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Comparative study of lipase inhibitory activity of some Georgian wines obtained through Kakhetian and European winemaking techniques

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ABSTRACT

The lipase inhibitory activity, total polyphenol content and antioxidant activity of some Georgian wines were studied. Fourteen commercially available samples of red and white wines were analysed, which differed by the production method (Kakhetian winemaking and Classic European winemaking methods). From the investigated wines, the highest total polyphenol content (TPC) was found in the wine made by Mukuzani Valley 2019 (3572.358 ± 153.111 mg Gallic Acid equivalent (GAE) per liter. This wine had the highest antioxidant activity (AOA), too, 4729.199 ± 88.162 mg ascorbic acid equivalent (AAE) L^{-1}.

It was recognised that wines made with the Kakhetian fermentation method contain more total polyphenols than those made by the classical European method. The differences between the samples were statistically significant. White wines made with this Kakhetian method have comparable TPC to some European-style red wines. Red wines in general are characterised on average by higher anti-lipase and antioxidant activity than white wines, although white wine had the most increased anti-lipase activity among the investigated samples (82.63% mL-1 of wine). Wines from Mukuzani microzone possess high anti lipase activity, which is ranged from 77.12 to 79.7 8% mL^{-1} of wine. No correlation between TPC and lipase inhibitory activity among the red and white wine samples was found, nor between lipase inhibitory activity and the winemaking method.

Keywords: Georgian wine, Red Wine, White wine, HPLC, Kakhetian fermentation method, Pancreatic lipase.

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Introduction

Obesity, which has been considered a twenty-first-century disease and the "New World Syndrome", is a global public health concern [1, 2]. Many studies have shown a positive relationship between obesity and the intake of a lipid-rich diet [3, 4]. Efficient absorption of dietary fats is highly reliant on the action of pancreatic lipase (PL) [5]; thus, the inhibition of this enzyme has become an attractive approach to manage and treat obesity [6 ,7]. Currently, the only available PL inhibitor at the market is Orlistat (Xenical) [6, 7], derived from the gram-positive bacterium [8] with adverse effects, such as fecal incontinence, stomach pain or discomfort, fatty/oily stools etc. [9-11]. Hence, the search for new substances with fewer undesirable effects and potent inhibitory activity against PL remains a hot topic in research [12,13].

A great deal of research showed that the class of polyphenols represents one of the essential sources to inhibit PL activity [14-16]. Grapes and their derivatives are considered one of the richest natural sources of phenolic compounds. The composition of phenolics is highly linked to wine quality properties, such as colour, flavour, and taste, as well to health-promoting properties, including antioxidants and cardioprotective properties among others [17, 18]. Furthermore, on account of health-promoting chemicals (e.g. phenolics), moderate wine con-

sumption is nowadays recognised as a risk-reducing factor in several human diseases, including type 2 diabetes [19, 20], several types of cancer [21, 22] and cardiovascular disease [21, 23]. However, the phenolic content in wine can be influenced by many factors such as grape cultivar, soil, climatic conditions, weather, winemaking procedure and conditions of maturation and storage [18, 24, 25].

In this manner, the country of Georgia is known to use the ancient method of winemaking (widely known as Kakhetian winemaking or Qvevri winemaking method), which differs from Western European techniques (referred to as the Classic winemaking, Industrial method). Kakhetian winemaking method involves placing crushed grapes and other parts, i.e., clusters (stem, skin, seeds) in a clay vessel called Qvevri, dug in the ground. Qvevri is then sealed, and the wine is left to mature. During fermentation, phenolic compounds are extracted from pomace, defining the composition and essence of Kakhethian wine [26]. This winemaking method has also been approved as an intangible cultural heritage convention by UNESCO [27]. Furthermore, Georgia is considered the "cradle of wine", as the earliest traces of winemaking have been found here [28]. Additionally, Georgia is home to over 500 varieties of indigenous grapes, many of which are unknown to the rest of the world [29].

Although a significant amount of research has been done about Georgian wines [30–32], to the best of our knowledge, only scarce information about their anti-lipase activity is available [33, 34]. Gulua et al. [33] studied chemical constituents, antioxidant and anti-lipase activity of some wines produced in Georgia. However, this study investigated the lipase inhibitory of six different wines, and it did not bring up any relationship between anti-lipase activity and technological methods.

In this matter, this study aims to investigate fourteen wines made from Georgian autogenous grapes (such as Rkatsiteli and Saperavi), obtained through Kakhetian and European winemaking methods and compare their PL inhibitory activity and polyphenol content and find a relationship, if any, between lipase inhibitory activity and winemaking method.

Materials and methods

Chemicals

Ascorbic acid, Sodium Hydroxide, Folin-Ciocalteu reagent, Potassium dihydrogen Phosphate, Olive Oil, Detergent Tween 80, Sodium carbonate, Ethyl acetate, glacial acetic acid and methanol were purchased from Sigma – Aldrich (Steinheim, Germany), TPTZ-2,4,6-Tris (2-pyridyl)-s-triazine (Sigma – Aldrich, Switzerland), hydrochloric acid, formic acid and phosphoric acid were provided by Merck (Darmstadt, Germany), lipase concentrate – HP was purchased from Integrative Therapeutics, LLC (USA). Orlistat® (trade name Xenical) by Roche (Italy) was purchased at the local pharmacy. All other reagents were commercially available at the local market and were of analytical grades.

Wine Samples

Samples of commercial wines made from autochthonous and leading grape varieties grown in the region of Kakheti were provided from local producers or purchased from wine stores.

A total of 14 red and white wines of the varieties Saperavi (n = 9) and Rkatsiteli (n = 5) were assessed. The wine samples for the experiment were chosen at random. We did it from the consumer's point of view. We did that because, as consumers would do, promising in vivo potent lipase inhibitory activity can be the definitive factor behind consumer decision making. Detailed information about the tested wines is included in Table 1. The wines, packed in glass bottles, were stored at room temperature until being analysed.

To exclude the impact of alcohol on the lipase, de-alcoholised red and white wine samples were used in the lipase inhibition assay, too.

Winemaking methods

The wines included in this study were either made based on the common "European methods" or by the Kakhetian method. Kakhetian method is one of the methods elaborated in Georgia. This style of wine is based on long period (up to 5 months) maceration and fermentation of must with the usage of 100% of grape pomace (skin, seeds, stems). Fermentation is carried in a clay vessel called "qvevri," buried underground. Qvevri is then sealed, and the wine is left to mature. During fermentation, phenolic compounds are extracted from pomace, defining the composition and essence of Kakhethian wine [26].

Titratable acidity

Titratable acidity (TA) was determined by titration with 0.1 N sodium hydroxide using an automatic titrator (ZDJ-4A, NASA Scientific Instrument Co., Ltd, Anting Shanghai, China). The TA results were expressed as g of tartaric acid equivalents 1000 mL-1 of the sample [35].

Total dry extract

For measurement of non-volatile dry matter, a 50 mL sample of wines were aliquoted into a porcelain dish. The dish was then placed onto a boiling water bath until the evaporation of water, alcohol, and other volatile compounds had occurred. The residual moisture was then evaporated from the samples by oven drying at 105^0C for 16h. The total dry extract was determined gravimetrically as the residue remaining after drying.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) was determined spectrophotometrically (UV 1609, A&E Lab Co LTD, UK), according to the Folin-Ciocalteu method [36]. Briefly, the diluted samples of each wine (1 mL) were pipetted into separate disposable test tubes and mixed with 5 mL Folin-Ciocalteu phenol reagent (1:10 v/v distilled water). 8 min after, 4 ml of Sodium Carbonate solution (7.5% (w/v)) was added into each test tube. The mixtures were stirred well, and the tubes were allowed to stand for another 60 minutes at room temperature for colour development. Subsequently, their optical densities against the water were read at 765 nm, with a 10 mm path length cell. Diluted Gallic acid (10-50 μg mL^{-1}) was used as a standard working solution. The calibration curve of absorbance vs concentration of a standard solution (equation y = 0.0123x + 0.0236, where Pearson's correlation coefficient: $R^2 = 0.9918$) was used to determine TPC. Results were expressed as mg of gallic acid equivalents (GAE) per litre.

Chromatographic determinationn of individual polyphenols

Individual polyphenols were separated and quantified by High-Performance Liquid Chromatography (HPLC) analysis performed on a Varian Prostar 500 series liquid chromatography (Varian Prostar 500, Walnut Creek, California, USA). Separation was achieved on a C18, 150 mm x 4.6 mm column (Waters Corporation, Milford, USA). Phenolic compounds were separated on an Acclaim® C18 (4.6 x 250 mm; 5μm) column (Dionex, USA), at 30°C using a temperature-controlled column compartment (TCC-3000). Data acquisition, peak integration, and calibrations were performed with Dionex Chromeleon software (Version 6.80 RS 10). Solvent A was 0.5% acetic acid, and solvent B was 100% methanol. Separation was achieved using the following gradient: isocratic 0% B and 100% A for 0 min; isocratic 40% B and 60% A over 40 min; 0% B and 100% A over 10 min; 0% B and 100% A over 10 min. The flow rate was 0.5 mL min^{-1} ,and eluent was monitored at 280 nm.

To prepare the sample for analysis, 4.0 mL of the wine sample was carefully deposited onto a C18 solid phase extraction cartridge (Agilent, Bond Elut, USA). Sugars and other polar substances were eluted using 2.0 mL of deionized water through the cartridge, whereas polyphenols were eluted using 2.0 mL of ethyl acetate, which was then evaporated under vacuum at 40-45 °C. Four mL of 50% ethanol was added to the dry extract. The extract was then filtered through 45μm filter paper (Whatman, Maidstone, UK) and 20 μL was injected onto the HPLC system.

Ferric reducing ability of plasma (FRAP) assay for total antioxidant activity

Ferric reducing ability of plasma (FRAP) assay has been applied for the evaluation of the total antioxidant activity (AOA), according to Benzie and Strain, 1996, with slight modifications [37]. The working FRAP reagent was prepared freshly by mixing acetate buffer (300 mM, pH 3.6), 2,4,6- tripyridyl-s-triazine (TPTZ) solution (10 mM, dissolved in 40 mM of HCl) and Ferric Chloride solution (20 mM) in the ratio 10:1:1. The FRAP reagent and ascorbic acid (1mM) were separately incubated for 15 min at 37 0C. 3 mL of working reagent was mixed with 100 microliters of the diluted sample. Ascorbic acid was used as a standard. The reduction was monitored at 593 nm, and the absorbance was recorded after 4 min. FRAP values of samples were compared to that of ascorbic acid and expressed as mg ascorbic acid equivalents (AAE) per 1 litre of wine.

Determination of Lipase inhibitory activity

Titrimetric assay method was used to determine lipase activity as reported by Stoytcheva et al., 2012, with minor modifications [38]. Briefly, the initial reaction mixture consisted of 2.5 mL of deionised water, 1 mL 200 mM Tris HCl buffer (pH 7.2), 3 mL of olive oil, and 0.5 mL of detergent (Tween 80). To obtain a good result, the solution was vigorously mixed on a magnetic stirrer for 15 min. Subsequently, 110 mg of the lipase concentrate was then added to the emulsified mixture, which was then incubated at 37 ^{0}C for exactly 30 min. At the end of the incubation, 3 mL of 95% ethanol was added, and the final reaction mixture was titrated with 50 mM NaOH until the value of pH 9 at automatic titrator (ZDJ-4A, NASA Scientific Instrument Co., Ltd, Anting Shanghai, China) was achieved. Blank titration was carried out as above, but potent inhibitors were involved without lipase in test samples. One unit of lipase activity is defined as the amount of enzyme that hydrolyses 1.0 micro equivalent of fatty acid from a triglyceride in one hour at pH 7.2 at 37 °C. Lipase activity was calculated using the following equation:

$$\text{Lipase Units} = \frac{(A - B)\ (1000)\ (2)\ (DF)}{(1)}$$

where

A = volume of 50 mM NaOH consumed by the test sample in mL;
B = volume of 50 mM NaOH consumed by the blank sample in mL;
1000 = conversion factor from milli equivalents to micro equivalents;
2 = time conversion factor from 30 min to 1 hour;
DF = dilution factor
1 = Volume (in millilitre) of enzyme used

The percentage of inhibition was calculated in the presence and absence of inhibitors. Orlistat was used as a standard inhibitor. Lipase activity was measured in the presence of Orlistat (10mg), and the per cent of inhibition was calculated per 1 mg of Orlistat.

To measure the percentage of lipase inhibition, 1 mL of potent inhibitor (i.e. wine) was added separately to the initial mixture, the following procedures were identical to those described previously. The effect of inhibition of the sample was calculated as the percent of Orlistat inhibition value.

Statistical analysis

The data represents the mean of a minimum of three replicates ± standard deviation (SD). Data were subjected to the one-way ANOVA and Tukey's HSD tests. One-way analysis of variance (ANOVA) was done to analyse the variation of the means between the experimental samples. Tukey's HSD test was used to differentiate between the mean values. All the analyses were done using XLSTAT (free trial version 2021, Addinsoft, Inc., Brooklyn, NY, USA)

Results and discussion

Chemical constituents

The characteristics of wines studied herein are shown in Table 1. Wines included in this study were either made based on the common "European methods" i.e. Classic technology or the Kakhetian method, i.e. Qvevri technology. Investigated samples were dry wines, except for late harvest wines. Most of the red wine samples were Mukuzani (S4-S9), Appellation Controlled Origin (AOC) dry red wine, produced from Saperavi grapes grown in the Mukuzani micro-viticulture area Kakheti region.

Table 1. *Wine characteristics*

Wine Code	Name of the bottle	Producer	Vintage	Grape variety	Type	Strength %	Making method
S1	Glekhuri - Khasmi Saperavi	Teliani Valley	2017	Saperavi	Dry Red	13	Kakhetian
S2	Matrobela	Matrobela Wines	2018	Saperavi	Dry Red	13.5	European
S3	Icewine - Saperavi - Guramishvilis Marani	Guramishvili's Marani	2017	Saperavi	Sweet Red	12	late-harvest European

S4	Mukuzani Valley - Mukuzani	Mukuzani Valley	2016	Saperavi	Dry Red	12.5	European
S5	Mukuzani Valley - Mukuzani	Mukuzani Valley	2019	Saperavi	Dry Red	12.5	European
S6	Rtvelisi - Mukuzani	Rtvelisi	2018	Saperavi	Dry Red	13	European
S7	Zurab Tsereteli - Mukuzani	Tsereteli Wine and Spirits	2015	Saperavi	Dry Red	13	European
S8	Zhamurashvili's wine - Mukuzani	Zhamurashvili's wine	2018	Saperavi	Dry Red	13	Kakhetian
S9	Nekresi Estate - Mukuzani	Nekresi winery	2016	Saperavi	Dry Red	13	Kakhetian
RK 1	Icewine Satrapezo	Telavi Wine Cellar	2013	Rkatsiteli	Sweet White	10.5	Late harvest European
RK 2	Rkatsiteli Vine Ponto	The Spirit of Georgia	2016	Rkatsiteli	Dry White	12.5	Kakhetian
RK 3	Mr Rkatsiteli from Gurjaani	Mr Wine	2018	Rkatsiteli	Dry White	13	Kakhetian
RK 4	Rkatsiteli – Vaziani	Vaziani company	2016	Rkatsiteli	Dry White	12.5	European
RK 5	Rkatsiteli	Kindzmaruli's Marani	2018	Rkatsiteli	Dry White	13	European

The total acidity varied between 4.791 and 7.986 g L^{-1} tartaric acid equivalent for white wines and between 5.25 - 8.794 g tartaric acid equivalent per liter for red wines. The established titratable acidity for Mukuzani wines provided by the legislation of Georgia is no less than 5 g L^{-1} (source); all our Mukuzani samples met this requirement.

The highest total dry extract was presented in late-harvest wines, 100.54 ± 0.06 g L^{-1} in Saperavi and 97.24 ± 0.04 in Rkatsiteli wine. This can be explained by using naturally dehydrated and completely frozen grape berries in winemaking. Icewine is a type of dessert wine produced from fully ripened grapes that have been frozen while still on the vine. Usually, grapes are left onto wine until the temperature drops below -9 degrees Celsius. This pre-harvest dehydration concentrates the soluble solids in grape berries. As a result, wine is rich in sugars, phenolic compounds and flavour (Moreno et al., 2008) [39].

Among the other samples, white wines made with the European method (Rk 4 and Rk5) had the lowest total dry extract, 13.46 ± 0.15 and 16.62 ± 0.18 g L^{-1}, respectively.

S5 (Mukuzani Valley, 2019) and S2 (Matrobela wines) samples showed the highest total polyphenolic content, i.e., 3572.358 ± 187.521 and 3482.927 ± 136.204 mg GAE L^{-1}, respectively. The TPC among the rest of Saperavi grape wines varied between the range of 2415 and 2930 mg GAE L^{-1}. Compared to the other red wine samples, Zurab Tsereteli's Mukuzani contained a statistically significant amount of TPC, 2415.176 ± 19.163 mg GAE L^{-1}, which could be caused by the winemaking method or vintage, or both. The TPC of this sample was statistically significant to the white wine sample RK 2, fermented by the Kakhetian method. This is a good example of how the production method can increase the TPC in wine. Usually, consumers consider that white wines do not have polyphenols present in such large quantities as red wine; however, because the Kakhetian method differs from the European method, a white wine made with this method can have comparable TPC to some red wines.

White wines made by the Kakhetian method (RK2 and RK 3) possessed significantly higher TPC than white wines prepared by the common European method (RK1, RK4, RK5). These results are in good agreement with Shalashvili et al. [25], Khatchapuridze et al. [34].

Individual polyphenols

Individual concentrations of 4 phenolic compounds (gallic acid, epicatechin, (+) catechin and caffeic acid, (-)) presented in each wine sample were quantified by HPLC-UV/Vis analysis (concentrations of compounds in all wine samples are shown in Table 2).

Table 2. *Chemical composition of wines*

Code	TA g L-1	Total dry extract g L-1	TPC mg Gallic acid equivalent L-1	Gallic acid mg /100mL	Epicatechin mg /100mL	Catechin mg/ 100m L	Caffeic acid mg/100 mL	AOA (mg AAE) L^{-1}
S1	6.588 ± 0.154	24.34 ± 0.02	2734.959 ± 59.002 bc	0.48 ± 0.02	6.22 ± 2.03	2.62 ± 0.32	2.73± 0.19	3397.031 ± 194.837 cd
S2	5.776 ± 0.054	25.88 ± 0.07	3482.927 ± 136.204 a	0.88 ± 0.04	2.71 ± 0.16	0.43 ± 0.08	ND	4160.465 ± 126.339 b
S3	5.254 ± 0.004	100.54 ± 0.06	1828.455 ± 28.455 e	0.5 ± 0.02	1.65 ± 0.15	0.5 ± 0.05	0.9 ± 0.07	1921.397 ± 119.724 f
S4	7.167 ± 0.0435	25.92 ± 0.02	2930.081 ± 74.809 b	5 ± 0.19	11.5 ± 0.31	2 ± 0.2	1.8 ± 0.21	3371.412 ± 240.218 cd
S5	6.984 ± 0.232	30.3 ± 0.01	3572.358 ± 153.111 a	5.26 ± 0.34	0.9± 0.02	4.05 ± 0.07	10.83± 0.31	4729.199 ± 88.162 a
S6	7.119 ± 0.301	25.80 ± 0.12	2810.840 ± 297.486 bc	7.37 ± 0.14	11.35 ± 0.51	2.3 ± 0.12	10.31± 0.28	3145.968 ± 113.186 cd
S7	7.15 ± 0.362	26.7 ± 0.09	2415.176 ± 19.163 cd	3.4 ± 0.1	7.62 ± 0.28	3.67 ± 0.13	8.58 ± 0.3	3012.751 ± 119.724 d
S8	8.413 ± 0.381	29.38 ± 0.07	2813.550 ± 49.823 bc	ND	4.4 ± 0.13	0.5 ± 0.01	ND	3299.678 ± 88.152 cd
S9	6.625 ± 0.002	30.70 ± 0.01	2965.312 ± 67.152 b	2.3 ± 0.11	9.19 ± 0.32	1.08 ± 0.02	1.37 ± 0.03	3494.381 ± 94.199 c
RK 1	7.455 ± 0.032	97.24 ± 0.04	149.594 ± 8.13 g	58.13 ± 0.45	0.2 ± 0.001	1.78 ± 0.09	1 ± 0.02	210.073 ± 28.984 g
RK 2	7.932 ± 0.055	27.48 ± 0.01	2515.477 ± 97.561 de	4.02 ± 0.2	11.38 ± 0.43	3.87 ± 0.22	11.17 ± 0.35	2413.275 ± 43.476 e
RK 3	4.961 ± 0.057	20.36 ± 0.02	1572.358 ± 56.912 e	13.6 ± 0.03	8.39 ± 0.26	4.52 ± 0.15	8.03 ± 0.23	1788.181 ± 69.123 f
RK 4	4. 909 ± 0.118	13.46 ± 0.15	489.577 ± 36.112 f	0.4 ± 0.001	0.4 ± 0.001	2.9 ± 0.11	6.78 ± 0.26	179.330 ± 50.722 g
RK 5	6.897 ± 0.226	16.62 ± 0.18	190.244 ± 8.13 g	1 ± 0.01	ND	2.2 ± 0.04	9.16 ± 0.32	199.825 ± 43.476 g

Means ± standard deviation (SD) in the same column with different alphabet letters indicate the significant difference at $p < 0.05$.
"ND" individual polyphenol was not detectable in the sample.
S1- Glekhuri, Khasmi Saperavi; S2 - Matrobela, Saperavi; S3 S3 - Icewine, Guramishvilis Marani, Saperavi; S4 -Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli,Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 - Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 - Vine Ponto, Rkatsiteli; RK3 Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 Rkatsiteli;

Due to the lack of corresponding standards, other individual polyphenols were not identified within this study. A reverse-phase HPLC separation profile of Mukuzani wine (Mukuzani Valley, 2016), is shown in Fig 1.

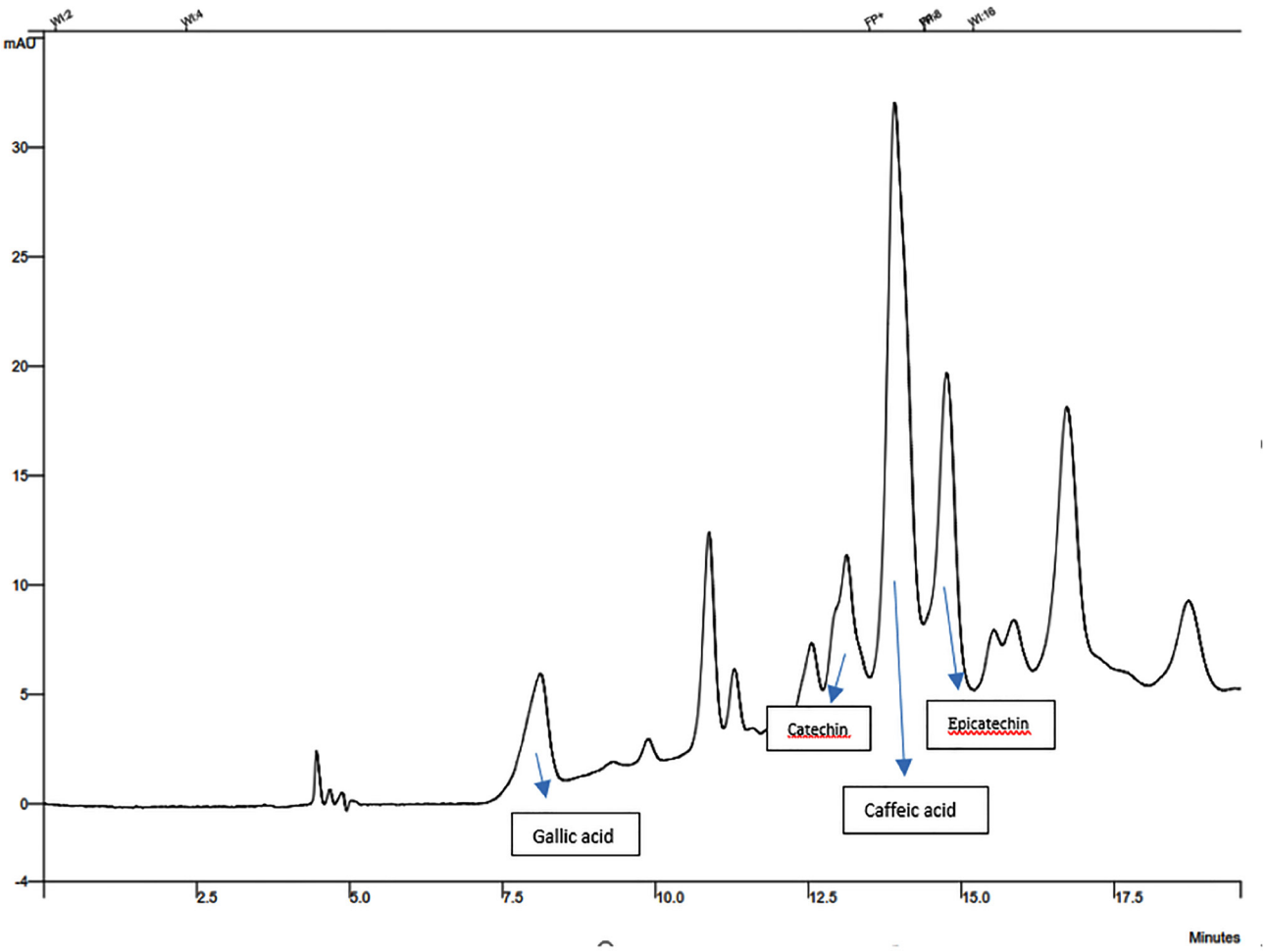

Fig 1. *A reverse-phase HPLC separation profile of Mukuzani wine (Mukuzani Valley, 2016)*

Highly significant differences in the four individual polyphenolic compounds quantified were observed in the wine samples analysed in this study. A high concentration of gallic acid could be found in Satrapezo Icewine, RK1 (58.13 ±0.46 mg / 100 mL), among the rest of Rkatsiteli samples, wines made with Kakhetian method (RK 2 and RK3) contained significantly higher amount of gallic acid (4.02 ± 0.02 and 13.6 ± 0.03 mg / 100 mL, respectively). Gallic acid was not detectable in Mukuzani Zhamurashvili's wine, S8 and in the rest of the Mukuzani samples, its content was varying between 2.19 - 7.52 mg /100 mL.

The highest caffeic acid content was presented in the following wines Rkatsiteli Vine Ponto 2016 (11.17±0.16 mg / 100 mL) and Mukuzani Valley 2019 (10.83±0.12 mg /100 mL), with no significant difference.

The concentration of catechin was varying between 0.5 - 4.52 mg /100 mL The highest content of epicatechin was found in wine S5 (Zhamurashvili Valley 2016) (11.5 ±0.1 mg /100 mL) RK2 (Rkatsiteli Vine Ponto samples) (11.38 ±0.15 mg /100 mL) and S6 (Rtvelisi) (11.35 ±0.16 mg /100 mL).

Samples S9, RK3, S7 and S1, contained intermediate levels of epicatechin, i.e., 9,19 ± 0.1; 8.39 ± 0.1; 7.62 ± 0.1; 6.22 ± 0.1 mg /100 mL, respectively. Epicatechin was not detectable in the RK 5 sample. In general, higher content of catechin and epicatechin was found in white wines made by the Kakhetian method than those prepared by the European method. These are the main flavonoids found in the skin and seed of grapes [32,33]. Probably due to this reason, wines made with the Kakhetian method resulted in richer wines. Samples S4 and S5 are made from the same producer, by the same technology and are different by vintage. As seen, the content of gallic acid, caffeic acid and catechin is decreased by vintage, whereas the content of epicatechin is significantly higher.

Antioxidant activity

White wines were significantly less effective in antioxidant assays than red wines. However, wines fermented using the Kakhetian method exhibited higher antioxidant activity and were comparable to some red wines made by the European method.

The wine S5 exhibited the highest AOA, 4729.199 ± 88.162 mg AAE L^{-1}. Wine S2 showed the second-highest antioxidant activity, 4160.465 ± 126.339 mg AAE L^{-1}. These two wines were statistically similar regarding TPC. No statistically significant difference between the samples S9, S1, S4, S8 and S6 was detected. The observed antioxidant activity was 3494.381 ± 94.199, 3397.031 ± 194.837. 3371.412 ± 240.218; 3299.68 ± 88.152; 3145.968 ± 113.186 mg AAE L^{-1}, respectively. The sample S7 showed no statistically significant difference compared to the sample S6, which was 3012.751 ± mg AAE L^{-1}. The antioxidant activity of white wine samples, RK2 (2413.275 ± 53.247 mg AAE L^{-1}), was higher than red wine sample S3 (1921.397 ± 146.631 mg AAE L^{-1}). Moreover, the AOA between these samples was statistically significant. However, the AOA of this late-harvested Saperavi wine was statistically similar to that of RK 3 (1788.181 ± 84.658 mg AAE L^{-1}). As stated earlier, wines fermented by the European method, RK1, RK5 and RK4 showed the lowest antioxidant activity; results were statistically similar and varying between 179 and 211 mg AAE L^{-1}.

We could demonstrate a high correlation (R^2= 0.9731) between the total polyphenol content and the antioxidant activity of wines (Fig 2). A significantly positive correlation was also reported other researches Han et al. [40], Paixão et al. [41].

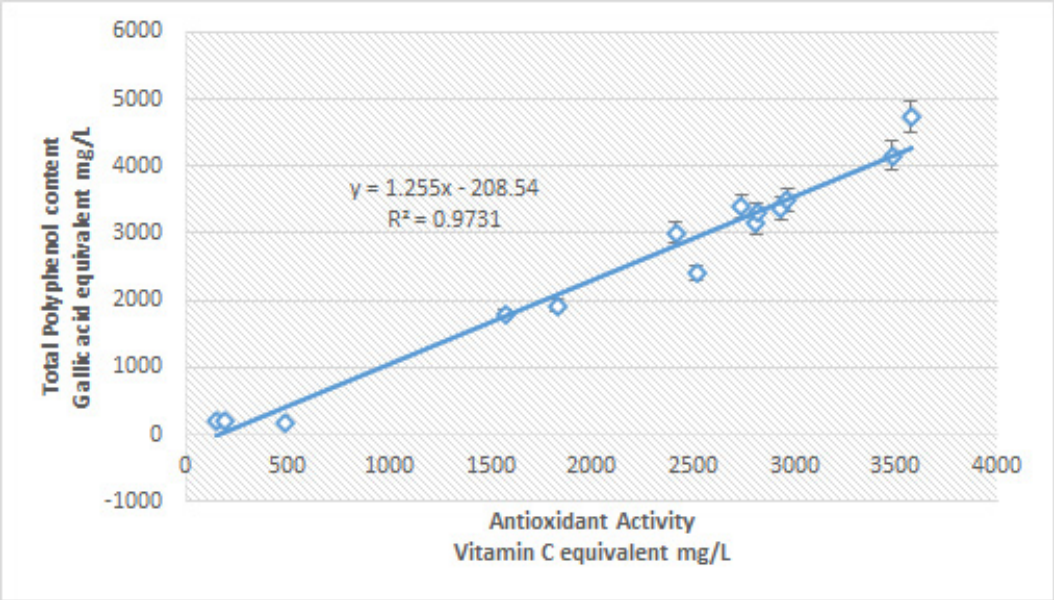

Fig. 2. *Correlation between the total phenolic content and antioxidant activity*

Lipase inhibition

Lipase activity and its inhibition by wines are shown in the Table 3. The highest level of lipase inhibition among the given samples was exhibited by the sample RK 3, 82.63% mL^{-1} of wine. This white wine sample was made by Qvevri winemaking method. It is noteworthy, that wines from Mukuzani microzone showed high anti lipase activity; their lipase inhibitory activity ranged from 77.12 to 79.7 8% mL^{-1} of wine. No significant difference was observed among these samples. Orlistat (10mg) itself showed 75.84% inhibition of lipase activity.

Table 3. *Lipase activity in the presence of various inhibitors*

Inhibitor	Lipase activity	Inhibition %	Inhibition % based on 1 mg inhibitor	Effect of 1 mg inhibitor as the percent of 1 mg Orlistat inhibition value
Orlistat	487.67 ± 21.142	75.84	7.58	100
S1	422.84 ± 17.26 fg	79.05 ± 0.86 ab	3.25 ± 0.04 b	42.82 ± 0.46 b
S2	562.71 ± 22.97 cd	72.13 ± 1.14 de	2.787 ± 0.04 def	36.75 ± 0.58 def
S3	504.63 ± 20.60 de	75 ± 1.02 cd	0.75 ± 0.01	9.84 ± 0.13 g
S4	461.81 ± 18.85 ef	77.12 ± 0.90 bc	2.98 ± 0.04 cd	39.23 ± 0.48 cd
S5	422.21 ± 17.24 fg	79.09 ± 0.85 ab	2.61 ± 0.03	34.41 ± 0.37 ef
S6	409.41 ± 16.71 fg	79.72± 0.83 ab	3.09 ± 0.03 bc	40.74 ± 0.42 bc
S7	449.64 ± 18.36 ef	77.73 ± 0.91 bc	2.91 ± 0.03 cd	38.38 ± 0.45 cd
S8	444.72 ± 18.16 ef	77.97 ± 0.90 bc	2.65 ± 0.03 ef	34.99 ± 0.40 ef
S9	408.14 ± 16.66 fg	79.78 ± 0.83 ab	2.60 ± 0.03 f	34.27 ± 0.35 f
RK 1	593.74 ± 24.24 c	70.59 ± 0.71 e	0.73 ± 0.01 g	9.57 ± 0.16 g
RK 2	469.50 ± 19.17 ef	76.74 ± 0.95 bc	2.793 ± 0.03 de	36.82 ± 0.de
RK 3	350.57 ± 14.31 g	82.63 ± 0.71 a	4.06 ± 0.03 a	53.51 ± 0.46a
RK 4	910.25 ± 37.16 a	54.91 ± 1.84 g	4.08 ± 0.14 a	53.79 ± 1.80a
RK 5	708.39 ± 28.92 b	64.91 ± 1.43 f	3.91 ± 0.09 a	51.49 ± 1.14a

Means ± standard deviation (SD) in the same column with different alphabet letters indicate the significant difference at $p < 0.05$.

S1- Glekhuri, Khasmi Saperavi; S2 - Matrobela, Saperavi; S3 S3 - Icewine, Guramishvilis Marani, Saperavi; S4 -Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli,Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 - Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 - Vine Ponto, Rkatsiteli; RK3 Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 Rkatsiteli;

The lowest lipase inhibitory activity was shown by the sample RK4 54.91% per mL wine. RK4 belongs to the class of dry white wines made with classic European technology. This sample also contained a low amount of dry extract, TPC and AOA compared to the other samples. The second sample with a low lipase inhibitory activity was also a white wine sample, RK5 (64.91%). The amount of TPC in it was the lowest compared to other samples - 190.24 mg GAE L^{-1}. At an average rate, white wines made by the classical technology possessed lower anti-lipase activity (69.96% per ml of wine) compared to red wines (77.51% per ml of wine). However, white wines made with Qvevri technology (RK1 and RK 2) showed higher lipase inhibitory activity than Saperavi samples (S2, S3), made with the European winemaking method. No significant difference was observed among the other Saperavi samples, which differed by the winemaking method.

No significant correlation was found between polyphenol content and anti-lipase activity in wine samples, nor between lipase inhibitory activity and winemaking method. In a previously published study, the R^2 correlation value between lipase inhibitory activity and TPC was also found to be low [34].

Some differences in the results were observed when calculating lipase inhibitory activity per mg dry extract. The highest level of lipase inhibition was shown by the white wine samples (RK 4, RK 3 and RK 5) and no statistically significant differences existed between the samples. A significant relationship was found between late harvest wines (S3 and RK 1), and they exhibited the lowest percentage of inhibition (0.75% and 0.73%) due to the high content of the dry extract. The anti-lipase activity of Orlistat® itself calculated per mg was equal to 7.58 %.

From obtained results, one can calculate the optimal dose required to achieve the same results as the intake of 120mg Orlistat gives. However, further studies are needed to define the mechanism of action and kinetics of inhibition in the wine samples.

Conclusion

In white wines produced by the Kakhetian method, antioxidant activity and TPC is significantly higher than in the white wines prepared by the common European method. No such effect was found in the samples of red wines. Probably because most of the studied samples of red wines belong to the Appellation of Controlled Origin and the Saperavi grapes grown in the Mukuzani micro-viticulture area are rich with polyphenols.

No significant correlation was found between polyphenol content and anti-lipase activity in wine samples, nor between lipase inhibitory activity and winemaking method.

Overall, it can be concluded that most of the wine samples we have examined, are characterised by noticeable or significant high anti-lipase activity. Regarding the results, we may conclude that the wines on the Georgian market made from local cultivars, i.e. Saperavi (red) and Rkatsiteli (white), are characterised by high anti-lipase and antioxidant activity and high polyphenol content.

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