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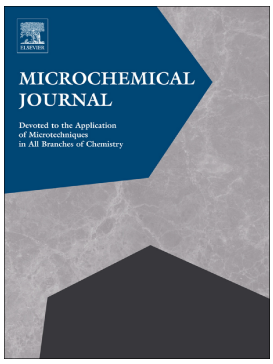

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PII: S0026-265X(18)30116-4
DOI: doi:10.1016/j.microc.2018.04.010
Reference: MICROC 3124

To appear in: *Microchemical Journal*

Received date: 30 January 2018
Revised date: 11 April 2018
Accepted date: 11 April 2018

Please cite this article as: Justyna Płotka-Wasylka, Vasil Simeonov, Jacek Namieśnik , Characterization of home-made and regional fruit wines by evaluation of correlation between selected chemical parameters. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Microc(2017), doi:10.1016/j.microc.2018.04.010

Characterization of home-made and regional fruit wines by evaluation of correlation between selected chemical parameters

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Keyword
Wine; biogenic amines; metals; chromatography; spectroscopy; cluster analysis

Abstract

Since the last decade of the 20th century, there has been rising interest in the production of fruit wines, as evidenced by the high number of published papers and books covering this matter. When aiming to produce quality fruit wines, it is essential to evaluate the analytical parameters of the beverage. In this context, there are a large number of analyses for the evaluation of wines and fruit wines. This article characterizes the fruit wines made from different fruits using selected parameters (BAs, metals, sulfates, phosphates) by the use of a traditional chemometric technique – hierarchical cluster analysis. To determine the organic compounds, an in situ derivatization coupled with dispersive liquid-liquid microextraction (DLLME) and gas chromatography-mass spectrometry (GC-MS) was used. The spectroscopy techniques such as flame photometers, AAS and GF-AAS were applied to determine the selected metals content. Wine made from grapes coming from Polish Vineyard (ANNA de Croy) were also analysed to compare the obtained results. The classification offered allows the identification of unknown wine samples with similar origin to be ordered in some of the patterns formed.

1. Introduction

Wine is an alcoholic beverage widely consumed throughout the world with a great social and economic importance. Wine is a distinctive product that influences major life events, from birth to death, victories, auspicious occasions, harvest and other events. The technique of winemaking is known since the dawn of civilization and has followed human and agricultural progress. In definition, wine is an alcoholic beverage produced from grapes, fermented without the addition of sugars, acids, enzymes, water, or other nutrients [1]. However, the word knows also other type of wine so called fruit wines which are fermented alcoholic beverages made from a variety of base ingredients (other than grapes), they may also have additional flavors taken from fruits, flowers and herbs. For historical reasons, mead, cider and perry are excluded from the definition of fruit wine [2,3].

Fruits produced by many indigenous trees are edible and can ripen within a very short span of time, generating surplus production [4]. Without a doubt many of fruits are consumed fresh, but unfortunately large quantities are wasted during peak harvest periods, due to many reasons such as high humidity fluctuations, temperature, improper handling, inadequate storage facilities, inconvenient transport and microbial infections [3]. Thus, utilization of ripe fruits or their juices for production of wines is considered to be an attractive means of utilizing surplus and overoripen fruits. Furthermore, fermentation helps to preserve and enhance the nutritional

value of foods and beverages. Currently, many researches assess the potential of fruit species which have been explored by the food industries to meet the growing needs of the ever increasing consumer market for several fruits by-products including wines [3].

Fruit wines have traditionally been popular with home winemakers. Nowadays, there is many wineries that produced fruits wine, however, in many countries so called home-made fruit wines still dominate on table at important family ceremonies. A big variety of fruits which differ in shape, taste, color and nutritive value [5], are available in the market and many are utilized widely for production of fermented beverages.

Although the wine is a fruit product, but fermentation produces a variety of chemical changes in the must (or fruit juice), and so wine is far from being juice with ethanol added [6,7]. It is important to know the characterization of wine due to many reasons with the most important being human health and life.

It is well documented that moderate consumption of wine (especially red variety) has been associated with several potential health benefits related to compounds with high antioxidant capacity like polyphenols, including trans-resveratrol [8,9]. On the other hand, red wines are known to be a source of biogenic amines (BAs) which can cause several physiological changes such as migraine headaches, nausea, cardiac palpitations, etc [10]. Another important issue for consumer wine quality perception is the presence of sulphates, an additive used for its antioxidant, antiooxidase and antiomicrobial properties [11]. Nevertheless, it is also a poisonous and allergenic substance. Moreover, daily consumption of wine in moderate quantities contributes significantly to the requirements of human organism for essential elements as Ca, Co, K, Fe, Mg, Ni, Zn and others[9]. However, special attention must to be given to other elements which are found in wine such As, Cd, Cr, Hg, Pb for their potential toxicity [9]. The presence of these hazardous species is regulated by health-protection laws [12].

Many factors impact on differences between wine and fruit wine especially substrates used to manufacturing, production process and additives added. These parameters may impact on the chemical characterization of fruit wine [13]. The question is how the parameters characterized the wine (e.g. BAs, metals, sulfates) are related to fruit wines? Does the correlation between these parameters exist or correlation between fruit wines made from different substrates takes place?

Although the number of publications about fruit wines has increased in recent years [14-16] the chemical characterization of these beverages has not been detailed. The purpose of this study was to characterize the fruit wines made from different fruits by selected parameters (BAs, metals, sulfates, phosphates) and to access the correlation between the selected factors and the samples. To determine the organic compounds, an in situ derivatization coupled with dispersive liquid-liquid microextraction (DLLME) and gas chromatography-mass spectrometry (GC-MS) was used. The spectroscopy techniques such as flame photometers, AAS and GF-AAS were applied to determine the selected metals content. Additionally other physic-chemical parameters were determined to characterize the samples. The correlation between the parameters and the samples were investigated by chemometric techniques. The application of multivariate statistics to the data set in consideration aims to reveal hidden relationships between the objects and their descriptive chemical variables. The schematic representation of the experiment way is presented in Figure 1.

Wine made from grapes coming from Polish Vineyard (ANNA de Croy) were also analysed to compare the obtained results.

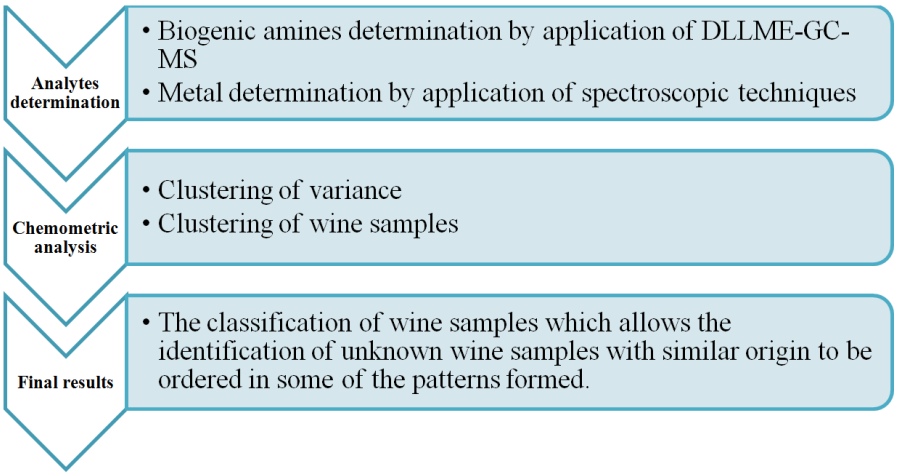

Figure 1. Schematic representation of the pathway of whole experiment

2. Experimental

2.1.Chemicals

The amine standards (histamine, cadaverine, putrescine, butylamine, tryptamine, dietyloamine, etyloamine, dimetyloamine, metyloamine, tyramine, propylamine, 2-phenyletyloamine,and spermine) and hexylamineas internal standard (IS) were purchased, mostly as hydrochloride salts, from Sigma Aldrich (Germany). The stock standard solutions of appropriate biogenic amine were prepared at concentration of 1.0 mg/mL in deionized water. The standard solutions were stored at 4 ºC. The working solutions ofstandard were made up by dilution and mixing of single compound solutions with deionized water. Isobutyl chloroformate (IBCF), and chloroform used in analysis as derivatizing reagent and extractive solvent, respectively, were supplied by Sigma Aldrich. Acetonitrile (MeCN) used as dispersive solvent, HCl and NaOH were obtained from Fluka.

For mineralization a mixture of oxidizing agents and acids were used: nitric acid, 65 %, Suprapur grade supplied by Merck company, hydrochloric acid, 36 %, Suprapur grade supplied by Merck company. Standards used for calibration solution preparations were as follows:

- Ca standard for AES, 10000 mg/L, supplied by BWB Technologies UK Limited,
- Cd standard solution for AAS, 1000 ± 4 mg/L in 2 % HNO_3, supplied by Fluka,
- Fe standard, 1000 mg/L in 2 % HNO_3, plasma grade supplied by SPEX CertiPrep,
- K standard for AES, 10000 mg/L, supplied by BWB Technologies UK Limited,
- Mg standard solution for AAS, 1001 ± 6 mg/L in 2 % HNO_3, supplied by Fluka,
- Pb standard solution for AAS, 1000 ± 4 mg/L in 2 % HNO_3, supplied by Fluka,
- Zn standard, 1000 mg/L in 2 % HNO_3, plasma grade supplied by SPEX CertiPrep,
- Hg standard-MSHG for CV-AAS, 100.48 ± 0.22 µg mL-1 in 3.3 % HCl purchased from Inorganic Ventures, INC (USA),

Firstly standard solution with intermediate concentration of 10 mg/L were prepared by diluting 1000 mg/L stock solution for all determined elements. For Flame Atomic Absorption Spectrometry (F-AAS) and Atomic Emission Analysis (AES) measurements, series of calibration solutions with proper concentrations were made. For Graphite Furnace Atomic Absorption Spectrometry (GF-AAS) measurement, one basic standard solution was prepared for every element. Concentrations of calibrations were as follows:

- Ca – 10, 20, 30, 40, 50 mg/L for AES analysis,
- Cd – 0,1, 0,3, 0,5, 0,7, 1,0 mg/L for F-AAS analysis,
- Cd – 0,002 mg/L for GF-AAS analysis,

- Fe – 0,5, 1,0, 1,5, 2,0, 2,5 mg/L for F-AAS analysis,
- K – 10, 20, 30, 40, 50, 60 mg/L for AES analysis,
- Mg – 0,4, 0,6, 0,8, 1,0, 1,2, 1,5 mg/L for F-AAS analysis,
- Pb – 0,5, 1,0, 1,5, 2,0, 2,5 mg/L for F-AAS analysis,
- Pb – 0,05 mg/L for GF-AAS analysis,
- Zn – 0,1, 0,3, 0,6, 0,8, 1,0, 1,2, 1,5 mg/L for F-AAS analysis.

For GF-AAS analysis proper modifiers were used:

- Phosphate modifier for graphite furnace AAS, $NH_4H_2PO_4$ 100 ± 2 g/L in H2O supplied by Merck company for Cd analysis.
- Magnesium nitrate - palladium nitrate matrix modifier 0,2 % Mg & 0,3 % Pd in 1 % HNO_3 supplied by MS Spektrum for Pb analysis.

2.2.Samples

Samples in number of 18 were fruits wine produced from different type of fruits such as apple, black lilac, quince, etc. These fermented alcoholic drinks were gained from regional shops as well as from people who manufactured it for their own consumption. Additional samples (4) made from grape were gained from Polish vineyard (Anna de Croy Vineyard). The samples were stored at -4^{o}C in a fridge where there was no light.

2.3.Biogenic amines analysis

The procedure reported by Płotka-Wasylka et al. [17] were used to determine BAs in obtained fruit wine samples. The sample preparation of the samples was based on DLLME method coupled with *in situ* derivatization process while the final determination technique was GC-MS. Different parameters affecting the extraction procedure were also studied. Efficient extraction procedures including dispersive (acetonitrile and methanol) and extractive (toluene and chloroform) solvent selection and its volume, derivatizing agent selection (isobutyl chloroformate and ethyl chloroformate), extraction and derivatization time were developed for the biogenic amines analysis if wine samples. The best extraction efficiency was obtained by application of methanol (500 µL), chloroform (500 µL) and isobuthyl chloroformate (85 µL). The derivatization procedure occurs in 15 min while extraction was performed in 1 min. The schematic diagram of the procedure is presented Figure 2.

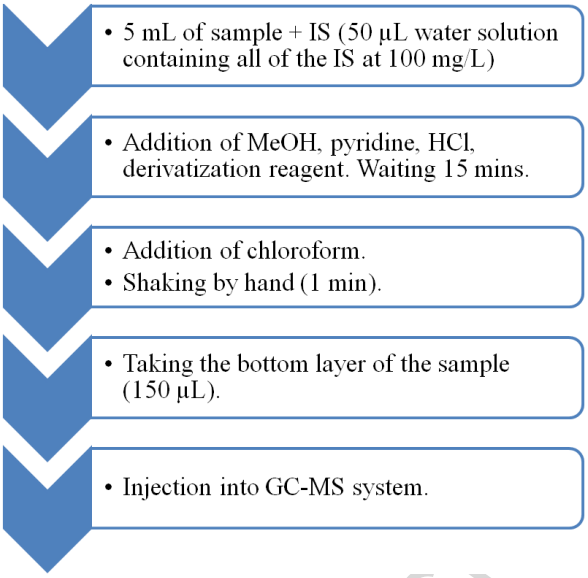

Figure 2. Simultaneous DLLME extraction and derivatization for biogenic amines determination by GC-MS.

The linearity of the method was determined by preparing 10 aqueous solutions containing all analytes at different concentrations ranging from 0.05 to 1.0 mg/L and 1.0 to 10.0 mg/L. To determine the recovery of procedure, the comparison of peak area obtained for unspiked wine samples and for speak samples of wine at one concentration level (0.15 mg/L). To determine the intra-day precision and inter-day precision, the analysing of wine samples spiked with standard solution at one concentration level (0.15 mg/L) was carried out four times in the same day and on two different days over a period of two weeks, respectively. The limits of detection (LODs)and the limits of quantification (LOQs) were determined. The information on these parameters are presented in Table 1.

Table 1. Information on important validation parameters (average recoveries (%), intra-day repeatability (%RSD), inter-day repeatability (%RSD), LOD, **(**µg/L), LOQ, **(**µg/L)) of DLLME-GC-MS (n = 4 at each level).

Analyte	**Interday (%RSD)**	**LOD** (µg/L)	**LOQ** (µg/L)	**Concentration levels**	
				0.15 mg/L	
				Recovery (%)	**Intraday (%RSD)**
BUT	2.7	3.2	9.6	91	2.9
CAD	2.6	1.5	4.5	86	2.7
DIET	3.8	2.2	6.6	82	2.7
DIMET	3.6	2.1	6.3	86	3.1
ET	3.6	3.4	10.2	81	2.6
HIST	2.7	4.0	12.0	74	2.6
MET	4.2	2.1	6.3	86	4.0
PROP	2.9	3.2	9.6	91	2.4
PUT	2.8	1.5	4.5	94	2.9
SPER	2.7	1.0	3.0	89	1.1
TRP	2.1	2.1	6.3	81	1.3
TYR	5	3.0	6.0	95	4
2-PE	6	3.5	10.5	86	6

2.4.Metals analysis

The procedure of the sample preparation for metal determination is presented in Supplementary Material (Figure 3). The wine samples were treated with hot HNO_3–H_2O_2 for decomposition of organic matrix. Two different samples were taken from each wine and therefore, after separate digestion, two different solutions were obtained for each sample all of which were analyzed three times with appropriate equipment. The original solutions of extracts were 1:1 diluted to measure the Ca, Fe, Pb, Zn, Cd content, 1:20 diluted to measure the Mg content (1:25 for sample no 10) and 1:10 diluted to measure K content.

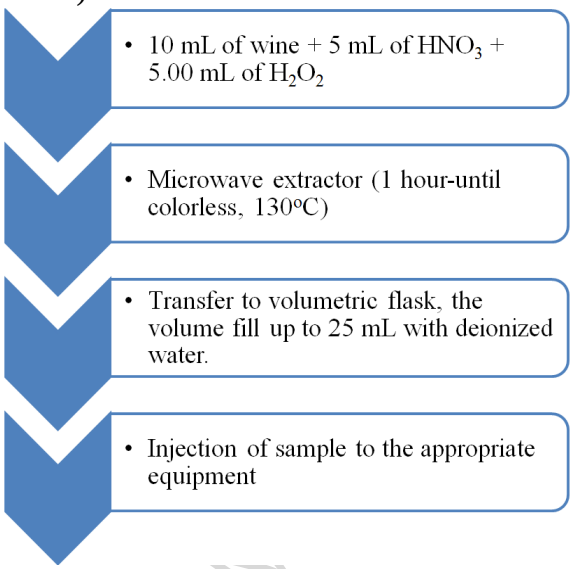

Figure 3. Procedure of sample preparation for spectroscopic analysis

The calibration of the measuring instrument was performed using one of the techniques of the external calibration - the calibration curve method using the appropriately prepared standard solutions of metal ions tested. Working calibration standard solutions were prepared by diluting standard stock solutions containing each of target compounds in the appropriate amounts of dionized water. Linear range for analytes of interest was studied by replicate analysis of the standard stock solutions. The linear regression values were calculated with the average absorbance of three replicate injections for each analyte. The calculated calibration curves showed good linearity range for all tested analytes. Coefficient of variation (CV) was the average value of different concentrations of examined compounds in the linear range. Sensitivity of the developed method was considered in terms of limit of detection (LOD). Limit of detection (LOD) and limit of the quantification (LOQ) of the methods have been set according to OIV recommended technique (OIV, 2007). The two limits were based on values of the standard deviation of the intercept (Sa) and they were deduced of mathematical expressions: LOD=(3,3*Sa)/b and LOQ=3*LOD. The obtained results are presented in the Table 2.

Table 2. Basic validation parameters obtained for each analyte by using developed method (n, number of standards in three replicates, R^2, Coefficient of determination).

Analyte	n	Equation	R^2	LOD	LOQ	Linearityrange	CV [%]
K	6	y = 654.68x + 1733.1	0.998	0.47 [mg/L]	1.41 [mg/L]	1.41-60 [mg/L]	2.6
Ca	5	y = 26.053x + 285.27	0.994	0.415 [mg/L]	1.245 [mg/L]	1.245-50 [mg/L]	2.4
Mg	5	y = 0.3746x + 0.0107	0.999	0.021 [mg/L]	0.063 [mg/L]	0.063-1.200 [mg/L]	1.5
Pb	5	y = 0.0207x – 0.0041	0.992	0.0031 [µg/L]	0.0093 [µg/L]	0.0093-2.5000 [µg/L]	2.0
Zn	7	y=0.1208x + 0.0023	0.998	0.027 [µg/L]	0.081 [µg/L]	0.081-1.500 [µg/L]	1.9
Cd	5	y=0.2805x + 0.0242	0.997	0. 0087 µg/L]	0.026 [µg/L]	0.026-2 [µg/L]	3.1
Fe	5	y = 0.0271x – 0.0059	0.991	0.009 [mg/L]	0.027 [mg/L]	0.027-5[mg/L]	1.7
Hg	5	y=0.0112x + 0.0045	0.989	0.012 [µg/L]	0.036 [µg/L]	0.036-0.8 [µg/L]	10.0
Sn	5	y = 0.0028x + 0,0101	0.990	9.9 [µg/L]	32.5 [µg/L]	32.5 – 100 [µg/L]	n.d

2.5.Equipment used

GC 7890A (Agilent Technologies) was interfaced to an inert mass selective detector (5975C, Agilent Technologies) with electron impact ionization (EI) chamber. Agilent Chemstation was used for data collection/processing and GC-MS control. The parameters of GC-MS were as follows: capillary column:ZB-5MS (30 m x 0.25 mm I.D., 0.25 µm film thickness); injection: pulsed splitless mode (injection pulse pressure 32 ps) at 230 ºC; temperature program: 50 ºC (1min), ramped to 280 ºC at 15 ºC/min (9 min); carrier gas: helium with a constant pressure of 30 psi; MS transfer line temperature: 280 ºC; ion source temperature: 250 ºC; electron impact ionization with 70 eV energy. The selective ion monitoring (SIM) mode was used. Analytes were quantified based on peak area using one target and one or more qualifier ion(s).

For samples mineralization Multiwave GO digestion system supplied by Anton Paar company was used. For Ca and K analysis Flame Photometer BWB-1 (AES) supplied by BWB Technologies UK Limited was used. For moderate concentration heavy metals determination, Flame Atomic Absorption Spectrometer (AAS) SensAA supplied by GBC Scientific equipment Pvt. ltd (Australia) with dual beam optical system and air acetyl flame was used. Deuterium lamp for background correction and hollow-cathode lamps as radiation source were installed. For low concentration heavy metals determination, Graphite Furnace Atomic Absorption Spectrometer Savant AAZ supplied by GBC Scientific equipment Pvt. ltd (Australia) with Zeeman background correction was used. As a carrier gas technical grade argon was supplied and hollow-cathode lamps were installed as radiation source. In case of AES analysis, K and Ca were determined jointly. In case of AAS analysis, subsequent measurement were carried out one element at a time using proper hollow cathode lamp for the specific wavelength. The wavelength used for Cd, Fe, Mg, Pb, Zn analysis were respectively: 228,8 nm, 248,3 nm, 285,2 nm, 217,0 nm and 213,9 nm. The linear regression method was used for the calibration curve. For the GF-AAS analysis proper furnace temperature programs were used.

Ion concentration was obtained by DIONEX 3000 chromatograph (DIONEX, USA) with application of Dionex Ion Pac AS22 analytical column (eluents: 4.5 mM Na2CO3 and 1.5 mM NaHCO3, flow rate: 0.3 mL min−1). Conductometric detection was applied.

2.6. Chemometric analysis (Hierarchical cluster analysis)

Cluster analysis is one of the most applied chemometric methods for multivariate data interpretation [18]. It is thoroughly described as a unsupervised pattern recognition approach which makes it possible to reveal groups of similarity (clusters) within a large and, generally, diffuse data set. The cluster formation could be achieved with respect to the objects of interest (described by various parameters, features, variables) or with respect to the variables identifying the objects. In order to perform this procedure several steps are necessary – data standardization

(in order to eliminate the role of variables dimension on the clustering), determination of the distances between the objects by some similarity measure equation (usually Euclidean distances), and linkage of the similar (close) objects in clusters (very often the Ward's method is preferred). The graphical output of the analysis is a tree-like diagram called dendrogram. Usually, statistical significance of the clusters has to be determined in order to better identify significant clusters. Missing data are replaced by the value LOD/2. The software package used was STATISTICA 8.0

The chemometric data interpretation in the present study is based on an input matrix consisting of 22 objects (wine samples) described by 22 chemical variables [22x22]. Hierarchical cluster analysis (HCA) was used to reveal patterns of similarity between the variables or between the wine samples. It could be of substantial help in data interpretation when specific markers (discriminating variables) for the different wine patterns are sought.

3. Results and discussion

3.1.Analysis of fruit wine samples

Samples of interest were analyzed with three replicates. Information on BAs content and metals content in samples calculated as mean (n=3) are given in Table 3. Moreover, other parameters characterized fruit wine samples are given.

All of the BAs analyzed were found in most of the wines, however, none of the BAs determined does not exceed the permissible (or toxic) concentration levels [19]. The most common analyte found in analysed samples was histamine (in 15 of 18 fruit wine samples and in all grape wine samples).The content of histamine differed depending on the fruits used to produce alcoholic beverage. The amine important as a potential precursor of the carcinogen dimethylnitrosamine, namely, dimethylamine, was determined in almost all samples with levels ranging from 0.210±0.009 to 0.743±0.020 mg/L. Also amines associated with sanitary conditions, namely putrescine and cadaverine, were determined in investigated samples, but the level was different depending on the analytes. Putrescine was found in 21 samples, while cadaverine was determined only in 11 samples. It need to be noted that the latest compound was found in all wine obtained from grape. The primary biogenic amines were found as follows: the methylamine in ten samples, ethylamine occurred in 15 samples, buthylamine occurred in 13 samples and propylamine occurred in 8 samples. The interesting issue is that methylamine, ethylamine and buthylamine were found in all wines produced from grape, while propylamine did not occur in any of these wines. Additionally analyzed BAs was spermine which was determined in 9 samples.

The appropriate remarks can be concluded after analysis of the sample in order to determine the metals content. The results reveal the amounts of Cd metals to be extremely low, however, how be found in all wine samples produced from grape. The content of Pb, Zn and Fe metals is also very low. In some cases, Cd, Pb and Fe concentrations remained below the limit of detection and could not be detected (iron was not determined in 10 samples). However, it can be concluded that in case of Pb, it was found at higher concentration in wine made from fruits growing on trees (apple) or higher bushes (quince, black lilac, grape). The results show that the content of Mg and Ca are at a similar concentration level, but was slightly low for grape wines. The results reveal the K content to be higher than the other elements in question.

The contents of the all metals in wine samples were considerably smaller than the maximum concentrations allowed according to the OIV [20-22]. In addition, the determined concentration of metals are much below the permissible concentrations, what is probably due to the fact, that the analysed wine are made from different kind of fruits except grapes.

3.2.Chemometric analysis of the analytical results

HCA is performed on z-standardized input data matrix (in order to eliminate dimension variability) by the use of squared Euclidean distances as similarity measure, Ward's method of linkage, Sneath's criterion for cluster significance and hierarchical dendrogram as graphical output. Our previous experience has proven that the mode used (squared Euclidean distances as similarity measure and Ward's method of linkage) is the most appropriate one (good separation of clusters, logical interpretation etc.). Missing data are replaced by the value LOD/2. It is accepted in multivariate statistical analysis to replace missing data by LOD/2 avoiding in this way the serious reduction of the dimension of the input data matrix. Very often it not a priory clear if the missing data are due to "not measured at all samples (analytes)" or to "not detected by the analytical method used" species. Thus, the replacement by LOD/2 is a good compromise for keepig the dimension of the data matrix. The interpretation of the chemometric expertise is sound and logic since it keeps in mind the low levels of certain variables.

3.2.1. Clustering of variables

In Figure 4A the hierarchical dendrogram for variables clustering is presented. Four clusters are formed at ½ D_{max} distance as follows:

K1 (*DIMET, MET, Fe, SPER, K, phosphates, sulfates*)
K2 (*Zn, 2-PE, ET, DIET*)
K3 (*TPR, PROP, Cd, HIST*)
K4 (*Ca, TYR, PUT, Pb, CAD, Mg, BUT*)

It could be stated that four major factors determine the chemical composition of the wine samples (respectively, the wine quality). They are related to dimethylamine and inorganic salts composition (K1), to ethylamine and Zn composition (K2), to histamine (K3) and to specific compounds like PUT, CAD, BUT and metal content (K4).

3.2.2. *Clustering of wine samples*

In Figure 4B the hierarchical dendrogram for the wine samples is given. Six major clusters are obtained as one of the samples is a typical outlier.

Taking into consideration the clusters presented above, it can be concluded that wine made from specific fruits have similar chemical characterization, e.g. plum wine (K1), red grape wine (K6). The K3 cluster is obtained for wine produced from black currant, strawberry and raspberry what may suggest that these fruits have similar characteristics responsible for fruit wine quality. Interesting results are obtained for wines made from apple. These wines are different in chemical composition, depending probably on the specific biotype of apple as a substrate, region of apple tree growing or year of production. Another specific result is found for wine made from white grape which has different chemical characteristic as compared to red wines made from grapes, but similar to those obtained from quinces. The analysis also shows that wine made from chokeberries has characteristics similar to those of red currant wine, and what is surprising, to apple wine.

Table 3. Information on characteristic parameters of fruit wine samples (substrates, alcohol level, year of production, biogenic amines, metals, sulfates and phosphates content calculated as mean (n=3)). **For chemometric analysis missing data are replaced by the value LOD/2.**

No	pH	Alco.	Year	Substrate	Color	*BUT [mg/L]*	*CAD [mg/L]*	*DIET [mg/L]*	*DIMET [mg/L]*	*ET [mg/L]*	*HIST [mg/L]*	*MET [mg/L]*	*PROP [mg/L]*	*PUT [mg/L]*	*SPER [mg/L]*	*TRP [mg/L]*	*TYR [mg/L]*	*2-PE [mg/L]*	*PO_4^{3-} [mg/L]*	*SO_4^{2-} [mg/L]*	K [mg/L]	Ca [mg/L]	Mg [mg/L]	Pb [µg/L]	Zn [µg/L]	Cd [µg/L]	Fe [mg/L]
1	3.46	16%	2013	apple	White	0.914	0.793	0.183	o	o	0.516	o	0.089	0.0011	o	0.049	0.991	0.070	7.763	14.834	330	17.9	19.0	88.5	86.9	3.72	0.432
2	4.16	14%	2014	Black lilac	Red	o	o	0.114	o	o	1.457	o	0.065	8.759	o	0.053	2.155	o	224.509	52.511	255	4.29	18.6	95.2	103	18.4	o
3	3.50	12%	2012	chokeberry	Red	0.582	o	0.200	0.245	0.320	1.119	0.099	0.097	7.560	o	0.033	3.099	0.055	26.587	6.550	165	30.9	18.0	35.6	276	0.509	0.508
4	3.55	14%	2010	Apple	White	0.902	o	o	o	o	0.789	o	0.054	2.214	0.044	o	1.764	0.037	7.989	15.458	353	28.1	13.03	116.3	105	1.11	o
5	3.49	14%	2015	Apple	White	o	o	o	0.545	0.101	0.611	0.133	0.087	2.540	o	0.026	0.0015	0.031	8.148	14.845	233	18.8	19.2	75.3	36.1	o	0.508
6	3.67	12%	2008	Plum	Rose	o	o	0.384	0.489	0.279	o	o	0.055	4.674	o	o	1.455	0.074	33.112	16.270	296	20.7	5.00	9.91	164	o	o
7	3.36	12%	2008	blackcurrant&mint	Red	o	0.688	0.302	0.627	0.116	0.677	o	o	9.015	0.033	o	3.982	0.068	110.130	105.886	441	27.0	13.05	43.5	99.1	0.795	o
8	3.51	14%	2015	chokeberry	Red	1.014	0.520	o	0.210	o	0.340	o	0.077	5.679	o	o	4.008	o	24.631	2.014	264	50.1	19.8	7.21	36.1	0.578	o
9	3.03	13%	2015	Red currant	Rose	o	0.734	o	0.240	0.097	0.641	0.111	o	5.674	o	o	0.0015	0.033	28.400	24.346	238	32.4	7.99	o	70.7	o	o
10	3.30	13%	2015	Raspberry	Rose	0.535	0.899	0.141	0.401	0.111	0.715	0.079	o	7.642	0.035	o	1.745	0.053	30.477	20.121	259	24.5	29.7	2.11	170	o	o
11	3.01	15%	2013	Strawberry	Rose	o	0.589	0.156	0.545	0.078	0.284	o	o	8.026	o	o	3.104	0.059	61.699	32.607	369	29.3	22.1	29.4	80.8	0.924	o
12	3.50	14%	2013	Red currant	Rose	o	0.910	0.189	0.677	0.131	o	0.087	o	6.464	o	0.037	1.671	0.031	102.665	13.782	411	23.7	11.97	8.97	146	o	0.407
13	3.60	14%	2011	Plum&wildrose&quince	Rose	o	o	0.100	0.450	0.087	0.219	0.076	o	6.111	0.043	0.061	2.996	0.033	7.074	13.326	233	35.0	10.58	6.62	132	o	0.432
14	3.47	11%	2008	red currant&mint	Rose	0.314	0.544	o	0.379	o	0.309	o	o	5.376	0.055	0.030	1.054	0.032	73.216	32.238	398	9.11	9.88	o	316	o	0.962
15	3.39	13%	2007	Black currant	Red	o	0.777	0.126	0.412	o	0.469	o	o	9.904	o	0.035	2.330	o	68.248	5.335	254	22.9	15.8	20.9	11.8	o	o
16	3.20	12%	2005	Quince	White	0.765	o	0.157	0.743	0.117	0.301	0.065	o	1.454	o	o	2.992	o	96.384	1.156	208	25.7	16.1	8.51	268	0.667	0.550
17	3.51	16%	2014	strawberry	Rose	0.930	o	0.109	0.544	o	0.488	o	o	6.045	0.036	0.030	3.786	0.048	74.974	13.856	402	22.9	11.86	10.98	138	0.423	o
18	3.66	12%	2014	plum	red	o	o	0.351	0.467	0.224	o	o	0.050	4.010	o	o	1.327	0.056	104.453	84.6156	310	20.6	5.12	9.23	156	o	o
19	3.48	14%	1012	red grape	red	0.308	0.612	0.130	0.456	0.097	0.688	0.099	o	5.229	0.033	o	2.013	0.035	95.862	38.260	432	18.6	10.64	24.56	97	0.912	1.231
20	3.54	11%	2008	red grape	red	0.324	0.656	0.151	0.512	0.113	0.715	0.105	o	6.009	0.035	o	2.135	0.043	23.238	53.635	467	21.3	11.56	26.40	101	0.897	1.278
21	3.34	12%	2008	whitegrape	white	0.567	0.213	0.210	0.634	0.134	0.547	o	o	2.154	o	0.033	1.235	o	234.217	56.366	345	11.7	15.7	25.78	112	0.678	0.978
22	3.47	13%	2010	red grape	red	0.278	0.567	0.164	0.534	0.124	0.746	0.119	o	6.234	0.041	o	2.434	0.033	26.565	46.968	478	22.8	11.9	26.78	132	0.879	1.456

Alco,- alcohol content,BUT, buthylamine, CAD, cadaverine, DIET, diethyloamine, DIMET, dimethyloamine, ET, ethylamine, HIST, histamine, MET, methyloamine, PROP, propylamine, PUT, putrescine, SPER, spermine, TRP, tryptamine, TYR, tyramine, 2-PE, 2-phenylethylamine, o-under limit of detection

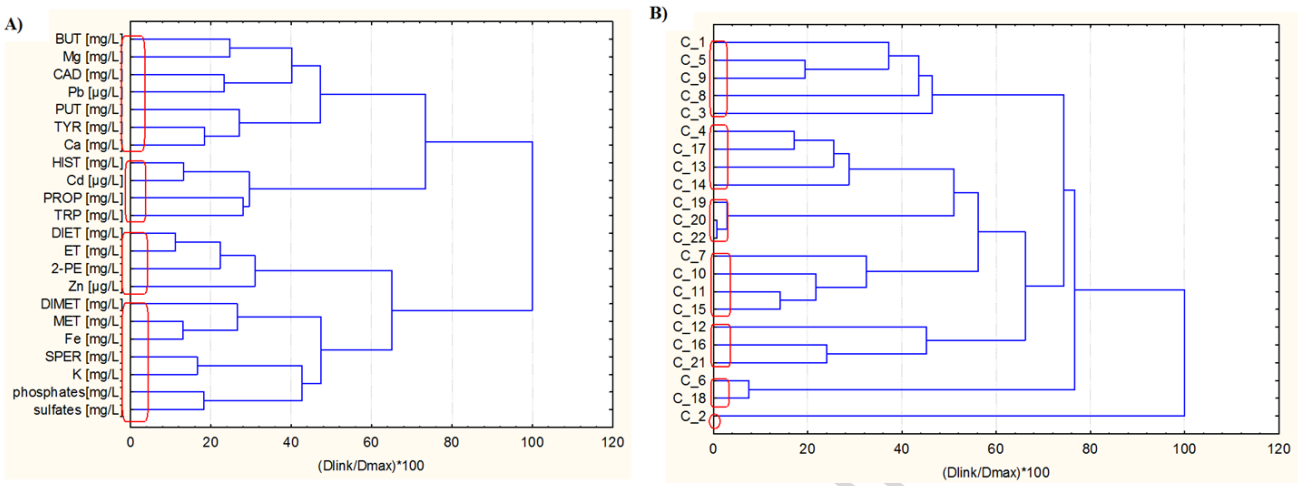

Figure 4. A) Hierarchical dendrogram for 22 chemical variables; B) Hierarchical dendrogram for 22 wine samples

It is important to use the clustering results as an option to introduce specific markers (indicators) able to discriminate one pattern of wine sample from the others. In order to reach conclusions for the pattern separation average values for each chemical variable for each of the identified 6 clusters are calculated (Table 4).

It is easy to find how the single patterns are separated. For instance, K1 which represents fruit wines made from plums is characterized by maximal values for diethylamine, ethylamine and 2-phenylethylamine and minimal values for butylamine, histamine, tryptamine, tyramine, Pb, Cd and Fe. Therefore, this group of wine samples is presented by high content of ethylenamines and low levels of histamine, butylamine, tyramine and selected metals. Thus, each wine pattern could be reliably described and interpreted (see Table 5).

For example, the specific role of wine sample 2 as an outlying object is due to the highest levels of quite many of the chemical components and the lowest levels of the rest of them. Thus, this is, indeed, a very different wine sort which does not resemble any of the other types. Additional descriptors of sample 2 are pH (the wine has the highest pH value, e.g. lower acidity), black lilac as fruit substrate, etc. It is also seen that the chemical variable namely propylamine is not quite significant as discriminating parameter since it has the same lowest values for most of the identified clusters.

We believe that the variables available (even less variables are used for distinguishing between different wine classes) are good enough to offer differentiation (classification): the necessary discriminating parameters are presented in Table 5 - six classes are identified and for each of them discriminating chemical indicators are offered. PCA confirmed entirely the results of cluster analysis.

Table 4. Calculated average values for each chemical variable for each of the identified clusters. Maximum acceptable limits for some analytes given by OIV (2015): Pb: 0.15 mg/l; Cd: 0.01 mg/L; Cd: 5.00 mg/L; SO_4^{2-}: 1500mg/L.

	BUT [mg/L]	*CAD [mg/L]*	*DIET [mg/L]*	*DIMET [mg/L]*	*ET [mg/L]*	*HIST [mg/L]*	*MET [mg/L]*	*PROP [mg/L]*	*PUT [mg/L]*	*SPER [mg/L]*	*TRP [mg/L]*	*TYR [mg/L]*	*2-PE [mg/L]*	*PO_4^{3-} [mg/L]*	*SO_4^{2-} [mg/L]*	K [mg/L]	Ca [mg/L]	Mg [mg/L]	Pb [µg/L]	Zn [µg/L]	Cd [µg/L]
K1	**0.002**	**0.001**	**0.368**	**0.477**	**0.254**	**0.002**	**0.002**	**0.052**	**4.345**	**0.001**	**0.001**	**1.391**	**0.062**	**68.783**	**50.443**	**303.000**	**20.650**	**5.060**	**9.570**	**160.000**	**0.004**
K2	**0.645**	**0.375**	**0.185**	**0.685**	**0.128**	**0.282**	**0.102**	**0.002**	**3.356**	**0.001**	**0.024**	**1.966**	**0.011**	**144.422**	**23.768**	**321.333**	**20.367**	**14.590**	**14.420**	**175.333**	**0.450**
K3	**0.135**	**0.737**	**0.181**	**0.490**	**0.077**	**0.535**	**0.021**	**0.002**	**8.644**	**0.018**	**0.009**	**2.789**	**0.045**	**67.639**	**40.987**	**330.750**	**25.925**	**20.163**	**23.978**	**90.425**	**0.432**
K4	**0.303**	**0.612**	**0.150**	**0.501**	**0.110**	**0.714**	**0.107**	**0.002**	**5.825**	**0.035**	**0.001**	**2.193**	**0.036**	**48.555**	**46.288**	**459.000**	**20.900**	**11.367**	**25.913**	**110.000**	**0.896**
K5	**0.536**	**0.136**	**0.054**	**0.344**	**0.027**	**0.450**	**0.020**	**0.015**	**4.935**	**0.045**	**0.029**	**2.400**	**0.037**	**40.813**	**18.720**	**346.500**	**23.778**	**11.338**	**33.475**	**172.750**	**0.385**
K6	**0.500**	**0.409**	**0.076**	**0.249**	**0.106**	**0.645**	**0.068**	**0.071**	**4.292**	**0.001**	**0.021**	**1.619**	**0.038**	**19.106**	**12.518**	**246.000**	**30.020**	**16.798**	**41.322**	**101.160**	**0.963**
outlier	**0.00165**	**0.0008**	**0.112**	**0.001**	**0.0075**	**1.456**	**0.0015**	**0.067**	**8.763**	**0.00055**	**0.052**	**2.156**	**0.00155**	**224.509**	**52.511**	**255**	**4.29**	**18.6**	**95.2**	**103**	**18.4**
max	K2	K3	K1	K2	K1	outl	K, K2	K6	outl	K5	outl	K3	K1	outl	outl	K3	K6	K3	outl	K2, K5	outl
min	K1, outl	outl	K5	outl	outl	K1	outl	K2-K5	K2	outl	K1, K4	K1	outl	K6	K6	K6, outl	outl	K4, K5	K1	K3	K1

Table 5. Specific discriminators (markers) for each wine pattern

discriminators								
K1 (6, 18)	DIET	ET	2_PE					high
	BUT	HIST	TPR	TYR	Pb	Cd	Fe	low
K2 (21, 16, 12)	BUT	MET	Zn					high
	PROP	PUT						low
K3 (15, 11, 10, 7)	CAD	TYR	K	Mg				high
	PROP	Zn	Fe					low
K4 (22, 20, 19)	MET	Fe						high
	PROP	TPR	Mg					low
K5 (14, 13, 17, 4)	SPER	Zn						high
	DIET	PROP	Mg					low
K6 (3, 8, 9, 5, 1)	PROP	Ca						high
	phosphate	sulfate	K					low
Outlier (2)	HIST	PUT	TPR	phosphate	sulfate	Pb	Cd	high
	BUT	CAD	DIMET	ET	MET	SPER	2-PE	low
	K	Ca	Fe					low

4. Conclusions

Today wines can be made from any fruit other than grape bringing the health benefits. The type wine or fruit wine can be chosen depending on taste, aroma and beneficial health expectations. In this paper, the home-made and regional fruit wines as well as grape wine were analysed in terms of selected biogenic amines and metals content. Additionally, other physico-chemical parameters were taken into consideration (sulfates, phosphates, alcohol content, color, year of production, ect.) to access the correlation between the selected factors as well as alcoholic beverage samples.

In this work, several parameters of fruit wine samples were determined including selected biogenic amines and metals, sulfates, phosphates and others and were used to investigate the correlation between these parameters. None of the biogenic amines determined did not exceed the permissible (or toxic) levels of concentration. The contents of the all metals determined by using spectroscopy techniques were considerably smaller than the maximum concentrations allowed according to the OIV.

Considering the correlation between the selected parameters as well as the samples, chemometric analysis allows to state that four major factors determine the chemical composition of the wine samples (respectively, the wine quality) being related to dimethylamine and inorganic salts composition, to ethylamine and Zn composition, to histamine and to specific compounds like putrescine, cadaverine and buthylamine and metal content. Moreover, taking into account the results obtained by chemometric analysis it can be concluded that wine made from specific fruits have similar chemical features, e.g. plum wine, red grape wine. It was also possible to separate the single wine patterns by some specific chemical markers.

Acknowledgments

The research is funded by Faculty of Chemistry, Gdańsk University of Technology within the mini-grant project (Decision no 4914/E-359/M/2017). The authors would like to thank Anna de Croy Vineyard from Słupsk, Poland. One of the authors (V. Simeonov) expresses his gratitude to European Union H2020 Programme (H2020) under grant agreement 692146-H2020-eu.4.b "Materials Networking" for financial support.

Conflict of interest

Authors decline no conflict of interest.

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Highlights

1. The fruit wines and wines are characterized by using selected parameters.
2. The correlation between the parameters and the samples were investigated by chemometric techniques.
3. None of the BAs did not exceed the permissible (or toxic) level of concentration.
4. The contents of all metals were considerably smaller than the maximum concentrations allowed according to the OIV.
5. Several internship between selected parameters were determined.