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Insight into Bioactive Compounds, Antioxidant and Anti-Diabetic Properties of Rosehip (*Rosa canina* L.)-Based Tisanes with Addition of Hibiscus Flowers (*Hibiscus sabdariffa* L.) and Saffron (*Crocus sativus* L.)

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Abstract: Tisane is a fruit or herbal infusion, commonly referred to as herbal tea. These products are consumed as part of a balanced diet, which is closely related to the trend of a healthier lifestyle. In this work, tisanes prepared from rosehip (R), and herbal mixtures containing rosehip/hibiscus flowers (R/H) and rosehip/hibiscus flowers/saffron (R/H/S) were studied. Rosehip was dried by the convective drying method at 40, 50 and 60 °C. Total phenolic content (TPC), total flavonoid content (TFC), total flavonol content (TFlC), total anthocyanin content (TAC), antioxidant properties ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$ assays) and in vitro inhibitory potential toward α-amylase of tisanes were examined. The highest TPC (based on dry weight (dw)) was measured in tisane obtained from rosehip dried at 60 °C (37.84 mg GAE/g dw). Tisanes prepared from a R/H/S mixture had the highest values of TFC (4.66–6.13 mg QUE/g dw), TFlC (2.67–3.98 mg QUE/g dw) and TAC (1.35–2.27 mg Cy 3-glc/g dw). The highest $DPPH^{\cdot}$ scavenging activity (53.42 mg TE/g dw) was measured in rosehip (dried at 60 °C) tisane, whereas tisane prepared from a rosehip (dried at 60 °C)/hibiscus mixture expressed the best $ABTS^{\cdot+}$ scavenging activity (107.44 mg TE/g dw). All tisane samples expressed high inhibitory potential toward α-amylase, with the highest activity of 85.03% and 89.90%, measured for tisanes prepared from rosehip/hibiscus flowers mixture (rosehip dried at 50 and 60 °C, respectively).

Keywords: herbal tea; rosehip; saffron; hibiscus; convective drying; phenols; flavonoids; α-amylase

1. Introduction

The consumption of plant-based diets is associated with several beneficial health outcomes and plants intended for food consumption represent a valuable source of different nutrients and bioactive compounds [1–5]. Some of the most represented bioactive compounds include polyphenols that are present in relatively small quantities and have strong

antioxidant properties. Furthermore, plant polyphenols are secondary plant metabolites reported to have a protective role in plants against radiation, mechanical damage, and microbial infection [6]. In addition to the potential health benefits due to their antioxidative properties, consumption of foods high in polyphenols is associated with number of other beneficial health effects such as anti-diabetic, anti-inflammatory, immunomodulating, antimicrobial, and promotion of gut health [1,4,5].

Although tea is one of the most commonly consumed beverages in the world [7], since relatively recently, the popularity of fruit and herbal infusions, 'tisanes', is on the rise [8–11]. Tisane is usually defined as an herbal tea or herbal infusion that is prepared using different morphological parts of plants such as leaves, stems, roots, fruit, buds and flowers [10,12,13]. The preparation of herbal infusions is based on a solid–liquid extraction process and involves the separation of bioactive compounds from plant tissues [11,13]. The materials used for the preparation of tisanes do not originate from the tea plant (*Camellia sinensis* L.) [14], but rather from over 200 different plant species [15]. Furthermore, tisanes were also reported to be a valuable source of several different bioactive compounds including polyphenols, phenolic acids, flavonoids, coumarins, alkaloids, polyacetylenes, saponins, terpenoids, and several major and trace elements [8,12–14,16–18]. The findings in the current literature also suggests that the consumption of tisanes is associated with a reduction in risk for the development of some non-communicable deceases (NCDs) such as cardiovascular diseases (CVD), type 2 diabetes mellitus (T2DM), various types of cancers, arthritis, autoimmune and neurodegenerative disorders [10,17–21].

Dog rose (*Rosa canina* L.) belongs to the family Rosaceae and the genus *Rosa* L. that includes nearly 250 species, nineteen of which can be found in the Republic of Serbia. The pseudo-fruit of the plant, often called 'rosehip', is one of the most important types of wild fruit used in the preparation of tisanes and other herbal teas [22]. This also provides a substantial potential for the use of rosehip on domestic and international markets [23]. Due to its nutritional value and sensory properties, rosehip is widely used in the food industry for the production of marmalade, jams, probiotics, various drinks, fruit yogurts, soups and rose hip extract are commonly used in the soft drink industry [24]. Furthermore, in several European countries, rosehips are used as components in food products that are declared as healthy food predominately due to its rich bioactives composition [25]. Rosehip is a rich source of phytochemicals, including flavonoids (quercetin, kaempferol, apigenin), flavan-3-ols (catechin), anthocyanins, proanthocyanidins (procyanidin), phenolic acids (gallic and ellagic acid), stilbenes (resveratrol) [26–28], vitamins (A, B3, C, D and E), carotenoids, tocopherols [29], and essential elements (Ca, Fe, K, Mn, Na, P and Zn) [30].

Hibiscus, also known as 'Chinese mallow' or 'Japanese rose', is a genus of nearly 220 species of plants from the mallow family (Malvaceae). The outer parts of the flower of the tropical plant *Hibiscus sabdariffa* L. are used to prepare hibiscus tea. Traditionally, the flower of *H. sabdariffa* L. was used in folk medicine [17], and the consumption of this product is associated with improvements in body weight reduction, inhibition of lipid accumulation and suppression of adipogenesis [20]. The bioactive properties and health benefits of hibiscus tea greatly contribute to its growing popularity [20,31,32]. Phenolic acids, flavonoids, organic acids, lutein, tannins, and various anthocyanins are some of the listed bioactive components of this plant [10,17,33]. Extracts of the plant have been found to show strong antioxidant and antimicrobial properties, and relatively recent research reveals potential benefits of hibiscus tea consumption in terms of beneficial effects on anti-modulating lipid profile, inflammation, oxidative stress and insulin resistance [10,17,34].

Saffron (*Crocus sativus* L.) is a plant predominately cultivated for the production of the culinary spice. It is mostly cultivated for its red flower marks (stigmas), which, after drying, form a highly valued spice ('red gold'), with unique organoleptic properties [9,35]. The phytochemical composition of saffron stigmas makes saffron spice a rich source of bioactive compounds. Among them, the most important are crocin, crocetin, picrocrocin and safranal, compounds that give saffron specific color, bitterness and aromaticity. Other bioactive compounds such as polyphenols, flavonoids, carotenoids and terpenoids were

identified in the saffron stigma, a commercially most important part of the plant with pronounced antioxidant properties [9,36–39]. Saffron also possesses a whole range of other biological characteristics (anti-inflammatory, antitumor, anticonvulsant, anti-diabetic and anti-hyperlipidemic) [35,40–42]. It is used for commercial products such as herbal teas, spice mixes, and as an addition to some of the culinary dishes (pasta and rice) in several different cultures [9,35]. In the current commercial market, herbal tea blends with saffron are placed in a category of functional foods and beverages [9].

The concept of functional foods and beverages has significantly contributed to the popularity of tisanes, primarily among consumers who are becoming increasingly aware of their potential health benefits individually and as a part of the healthy dietary pattern. Tisanes are also reported to offer a variety of tastes (fruity, minty, flowery, spicy, sweet) and colors that can satisfy demands of customers. Moreover, they are predominately caffeine-free beverages that can be served hot or cold and can be consumed for enjoyment and potential medicinal purposes [9,10,14,15,43]. One of the widely consumed herbal teas is rosehip tea that has substantial commercial value in Europe and the United States [44].

The aim of this study was to assess the phytochemical composition (total phenolics, total flavonoids, total flavonols and total anthocyanins), antioxidant properties and in vitro inhibitory potential toward α-amylase of tisanes prepared from pseudo-fruit of dog rose (rosehip), as well as from herbal mixtures containing rosehip, hibiscus flowers and saffron.

2. Materials and Methods

2.1. Plant Material

The rosehips (*Rosa canina* L.) at the stage of full physiological maturity were obtained from the "Rosehip plantation Petrović" (near Mladenovac, Serbia (coordinates: 44°30′ N/ 20°42′ E) in September 2021. Dried hibiscus (*Hibiscus sabdariffa* L.) flowers and saffron (*Crocus sativus* L.) dried stigmas (producer KOTANYI GmbH, Austria) were purchased from a local commercial supplier in 2022.

2.2. Drying of Rosehip

The measurements were performed as described in our previous paper [45] with some modifications; an experimental dryer by the convective drying method was used (Supplementary 1, Figure S1). Mass change in time of the samples was monitored for air velocity of 2 m/s. Temperatures for the experiments (40, 50 and 60 °C) were chosen based on the recommendations for rosehip drying in the previous literature [16,46]. The change in mass of the samples was weighted by the electronic balance (Kern precision Balance, Germany) with an accuracy of ±0.01 g and monitored at 5 to 10 min intervals. The drying experiments ended until the final moisture content of the samples was 12%. An experimental installation was used for the experiments and it is described in more detail in the SI. The dimensionless moisture ratio depending on the drying time for rosehip samples is shown in Figure S2.

2.3. Experimental Design and Preparation of Tisanes

Dried rosehips (with seeds) were ground using a blender (BOSCH MKM6000, 180 W, Slovenia). For the preparation of tisanes, rosehip samples (dried at 40, 50 and 60 °C) were mixed with dried hibiscus flowers and saffron stigmas. The composition of herbal mixtures (weight:weight (*w*/*w*) expressed in percentage (%)), drying temperatures of rosehip (40, 50 and 60 °C), and tisane labels (R-rosehip, R/H-rosehip/hibiscus, R/H/S-rosehip/hibiscus/saffron) are shown in Table 1. Based on the literature and data collected from labels of marketed herbal teas, for the preparation of tisanes (total of 9 samples), herbal material (1.6 g) was brewed in 100 mL of boiling distilled water (100 °C) for 10 min (covered with a glass) and stirred occasionally. Stirring was applied to enhance the extraction of bioactive compounds. Tisane samples (Figure S3) were filtered through the filter paper (Whatman No 1) in plastic test tubes and stored in a refrigerator (4 °C) until analysis.

Table 1. Herbal constituents, mass ratio and tisane labels.

Herbal Constituents	Mass Ratio [1] (*w*/*w*)	Tisane Label [2]
Rosehip 40 °C [3]	100%	R40 °C
Rosehip 50 °C	100%	R50 °C
Rosehip 60 °C	100%	R60 °C
Rosehip 40 °C/Hibiscus	60:40%	R40 °C/H
Rosehip 50 °C/Hibiscus	60:40%	R40 °C/H
Rosehip 60 °C/Hibiscus	60:40%	R40 °C/H
Rosehip 40 °C/Hibiscus/Saffron	60:35:5%	R40 °C/H/S
Rosehip 50 °C/Hibiscus/Saffron	60:35:5%	R50 °C/H/S
Rosehip 60 °C/Hibiscus/Saffron	60:35:5%	R60 °C/H/S

[1] Mass ratio (*w*/*w*) of herbal constituents expressed in percentage (%); [2] rosehip (R), hibiscus (H), and saffron (S); [3] drying temperatures of rosehip (40, 50 and 60 °C).

2.4. Determination of Total Phenolic Content in Tisanes Preparations

Total phenolic content (TPC) of the hot water extracts was determined using the spectrophotometric Folin–Ciocalteu method previously described by Singelton et al. [47]. In brief, 2.5 mL of ten-fold diluted Folin–Ciocalteu reagent and 2 mL of 7.5% $NaHCO_3$ were added to the 0.5 mL of the sample. After 15 min of incubation the absorbance of the mixture was measured at 765 nm. The values were expressed, using the gallic acid equivalents (mg GAE/g) of dry weight (dw) of the plant material. The concentration interval for gallic acid was from 0.02 to 0.1 mg/mL. The obtained results for the samples were included in the calibration curve of gallic acid (y = 8.0052x + 0.0147). All spectrophotometric measurements were performed using UV–Vis double beam spectrophotometer Halo DB-20S (Dynamica GmbH, Dietikon, Switzerland).

2.5. Determination of Total Flavonoid Content in Tisanes Preparations

To determine total flavonoid content (TFC) used the $AlCl_3$ method [48]. The same volume of the sample solution and 2% $AlCl_3$ were mixed and incubated for 1 h. Thereafter, the absorbance was measured at 415 nm. TFC content was expressed as quercetin equivalents (mg QUE/g dw). The concentration interval for quercetin was from 0.02 to 0.1 mg/mL. The obtained results for the samples were included in the calibration curve of quercetin (y = 27.219x − 0.0311).

2.6. Determination of Total Flavonol Content in Tisanes Preparations

The method reported by Yermakov et al. [49], with $AlCl_3/CH_3COONa$, was used to estimate total flavonol content (TFlC). The methanol solution of 2% $AlCl_3$ (1 mL) and 3 mL of sodium acetate (50 mg/mL) were added to the 1 mL of the tested sample followed by 180 min of incubation at room temperature. The absorbance was measured at 440 nm. TFlC results were expressed as quercetin equivalents (mg QUE/g dw). The concentration interval for quercetin was from 0.02 to 0.1 mg/mL. The obtained results for the samples were included in the calibration curve of quercetin (y = 18.637x − 0.0895).

2.7. Determination of Total Anthocyanin Content in Tisanes Preparations

Total anthocyanin content (TAC) was evaluated with the pH differential method reported in [50]. Dilution factor (F) was determined by dilution sample with KCl buffer (pH 1) to reach absorbance at λ_{max} 520 nm between 0.4 and 0.7. In the 2.5 mL of tested sample, 7.5 mL of KCl-buffer and 0.025 M KCl-buffer (pH 1.0) were added. The absorbance was measured at 520 and 700 nm after incubation the mixture for 15 min. TAC results were expressed as equivalents of cyanidin-3-*O*-glycoside per gram of dry extract (mg Cy 3-glc/g extract). The results were calculated according to the following equation:

$$c = (A \times M \times F \times 1000)/(\varepsilon \times l), \quad (1)$$

where c—concentration of total anthocyanins; A—absorbance calculated as (A 520 nm–A 700 nm) pH 1.0; M—molar weight of cyanidin-3-*O*-glycoside (449.2 g/mol); F—dilution factor; ε—molar absorptivity (26,900 L/mol × cm); l—cell length (1 cm).

2.8. Evaluation of Antioxidant Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined using the method of Kumarasamy et al. [51]. The DPPH solution (1 mL, 80 µg/mL) was added to the series of double dilutions of the sample (1 mL) and incubated for 30 min at room temperature. The absorbance of the mixture was read at 517 nm.

2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium (ABTS) radical-cation scavenging activity was determined using the method of Re et al. [52]. The method was based on preparing the radical cation ($ABTS^{\cdot+}$) in reaction of 7 mM ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and 2.45 mM potassium for 16 h before use. The $ABTS^{\cdot+}$ solution for the use in the method was prepared by dilution with 5 mM phosphate-buffered saline (pH 7.4) in order to obtain the absorbance values of 0.70 ± 0.02 at 734 nm. This solution (900 µL) was added to the 100 µL of the tested sample and mixture was incubated for 30 min. The absorbance was measured at 734 nm.

The scavenging activity of tested samples (%) in both assays was calculated using the following equation:

$$\text{Scavenging activity (\%)} = [(\text{Ac} - \text{As}) - \text{Ac}] \times 100 \quad (2)$$

where Ac is the absorbance of control sample ($DPPH^{\cdot}$ or $ABTS^{\cdot+}$ in methanol) and As is the absorbance of the samples. The results of DPPH and ABTS assays were expressed as mg of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents per gram of dry weight (mg TE/g dw). The concentration interval for Trolox in both assays was from 0.02 to 0.1 mg/mL. The Trolox calibration curve for antioxidant activity assays were y = −28.54x + 1.0978 and y = −9.968x + 0.6277, respectively.

2.9. Inhibition of α-Amylase

In vitro α-amylase inhibition assay adapted for microplates was performed according to a previously described method, with some modifications [53]. α-Amylase (EC 3.2.1.1) from porcine pancreas was used for the experiment. Briefly, 30 µL of the sample and 80 µL of phosphate buffer (0.1 M, pH 6.0) was added to the microtiter plate. This was followed by the addition of 80 µL of the enzyme solution prepared in phosphate buffer (the concentration of α-amylase in the well of the microplate was 0.05 U/mL). Finally, 20 µL of a substrate 2-chloro-4-nitrophenyl-α-D-maltotrioside (Sigma Chemical Co., St. Louis, MO, USA) was added. The blank was carried out in a similar manner, with the test sample replaced by water. The reaction was monitored for 30 min at a wavelength of 405 nm, and the values were recorded every three minutes. A microplate reader (ELx808, BioTek Instruments, Inc., Madison, WI, USA) controlled by Gen5TM Software was used. The ability of tisane samples to inhibit α-amylase was calculated based on the equation:

$$\text{\% of inhibition} = [(Slope_{\text{blank}} - Slope_{\text{sample}})/Slope_{\text{blank}}] \times 100 \quad (3)$$

All measurements were performed in triplicates.

2.10. Statistical Analysis

Statistical analysis was performed using Origin 2019b statistical software (OriginLab Corporation, Northampton, MA, USA) by one-way analysis of variance (ANOVA), and the means were compared by Tukey and Fisher LSD tests. Pearson's correlation analysis was performed using Microsoft Excel 2013. The level of statistical significance was set at $p < 0.05$. All measurements were performed in triplicate.

3. Results and Discussion

3.1. Drying of Rosehip

Drying is one of the most cost-effective ways of preserving foods and dried fruits and other parts of the plants are usually used the preparation of tisanes [3,54]. Drying of moist foods involves simultaneous monitoring of heat and mass transfer within the material being dried [55–57]. Convective drying of fresh rosehips was performed with hot air (airflow rate of 2 m/s), and different temperatures (40, 50 and 60 °C). The obtained results suggest that the optimal temperature was 60 °C, as the change in mass of the sample was most uniform and constant over time and the stationary drying time was the shortest. Continuous monitoring of the moisture content in the material during the entire drying period is a common approach in the drying technique [58,59]. Figure 1 represents the change in the sample mass depending on the drying process time at an airflow velocity of 2 m/s and at different drying temperatures.

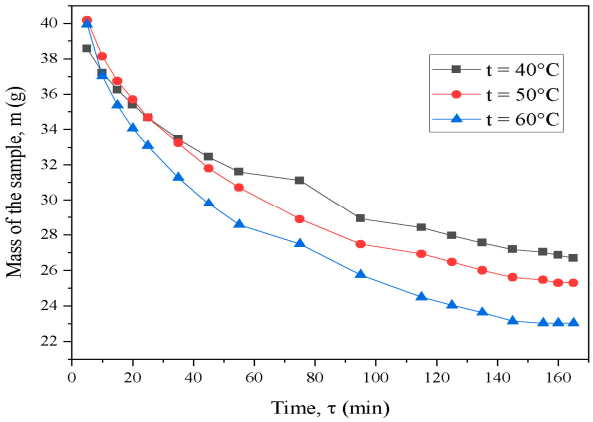

Figure 1. Change in the sample mass depending on the drying process time at an airflow velocity of 2 m/s and different drying temperatures of 40, 50 and 60 °C.

The time required to reach the uniform dry stage was reported at 165 (40 °C), 160 (50 °C) and 145 min (60 °C). From drying curves (Figure 1), it can be observed that the highest drying velocity decreasing of the moisture content occurs at the beginning of the drying process when the moisture content of the drying material is the highest. At higher drying temperatures, the driving force of the heat transfer process is greater, as well as the rate of evaporation and heating of the dried material. The values of the mass transfer coefficient, the heat transfer coefficient, and the thermal coefficient conductivity are also higher, which affects the shortening of drying time. This indicates that the velocity extraction of moisture from the material increases with increasing drying temperature, due to a more intensive exchange of substances on the surface of the material. In the drying process of the rosehips samples, the moisture loss at beginning was faster comparing it with the end of drying process (Figure 1). The drying process of rosehips is more efficient at higher drying temperatures (60 °C). These findings are similar with the previous results on thin-layer drying of biological products reported in the literature [46,58,59].

3.2. Effect of Rosehip Drying Temperature on the Phenolic Composition and Antioxidant Properties of Tisanes

The levels of TPC in prepared samples ranged between 14.47 and 37.84 mg GAE/g dw. Drying temperatures applied to rosehip, significantly affected the concentrations of TPC in most tisane samples (Figure 2A–C, $p < 0.05$). The highest TPC was determined in tisanes prepared from rosehip (R) sample dried at 60 °C (37.84 ± 0.96 mg GAE/g dw), followed by R sample dried at 50 °C (28.75 ± 5.22 mg GAE/g dw), whereas the lowest value of this parameter was found in tisane prepared from the R dried at 40 °C (21.74 ± 1.80 mg GAE/g dw). The drying method of a plant material, the extraction method and the brewing time may affect the content of total phenolics and total anthocyanins, as well as the antioxidant

activity of herbal infusions [11,12,16,46]. The results of our study are in contrast to previous research of Demasi et al. [16] where lower drying temperatures had a positive effect on the content total phenolics in decotations prepared from *R. canina* flowers. This could be due to the addition of hibiscus to rosehip to form the herbal mixture-based tisanes, resulting in a TPC value similar to the samples based on rosehips dried at 40 to 60 °C. The values were in the range from 21.69 to 23.65 mg GAE/g dw, and were not significantly different (Figure 2B, $p > 0.05$). The TPC content in the tisanes prepared with herbal mixtures of rosehip, hibiscus and saffron increased with the temperature of rosehip drying. Significant differences ($p < 0.05$) were observed between tisane based on rosehip dried at 40 °C and the other two tisanes (Figure 2C).

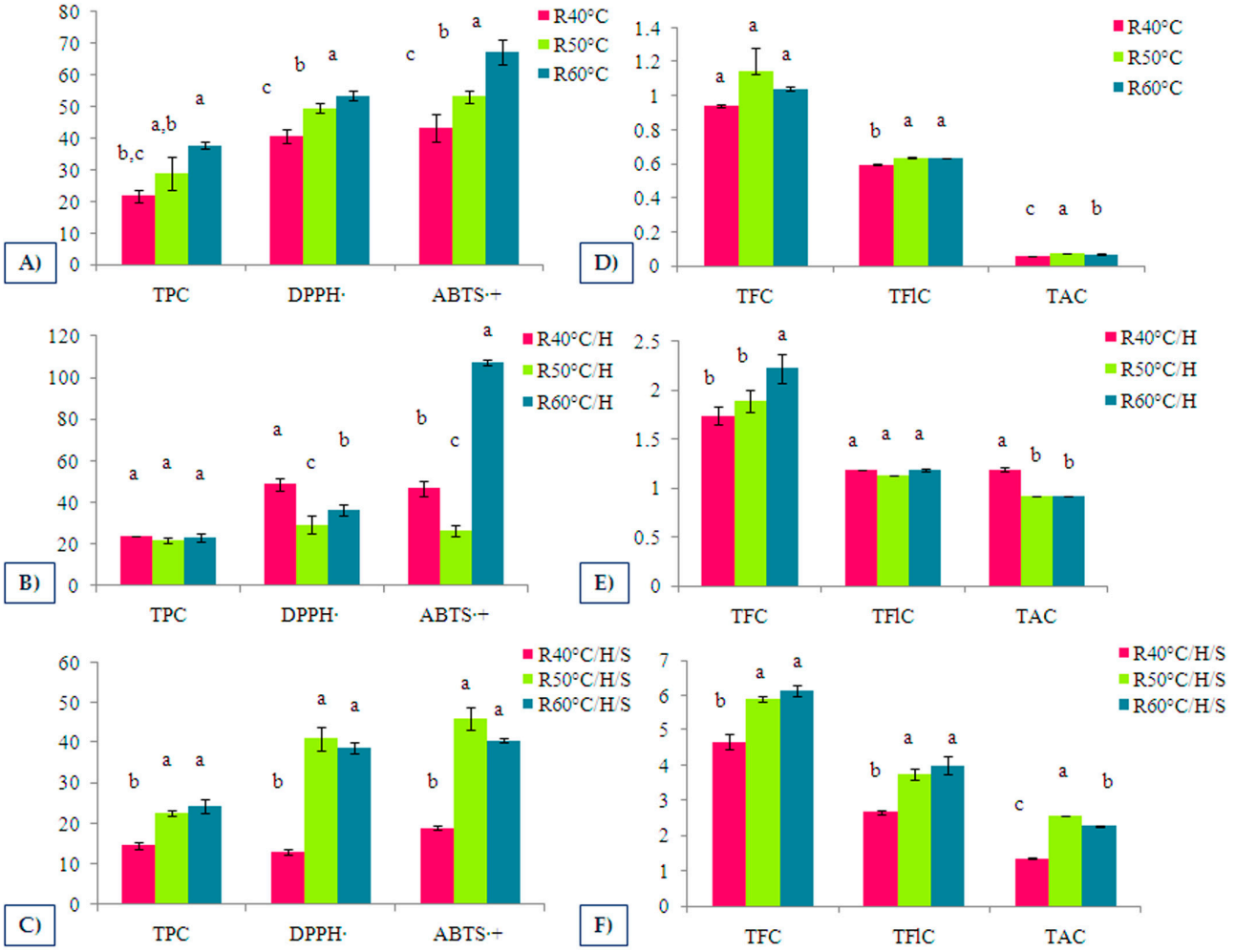

Figure 2. The effect of tisane ingredients on the content of phenolic compounds of rosehip-based tisanes; (**A**–**C**). TPC—total phenolic content, $DPPH^{\cdot}$—scavenging activity, $ABTS^{\cdot+}$—scavenging activity; (**D**–**F**). TFC—total flavonoid content, TFlC—total flavonol content, TAC—total anthocyanin content; the values are represented as mean ± SD (n = 3); different letters (a, b, c) indicate a significant difference at $p < 0.05$; one-way ANOVA analysis with the Fisher LSD test, in each additive dependent group of samples separately, as groups were not compared mutually.

The lowest TFC (0.94–1.04 mg QUE/g dw) was recorded in rosehip tisanes with no significant differences between samples (Figure 2D, $p > 0.05$). On the other hand, the highest TFC content (4.66–6.13 mg QUE/g dw) were observed in tisanes prepared with herbal mixtures of rosehip (dried at all three temperatures), hibiscus and saffron (Figure 2F). Significantly higher TFC was noticed in tisane prepared from rosehip dried at 60 °C with hibiscus (Figure 2E, $p < 0.05$). In tisanes prepared from mixtures of rosehip (dried at 50 and

60 °C), the hibiscus and saffron content of flavonoids was significantly higher compared to tisane that was prepared with herbal mixture that contained rosehip dried at 40 °C (Figure 2F, $p < 0.05$).

The results obtained for the TFlC followed a similar pattern to the TFC contents. Tisanes prepared from herbal mixtures R/H/S were the richest in TFlC (2.67–3.98 mg QUE/g dw), followed by tisanes prepared from R/H mixture (1.13–1.18 mg QUE/g dw) and finally those prepared with rosehip only (0.60–0.64 mg QUE/g dw), (Figure 2D–F). Significant differences were observed for tisane samples prepared from rosehip as well as for tisanes prepared from R/H/S mixture (Figure 2D,F, All p's < 0.05).

The values for TAC significant differences were observed between samples within all three types of tisanes (Figure 2D–F, $p < 0.05$). As expected, the highest TAC values were recorded in tisanes prepared by adding hibiscus and saffron to the herbal mixture (Figure 2F). The highest content of anthocyanins was observed in mixture R50 °C/H/S (2.55 mg Cy 3-glc/g dw), followed by R60 °C/H/S and R40 °C/H/S (2.27 and 1.35 mg Cy 3-glc/g dw, respectively). The TAC values in rosehip tisanes were in range from 0.057 to 0.076 mg Cy 3-glc/g dw, with significant differences observed between the samples (Figure 2D, $p < 0.05$).

Among all samples, the highest antioxidant activity of tisanes (Figure 2A–C), measured via DPPH assay, was found for tisanes that were prepared from R50 °C and R60 °C (49.53 and 53.42 mg TE/g dw, respectively). However, significant differences were observed between those two samples (Figure 2A, $p < 0.05$). In the ABTS analysis, tisane prepared from the mixture of R60 °C/H had the highest value (107.44 mg TE/g dw). In both assays, the lowest antioxidant activity (DPPH; 13.03 and ABTS; 18.82 mg TE/g dw) showed tisanes prepared from the R40 °C/H/S (Figure 2C).

Based on the current findings, the highest content of phenolics was detected in 100% rosehip tisanes, particularly in those prepared from the material dried at 50 and 60 °C. In the remaining tisanes that were prepared with a lower proportion of rosehip, with the addition of hibiscus and saffron, a decrease in the amount of phenolic compounds was noticed. The obtained results further support the findings from a study by Veljković et al. [60], where commercially available herbal teas in the Republic of Serbia contained substantially higher TPC and DPPH antioxidant activity in rosehip infusion then in hibiscus infusion. Gallic acid, caffeic acid, (+)-catechin, (−)-epicatechin, and (−)-epigallocatechin strongly influence the antioxidant activity of infusions [60]. The antioxidant activity mostly followed the TPC values of the samples with the exception, of the sample R60 °C/H that had the highest ABTS antioxidant potential in comparison to all other samples. Similar results were reported relatively recently by Akar et al. [44] where the effects of different temperatures of infusions (0, 15, 30, 45, 60, 75, 90 °C) on the antioxidant activities of rosehip tea bags were examined. In this study, it was found that levels of total phenolic compounds increased in proportion to the increase in preparation temperature. Due to loss of water during the drying process, dried plant products are known to have higher concentrations of phenolic compounds and antioxidant activity than fresh. However, higher temperatures of drying can also cause decrease in total phenolic content, anthocyanins, flavonoid glycosides and vitamin C [16]. Furthermore, in some fruits, the TPC can be increased by higher drying temperatures, as along with the content of flavonoids, catechins, and phenolic acids, depending of treatment conditions and pretreatment parameters [3]. In a study by Pashazadeh et al. [46], the optimization of fresh rosehip drying conditions (from 50 up to 90 °C) was investigated. The findings have indicated that the highest content of TPC was observed in samples dried at 60 °C and total flavonoid content and the antioxidant activity followed a similar trend which is consistent with the present study. A study by Vuong et al. [11] showed that the extraction of phenolic compounds from herbal material is influenced by the brewing time, suggesting that most of the active substances were extracted in the first 5 min and levels of TPC and TFC were stabilized after the 7 min of brewing. Based on this, it can be proposed that during the preparation of the tested tisane samples (brewing for 10 min), the extraction of the maximum amount of phenolic

compounds was ensured. The brewing time as an effect on extraction of phytochemicals was also reported in another study where 11.62 min was the most optimal time for the extraction of phenolics from rosehip [46]. Petkova et al. [13] reported the influence of different preparation methodologies (infusion and decoction) on composition and bioactivity of rosehip herbal teas. By comparing the two herbal mixtures (rosehip: hibiscus, 1:1, and rosehip: dried apple: pear fruit particles: St. John's wort aerial parts, 7:1:1:1) with pure rosehip fruits infusions and decoctions it was shown that decoction was much better option in terms of TPC and TFC in all three samples, which further reflects on the antioxidant activity. The trend that was observed in the present study in the terms of comparison of a rosehip-based tisane and tisane prepared from a R/H mixture, was in line with literature [13]. The authors reported that tisane obtained from a R/H (1:1, *w*/*w*) mixture had a lower amount of TPC (44.8 GAE/250 mL) and a higher amount of TFC (6.7 QE/250 mL) in comparison to rosehip tisane (68.2 GAE/250 mL and 4.5 QE/250 mL, respectively).

3.3. Effect of Hibiscus and Saffron Addition on the Phenolic Composition and Antioxidant Properties of Tisanes

Comparison of tisanes prepared from the rosehip dried at each temperature (40, 50 and 60 °C) and the relevant herbal mixtures (R/H and R/H/S) revealed that the TPC and antioxidant properties were not affected by the addition of hibiscus and saffron to rosehip. In contrast, TFC, TFlC and TAC were higher in tisanes when hibiscus and saffron were added to the mixture (Figure 3). Compared to hibiscus infusion, rosehip infusion contains higher levels of gallic acid and caffeic acid [50], and it can be assumed that differences in antioxidant properties of studied tisanes are attributed to the composition of individual phenolics in tisanes. Tisane prepared from the R40 °C/H mixture had no significantly higher TPC compared to tisane prepared from rosehip dried at the same temperature (Figure 3A, $p > 0.05$). The addition of hibiscus and saffron in herbal mixtures with rosehip dried at 50 and 60 °C resulted in significantly lower TPC (Figure 3B,C, $p < 0.05$). The same trend was observed for the antioxidant properties of tisanes measured via the $DPPH^{\cdot}$ test. Namely, enrichment of rosehip with hibiscus and saffron had a significantly negative impact on the $DPPH^{\cdot}$ ($p < 0.05$) with the exception of tisane prepared from rosehip (dried at 40 °C)/hibiscus mixture (Figure 3A–C). The results of the antioxidant activity of tisanes measured via $ABTS^{\cdot+}$ test indicated significantly higher values for tisanes prepared from rosehip dried at 40 °C, and rosehip R40 °C/H mixture than for R40 °C/H/S tisane (Figure 3A, $p < 0.05$), rosehip dried at 50 °C and tisane prepared from R60 °C/H mixture compared to other two samples that used rosehip dried at 50 and 60 °C, respectively (Figure 3B,C, $p < 0.05$). The reaction kinetics between phenolics and the ABTS radical cation and DPPH radical may cause the differences in results from two methods [60]. On the other hand, tisanes prepared from R/H and R/H/S herbal mixtures had significantly higher values of TFC (R: 0.94–1.14, R/H: 1.74–2.22, R/H/S: 4.66–6.13 mg QUE/g dw), TFlC (R: 0.6–0.64, R/H: 1.13–1.19, R/H/S: 2.67–3.98 mg QUE/g dw) and TAC (R: 0.06–0.08, R/H: 0.92–1.19, R/H/S: 1.35–2.55 mg Cy 3-glc/g dw) regardless of the rosehip drying temperature (Figure 3D–F, $p < 0.05$).

Finally, all samples were mutually compared, and the results of various groups of phenolic compounds and antioxidant activity are presented in Figure S4. It was observed that drying temperatures applied in this study significantly affected the TPC, TFC, TFlC and TAC in most samples (Figure S4A–D, $p < 0.05$). The free radical scavenging capacity of rosehip-based tisanes was evaluated by two individual assays (Figure S4E,F). The highest antioxidant activity of tisanes, measured via $DPPH^{\cdot}$ assay was found for rosehip (dried at 60 °C) tisane, 53.42 mg TE/g dw. For tisane prepared from a R60 °C/H mixture the highest $ABTS^{\cdot+}$ scavenging activity (107.44 mg TE/g dw) was measured. Significant differences were observed between samples R60 °C and R60 °C/H and other samples in applied antioxidant assays (Figure S4E,F, $p < 0.05$).

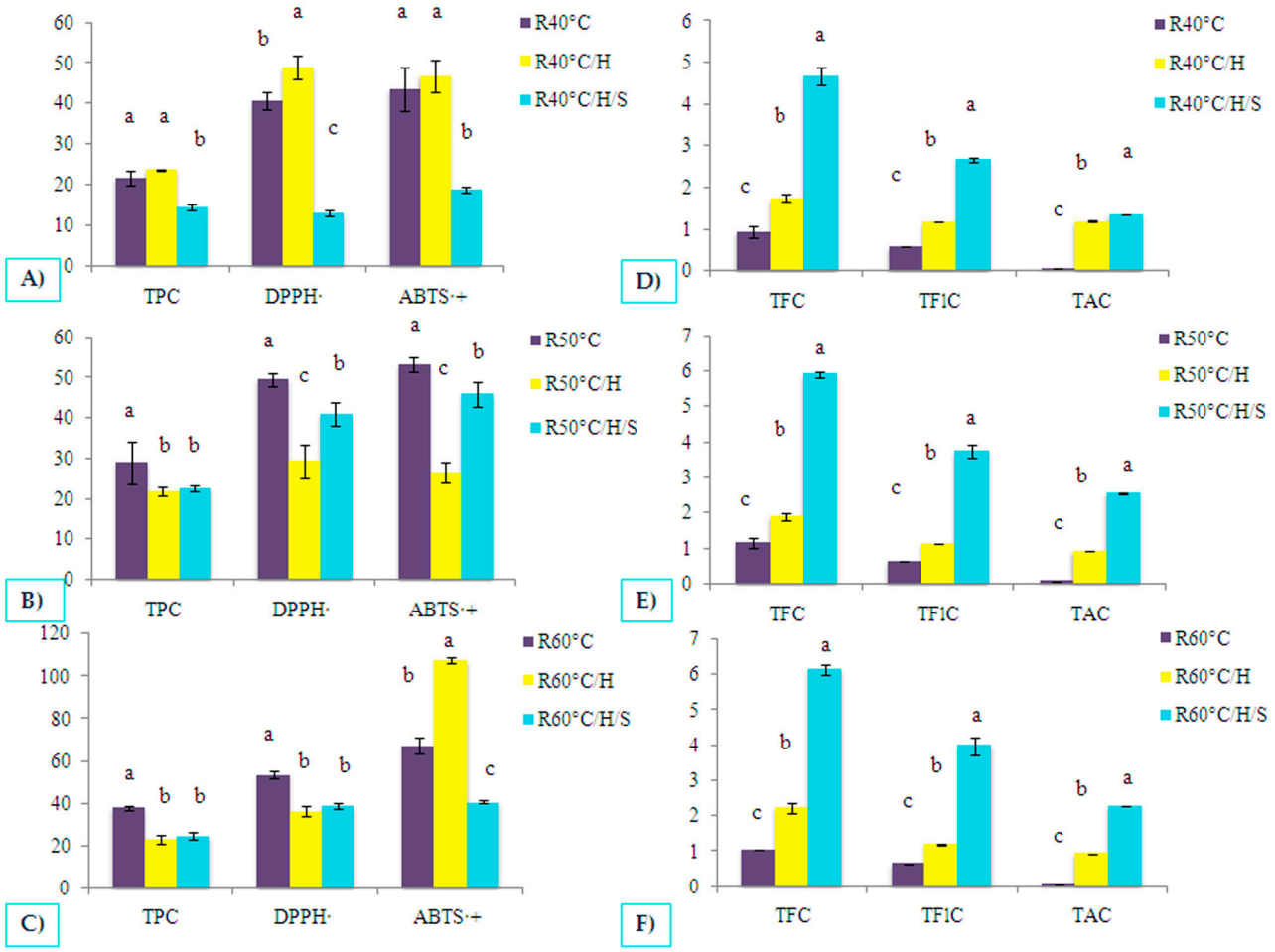

Figure 3. The effect of tisane ingredients on the content of phenolic compounds of rosehip-based tisanes (**A**–**C**) TPC—total phenolic content, $DPPH^{\cdot}$—scavenging activity, $ABTS^{\cdot+}$—scavenging activity; (**D**–**F**) TFC—total flavonoid content, TFlC—total flavonol content, TAC—total anthocyanin content; the values represented are the means ± SD; different letters (a, b, c) mean a significant difference at $p < 0.05$; in one-way ANOVA analysis with the Fisher LSD test, in each temperature-dependent group of samples separately, as groups were not compared mutually.

3.4. Inhibition of α-Amylase

Plants are an important source of chemical constituents with potential for inhibition of α-amylase and can be used as therapeutic or functional food sources [61]. In this study, several individual and combinations of rosehip, hibiscus and saffron tisanes were tested for the in vitro inhibition of α-amylase (Figure 4). The results revealed that all tisane samples expressed inhibitory potential toward α-amylase by the prevention of CNP-G3 hydrolysis and release of the 2-chloro-4-nitrophenol (CNP), a colored by-product. Among analyzed tisanes, the highest activity was measured in tisanes prepared from R60 °C/H and R50 °C/H mixtures (89.90%—R60 °C/H and 85.03%—R50 °C/H, Figure 4). The R60 °C/H and R50 °C/H tisanes showed significantly higher ($p < 0.05$) inhibition compared to tisanes prepared from R60 °C (75.51%) and the R60 °C/H/S mixture (72.11%). On the other hand, the lowest inhibitory potential was observed for tisanes prepared from R60 °C/H/S mixture (56.46%) and R40 °C (61.22%) (Figure 4, $p < 0.05$).

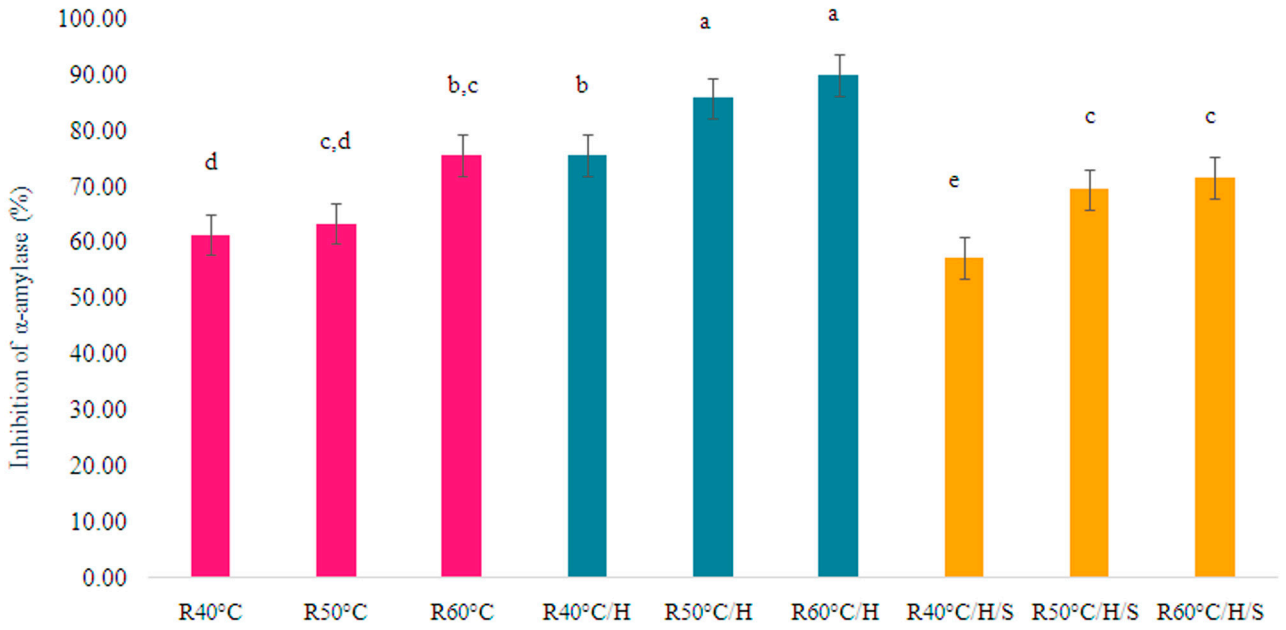

Figure 4. Inhibitory potential of tisanes toward α-amylase (in percentage); R-rosehip, R/H—rosehip/hibiscus, and R/H/S—rosehip/hibiscus/saffron (40, 50, 60 °C—drying temperatures of rosehip). the values are the means ± SD; different letters (a–e) indicate significant differences at $p < 0.05$.

3.5. Relationship between the Antioxidant Capacity, Total Phytochemical Composition and Inhibition of α-Amylase

Among examined parameters, positive correlations were observed between TPC, TAC and antioxidant ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$) scavenging activity. Moreover, the contribution of $ABTS^{\cdot+}$ radical-cation scavenging activity to the inhibition of α-amylase was most pronounced. As shown in Table 2, the high positive correlations were observed for the parameters: $DPPH^{\cdot}$/TPC (r = 0.83, $p < 0.05$), TAC/TFC (r = 0.93, $p < 0.05$) and TAC/TFlC (r = 0.94, $p < 0.05$). In contrast, negative correlations were noticed between antioxidant activity (both assays) and polyphenolic subclasses (TFC, TFlC and TAC). These correlations are also in line with the previous literature [62]. Further, correlation between $DPPH^{\cdot}$ scavenging activity and α-amylase was positive but negligible (r = 0.16, $p < 0.05$), whereas a positive moderate correlation was observed for $ABTS^{\cdot+}$ and α-amylase (r = 0.57, $p < 0.05$). These findings could be potentially explained as certain phenolic compounds with appropriate structure are effective α-amylase inhibitors [63,64]. For example, some dihydrochalcones and flavanones did not react with the $DPPH^{\cdot}$ in contrast to the $ABTS^{\cdot+}$ [65].

Table 2. Pearson's correlation between studied traits in tisanes.

	TPC [1]	TFC	TFlC	TAC	$DPPH^{\cdot}$	$ABTS^{\cdot+}$	α-AmyI
TPC	1						
TFC	−0.44	1					
TFlC	−0.40	1.00	1				
TAC	−0.44	0.93	0.94	1			
$DPPH^{\cdot}$	0.83	−0.43	−0.37	−0.32	1		
$ABTS^{\cdot+}$	0.44	−0.31	−0.32	−0.24	0.44	1	
α-AmyI	0.23	−0.19	−0.19	0.04	0.16	0.57	1

[1] Parameters: TPC—total phenolic content; TFC—total flavonoid content; TFlC—total flavonol content; TAC—total anthocyanin content; $DPPH^{\cdot}$ scavenging activity; $ABTS^{\cdot+}$ radical-cation scavenging activity; α-AmyI—α-amylase inhibition; bolded values designate high (>0.80) and moderate (0.50–0.80) positive correlations.

Regression analysis (Table 2) did not reveal significant correlation (All p's > 0.05) among inhibition of α-amylase activity of investigated tisanes and total content of analyzed

phytochemicals (TPC, TFC, TFlC and TAC). This may suggest that the presence of specific phenolic compounds is of greater importance than their total content in the ability to inhibit α-amylase.

In recent years, the activity of different phenolic compounds has been intensively studied concerning the modulation of T2DM regarding the inhibition of isolated enzymes such as α-amylase [61]. Some of the phenolic acids and flavonoids, often present in tea infusions, such as gallic acid, quercetin, catechin, and epigallocatechin gallate, have been proposed as effective inhibitors of α-amylase [61]. It is reported in the literature that rosehip infusions are sources of polyphenolic acids (gallic, chlorogenic, caffeic, rosmarinic and coumaric), quercetin, catechin, procyanidin dimer, rutin, catechin, epicatechin and epigallocatechin gallate [60,66]. Moreover, a study by Sun et al. [63] explored the interactions between polyphenols and α-amylase and shown that the galloyl moiety was an important substituent group in the binding of catechins with α-amylase. Furthermore, the molecular docking studies revealed that phenolic compounds bind at both the active sites and allosteric sites of α-amylase, resulting in structural changes and activity inhibitors [67]. On the other hand, hydrogen bonds, hydrophobic interactions, and van der Waals interactions are the predominant force involved in the complexation of the phenolic compounds with amylase [67].

4. Conclusions

The present study revealed that the drying temperature of rosehip as a main herbal component used for preparation of tisanes, and composition of the herbal mixtures affected the phytochemical composition, antioxidant activity and inhibitory potential toward α-amylase of studied herbal infusions. A drying temperature of 60 °C appears to be the most appropriate in obtaining rosehip for preparation of tisanes with high content of phenolic compounds and good antioxidant properties. An increase in the total amount of flavonoids, flavonols, and anthocyanins was observed particularly in tisanes prepared from the herbal mixtures (rosehip/hibiscus and rosehip/hibiscus/saffron), that could also be attributed to the addition of hibiscus flowers and saffron. In the ABTS assay analysis, tisane prepared from rosehip dried at 60 °C with addition of hibiscus stood out with the highest antioxidant activity. The antioxidant activity of tisanes was positively correlated to the TPC and negatively to phenolic subclasses (TFC, TFlC, TAC). All tisane samples showed good inhibitory potential toward α-amylase (56.5–89.8%), whereas ones prepared from herbal mixture of rosehip (dried at 50 and 60 °C) and hibiscus were the most effective. The α-amylase inhibitory potential of all tisane samples was moderately correlated to $ABTS^{\cdot+}$ scavenging activity, whereas no significant correlation was observed between α-amylase, $DPPH^{\cdot}$ scavenging activity, TPC, and TAC. This indicates that the structure of individual phenolic compounds is more important for α-amylase inhibition than the total phenolic content and the total content of different subclasses of phenolic compounds. All abovementioned emphasizes the necessity of a detailed quantification analysis of the phenolic compounds in the investigated tisane samples. Furthermore, for dietary application of tisane polyphenols in the prevention of T2DM, hyperglycemia and obesity, further human studies will be also required to discover if the findings of this study can be transferred to the in vivo systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages10010001/s1, Supplementary 1. Experimental installation and procedure. Figure S1: Schematic presentation of the experimental dryer. Figure S2: The dimensionless moisture ratio depending on the drying time for rosehip samples at an airflow velocity of 2 m/s and drying temperatures of 40, 50 and 60 °C. Figure S3: Tisane samples. Figure S4: The content of polyphenols and antioxidant activity in rosehip-based tisanes: (A) TPC—total phenolic content, (B) TFC—total flavonoid content, (C) TFlC—total flavonol content, and (D) TAC—total anthocyanin content; (E) $DPPH^{\cdot}$ scavenging activity, and (F) $ABTS^{\cdot+}$ scavenging activity; dry weight (dw) of plant parts; R—rosehip, R/H—rosehip/hibiscus mixture, and R/H/S—rosehip/hibiscus/saffron mixture (40, 50, 60 °C—drying temperatures of rosehip).

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Abbreviations

α-AmyI	α-amylase inhibition
$ABTS^{\cdot+}$	2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium (ABTS) radical-cation scavenging activity (mg TE/g dw)
Cy 3-glc	cyanidin-3-glycoside equivalents
DPPH	2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (mg TE/g dw)
dw	dry weight
GAE	gallic acid equivalents
R	rosehip
R/H	rosehip/hibiscus mixture
R/H/S	rosehip/hibiscus/saffron mixture
QUE	quercetin equivalents
TAC	total anthocyanin content (mg Cy 3-glc/g dw)
TE	Trolox equivalents
TFC	total flavonoid content (mg QUE/g dw)
TFlC	total flavonol content (mg QUE/g dw)
TPC	total phenolic content (mg GAE/g dw)

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