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ArticleTitle	Properties of some fruit wines	
Article Sub-Title		
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Journal Name	European Food Research and Technology	
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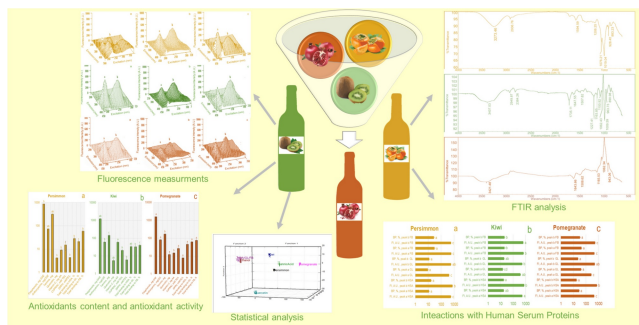
Schedule	Received	22 May 2023
	Revised	7 Oct 2023
	Accepted	13 Oct 2023



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:

Keywords (separated by '- Polyphenols - Pomegranate - Kiwifruit - Persimmon - Fluorescence - FTIR bands - Antioxidants - Human serum proteins - Quenching')

Footnote Information



2 Properties of some fruit wines

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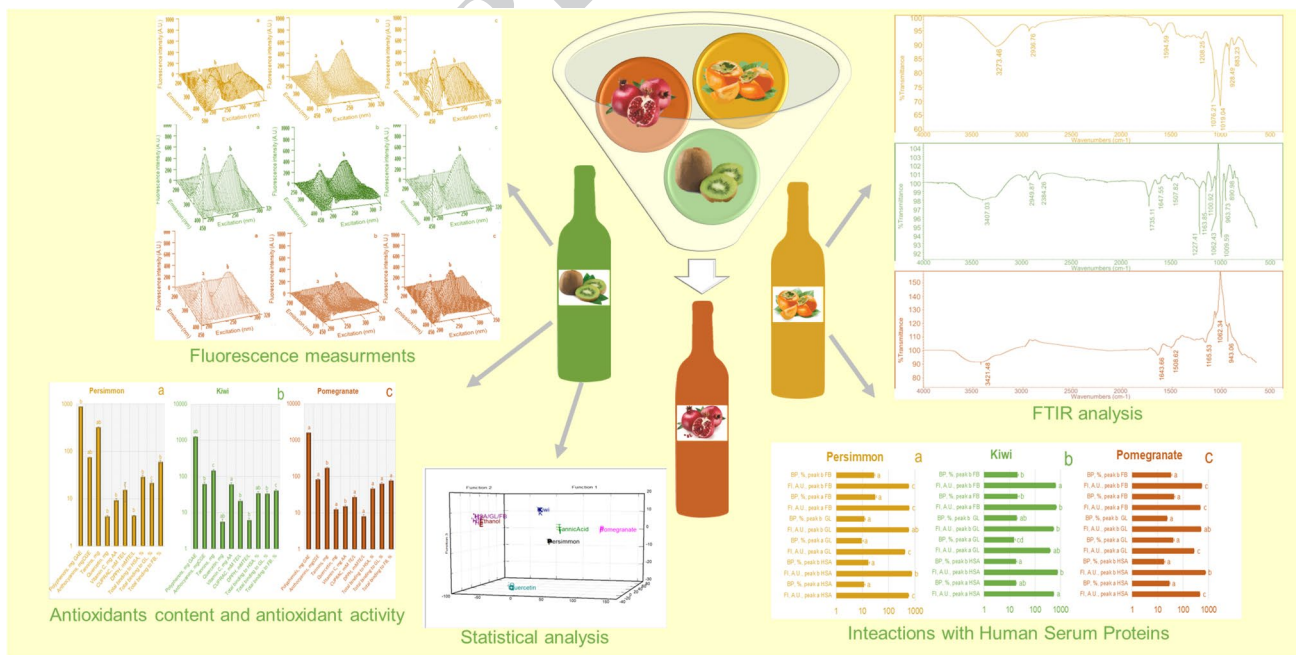
6 Received: 22 May 2023 / Revised: 7 October 2023 / Accepted: 13 October 2023
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22 Graphical Abstract

23



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25 **Keywords** Polyphenols · Pomegranate · Kiwifruit ·
 26 Persimmon · Fluorescence · FTIR bands · Antioxidants ·
 27 Human serum proteins · Quenching

28 Introduction

29 Most of the tropical and traditional fruits are important
 30 sources of antioxidants, vitamins, and minerals and form a
 31 very healthy part of a diet [1, 2]. Dietary persimmon (peel
 32 and pulp) was reported to exert hypolipidaemic properties in
 33 some hyperlipidemic animal models [3–5]. Kiwifruits exhibit
 34 mostly antioxidative and antihypercholesterolemic properties.
 35 Analytical and processing methods affect the physicochemi-
 36 cal and biological properties of kiwifruit-derived ingredients
 37 and depend on different cultivars which were intensively
 38 studied and compared with persimmon [6–8]. Pomegranate
 39 is rich in colored and colorless phenolic compounds, varied
 40 depending on cultivars, and contains a high amount of poly-
 41 phenols, anthocyanins, catechins, tannins, gallic and ellagic
 42 acids, and possesses high health properties [9, 10]. Concerning
 43 the antioxidant and healthy properties of presented fruits, the
 44 manufacture of wine from fruits, other than grapes, has been
 45 developed in recent years [11]. As it was shown above, kiwi-
 46 fruits, persimmons, and pomegranates contain high levels of
 47 bioactive compounds, especially polyphenols, and it is impor-
 48 tant to preserve them in the preparation of wines, juices and
 49 other varieties of food products [12–14]. The fruit wines are
 50 less popular compared to grape wines, but the raw materials
 51 of these wines, are rich in phenolic antioxidants. Polyphenols
 52 have a strong bioactivity as an active element in foods, fruits,
 53 cereals, vegetables and beverages (beers and wines). Many
 54 studies [2, 3, 5, 8] have demonstrated that polyphenols have
 55 also strong effects on the vascular system by lowering blood
 56 pressure, increasing antioxidant defenses, inhibiting platelet
 57 aggregation and low-density lipoprotein oxidation [15–17].
 58 These properties are extensively used in the prevention and
 59 treatment of coronary artery disease (CAD) [1–3]. The pur-
 60 pose of the present report was to study the antioxidant, bind-
 61 ing and healthy properties of bioactive compounds, which
 62 are present in kiwifruit, persimmon, and pomegranate wines,
 63 applying the advanced analytical methods, such as FTIR and
 64 fluorescence. The antioxidant properties of wines were deter-
 65 mined by cupric ion reducing antioxidant capacity (CUPRAC)
 66 and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The
 67 polyphenol spectrum of wines was determined by Fourier
 68 transform infrared (FTIR) spectroscopy. The binding abilities
 69 of wines with human serum albumin (HSA), globulin (GL)
 70 and fibrinogen (FB) were estimated by 3D- fluorescence and
 71 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-picrylhy-
 drazyl (DPPH), lanthanum(III) chloride heptahydrate,
 $\text{CuCl}_2 \times 2\text{H}_2\text{O}$, 2,9-dimethyl-1,10-phenanthroline (neo-
 cuproine), 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 Rimón wineries, Israel.
 Persimmon wine (Persimmon wine (Regular) with 12.0% alco-
 hol was delivered from Agricultural Corporation Cheongdo
 Persimmon 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 ripen-
 ing, 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).

115 The anthocyanin content (AC) in wines was measured in
 116 aliquots of 250 μL of the wine sample which was poured into
 117 a tube with 2 mL of potassium chloride solution (0.025 M),
 118 adjusted to pH 1 with concentrated HCl. The mixture was
 119 incubated at room temperature for 20 min. In another tube,
 120 250 μL of wine was mixed with 2 mL of sodium acetate
 121 solution (0.4 M, pH 4.5) and incubated at room temperature
 122 for 20 min. The absorbance of an aliquot of 300 μL of each
 123 wine sample was measured at 520 and 700 nm. The results
 124 were expressed as milligrams of cyanidin 3-glucoside equiv-
 125 alent per L (mg C3G/L) [19, 20]. The total tannins (TNs)
 126 were estimated by using spectrophotometric measurements
 127 of 0.5 mL of wine, where 3 mL of a 4% methanol vanillin
 128 solution and 1.5 mL of concentrated hydrochloric acid were
 129 added. The mixture was allowed to stand for 15 min. The
 130 absorption of the samples and a blank against water was
 131 measured at 500 nm [20, 21]. Some bioactive compounds,
 132 such as quercetin, were determined with a high-performance
 133 liquid chromatography HPLC system [20, 22]. A volume of
 134 50 mL of each of the wine samples was extracted three times
 135 with 25 mL of diethyl ether and then three times with 25 mL
 136 of diethyl acetate, and the organic fractions were combined.
 137 After 30 min of drying with anhydrous Na_2SO_4 , the extract
 138 was filtered through a Whatman-40 filter and evaporated to
 139 dryness in a rotary evaporator. The residue was dissolved in
 140 2 mL of methanol/water (1:1, v/v) and analyzed by HPLC.
 141 A Waters (Milford, MA, USA) chromatograph equipped
 142 with a 600-MS controller, a 717 plus autosampler, and a
 143 996 photodiode-array detector was used [20]. For the HPLC
 144 analysis, an aliquot (50 μL) was injected into the column and
 145 eluted at the temperature of 20 $^\circ\text{C}$. Total ascorbic acid con-
 146 tent [TAAC, mg ascorbic acid (AA) per L] was evaluated in
 147 water wine extracts, where 100 mg of the freeze-dried wine
 148 sample was extracted with 5 mL water. Then, the CUPRAC
 149 method was conducted and formed bis (Nc)-copper (I) che-
 150 late was determined spectrophotometrically at 450 nm [23].

151 Antioxidant capacity assays

152 For the cupric-reducing antioxidant capacity (CUPRAC)
 153 assay [23, 24] fruit wines were diluted in a ratio of 1:10
 154 (v/v) with dH_2O . About 1.0 mL of each of the three solutions
 155 containing 0.010 M Cu (II), ammonium acetate buffer at pH
 156 7.0, and 0.0075 M neocuproine (2,9-dimethyl-1,10-phenan-
 157 throline) in EtOH was mixed with 0.5 mL of the appropri-
 158 ately diluted sample together with 0.6 mL of dH_2O in a tube.
 159 The reaction mixture was left for 1 h in the dark, and then
 160 the absorption was measured at 450 nm [20, 23, 24]. The
 161 antioxidant capacity was measured by using 1, 1-diphenyl-
 162 2-picrylhydrazyl (DPPH) in 25 μL of the sample, which was
 163 mixed with 180 μL of DPPH radical at 6 mM and meas-
 164 ured at 517 nm every 30 s for 10 min. Trolox was used as a
 165 standard for two antioxidant methods, and the results were

expressed as millimoles of Trolox equivalent per liter (mmol
 TE/L) [20, 25].

168 Fourier transform infrared spectra of polyphenols 169 in wines

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

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

182 Fluorometric studies

183 The properties of bioactive substances in wines were deter-
 184 mined by using three-dimensional (3D-FL) fluorescence
 185 (model FP-6500, Jasco spectrofluorometer, serial N261332,
 186 Tokyo, Japan). The 3D-FL was measured at emission wave-
 187 lengths between 200 and 795 nm, and the initial excitation
 188 wavelength was 200 nm. For comparison of the obtained
 189 results quercetin and tannic acid were used [20]. Standard
 190 phenolic solutions, such as tannic acid and quercetin were
 191 prepared daily by dissolving at a concentration of 10 mM in
 192 methanol and then diluting with 10 mM phosphate buffer
 193 at pH 7.4. The initial fluorescence intensities of fibrinogen,
 194 albumin, and globulin were measured before their interac-
 195 tions with the investigated wines. As mentioned above, the
 196 changes in the fluorescence intensities were used in the esti-
 197 mation of the binding activities [17, 20].

198 Statistical analysis

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

206 For the purposes of distinguishing the fruit wines, the dis-
 207 criminant procedure was realized by means of the Unistat[®]
 208 statistical package (Unistat, London, United Kingdom)
 209 using the entire data, involving methods of principal com-
 210 ponent analysis (PCA), principal component factoring with
 211 varimax rotation (PCF) and canonical discriminant analysis
 212 (CDA). The convergence criteria of discriminant analysis

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

217 Results and discussion

218 Bioactivities of wine samples

219 The bioactivities of the main compounds in wines and their
220 antioxidant activities were determined (Fig. 1). As can be
221 seen (Fig. 1c) that pomegranate wine contains the highest
222 amount of polyphenols and anthocyanins, followed by kiwi
223 (Fig. 1b) and persimmon wines (Fig. 1a). Such results are in
224 agreement with some recent reports [14], where the amount
225 of total polyphenols in pomegranate wine was similar to
226 the present results (Fig. 1c). Quercetin in pomegranate wine
227 samples was 12.1 mg/L. The amount of anthocyanins was
228 about 105.4 mg C3G/L. The amount of anthocyanin's con-
229 centration oscillated between 136 and 23 mg/100 mL for
230 Wonderful and Mollar de Elche juices, respectively [27].
231 The amount of anthocyanins, as other bioactive compounds,
232 varied in several genotypes and showed different values in
233 the variety of cultivars, as cyanidin-3,5-O-diglucoside and
234 pelargonidin-3,5-O-diglucoside in Santa Tecla population
235 and were 97.64 mg/L and 40.29 mg/L, respectively. The

amount of bioactive compounds (polyphenols, anthocya-
nins, 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 $\mu\text{mol TE}/100\text{ mL}$ 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 per-
simmon pulp as test materials. It is well known that persim-
mon 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 plas-
matic lipids (cholesterol, triglycerides, LDL) after 4 weeks
compared to control rats [1]. As it was shown previously
that persimmon, kiwifruit and pomegranate have high anti-
oxidant activities, then the wines prepared from the same
fruits possess health properties. Due to the high content

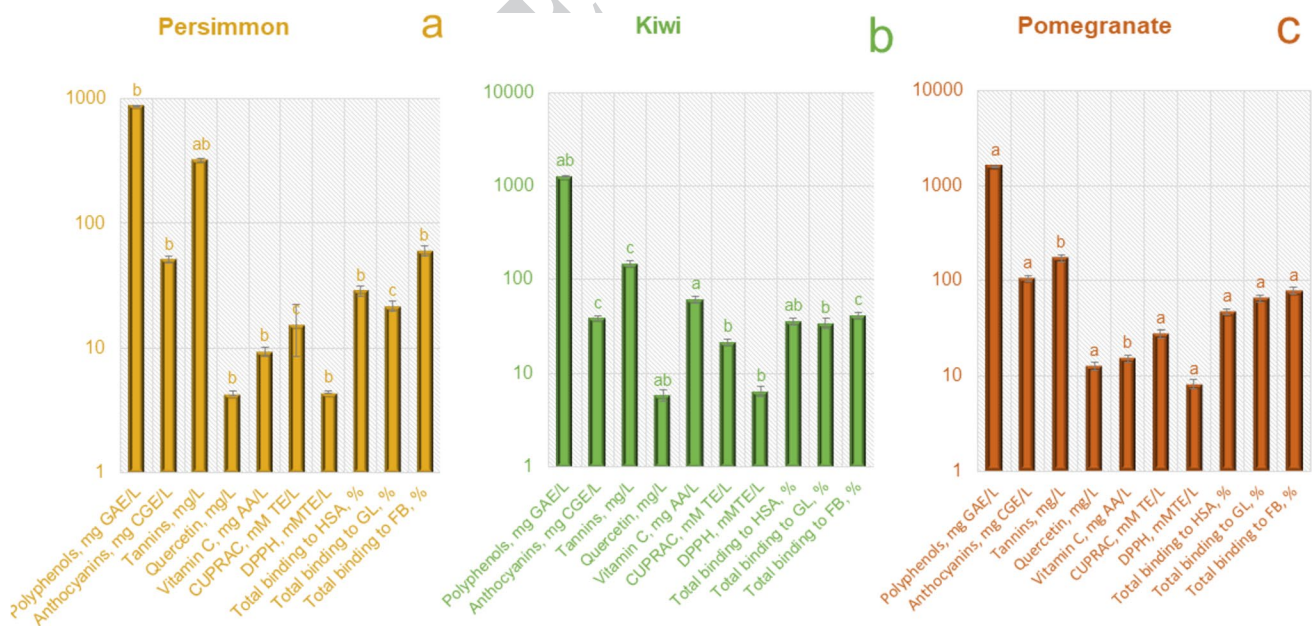


Fig. 1 Bioactive compounds, antioxidant activities and total binding properties of fruit wines **a** persimmon, **b** kiwi, **c** and pomegranate/L. Values are means \pm SD of 5 measurements; Means within bars with different superscripts are statistically different ($p < 0.05$; Student's *t* test). Abbreviations: 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

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 $\mu\text{mol/L}$ [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.

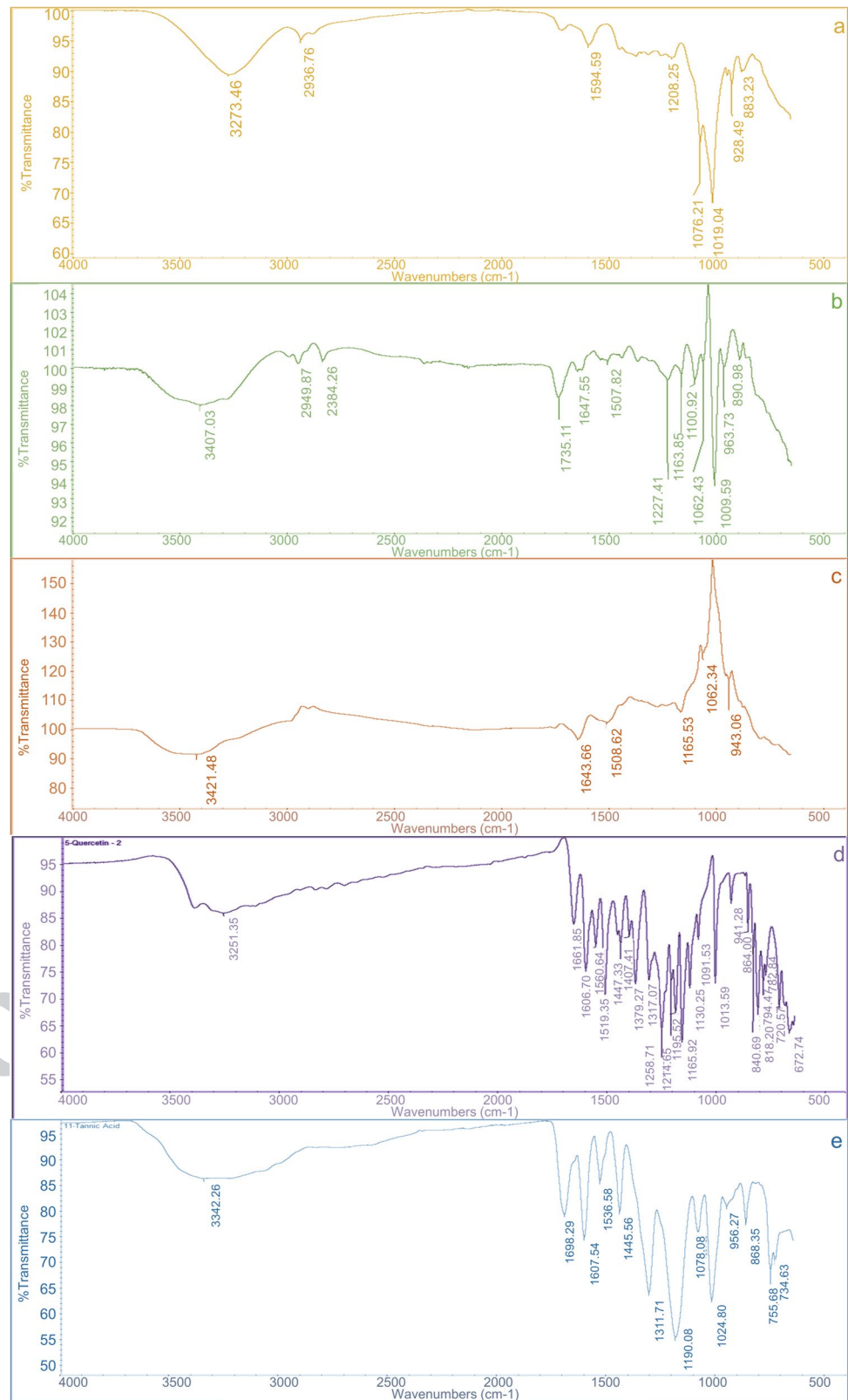
Kiwifruit possesses similar properties as pomegranate with the highest amount of vitamin C (Fig. 1b). Cited reports described that kiwifruit is used as a juice, vinegar, dried slices, jam, and wine [38, 39]. The quality characteristics of kiwifruit wine made from over-ripened fruit treated with pectinase 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, CH₂, and CH₃ 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 NO₂ 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

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

370
371
372

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

386 **Fluorescence properties of wines**

387 The antioxidant's strong affinity to human serum proteins
 388 and synergism in bioactivity are the main indices in the
 389 health application of wines [4, 5, 10, 20]. The chemical,
 390 phenolic and antioxidant characteristics of the wines were
 391 assessed by measurement of tannins, anthocyanins, and phe-
 392 nolic compounds (Figs. 1, 2). Our results were similar to the
 393 wines prepared by classical maceration. These properties of
 394 the wine bioactive compounds, mainly polyphenols, in inter-
 395 action with the main human serum proteins are shown for all
 396 investigated samples (Fig. 3). As it was found previously that

persimmon wine has the lowest antioxidant activity in com-
 397 parison with other wines, it is expected from our previous
 398 reports that the quenching properties of persimmon wine are
 399 lower than in other samples [7, 8, 17, 20]. The calculations
 400 were done on the basis of decreasing the initial fluorescence
 401 intensity (FI) of human serum proteins before interaction
 402 with bioactive wine substances (Fig. 3a, Fig. 4a,b,c) and
 403 after interaction with wine samples, using the intensity of
 404 peaks a and b.

The initial fluorescence intensities for fibrinogen [(FI, Arbitrary Units (A.U.) and maximum wavelength ($\lambda_{em/ex}$) nm] were the following: peak a with FI=861.1 ± 10.4 and $\lambda_{em/ex}$ =229/342. Peak b was estimated as FI=809.7 ± 10.3 and $\lambda_{em/ex}$ =282/341 (Figs. 3a and 4a). The initial fluorescence intensities for globulin [(FI, Arbitrary Units (A.U.) and maximum wavelength ($\lambda_{em/ex}$) nm] were the following: peak a with FI=457.3 ± 9.3 311 and $\lambda_{em/ex}$ =231/335. Peak b was estimated as FI=661.1 ± 10.3 and $\lambda_{em/ex}$ =280/334 (Figs. 3a and 4b). The initial fluorescence intensities for HSA [(FI, Arbitrary Units (A.U.) and maximum wavelength ($\lambda_{em/ex}$) nm] were the following: peak a with FI=643.0 ± 7.9 and $\lambda_{em/ex}$ =228/353. Peak b was estimated as FI=920.1 ± 10.4 and $\lambda_{em/ex}$ =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

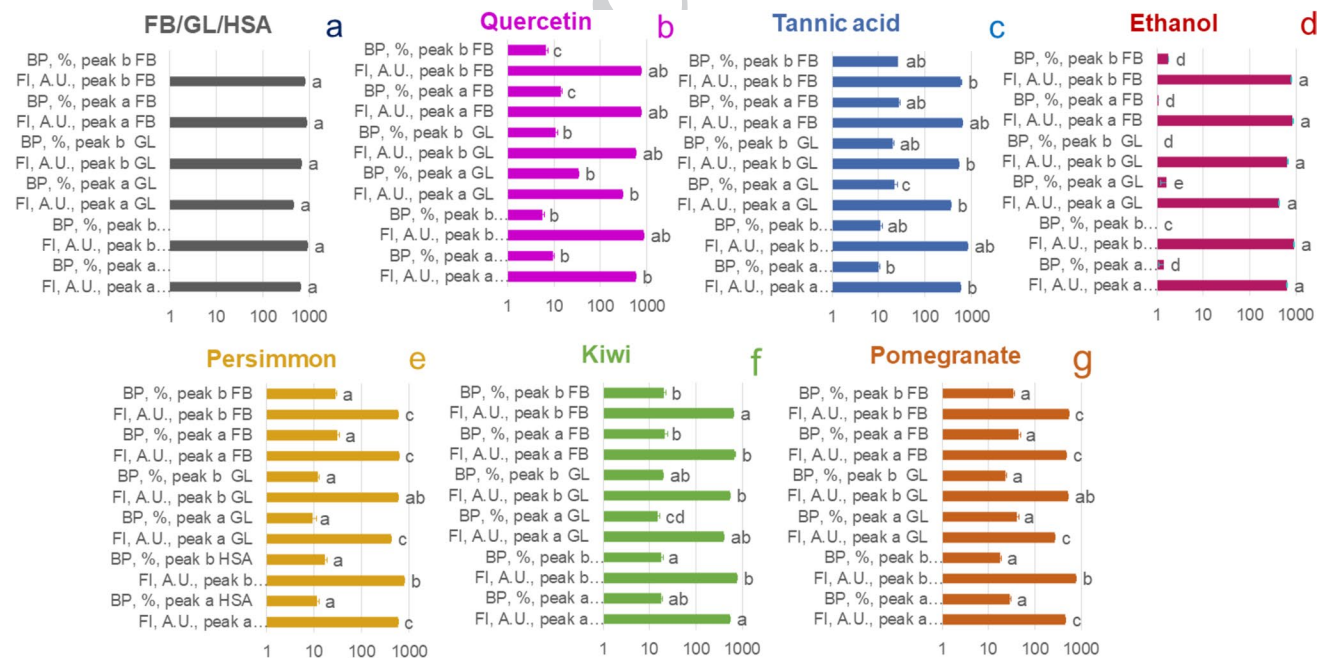


Fig. 3 Change in the values of a fibrinogen/globulin/human serum albumin before the interaction with initial values of fluorescence intensities and after interaction with b quercetin, c tannic acid, d ethanol, e persimmon, f kiwi, g pomegranate wines. Values are means ± SD of 5 measurements; n=5 samples, 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); Arbitrary 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

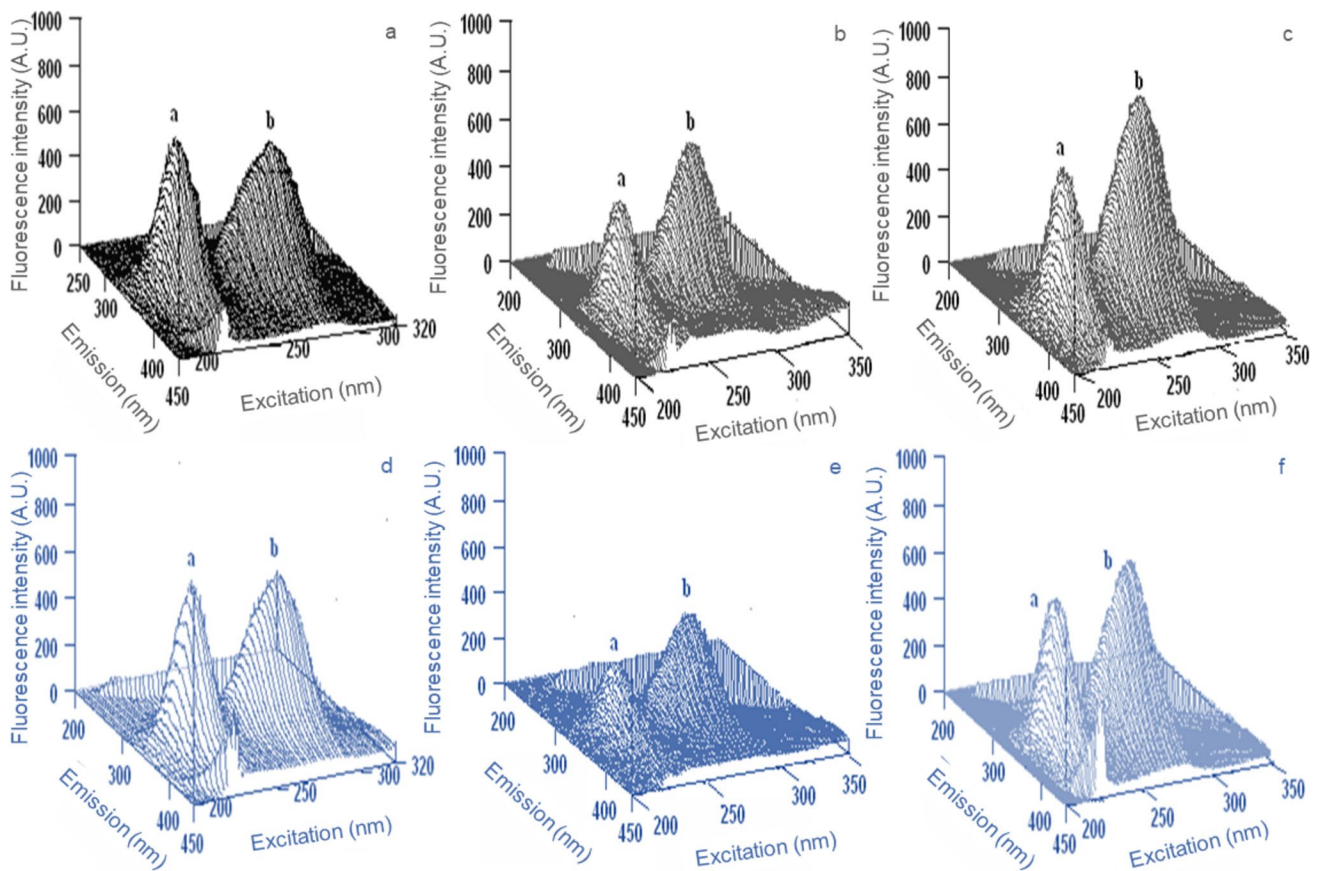


Fig. 4 Fluorometric measurements in a three-dimensional fluorescence analysis (3D-FL) of **a** fibrinogen, **b** globulin, **c** human serum albumin and tannic acid after interaction with **d** fibrinogen, **e** globulin, **f** human serum albumin. The locations of peaks **a** and **b** are shown in this figure: for fibrinogen with fluorescence intensity of Arbitrary 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)

423 the fluorescence intensities and a change in the maximum
424 wavelengths during the reaction of fibrinogen, globulin, and
425 HSA with bioactive substances of persimmon, kiwifruit,
426 and pomegranate wines. The total binding properties (%) of
427 quercetin for fibrinogen, globulin, and HSA of peaks **a** and
428 **b** were estimated as 20.7 ± 1.1 , 44.6 ± 3.5 , and 15.1 ± 0.8 ,
429 respectively (Fig. 3b). The total binding properties (%) of
430 tannic acid for fibrinogen, globulin and HSA of peaks **a** and
431 **b** were estimated as 54.3 ± 4.3 , 42.5 ± 3.8 , and 20.9 ± 1.7 ,
432 respectively (Fig. 3c and Fig. 4d–f). The total binding prop-
433 erties (%) of ethanol for fibrinogen, globulin, and HSA of
34 peaks **a** and **b** were calculated as 2.9 ± 0.3 , 2.6 ± 0.5 , and
35 2.4 ± 0.3 , respectively (Fig. 3d). The total binding prop-
36 erties (%) of persimmon wines for fibrinogen, globulin, and
37 HSA of peaks **a** and **b** showed the following data: 59.6 ± 5.7 ,
38 21.6 ± 2.1 , and 28.8 ± 2.7 , respectively (Figs. 1a, 3e, and
39 5a,b,c). The total binding properties (%) of kiwifruit wines
40 for fibrinogen, globulin, and HSA of peaks **a** and **b** were cal-
41 culated 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 (%) 442
of pomegranate wines for fibrinogen, globulin, and HSA 443
of peaks **a** and **b** were estimated as 78.2 ± 6.3 , 64.8 ± 5.8 , 444
and 47.0 ± 3.9 , respectively (Figs. 1c, 3g, and 5g, h, i). The 445
interactions of quercetin with fibrinogen, globulin, and HSA 446
showed the following total binding properties (%) of peaks **a** 447
and **b** such as 20.7 ± 1.1 , 44.6 ± 3.5 , and 15.1 ± 0.8 , respec- 448
tively (Fig. 3f). The total binding properties (%) of ethanol 449
for fibrinogen, globulin, and HSA of peaks **a** and **b** were 450
calculated as 2.9 ± 0.3 , 2.6 ± 0.5 , and 2.4 ± 0.3 , respectively 451
(Fig. 3g). All the presented results after the interaction of the 452
main serum proteins were connected to the amount of poly- 453
phenols, anthocyanins, tannic acid, and antioxidant activities 454
of the samples. The highest total binding properties were 455
estimated for pomegranate, followed by kiwifruit and per- 456
simmon wines (Fig. 1). The results of bioactive compounds 457
and fluorescence quenching show that these wines possess 458
multiple properties that have a great potential to be used for 459
human health and show similar data as the used fruits [8, 460

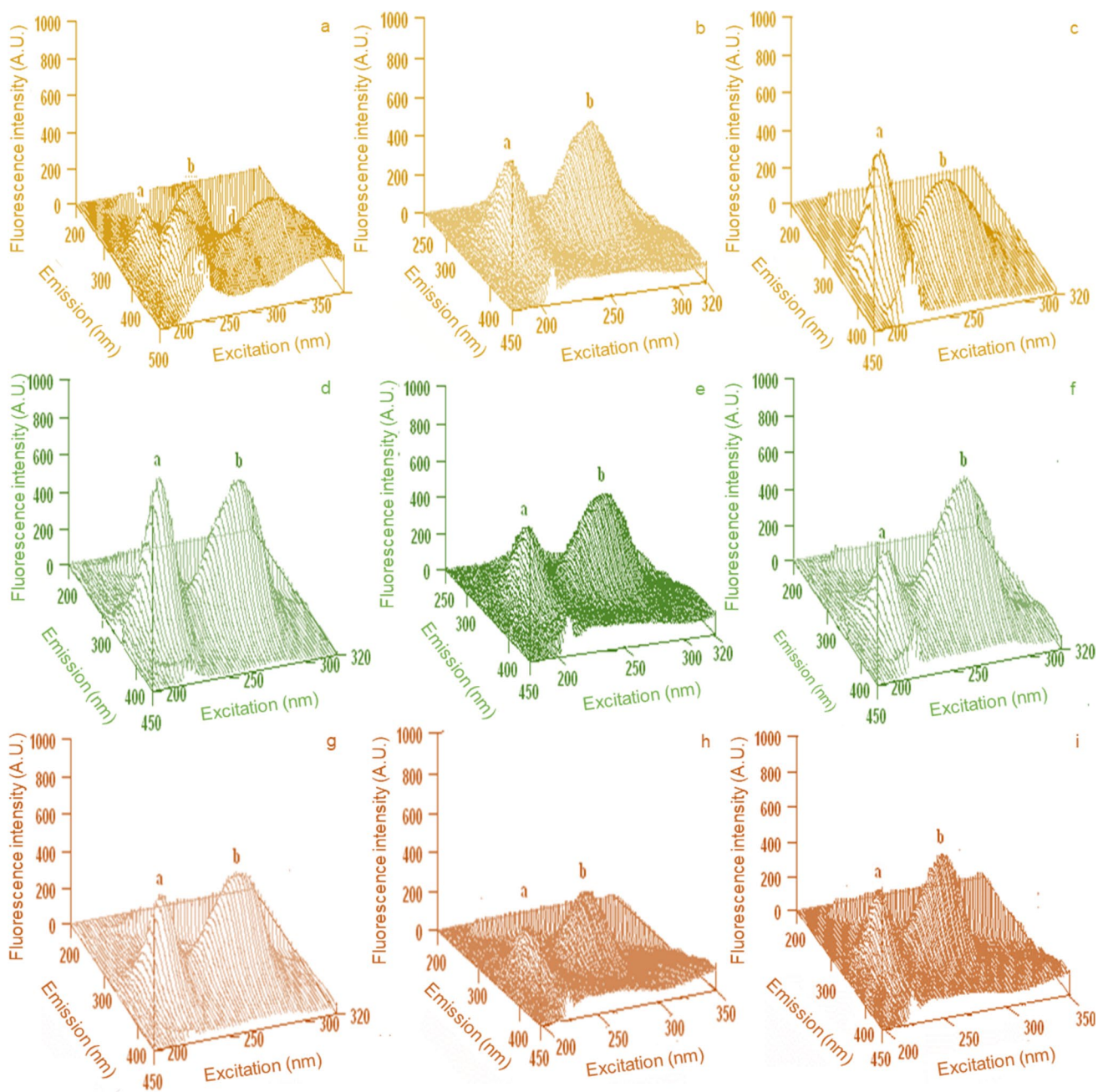


Fig. 5 Fluorometric measurements in a three-dimensional fluorescence analysis (3D-FL) of wines: **a–c** persimmon, **d–f** kiwi fruit, **g–i** pomegranate after interaction with (**a, d, g**) fibrinogen, (**b, e, h**)

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

461 10]. The beneficial effect of fruit- and vegetable-rich diets
 32 on cardiovascular health is partly attributed to the effect of
 33 their bioactive compounds [46] and bioactive-rich extracts
 34 of kale and pomegranate that are consumed as traditional
 35 plant foods of Black Sea area countries were effective in
 36 modulating platelet function. Comparison of the present
 37 results with the reported ones [10, 20, 47, 48] showed the
 38 following antioxidant activities: 9.6–29.9 mM TE in red
 39 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 470
 in white wines from South Africa. The present results of 471
 antioxidant activities of 19.4–28.3 367 mM TE by CUPRAC 472
 assay showed that the fruit wines had relatively high anti- 473
 oxidant capacities that give a strong possibility of their use 474
 as a promising source of phenolic antioxidants. In vitro and 475
 in silico interactions of red grape wine polyphenols with 476
 human serum albumin, fibrinogen, glutathione peroxidase 477
 3 and C-reactive protein enhance their biological activity 478

[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

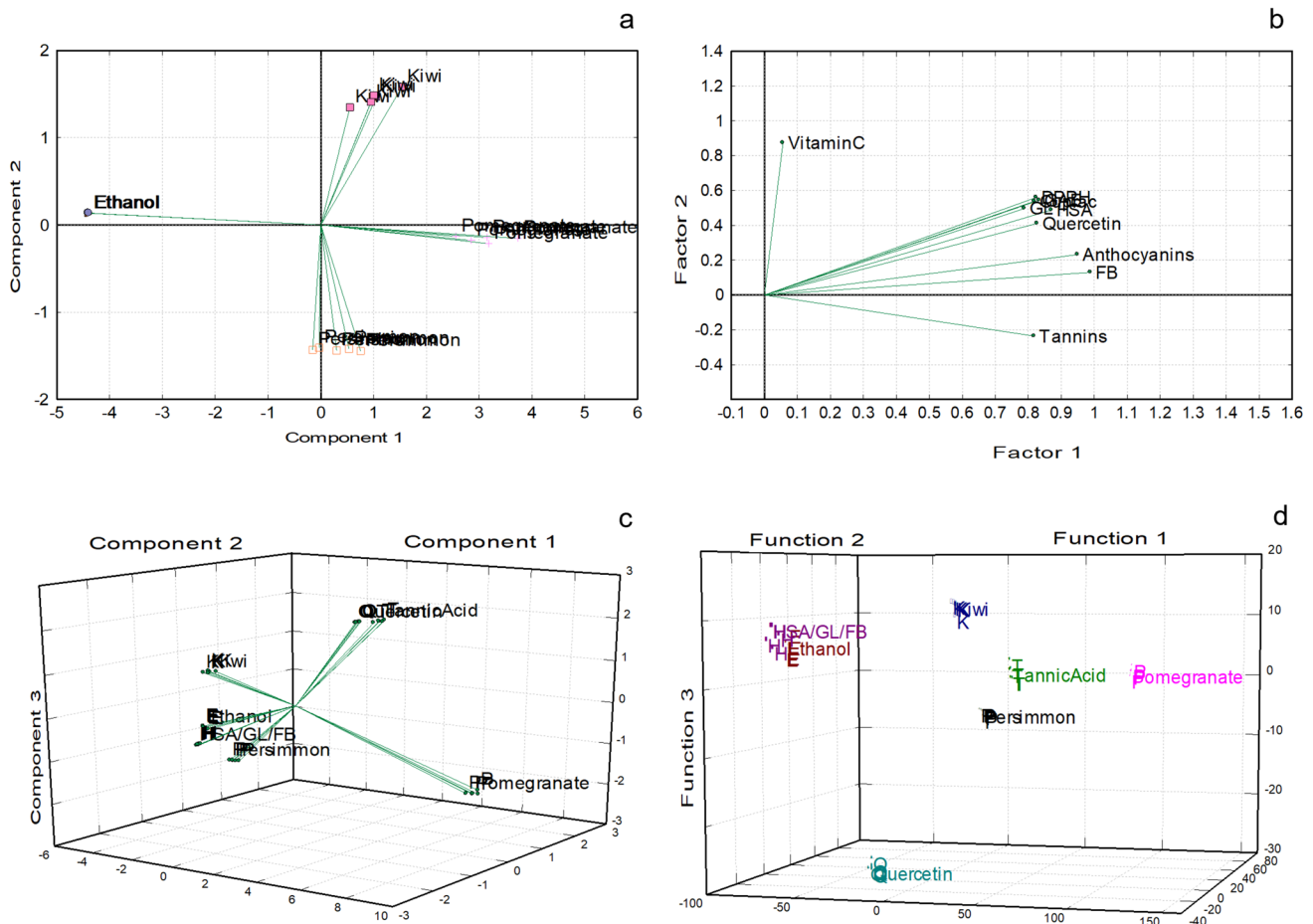


Fig. 6 Relationships between antioxidant, spectral, and binding properties of fruit wines. **a**, Principal component analysis of fruit wines based on antioxidant and binding properties of fruit wines. **b**, Plot of factors (varimax rotation) indicating mutual correlation and the importance of antioxidant 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

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580 fingerprint showing the real composition of the products.
 581 The fluorescence spectra, resulting in the binding prop-
 582 erties during interaction of different phenolic compounds
 583 with human serum proteins, can be used as a fast and reli-
 584 able in vitro analysis for health-promoting benefits of the
 585 food products.

586 **Acknowledgements** Thanks from all authors to Dr. Elena Katrich for
 587 her assistance in the measuring of some indices in wines. Thanks to
 588 Rachel Aviv from the neighborhood wine shop, “Alciolimi”, Tagore 32,
 589 Tel Aviv, Israel, for collecting samples of wines.

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

Declarations

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

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