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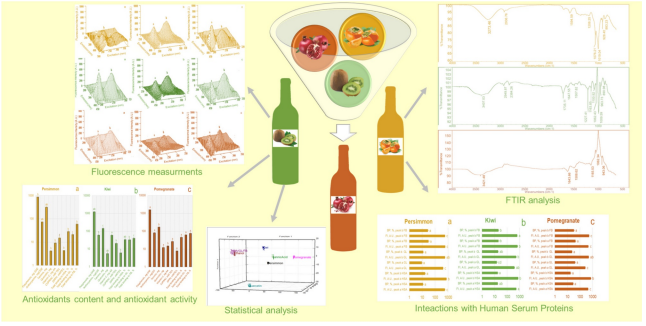
Abstract	*Abstract:* Recently we reported about the consumption of red wines from grapes, having several health properties. There are different types of wines that originated from grapes and other fruits. In the present study fruit wines from persimmon, kiwifruit and pomegranate were investigated and compared for their antioxidant ability, using cupric ion reducing antioxidant capacity (CUPRAC) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. To the fruit wines were applied the same methods of investigation as to the traditional ones made from grapes. The results showed the highest antioxidant activity of pomegranate, followed by kiwifruit and persimmon wines. Fourier transform infrared (FTIR) spectroscopy was used in order to correlate these results. The interaction of wine bioactive compounds with the main serum proteins in the human metabolism, such as human serum albumin (HSA), globulin (GL), and fibrinogen (FB), showed that pomegranate wine possesses higher quenching properties than kiwifruit and persimmon wines. All determined fluorescence indices have a direct correlation with the bioactivity of polyphenols and not with the content of alcohol. We hypothesize that the results of the interaction of main human serum proteins with bioactive compounds of wines can be additional predictors of their health properties. The used analytical methods for quality of fruit wines can be applied to a wide range of fruits and vegetables. *Graphical Abstract:* 
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ORIGINAL PAPER

Properties of some fruit wines

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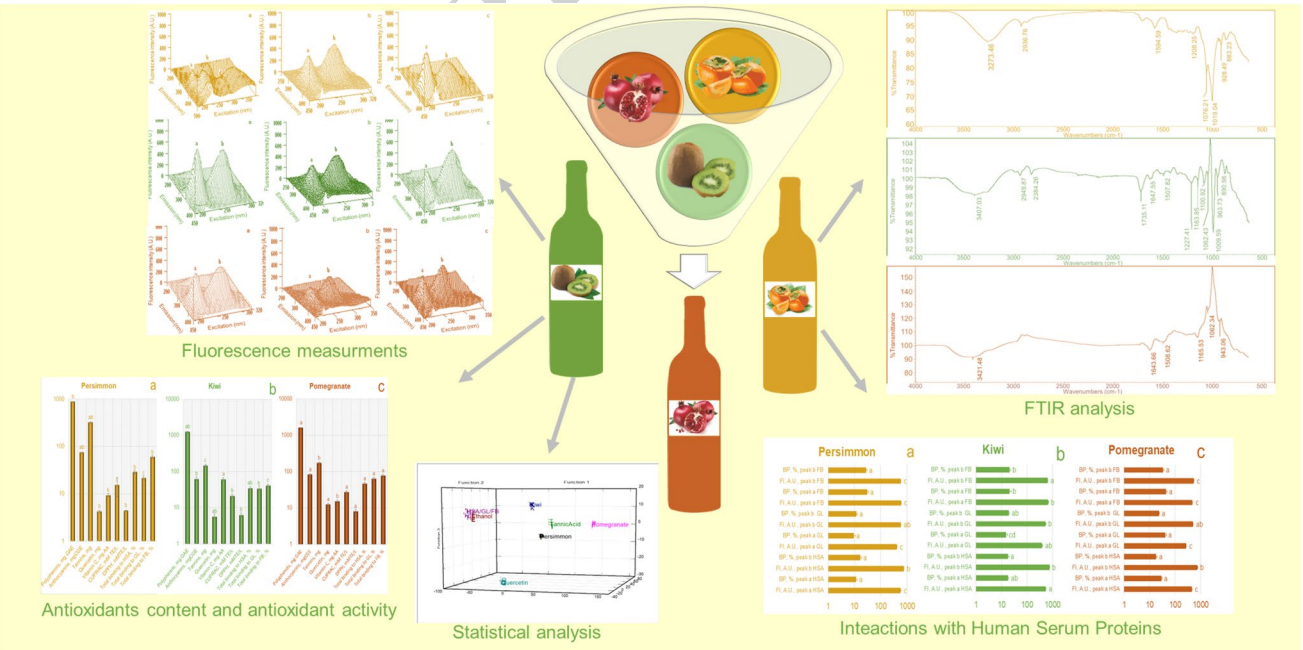
Abstract

Recently we reported about the consumption of red wines from grapes, having several health properties. There are different types of wines that originated from grapes and other fruits. In the present study fruit wines from persimmon, kiwifruit and pomegranate were investigated and compared for their antioxidant ability, using cupric ion reducing antioxidant capacity (CUPRAC) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays.

To the fruit wines were applied the same methods of investigation as to the traditional ones made from grapes**.** The results showed the highest antioxidant activity of pomegranate, followed by kiwifruit and persimmon wines. Fourier transform infrared (FTIR) spectroscopy was used in order to correlate these results. The interaction of wine bioactive compounds with the main serum proteins in the human metabolism, such as human serum albumin (HSA), globulin (GL), and fibrinogen (FB), showed that pomegranate wine possesses higher quenching properties than kiwifruit and persimmon wines. All determined fluorescence indices have a direct correlation with the bioactivity of polyphenols and not with the content of alcohol. We hypothesize that the results of the interaction of main human serum proteins with bioactive compounds of wines can be additional predictors of their health properties. The used analytical methods for quality of fruit wines can be applied to a wide range of fruits and vegetables.

Graphical Abstract



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Keywords Polyphenols · Pomegranate · Kiwifruit · Persimmon · Fluorescence · FTIR bands · Antioxidants · Human serum proteins · Quenching

Introduction

Most of the tropical and traditional fruits are important sources of antioxidants, vitamins, and minerals and form a very healthy part of a diet [1, 2]. Dietary persimmon (peel and pulp) was reported to exert hypolipidaemic properties in some hyperlipidemic animal models [3–5]. Kiwifruits exhibit mostly antioxidative and antihypercholesterolemic properties. Analytical and processing methods affect the physicochemical and biological properties of kiwifruit-derived ingredients and depend on different cultivars which were intensively studied and compared with persimmon [6–8]. Pomegranate is rich in colored and colorless phenolic compounds, varied depending on cultivars, and contains a high amount of polyphenols, anthocyanins, catechins, tannins, gallic and ellagic acids, and possesses high health properties [9, 10]. Concerning the antioxidant and healthy properties of presented fruits, the manufacture of wine from fruits, other than grapes, has been developed in recent years [11]. As it was shown above, kiwifruits, persimmons, and pomegranates contain high levels of bioactive compounds, especially polyphenols, and it is important to preserve them in the preparation of wines, juices and other varieties of food products [12–14]. The fruit wines are less popular compared to grape wines, but the raw materials of these wines, are rich in phenolic antioxidants. Polyphenols have a strong bioactivity as an active element in foods, fruits, cereals, vegetables and beverages (beers and wines). Many studies [2, 3, 5, 8] have demonstrated that polyphenols have also strong effects on the vascular system by lowering blood pressure, increasing antioxidant defenses, inhibiting platelet aggregation and low-density lipoprotein oxidation [15–17]. These properties are extensively used in the prevention and treatment of coronary artery disease (CAD) [1–3]. The purpose of the present report was to study the antioxidant, binding and healthy properties of bioactive compounds, which are present in kiwifruit, persimmon, and pomegranate wines, applying the advanced analytical methods, such as FTIR and fruorescence. The antioxidant properties of wines were determined by cupric ion reducing antioxidant capacity (CUPRAC) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The polyphenol spectrum of wines was determined by Fourier transform infrared (FTIR) spectroscopy. The binding abilities of wines with human serum albumin (HSA), globulin (GL) and fibrinogen (FB) were estimated by 3D- fluorescence and correlated with their polyphenol contents.

Materials and methods

Materials

The chemicals 6-Hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid (Trolox), 1,1-diphenyl-2-picrylhydrazyl (DPPH), lanthanum(III) chloride heptahydrate, $CuCl_2 \times 2H_2O$, 2,9-dimethyl-1,10-phenanthroline (neocuproine), quercetin, tannic acid, human serum albumin (HSA), fibrinogen (FB), globulin (GL), phosphate buffer and Folin-Ciocalteu reagent (FCR) were purchased from Sigma (St. Louis, MO, USA) and Fluka Chemie GmbH (Buchs, Switzerland).

Wine samples

Wines were bought in Israel and South Korea and were used in the present investigation study. Each kind of wine was purchased in the amount of five samples in several places, but from the same year of vintage and showed the same shelf life. The bottles with a range of alcohol in the same volume were frozen at − 80 °C to assess their antioxidant status and bioactivity. Pomegranate dry wine with 13.8% alcohol of 2018 vintage was purchased from Rimon wineries, Israel. Persimmon wine (Persimun wine (Regular) with 12.0% alcohol was delivered from Agricultural Corporation Cheongdo Persimun wine, Cheongdo, Gyeongsangbuk-do, Korea. Kiwi (Darae) wine with 8.0% alcohol was made from kiwis (chamdarae in Korean) grown in an environmentally friendly manner from farms in Sacheon, Gyeongsangnam-do (South Gyeongsang Province). As kiwis are consumed after ripening, a bottle of Darae Wine is made after going through a 10 months maturation. The samples were produced by Darae wine shop, GyeongnamSacheon-siMiryong-Gil, Korea. Samples for analysis were taken out of the refrigerator and were diluted according to the methods explained below.

Methods

Determination of bioactive compounds

The total phenolic amount (TP) was measured by using the Folin–Ciocalteu method [18], using 250 µL of wine mixed with 1000 µL of sodium carbonate (7.5%) and 1250 µL of Folin–Ciocalteu's (10% in water) reagent. The mixture was incubated for 15 min at 50 °C in the dark (water bath) and measured at 765 nm, using a spectrophotometer (Hewlett-Packard, model 8452A, (Rockville, MD, USA). Gallic acid was used as the standard, and the results were expressed as milligrams of gallic acid equivalent per liter (mg GAE/L).

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The anthocyanin content (AC) in wines was measured in aliquots of 250 µL of the wine sample which was poured into a tube with 2 mL of potassium chloride solution (0.025 M), adjusted to pH 1 with concentrated HCl. The mixture was incubated at room temperature for 20 min. In another tube, 250 µL of wine was mixed with 2 mL of sodium acetate solution (0.4 M, pH 4.5) and incubated at room temperature for 20 min. The absorbance of an aliquot of 300 µL of each wine sample was measured at 520 and 700 nm. The results were expressed as milligrams of cyanidin 3-glucoside equivalent per L (mg C3G/L) [19, 20]. The total tannins (TNs) were estimated by using spectrophoto-metric measurements of 0.5 mL of wine, where 3 mL of a 4% methanol vanillin solution and 1.5 mL of concentrated hydrochloric acid were added. The mixture was allowed to stand for 15 min. The absorption of the samples and a blank against water was measured at 500 nm [20, 21]. Some bioactive compounds, such as quercetin, were determined with a high-performance liquid chromatography HPLC system [20, 22]. A volume of 50 mL of each of the wine samples was extracted three times with 25 mL of diethyl ether and then three times with 25 mL of diethyl acetate, and the organic fractions were combined. After 30 min of drying with anhydrous Na_2SO_4, the extract was filtered through a Whatman-40 filter and evaporated to dryness in a rotary evaporator. The residue was dissolved in 2 mL of methanol/water (1:1, *v/v*) and analyzed by HPLC. A Waters (Milford, MA, USA) chromatograph equipped with a 600-MS controller, a 717 plus autosampler, and a 996 photodiode-array detector was used [20]. For the HPLC analysis, an aliquot (50 µL) was injected into the column and eluted at the temperature of 20 °C. Total ascorbic acid content [TAAC, mg ascorbic acid (AA) per L] was evaluated in water wine extracts, where 100 mg of the freeze-dried wine sample was extracted with 5 mL water. Then, the CUPRAC method was conducted and formed bis (Nc)-copper (I) chelate was determined spectrophotometrically at 450 nm [23].

Antioxidant capacity asssays

For the cupric-reducing antioxidant capacity (CUPRAC) assay [23, 24] fruit wines were diluted in a ratio of 1:10 (*v/v*) with dH_2O. About 1.0 mL of each of the three solutions containing 0.010 M Cu (II), ammonium acetate buffer at pH 7.0, and 0.0075 M neocuproine (2,9-dimethyl-1,10-phenanthroline) in EtOH was mixed with 0.5 mL of the appropriately diluted sample together with 0.6 mL of dH_2O in a tube. The reaction mixture was left for 1 h in the dark, and then the absorption was measured at 450 nm [20, 23, 24]. The antioxidant capacity was measured by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in 25 µL of the sample, which was mixed with 180 µL of DPPH radical at 6 mM and measured at 517 nm every 30 s for 10 min. Trolox was used as a standard for two antioxidant methods, and the results were expressed as millimoles of Trolox equivalent per liter (mmol TE/L) [20, 25].

Fourier transform infrared spectra of polyphenols in wines

Total phenols in the investigated fruit wines extracts were studied by IR spectroscopy.

The fruit wines were evaporated from the amount of alcohol, transferred under the liquid nitrogen in order to prevent their oxidation, and then lyophilized. The dry powder was extracted with methanol (25 mg/mL), then evaporated and lyophilized. The polyphenol extracts were used for FTIR measurements. A Nicolet iS 10 Fourier transform infrared (FTIR) Spectrometer (ThermoScientific Instruments LLC, Madison, (WI, USA)), with the smart iTRTM attenuated total reflectance (ATR) accessory was used to record IR spectra [26].

Fluorometric studies

The properties of bioactive substances in wines were determined by using three-dimensional (3D-FL) fluorescence (model FP-6500, Jasco spectrofluorometer, serial N261332, Tokyo, Japan). The 3D-FL was measured at emission wavelengths between 200 and 795 nm, and the initial excitation wavelength was 200 nm. For comparison of the obtained results quercetin and tannic acid were used [20]. Standard phenolic solutions, such as tannic acid and quercetin were prepared daily by dissolving at a concentration of 10 mM in methanol and then diluting with 10 mM phosphate buffer at pH 7.4. The initial fluorescence intensities of fibrinogen, albumin, and globulin were measured before their interactions with the investigated wines. As mentioned above, the changes in the fluorescence intensities were used in the estimation of the binding activities [17, 20].

Statistical analysis

All data obtained were calculated on the basis of a statistical analysis of Duncan's multiple range test. Values were mean s ± SD per liter of 25 measurements, representing the commercial status of the wines and their replicates. Five replications of five wine samples were used. To determine the statistical significance at the 95% interval of reliability, a one-way analysis of variance (ANOVA) was used.

For the purposes of distinguishing the fruit wines, the discriminant procedure was realized by means of the Unistat® statistical package (Unistat, London, United Kingdom) using the entire data, involving methods of principal component analysis (PCA), principal component factoring with varimax rotation (PCF) and canonical discriminant analysis (CDA). The convergence criteria of discriminant analysis

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were chosen for a standardized proximity matrix with the maximum number of iterations, 50. The following stepwise selection criteria were used: tolerance – 0.001, F statistic: F to enter – 3.8416, F to remove – 2.7056.

Results and discussion

Bioactivities of wine samples

The bioactivities of the main compounds in wines and their antioxidant activities were determined (Fig. 1). As can be seen (Fig. 1c) that pomegranate wine contains the highest amount of polyphenols and anthocyanins, followed by kiwi (Fig. 1b) and persimmon wines (Fig. 1a). Such results are in agreement with some recent reports [14], where the amount of total polyphenols in pomegranate wine was similar to the present results (Fig. 1c). Quercetin in pomegranate wine samples was 12.1 mg/L. The amount of anthocyanins was about 105.4 mg C3G/L. The amount of anthocyanin's concentration oscillated between 136 and 23 mg/100 mL for Wonderful and Mollar de Elche juices, respectively [27]. The amount of anthocyanins, as other bioactive compounds, varied in several genotypes and showed different values in the variety of cultivars, as cyanidin-3,5-O-diglucoside and pelargonidin-3,5-O-diglucoside in Santa Tecla population and were 97.64 mg/L and 40.29 mg/L, respectively. The amount of bioactive compounds (polyphenols, anthocyanins, and quercetin) was in correlation with the antioxidant activity and showed the value of total antioxidant activity by CUPRAC of 27.7 mM TE/L and by DPPH of 8.16 mM TE/L. These data are comparable with the same report [27], where the antioxidant activity was estimated at 9.8 mM/L. The total antioxidant activity values ranged between 221.5 and 36.73 µmol TE/100 mL of juice [28]. Such variety in the amounts of bioactive substances can be explained by the differences in cultivars, climate, and production processes. Pomegranate wines usually have high total phenolics and were 1.5 times higher than the amount of phenolics in green tea of 1029 mg/kg [29]. Persimmon wines showed the lowest polyphenol content in comparison with the other two wines (Fig. 1a). The obtained results depend on the cultivar used for wine production [30]. Most of the cited studies were conducted with whole persimmon or persimmon peel or persimmon pulp as test materials. It is well known that persimmon fruit contained a large number of components such as condensed tannin and polyphenols [1, 3, 31]. According to some authors, Wistar rats fed with a hypercholesterolemic diet enriched in persimmon (7%) had lower values of plasmatic lipids (cholesterol, triglycerides, LDL) after 4 weeks compared to control rats [1]. As it was shown previously that persimmon, kiwifruit and pomegranate have high antioxidant activities, then the wines prepared from the same fruits possess health properties. Due to the high content

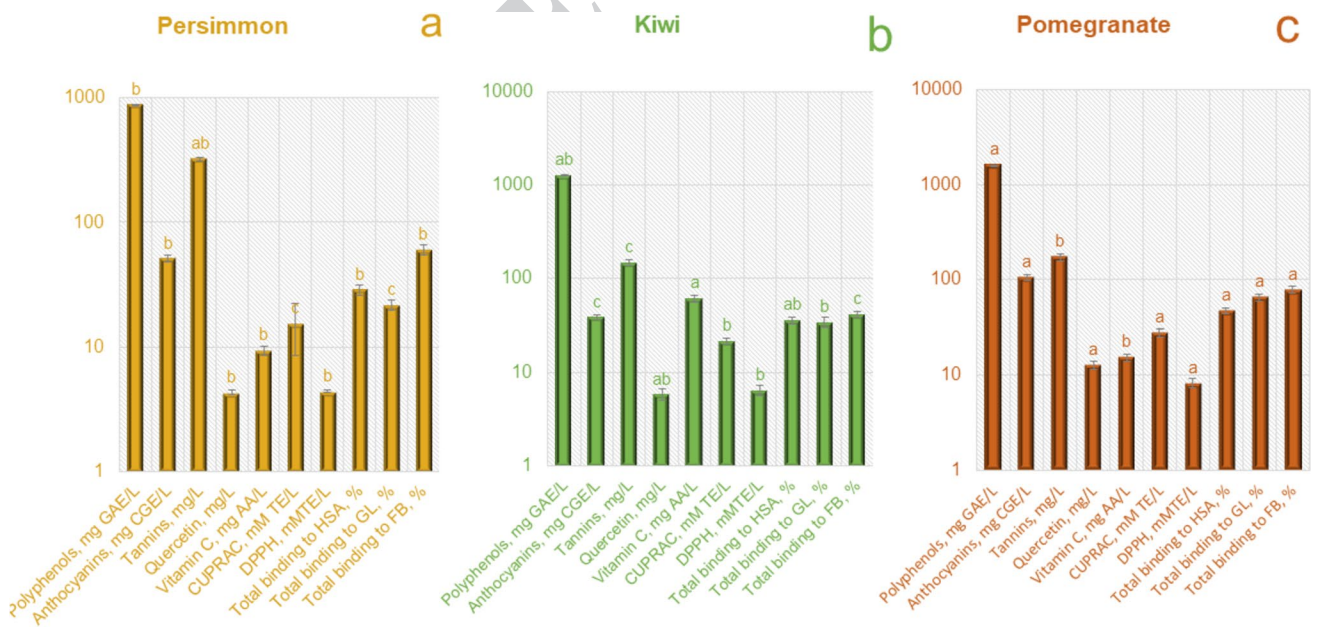

Fig. 1 Bioactive compounds, antioxidant activities and total binding properties of fruit wines **a** persimmon, **b** kiwi, **c** and pomegranate/L. Values are means ± SD of 5 measurements; Means within bars with different superscripts are statistically different ($p < 0.05$; Student's t test). Abbreviations: GAE, gallic acid equivalent; C3G, cyanidin 3-glucoside equivalent; AA, ascorbic acid; CUPRAC, Cupric reducing antioxidant capacity; DPPH, 1, 1-Diphenyl-2-picrylhydrazyl method; TE, Trolox equivalent; HSA, human serum albumin; GL, human serum globulin; FB, fibrinogen

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of antioxidants, persimmon could be of help in reducing or preventing LDL oxidation and thus the development of atherosclerosis [32]. It was shown in some reports that the high molecular weight of persimmon tannins is responsible for the hypocholesterolemic effect of persimmon fruit and it might exert the hypolipidemic effect, improving the antioxidant profile of human serum [3, 4, 33]. It is obvious that the properties of fruits have to be prevented during processing. The high temperature increased the contents of phenolic and polymeric pigment in wine: with polyphenols of 871.3 mg/L, quercetin of 0.04 mg/L, and total tannins of 311.29 mg/L, which are similar to the one shown in this report (Fig. 1a). The values of antioxidant activity by DPPH were slightly lower than in the present report of 775.1–1326.0 µmol/L [34]. It is generally approved that a moderate consumption of fermented beverages prevents metabolic disorders due to the antioxidant properties of phenolic compounds. Persimmon liqueur was prepared from fresh or dry fruit by: (1) extraction with alcohol, (2) fermentation of fresh fruit, and (3) extraction of dry fruit with distilled alcohol from an extract. Alteration in the ratio of raw and dry materials to a solvent, conditions of fermentation, and the degree of distillation resulted in a beverage with high aroma and taste, polyphenols, and proteins. Similar results were obtained with and without fermentation [13, 35]. Persimmon wine may offer nutritional and medicinal value as it contains compounds that may be beneficial to health. Phenolic compounds are important to wine quality as they contribute to antioxidant activity, aroma formation, colloidal stability, and sensory properties. High temperature also induced the increase of total tannins, compounds that contribute to antioxidant activity [36]. Kiwifruit has a beneficial impact on inflammatory processes, atherogenesis, and thrombogenesis, exerting also hypolipidaemic activity, and preventing diabetes development. Such properties were only found for kiwifruit and not for other fruits according to the EVIDENT study [37]. These properties of kiwifruit characterize the wines from this fruit.

Kiwifruit possesses similar properties as pomegranate with the highest amount of vitamin C (Fig. 1b). Cited reports described that kiwifruit is used as a juice, vinegar, dried slices, jam, and wine [38, 39]. The quality characteristics of kiwifruit wine made from over-ripened fruit treated with pectinase showed higher values of wine in many aspects such as sensory value, alcohol and total phenolics content, antioxidant activity, minerals, and production yield [12]. Several domestic varieties of kiwifruit were utilized for the processing of wines. It was shown that the wines have high antioxidant activity. The effects on human health depend on the amount of consumed wines and on their bioavailability. 'Daeheung' had total phenols of 790 mg/L, which was the highest among wines, followed by 'Haenam' and 'Golden King'. 'Daeheung' also showed the highest antioxidant activity (22.55 mMTE/L), while 'Arimold' showed the lowest one (10.91 mMTE/L). These values are in accordance with the present results (Fig. 1b). Two fruit wines (kiwi and persimmon) were delivered from South Korea, where the consumption of fruit wines is high. Various fruits such as raspberry (3,106 233 tons/year), plum (579 tons/year), and mulberry (434 tons/year), and relatively low amounts such as persimmon (83 tons/year) and kiwifruit (70 tons/year) are processed 235 into wine [40, 41].

FTIR spectra of wines

The infrared spectra of persimmon, kiwifruit, and pomegranate wines were measured in the frequency range of 4000–800 cm^{-1} (Fig. 2a, b, c). Standards such as quercetin and tannic acid (Fig. 2d, e) were done in order to identify the peaks. The O–H and C–H stretching frequencies in the polyphenols are found in the 3500–2600 cm^{-1} region and C–H stretching vibration occurred in the region of 2900 to 2800 cm^{-1} (for kiwi and persimmon wines as 2936.76; 2949.87; 2834.26 cm^{-1}). In the region of 2937–2950 cm^{-1}, the CH, CH_2, and CH_3 stretching vibrations, derived from carbohydrates and sugars, which are shown for kiwi and persimmon wines [42]. In quercetin, the peak obtained in the range of 3261 cm^{-1} represented the O–H stretching vibration due to the intra-molecular hydrogen bonding. The band observed at 1662 and 1607 cm^{-1} assigned to carbonyl C=O stretching vibration. The band obtained at 1519 cm^{-1} assigned for NO_2 bending vibration, the peak at 1447–1407 cm^{-1} for C–O, and the band at 1259–1215 cm^{-1} allocated to C–O–C of ester for quercetin compound. The prominent peak at 1166–1092 cm^{-1} indicated the stretching vibration of the C–O–C group. The presented results were similar to the reported [43]. Pomegranate wine does not show vibration in this region. In tannic acid, one characteristic peak was found at 3344 cm^{-1} which shows the stretching vibration of the O–H group. The peak at 1698 cm^{-1} represented the stretching of the carbonyl (C=O) group. The peak at 1537–1446 cm^{-1} allocated the carboxylic acid (O–C–O) and at 1312–1190 cm^{-1} showed the bending vibration of the O–H group. The band between 1024 and 956 cm^{-1} is assigned to the C=O group of molecules. The band at 756–868 cm^{-1} is due to the meta-substitution of the aromatic protons. The presented results of the peaks were equal to other reports [43, 44]. The major protein bands include amides I (C=O stretching coupled with N–H bending) vibrations at approximately 1650 cm^{-1} which appeared in kiwi and pomegranate wines at 1644 and 1648 cm^{-1} (Fig. 2b,c). The in-plane bending vibration of aromatic CH is detected at 1100 cm^{-1}, in kiwi wine and a CO stretching vibration is produced at 1062 cm^{-1} for kiwi and pomegranate wines. For signals with wavelengths smaller than 900 cm^{-1}, the aromatic CH stretching vibration is detected at 891 and 883 cm^{-1} for kiwi and persimmon wines. The

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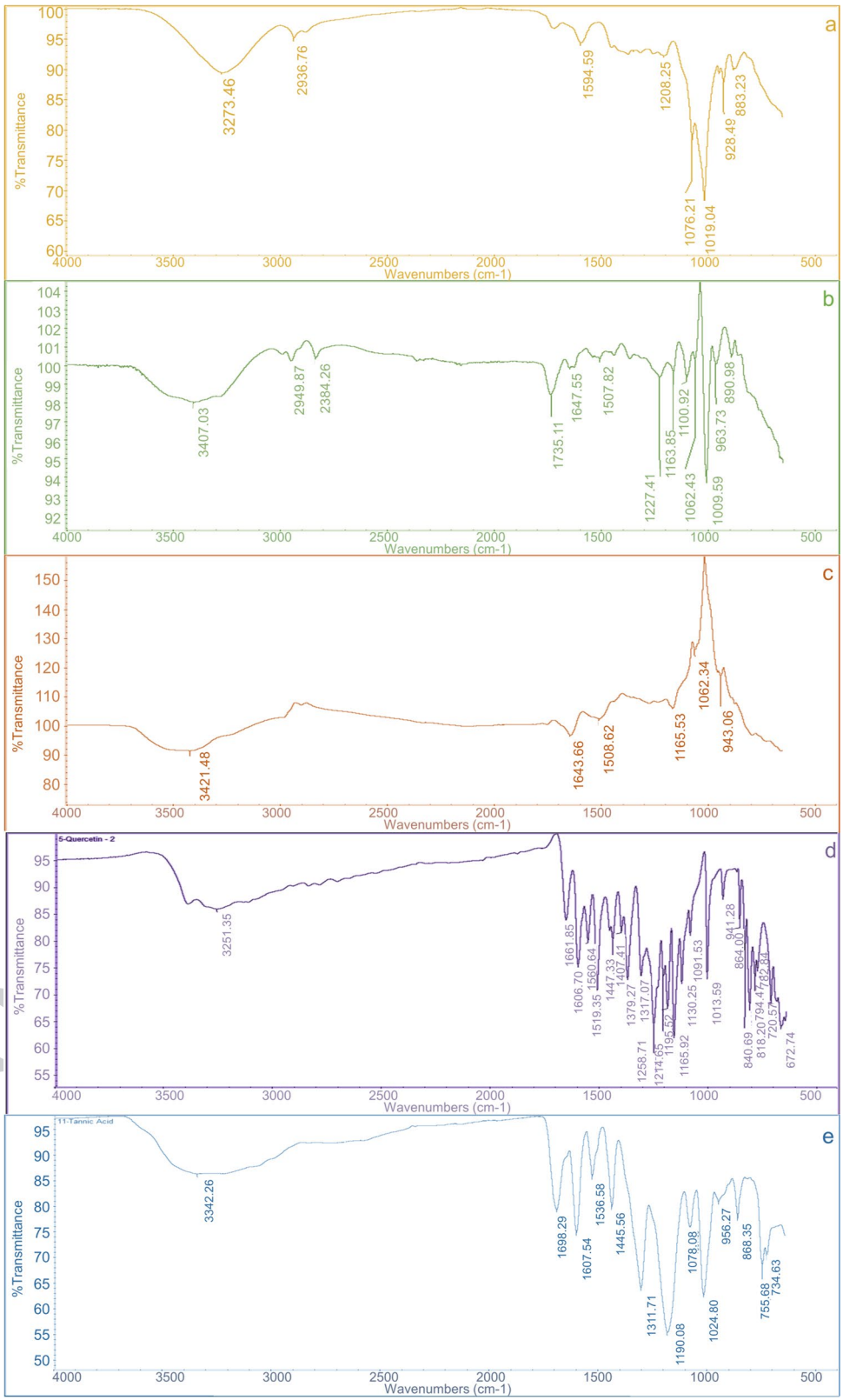

Fig. 2 Fourier Transform Infrared Spectra (FTIR) of polyphenols in wines: **a** persimmon, **b** kiwi, **c** pomegranate, **d** quercetin **e** tannic acid

peaks appeared in the standards and in the wines mostly in the range 1735–900 cm^{-1} for phenolics. The spectra of kiwifruit wine exhibit a shoulder around 1735 cm^{-1} due to the stretching of the carbonyl C=O group. Peaks at 1062 cm^{-1} (for pomegranate and kiwi wines) are ascribed to the –COH group of sugars in glycosylated phenols. The

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peak at 1019 cm^{-1} was ascribed to the phenolic C–OH in persimmon wine. The peak at 1735 cm^{-1} was assigned to the carbonyl C=O stretching band of protonated carboxylic acid, characteristic of the galloyl unit of hydrolysable tan-nins for kiwi wine [45]. Peaks at 1648 and 1644 cm^{-1} were assigned to the –COO– stretching for pomegranate and kiwi wines. The peak at 1227 cm^{-1} corresponds to the C–OH of phenols. Peaks at 1163 and 1166 cm^{-1} were ascribed to the C–OH stretching in glycosylated phenols for pomegranate and kiwi wines. Pomegranate and kiwi wines have in common absorptions at 1062, 1165, 1508, and 1647 cm^{-1}. The FTIR spectra in the region between 1735 and 900 cm^{-1} can serve as fingerprint and comparison of the investigated wines.

Fluorescence properties of wines

The antioxidant's strong affinity to human serum proteins and synergism in bioactivity are the main indices in the health application of wines [4, 5, 10, 20]. The chemical, phenolic and antioxidant characteristics of the wines were assessed by measurement of tannins, anthocyanins, and phenolic compounds (Figs. 1, 2). Our results were similar to the wines prepared by classical maceration. These properties of the wine bioactive compounds, mainly polyphenols, in interaction with the main human serum proteins are shown for all investigated samples (Fig. 3). As it was found previously that persimmon wine has the lowest antioxidant activity in comparison with other wines, it is expected from our previous reports that the quenching properties of persimmon wine are lower than in other samples [7, 8, 17, 20]. The calculations were done on the basis of decreasing the initial fluorescence intensity (FI) of human serum proteins before interaction with bioactive wine substances (Fig. 3a, Fig. 4a,b,c) and after interaction with wine samples, using the intensity of peaks a and b.

The initial fluorescence intensities for fibrinogen [(FI, Arbitral Units (A.U.) and maximum wavelength (λem/ex) nm] were the following: peak a with FI = 861.1 ± 10.4 and λem/ex = 229/342. Peak b was estimated as FI = 809.7 ± 10.3 and λem/ex = 282/341 (Figs. 3a and 4a). The initial fluorescence intensities for globulin [(FI, Arbitral Units (A.U.) and maximum wavelength (λem/ex) nm] were the following: peak a with FI = 457.3 ± 9.3 311 and λem/ex = 231/335. Peak b was estimated as FI = 661.1 ± 10.3 and λem/ex = 280/334 (Figs. 3a and 4b). The initial fluorescence intensities for HSA [(FI, Arbitral Units (A.U.) and maximum wavelength (λem/ex) nm] were the following: peak a with FI = 643.0 ± 7.9 and λem/ex = 228/353. Peak b was estimated as FI = 920.1 ± 10.4 and λem/ex = 280/357 (Figs. 3a and 4c). The measured data were changing during the interaction of these proteins with wines, tannic acid, quercetin, and ethanol (Figs. 3, 4, 5). It was determined a decrease in

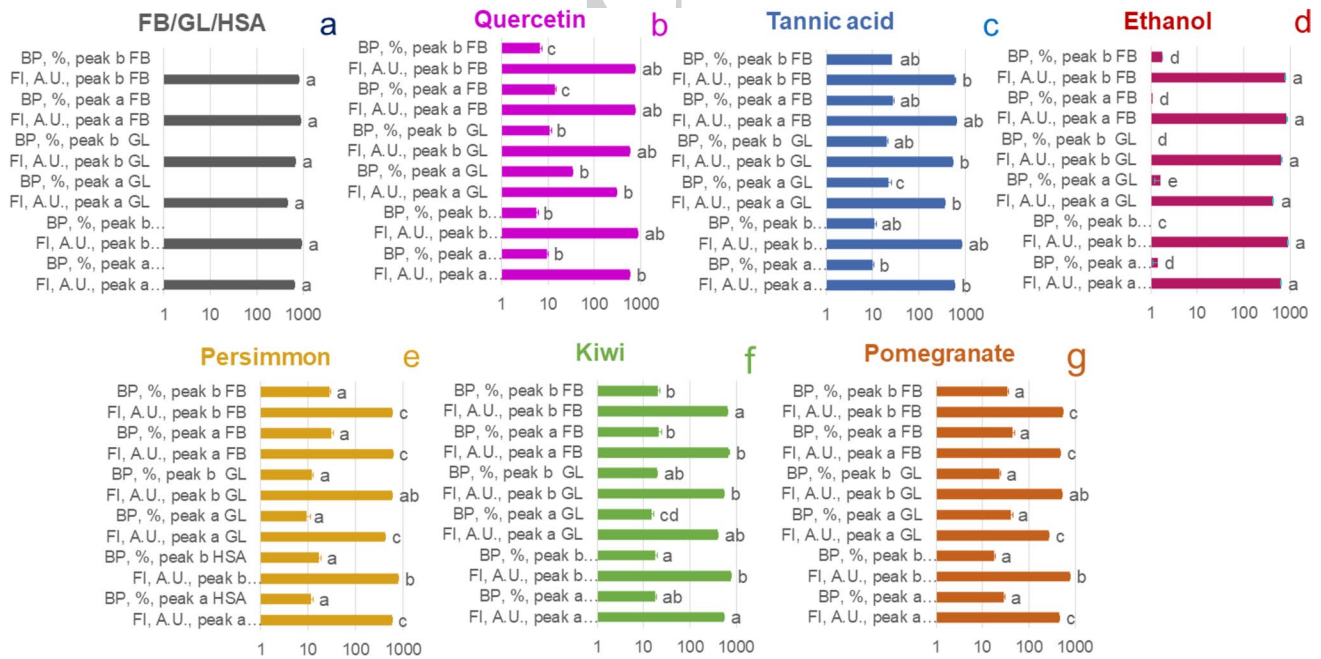

Fig. 3 Change in the values of **a** fibrinogen/globulin/human serum albumin before the interaction with initial values of fluorescence intensities and after interaction with **b** quercetin, **c** tannic acid, **d** ethanol, **e** persimmon, **f** kiwi, **g** pomegranate wines. Values are means ± SD of 5 measurements; n = 5 samples, each subsampled and analyzed 5 times. Means within bars with the different superscripts are statistically different ($p < 0.05$; Student's t test). Abbreviations: fluorescence; intensity (FI); Arbitral Units (A.U.); fibrinogen (FB); human serum globulin (GL); human serum albumin (HSA), Binding properties (BP, %): binding to FB, to GL and HSA is the % decrease of fluorescence emission intensity of the fractions of the binding sites of the proteins occupied by the ligand to the initial ones

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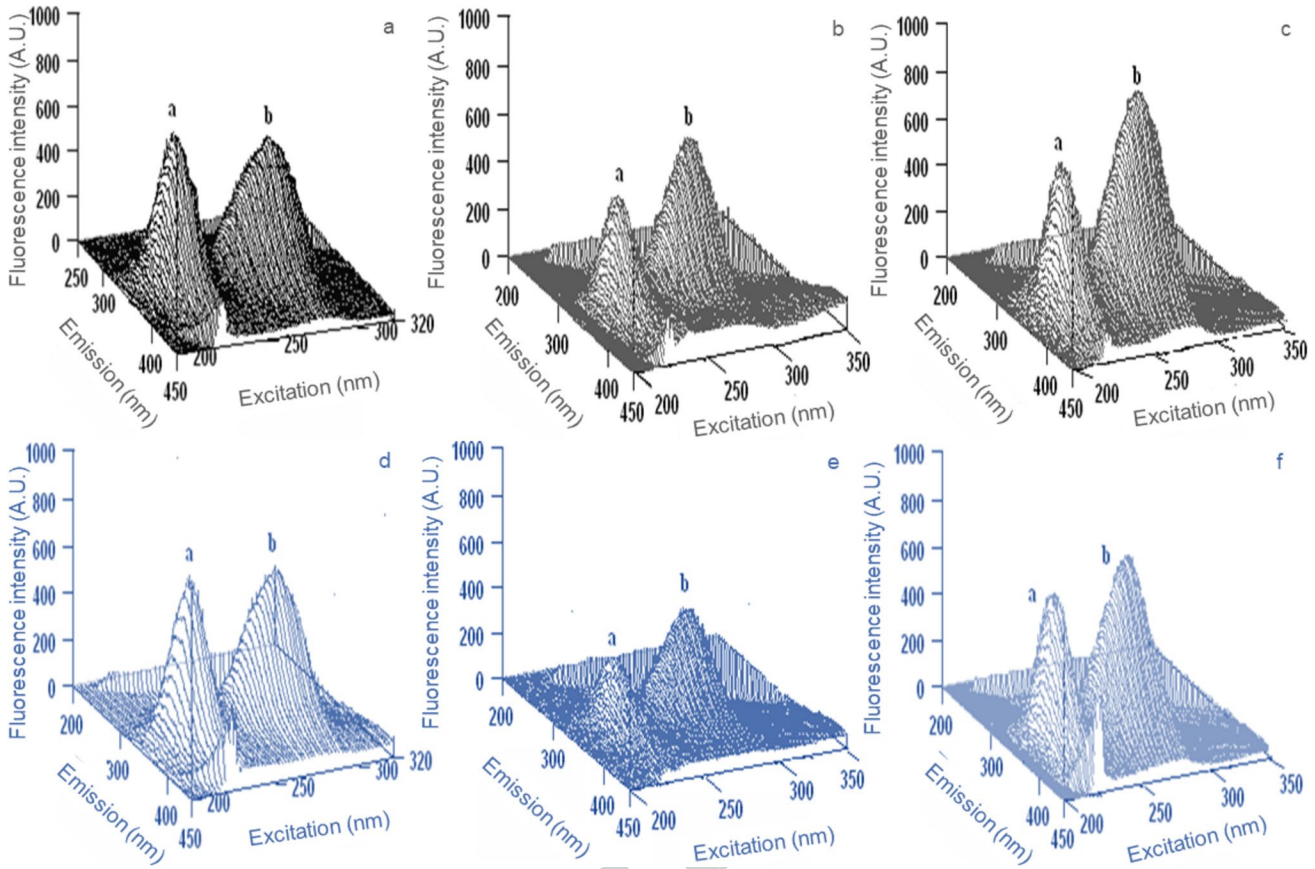

Fig. 4 Fluorometric measurements in a three-dimensional fluorescence analysis (3D-FL) of **a** fibrinogen, **b** globulin, **c** human serum albumin and tannin acid after interaction with **d** fibrinogen, **e** globulin, **f** human serum albumin. The locations of peaks **a** and **b** are shown in this figure: for fibrinogen with fluorescence intensity of Arbitral Units (A.U.): peak **a** = 861.1 ± 10.4, peak 339 **b** = 809.7 ± 10.3; for globulin: peak **a** = 457.3 ± 9.3, peak **b** = 661.1 ± 10.3; and for HSA: peak 340 **a** = 643.0 ± 7.9; peak **b** = 920.1 ± 10.4 and the locations of peaks **a** and **b** for tannic acid are shown in this (**c**) (for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

the fluorescence intensities and a change in the maximum wavelengths during the reaction of fibrinogen, globulin, and HSA with bioactive substances of persimmon, kiwifruit, and pomegranate wines. The total binding properties (%) of quercetin for fibrinogen, globulin, and HSA of peaks a and b were estimated as 20.7 ± 1.1, 44.6 ± 3.5, and 15.1 ± 0.8, respectively (Fig. 3b). The total binding properties (%) of tannic acid for fibrinogen, globulin and HSA of peaks a and b were estimated as 54.3 ± 4.3, 42.5 ± 3.8, and 20.9 ± 1.7, respectively (Fig. 3c and Fig.4d–f). The total binding properties (%) of ethanol for fibrinogen, globulin, and HSA of peaks a and b were calculated as 2.9 ± 0.3, 2.6 ± 0.5, and 2.4 ± 0.3, respectively (Fig. 3d). The total binding properties (%) of persimmon wines for fibrinogen, globulin, and HSA of peaks a and b showed the following data: 59.6 ± 5.7, 21.6 ± 2.1, and 28.8 ± 2.7, respectively (Figs. 1a, 3e, and 5a,b,c). The total binding properties (%) of kiwifruit wines for fibrinogen, globulin, and HSA of peaks a and b were calculated as 41.7 ± 3.2, 34.4 ± 4.6, and 35.7 ± 2.9, respectively (Figs. 1b, 3f and 5d, e, f). The total binding properties (%) of pomegranate wines for fibrinogen, globulin, and HSA of peaks a and b were estimated as 78.2 ± 6.3, 64.8 ± 5.8, and 47.0 ± 3.9, respectively (Figs. 1c, 3g, and 5g, h, i). The interactions of quercetin with fibrinogen, globulin, and HSA showed the following total binding properties (%) of peaks a and b such as 20.7 ± 1.1, 44.6 ± 3.5, and 15.1 ± 0.8, respectively (Fig. 3f). The total binding properties (%) of ethanol for fibrinogen, globulin, and HSA of peaks a and b were calculated as 2.9 ± 0.3, 2.6 ± 0.5, and 2.4 ± 0.3, respectively (Fig. 3g). All the presented results after the interaction of the main serum proteins were connected to the amount of polyphenols, anthocyanins, tannic acid, and antioxidant activities of the samples. The highest total binding properties were estimated for pomegranate, followed by kiwifruit and persimmon wines (Fig. 1). The results of bioactive compounds and fluorescence quenching show that these wines possess multiple properties that have a great potential to be used for human health and show similar data as the used fruits [8,

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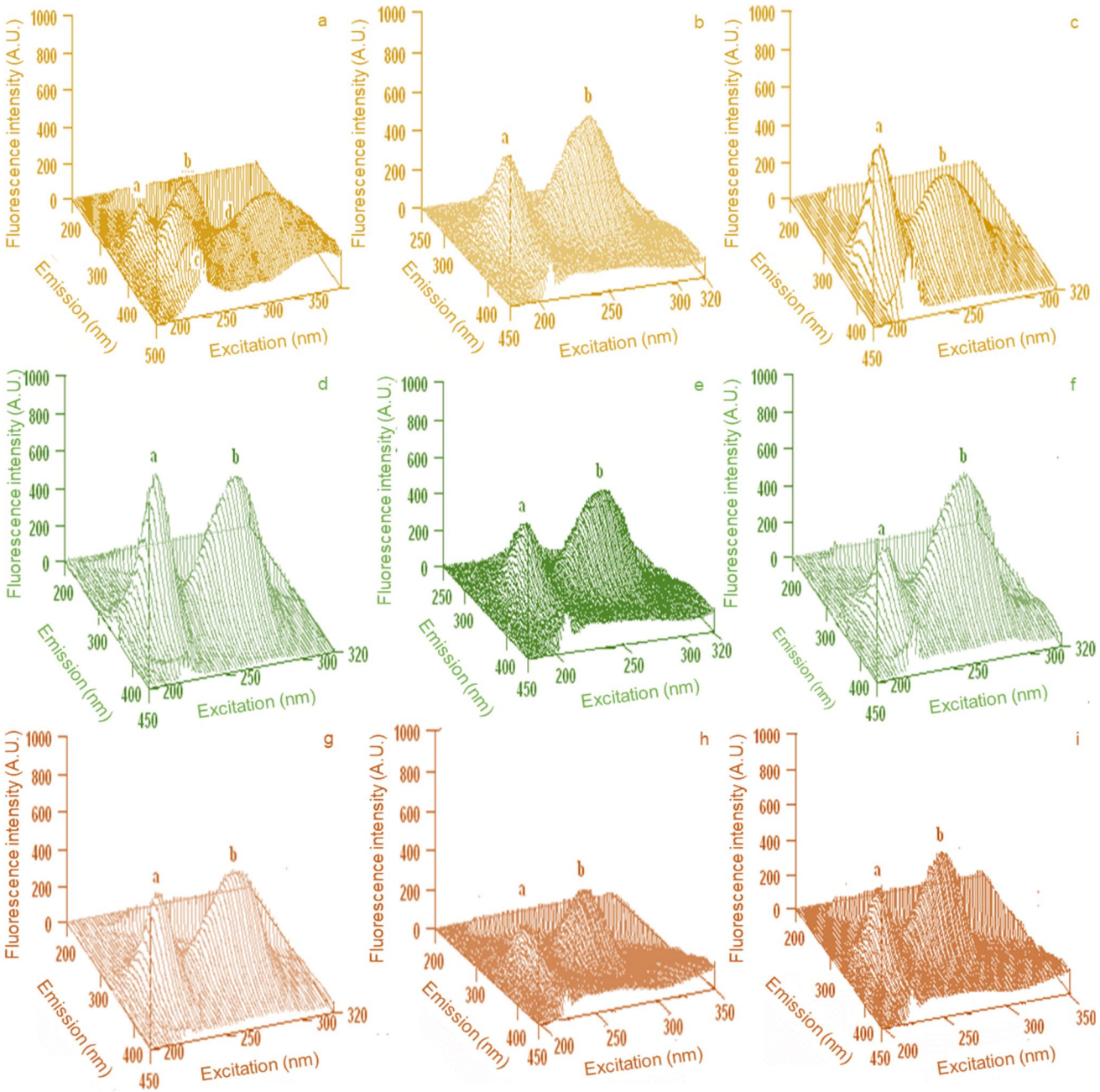

Fig. 5 Fluorometric measurements in a three-dimensional fluorescence analysis (3D-FL) of wines: **a**–**c** persimmon, **d**–**f** kiwi fruit, **g**–**i** pomegranate after interaction with (**a**, **d**, **g**) fibrinogen, (**b**, **e**, **h**) globulin, (**c**, **f**, **i**) human serum albumin, respectively. The locations of peaks **a** and **b** are shown in this figure and for persimmon, kiwi, and pomegranate wines in Fig. 4e, f, and g**,** respectively

10]. The beneficial effect of fruit- and vegetable-rich diets on cardiovascular health is partly attributed to the effect of their bioactive compounds [46] and bioactive-rich extracts of kale and pomegranate that are consumed as traditional plant foods of Black Sea area countries were effective in modulating platelet function. Comparison of the present results with the reported ones [10, 20, 47, 48] showed the following antioxidant activities: 9.6–29.9 mM TE in red grape wines and 1.7–3.7 365 mM TE in white wines from France; 9.2–19.5 mM TE in red wines, and 0.5–1.4 mM TE in white wines from South Africa. The present results of antioxidant activities of 19.4–28.3 367 mM TE by CUPRAC assay showed that the fruit wines had relatively high antioxidant capacities that give a strong possibility of their use as a promising source of phenolic antioxidants. In vitro and in silico interactions of red grape wine polyphenols with human serum albumin, fibrinogen, glutathione peroxidase 3 and C-reactive protein enhance their biological activity

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[20]. A comparison of the binding properties of ethanol and bioactive substances in fruit wines once more showed the natural oxidative properties of polyphenols and their health properties. This can be explained only in the presence of bioactive substances in wines (the total binding properties of wines with fibrinogen ranged from 78.2 to 41.7% in comparison with ethanol of 2.9% [48]. The highest binding values were with fibrinogen, which is a very important protein and one of the indices of coronary artery disease [10, 49]. The binding to HSA in pomegranate wine was slightly lower than with fibrinogen and globulin, and only in kiwifruit and persimmon wines was slightly higher than for globulin. HSA [20, 50] is the main carrier in human metabolism for drugs, such as antibiotics and a big number of drugs. The binding of HSA resulted in the fluorescence quenching of HSA, as it is in the presented results. Our study for the first time unveils the differential binding properties of kiwifruit and persimmon phytoconstituents with HSA. Although cultivars possess virtually the same amount, the presence of one unique compound significantly alters the binding properties of HSA. The results of fluorescence quenching and molecular docking showed that these fruits possess multiple properties, which have a great potential to be used as functional foods [7]. Synergism was shown in the obtained measurements of quercetin, where the binding was lower than in used wine samples. Oppositely tannic acid showed high results of quenching in comparison with wines, especially persimmon wine which has a high amount of tannins. The binding properties of tannic acid with fibrinogen were higher than in kiwifruit wine but lower than in pomegranate and persimmon wines, which showed equal values for fibrinogen. The interaction between proteins and tannins was strong, as expected, leading to the precipitation of protein-tannin complexes. These results were in agreement with previously published data showing that high levels of precipitation of BSA by tannin can occur at low pH when the tannin to protein ratio is high [51, 52].

Relationship between antioxidant and binding properties of fruit wines

To assess the relationship between antioxidant and binding properties of fruit wines methods of multivariate statistics were applied. Principal component analysis (PCA) brings clear differentiation of eigenvectors according to the wine type (Fig. 6a). As regards the numerical values, the first two principal components (PC) cumulatively explained more than 91% of the whole dataset variability, with the dominant role of total binding to HSA, CUPRAC, and polyphenols (first PC, > 34% all), and vitamin C, tannins, and total binding to FB (second PC, > 69%, > 56% and > 30%, respectively). Results of PCA also confirm principal component factoring (PCF) and the stepwise discriminant analysis (CDA, data not presented) resulting in 100% correct classification of fruit wines. The plot of factors (varimax rotation, Fig. 6b) shows mutual strong positive correlations among binding properties to main serum proteins and polyphenols, anthocyanins, quercetin, CUPRAC, and DPPH. On the other hand, weak and moderate correlations between binding properties, vitamin C, and tannins are obvious from Fig. 6b, which correspond with Pearson's correlation coefficients.

Methods of multivariate statistics were utilized also to process the results of the fluorescence measurements described above. Principal component analysis based on fluorescence data led to the successful differentiation of fruit wines and selected standards (Fig. 6c). First three PCs cumulatively explained more than 81% of the variability of the experimental characteristics, recognizing the variables binding properties and fluorescence intensities of peaks a and b for fibrinogen as the most important for PC1 construction, whereas for the second PC, fluorescence intensity and binding properties of peak a for GL and maximum wavelengths ($\lambda_{em/ex}$) of peaks a and b for HSA, and for the third PC, maximum wavelengths ($\lambda_{em/ex}$) of peak b for GL and FB, were identified as parameters with the highest eigenvalues. Similarly to PCA, the stepwise discriminant analysis resulted in 100% correct classification of samples (Fig. 6d). By means of the first discriminant function (DF) > 69.5% of the cases were correctly classified, whereas by the first and second DF > 97.4% of the cases and by the first three DFs, 100% of the cases were classified. As the most discriminating characteristics, in the first DF, fluorescence intensities of peaks a and b for FB were identified. In the second DF, fluorescence intensities of peak b for FB and peak a for GL, and in the third DF, fluorescence intensities of peaks a and b for FB reached the highest discriminant coefficients.

Conclusions

In this study, three-dimensional fluorescence spectroscopy in combination with FTIR was used in the investigation of antioxidant profiles in fruit wines. This is the first report showing differences and similarities in fruit wines, using their binding properties. The fluorescence spectral methods, which were applied as a powerful tool showing the quenching properties of intrinsic fluorophores in protein molecules in the presence of fruit wine polyphenols, can contribute to the interaction with drugs. Based on the quenching properties of human serum proteins with wines and recent reports in vivo on human studies, we hypothesize that the used human proteins can be predictors of coronary artery disease (CAD). The applied analytical methods are universal not only for the authentication of fruit wines, but also for a variety of fruits and vegetables. The application of FTIR measurements can be used as a

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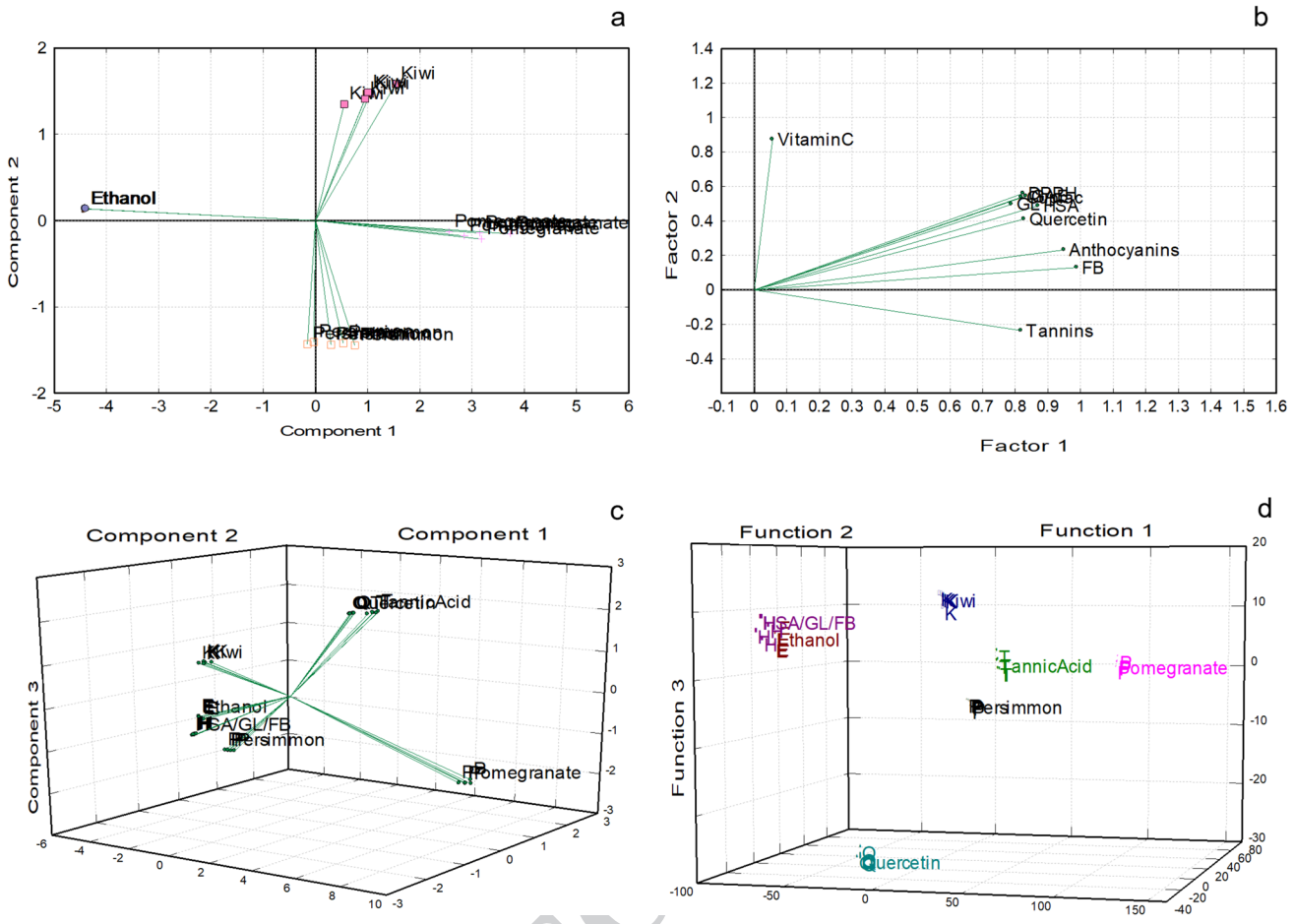

Fig. 6 Relationships between antioxidant, spectral, and binding properties of fruit wines. **a,** Principal component analysis of fruit wines based on antioxidant and binding properties of fruit wines. **b,** Plot of factors (varimax rotation) indicating mutual correlation and the importance of antioxidant and binding properties for the fruit wines differentiation. **c,** Principal component analysis of fruit wines and selected standards based on fluorescence data. **d,** Stepwise discriminant analysis of fruit wines and selected standards based on fluorescence data

fingerprint showing the real composition of the products. The fluorescence spectra, resulting in the binding properties during interaction of different phenolic compounds with human serum proteins, can be used as a fast and reliable in vitro analysis for health-promoting benefits of the food products.

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Author contributions SG; YMK; ML-S; MP; BT: conceptualization; YMK; YSP; KSH: statistical evaluation; YKP; SGK: data curation; KSH; YKP; SGK: formal analysis; MP; BT: investigation; DB; AN; SG; ML-S: methodology; YSP; KSH: software; ML-S; PT; YSP; DB; AN: validation; SG: supervision; YMK; ML-S; SG; DB; AN; MP; BT: writing—original draft preparation, YMK; SG; ML-S; PT: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interests.

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