CP2.0 compared to GTL, WHF and RHF, respectively. Increasing the share of pomace caused a further decrease in lightness. Although these changes reached 13–15%, comparing the samples with 2.0 to 3.0 g of dried chokeberry pomace addition, they were not statistically significant. The lowest L* values were recorded for WHF + CP3.0 and RHF + CP3.0 infusions, and they were close to the lightness (the L* value was in the range of 46.65–49.67) of infusions obtained from chokeberry pomace by Bober and Oszmiański [15].

In infusions of GTL and WHF, no red color was recorded, which is indicated by the low negative values of the color parameter a*. In turn, infusions from RHF were characterized by a positive value (a* = 22) of the above-mentioned color parameter that indicated the red tone of the liquid. The incorporation of dried chokeberry pomace to green tea leaves and white hibiscus flowers resulted in a significant increase in the value of the a* parameter in the infusions. In the case of hibiscus flowers, this increase was significantly higher than that for green tea infusions. The addition of 2.5 g or 3.0 g of pomace caused a further increase in the value of parameter a*; however, these changes were not statistically significant. It is also worth noting that the value of the a* parameter in RHF + CP infusions was not significantly higher than that in WHF + CP (Table 2). It can be concluded that the value of the a* parameter in WHF and RHF infusions with the addition of CP is mainly affected by the by-product addition, which is a good source of anthocyanins. This is confirmed by the results presented in Table 3, which indicate a relatively low content of anthocyanins in RHF infusions. Also, the chemical structure of anthocyanins and the self-association phenomenon could have affected the color of the infusions. For example, the maximum wavelength for delphinidine derivatives at pH 3.6 is 522 nm, while cyanidin under the same conditions has a maximum at 514 nm [36]. As studied by González-Manzano et al. [37], the presence of disubstituted B-ring anthocyanins (such as cyanidin glicoside, mainly coming from CP) could tend to self-associate, resulting in an increasing color hue shift towards reddish. The value of parameter b* did not differ significantly for infusions of GTL, WHF and RHF without dried pomace addition, and ranged from 4.4 to 6.6. In green tea leaf infusions, the addition of chokeberry pomace slightly increased the b* parameter, but this effect was not statistically important. In the case of hibiscus, both white and red, after dried CP addition, a significant increase in the value of the b* parameter was observed. Infusions WHF + CP and RHF + CP possessed b* values in the range of 28–42. A further increase in the addition of CP from 2.0 to 3.0 resulted in an increase in the value of this parameter, although these changes were not statistically significant (Table 2).

Color differences ΔE after the addition of dried chokeberry pomace were very high and ranged from 38 to 84. Those differences could be easily noticed by the human eye. The highest color changes were observed in the case of WHF samples. Here, even in the sample with the lowest dried pomace addition (WHF + CP2.0), the ΔE value was 71, while this level was impossible to achieve in the case of GTL + CP3.0 or RHF + CP3.0.

Dried chokeberry fruit or its pomace may be an attractive food ingredient, especially for creating the red color of the product. As was found by Bober and Oszmiański [15], water infusions of chokeberry fruit could possess a* values in the range of 64.58–65.48. Galus and Podolska [38] also produced infusions from chokeberry pomace. They found the color parameters L*, a* and b* at a level of 45, 42 and 32, respectively, and observed slight changes in the values of the color parameters, despite the doubling of the concentration of chokeberry pomace for the preparation of the infusion.

The sensory evaluation of the color was carried out on samples with the addition of chokeberry pomace. Infusions from hibiscus flowers with pomace were characterized by a high color desirability with an intense red color (score 5.0), regardless of the addition level used. In the case of green tea leaf infusions, the addition of 2.5 g and 3.0 g of pomace to the mixture allowed us to obtain a color with a tone and acceptability corresponding to those of hibiscus infusions. A significantly lower score for color was obtained in infusions of dried green tea leaves with 2.0 g of pomace (Figure 2). A statistically significant positive correlation was found between the color parameters a*, b* and the color sensory scores (r = 0.61, r = 0.55, respectively). In the case of the color parameter L* and the color evaluation notes, a negative correlation was noted (r= −0.56).

Table 3. Influence of dried chokeberry pomace addition to green tea leaves or hibiscus flowers on the anthocyanin content of infusions.

Infusions	Delphinidin-3-*O*-Sambubioside	Cyjanidin-3-*O*-Sambubioside	Cyjanidin-3-*O*-Galactoside	Cyjanidin-3-*O*-Glucoside	Cyjanidin-3-*O*-Arabinoside	Cyjanidin-3-*O*-Xyloside	Total
GTL	-	-	-	-	-	-	-
GTL + CP2.0	-	-	3.9 ± 0.1^{bcd}	0.10 ± 0.01^{a}	2 ± 0.1^{b}	0.20 ± 0.02^{a}	6.2 ± 0.2^{b}
GTL + CP2.5	-	-	5.0 ± 0.2^{ab}	0.10 ± 0.01^{a}	2.5 ± 0.1^{a}	0.20 ± 0.02^{a}	7.8 ± 0.4^{a}
GTL + CP3.0	-	-	4.8 ± 0.1^{a}	0.10 ± 0.01^{a}	2.4 ± 0.1^{a}	0.20 ± 0.01^{a}	7.5 ± 0.3^{a}
WHF	-	-	-	-	-	-	-
WHF + CP2.0	-	-	3.0 ± 0.1^{cd}	0.10 ± 0.01^{a}	1.3 ± 0.1^{c}	0.07 ± 0.01^{a}	4.4 ± 1.0^{c}
WHF + CP2.5	-	-	3.5 ± 0.3^{bcd}	0.04 ± 0.04^{a}	1.7 ± 0.1^{b}	0.12 ± 0.01^{a}	5.3 ± 0.3^{bc}
WHF + CP3.0	-	-	4.2 ± 0.3^{bc}	0.07 ± 0.01^{a}	1.9 ± 0.2^{b}	0.16 ± 0.01^{a}	6.4 ± 0.4^{ab}
RHF	0.52 ± 0.01^{a}	0.20 ± 0.00^{a}	-	-	-	-	0.72 ± 0.01^{d}
RHF + CP2.0	0.50 ± 0.01^{a}	0.10 ± 0.00^{b}	2.6 ± 0.2^{d}	0.11 ± 0.01^{a}	1.2 ± 0.1^{c}	0.10 ± 0.01^{a}	4.6 ± 0.3^{c}
RHF + CP2.5	0.51 ± 0.01^{a}	0.14 ± 0.01^{b}	3.9 ± 0.4^{bcd}	0.10 ± 0.01^{a}	1.7 ± 0.1^{b}	0.13 ± 0.01^{a}	5.3 ± 0.5^{bc}
RHF + CP3.0	0.32 ± 0.1^{a}	0.10 ± 0.01^{b}	4.2 ± 0.3^{bc}	0.06 ± 0.01^{a}	1.9 ± 0.2^{b}	0.14 ± 0.01^{a}	6.4 ± 0.5^{ab}

Mean ± standard deviation; the different letters indicate a significant difference at $p < 0.05$ for the two-way ANOVA and Tukey post hoc test. GTL—dried green tea leaves (0.5 g); WHF—dried white hibiscus flowers (0.5 g); RHF—dried red hibiscus flowers (0.5 g); CP—dried chokeberry pomace; 2.0, 2.5 and 3.0—indicate pomace addition in g.

The following anthocyanins derived from chokeberry pomace were identified in the infusions: cyanidin-3-*O*-galactoside, cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-xyloside and cyanidin-3-*O*-glucoside. The increasing addition of dried chokeberry pomace to the infusion composition caused an increase in cyanidin-3-*O*-galactoside and cyanidin-3-*O*-arabinoside content (Table 3). The anthocyanin composition of chokeberry pomace was studied by others. The same profile was confirmed by Roda-Serrat et al. [39], with a dominant contribution of cyanidin-3-*O*-galactoside (62%) and cyanidin-3-*O*-arabinoside (30%), together representing about 92% of chokeberry anthocyanin. Bober and Oszmiański [15] determined anthocyanins in chokeberry pomace infusions at a much higher level (27.2–34.2 mg/100 mL) than in our study. The content of pigments in the infusion may depend on many factors. The initial content of these compounds in the by-product is very important. This, in turn, may depend on the content of anthocyanins in the raw material, the processing method of the fruit into juice and, finally, the conditions of drying the pomace. Thus, obtaining the highest content of anthocyanins in the pomace infusion also requires further research related to the optimization of the process of their sourcing.

In RHF infusions, the following anthocyanins have been identified: delphinidin-3-*O*-sambubioside and cyjanidin-3-*O*-sambubioside (Table 3). Literature data indicate the presence of four compounds. Apart from the two compounds previously mentioned, two more were found: delphinidin-3-*O*-glucoside, and cyanidin-3-*O*-glucoside [40,41]. The former two were mainly extracted from the calyx [42]. The content of these compounds was relatively low (0.7 mg/100 mL); therefore, there was no significant difference between the content of anthocyanins in the infusions of white and red hibiscus flowers with the addition of chokeberry pomace. Bechoff et al. [43] determined the content of anthocyanins in infusions of red hibiscus flowers at a level of 7.3 mg/100 mL. However, the brewing time used by them was longer and lasted 20 min.

The addition of dried pomace to both green tea leaves and hibiscus flowers caused a significant increase in anthocyanins in the obtained infusions. In the case of green tea leaves, the highest content in infusions was determined when 2.5 g of by-product was added. In turn, for hibiscus flowers, a comparable content of anthocyanins was obtained after adding 3.0 g of chokeberry pomace (Table 3). The higher content of anthocyanins in the infusions obtained from green tea leaves with 2.5 g of chokeberry pomace added may be the result of co-pigmentation with phenolic compounds. Such an effect was studied by Klisurova et al. [27], who indicated the participation of phenols present in green tea, such as catechin, epicatechin or chlorogenic acid, in the co-pigmentation of anthocyanins from chokeberry pomace. Co-pigmentation with a phenolic compound consists of creating complexes by charge transfer or π–π interactions. These processes result in a greater stability of anthocyanins, as well as bathochromic (a wavelength shift to longer wavelengths) and hyperchromic effects (an increase in absorption intensity). However, taking into account the instrumental and sensory assessment of the color, the higher content of anthocyanins did not result in a better color of the infusions compared to the hibiscus infusions, especially with the addition of 2 g of chokeberry pomace to the green tea leaves (Figure 2). This may be the result of both a greater share of yellow and green colors in green tea infusions (Table 2), as well as a higher pH value (Figure 1). At a lower pH, which is characterized by hibiscus infusions, the proportion of red flavylium forms is dominant [44]. Also, the presence of minerals in tea infusions (from used ingredients), such as metal ions, could affect the spectral characteristics of anthocyanins and, as a result, their content. As investigated by Sigurdson and Giusti [45], anthocyanins demonstrated bathochromic and hyperchromic shifts when exposed to the metal ion.

In this study, a statistically significant positive correlation between the content of anthocyanins and the value of the color parameter a* (r = 0.79) was found. The same relationship (r = 0.90) between the above parameters was also noted in the research of Bober and Oszmianski [15].

3.3. Content of Total Phenolic Compounds (TPC)

Phenolic compounds are very important plant-derived antioxidants. A total phenolic compound (TPC) analysis conducted by the Folin-Ciocaltau method has been widely used to assess and compare their content in different food matrices. Figure 3 shows the effect of the addition of chokeberry pomace to green tea leaves and hibiscus flowers on the content of total phenolic compounds in infusions.

The content of phenolic compounds in green tea leaf infusions was significantly higher (23 mg GAE/100 mL) than in hibiscus flower infusions (2.3–3.6 mg GAE/100 mL). Kodama et al. [46] determined the content of phenols in green tea leaf infusions at a level of 57–84 mg of catechin equivalents/100 mL. However, they prepared infusions with 1.5 g of dried leaves and used different phenolic standards. In turn, Preciado-Saldaña et al. [47] optimized the conditions for the preparation of infusions with calyces hibiscus (tested ranges: calyces/water ratio of 1–20 g/100 mL; temperature of 7–100 °C; and brewing time of 1–1200 min) and determined phenolic compounds from 5.48 to 21.47 mg GAE/100 mL. The content of phenols determined in our studies was lower, which may be related to both the method of flower drying and the brewing conditions [48]. The addition of dried pomace to green tea leaves or hibiscus flowers, regardless of the proportion, increased the content of phenols by an average of 16 mg/100 mL–23 mg/100 mL. The highest content was found in infusions of green tea leaves, although greater increases were noted for infusions of hibiscus. This suggested better extractability of pomace phenolics in the presence of compounds from hibiscus flowers. A greater increase in the content of phenolic compounds in hibiscus infusion may be associated with a significantly lower pH of this solution compared to green tea leaf infusions. Also, Friedman and Jürgens [49] determined the effect of pH in the range of 3 to 6 on the stability of chlorogenic and caffeic acids. They found that an increase in pH value causes a decrease in absorbance in spectrophotometric assays; however, they did not identify the nature of these changes.

An increase in the addition of chokeberry pomace (x) to hibiscus flowers, irrespective of their type (red or white), resulted in an increase in total polyphenol levels (y) according to the equation $y = 7.5x + 3.9667$ ($R^2 = 0.889$).

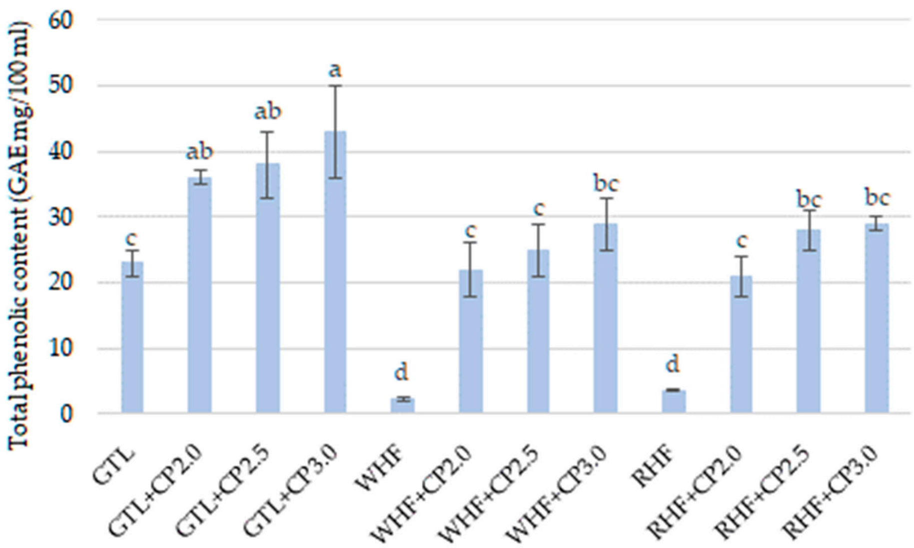

Figure 3. Influence of dried pomace addition to green tea leaves or hibiscus flowers on the total phenolic compound content in infusions (the different letters above the bars indicate a significant difference at $p < 0.05$ for the two-way ANOVA and Tukey post hoc test. GTL—dried green tea leaves (0.5 g); WHF—dried white hibiscus flowers (0.5 g); RHF—dried red hibiscus flowers (0.5 g); CP—dried chokeberry pomace; 2.0, 2.5 and 3.0—indicate pomace addition in g.

3.4. Principal Component Analysis (PCA)

Two principal components were identified, which explain 95.5% of the variability. The first component (PC1) explains 63.19%, including negatively correlated content of anthocyanins, color parameters a* and b* and positively—lightness L*. While the second component (PC2) explains 32.31% of the variation. PC2 was negatively correlated with phenol content and pH value (Figure 4). The PCA score plot indicates the beneficial effect of the by-product addition to both dried green tea leaves and hibiscus flowers on the content of phenolic compounds (Figure 5). The highest content of the tested bioactive compounds was obtained in infusions of green tea leaves with the addition of chokeberry pomace.

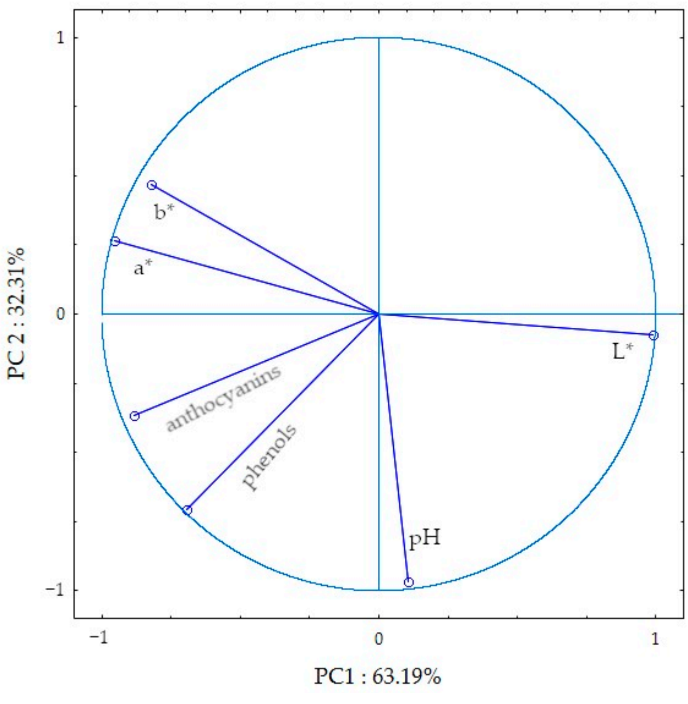

Figure 4. PCA loading plot.

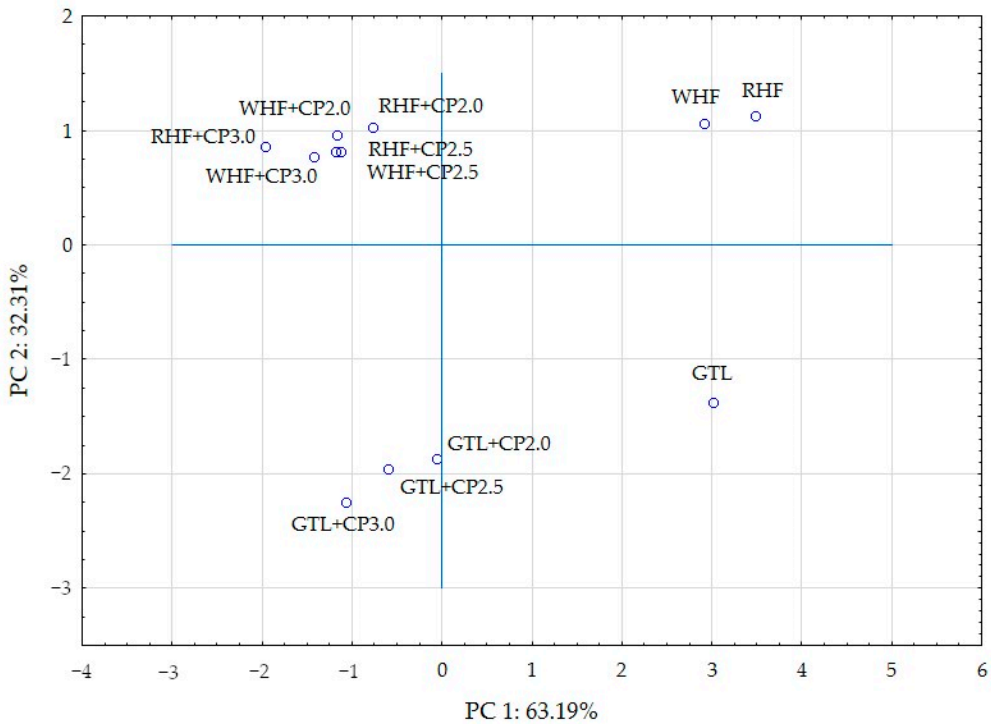

Figure 5. PCA score plot. GTL—dried green tea leaves (0.5 g); WHF—dried white hibiscus flowers (0.5 g); RHF—dried red hibiscus flowers (0.5 g); CP—dried chokeberry pomace; 2.0, 2.5 and 3.0—indicate pomace addition in g.

4. Conclusions

Infusions obtained from hibiscus flowers, both with and without the addition of by-products, had a lower pH value and a darker color with a greater share of red and yellow tones than infusions from dried green tea leaves. The addition of chokeberry pomace to infusions caused a significant increase in the content of phenols, including anthocyanins. From the point of view of health-beneficial potential, the best infusion among the tested is obtained from green tea leaves (0.5 g) with 3.0 g of dried chokeberry pomace.

The conducted research indicates the significant role of the ingredients of the infusion (green tea leaves or hibiscus flower) on the level of extractability of anthocyanins and phenols from chokeberry pomace. For green tea leaf infusions, the highest content of anthocyanins was obtained. Despite the higher content of anthocyanins in green tea leaves, the color was less desirable. Infusions prepared from the mixture containing dried chokeberry pomace and green tea leaves also contained a higher content of phenols than other samples. In this case, the content of phenols was associated with the incorporation of a greater amount of these compounds from tea leaves than from hibiscus flowers. However, better extractability was found in infusions of hibiscus flowers, which may be related to their lower pH.

The use of by-products to obtain infusions seems to also be an interesting way to utilize them. Obtaining the optimal effect, taking into account the improvement of the quality of these beverages, requires further research. They should focus on both optimizing the pomace sourcing process and the appropriate selection of ingredients for infusions.

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