Identification of Prohibited Additives in Wines

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Abstract. There is no uniform test or "black box method" to establish definitely the authenticity of wine samples. Methods and types of adulteration are constantly being modernized, requesting to improve and develop analytical methods of increasing the efficiency of identification and detection of counterfeits. The goal of the research is to create a system for diagnostics of prohibited process technologies and additives during the wine identification. The studies were carried out in 2016-2023. The objects of research are: wines; model systems obtained by introducing of permitted and prohibited additives. A total of 3.500 samples were examined. Enochemical indicators based on the principles of high-performance and gas-liquid chromatography, potentiometry and colorimetry were identified in the objects. The developed classifying system covers the range of detection of prohibited additives and a number of process technologies: coloring agents of different origin, flavoring agents, organic acids, water, glycerol; sugar-containing substances of non-grape origin, additives of non-grape raw materials. Each element of the system can be used to diagnose several types of counterfeits. The system is open for seeding and spreading. Methods of analysis of classifying indicators are quite simple and available for industrial laboratories of various levels.

1 Introduction

Adulteration of wine constitutes consumer fraud by providing inaccurate or fake information about the product on the label. Product adulteration has an extremely negative effect on the image policy of wineries. In addition, such wines cause material and moral damage to consumers and, in some cases, can threaten their health and life. Adulteration of wines is a significant global problem: according to official data, the share of counterfeit and adulterated products is 30% and 20%, respectively [1]. According to the published information, in two months of 2019 in 78 countries there were seized more than 33 million liters and 16,000 tons of potentially dangerous adulterated drinks and food [2]. The proportion of wine export of some countries may be several times higher than the potential volume of their production [3].

When counterfeiting wines, adulterators use such prohibited methods of correcting organoleptic and physicochemical parameters as unregulated adding of ethanol, sweetening, acidifying, aroma-producing and coloring agents, glycerol introduction, as well as watering

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the wine to increase its volume [4]. Taking into account global level of the problem, scientists are actively looking for methodological solutions to detect counterfeit wines. Researchers developed various indicators of technochemical control, including traditional methods (chemical analysis, gas and liquid chromatography), as well as methods based on modern scientific achievements (biomolecular and isotopic analyses, nuclear magnetic resonance) [5-10].

However, every proposed approach concerns the detection of only a certain type of wine adulteration. The information obtained during the study of wines in different countries also do not take into account special aspects of regional regulatory documents, wine production technology and chemical composition, making difficult using of identification systems to detect adulteration of local wines.

Thus, a wide range of counterfeiting methods necessitate the scientific research aimed at substantiating criteria indicators for detecting any prohibited components, developing new analytical methods of identification, as well as systematizing the results obtained to effectively detect adulterated products.

This work systematizes the research of authentic and adulterated wines, gives the table of classification indicators to detect counterfeits and presents conclusions and directions for further research.

2 Materials and Methods

The goal of this research is to create a system for diagnosing of prohibited technological methods and additives for wine identification. The objectives are: selection and substantiation of enochemical indicators characterizing the authenticity of wines and determining the range of their variation; modeling of counterfeit wines; studying of experimental samples; mathematical data processing. The studies were conducted from 2016 to2 023.

The objects of research are: wines from grapes of white and red varieties (Vitis vinifera); model systems obtained by introducing prohibited additives. A total of 3,500 samples were examined.

The imitation of red wine color was achieved by adding coloring agents to white dry wines (natural (E163) and synthetic (carmoisine E122, tartrazine E102, indigo carmine E132)) agents; blueberry, black currant, cherry, elderberry and beet juices and extracts. Synthetic flavoring agents were used replicate the aroma of Muscat wines. Flavor modification was accomplished by adding concentrated grape must, sucrose and glucose-fructose syrups in doses of 1-200 g/l, glycerol - 2-12 g/l, organic acids 0.5-2 g/l.

The content of phenolic substances was determined using the colorimetric method with the Folin&Ciocalteu reagent, the profile of phenolic substances using the HPLC method and synthetic coloring agents using method of fixing on woolen thread [11]. The content of potassium, calcium, sodium and magnesium was determined by atomic absorption spectroscopy, of chlorides - by potentiometry [11]. The aroma-producing complex of wines was determined using gas-liquid chromatography (chromatograph Agilent Technologies 6890 Network with flame ionization and mass selective detector) [11].

To determine the content of glycerol, the profile of organic acids and sugars, methods based on high- performance liquid chromatography (chromatograph Shimadzu LC Prominence, Japan) with the use of refractometric and spectrophotometric detectors of the system (organic acids and sugars, respectively) were supplied. Determination of organic acids was carried out at a wavelength of 210 nm. Sample separation was provided on Supelcogel C610H column, filled with a sorbent based on sulfited divinyl-polystyrene (column size 300 × 7.8, sorbent graining is no more than 10.0 μm, Supelco®, Sigma-

Aldrich), in isocratic mode with an eluent supply of 0.1% aqueous solution of orthophosphoric acid, at the rate of 0.5 ml/min [11, 12].

The glucose-fructose index was calculated as a content ratio of glucose to fructose, the error of the final result did not exceed 0.01. The glycerol factor was calculated as a ratio of mass concentrations of glycerol and ethyl alcohol, with the result multiplied by 100 [11].

The results of analytical studies were processed using methods of mathematical statistics at a significance level of $\alpha = 0.05$; the difference between groups of indicators of authentic and counterfeit wines was established according to Fisher's ratio test (F>Frt).

3 Results

When identifying wines, the problem of classification is actually solved by systematically distributing of the studied subjects in accordance with essential characteristics and specific category labels. To solve the problem of identifying wine products, the dichotomy principle, in which objects divide into two antipodal categories: "authentic ↔ not authentic", "adulterated ↔ not adulterated" is applied.

The research is based on our ideas that wine is a complex multicomponent system with typical buffering properties and ranges of enochemical indicators, and introduction of extraneous ingredients of non-grape origin leads to imbalance in the composition of the beverage and its physicochemical and organoleptic characteristics.

The first stage of the research involved summarizing previously obtained data and systematizing literary sources to classify the types of wine counterfeiting. As a result, 15 types of counterfeit wines were classified. We have systematized the existing types and methods of adulteration wine products with conditional indices assigned (Table 1).

Table 1. Counterfeit indices

Code	Type of adulteration
1	Adding of non-grape raw materials
2	Unrestricted using of sucrose
3	Using of sugar-containing components of non-grape origin
4	Adulteration of concentrated grape must
5	Unlimited adding of organic acids
6	Adding of acids of non-grape origin
7	Substitution of grape variety
8	Adding of coloring agents of various origin
9	Fake vintage
10	Adding water
11	Adding glycerol
12	Fake origin
13	Imitation of flavor
14	Imitation of color
15	Imitation of aroma/bouquet

Falsification of wine products means any changes in the type or composition and properties of wine due to the modification of raw material composition, as well as production use of techniques, methods or substances that are prohibited by regulatory documentation. The addition of prohibited substances alters the content and qualitative composition of certain components, as well as their ratios typical of grape wines, making it impossible to identify them as authentic. It has been shown that the use of single-component criteria for identifying the addition of prohibited components in wines is insufficient.

At the next stage of the research, we investigated the changes in the physicochemical parameters of wines and model systems upon the introduction of various components, such as coloring agents, sweeteners, organic acids, water, and alcohol.

As a result, ranges of variation in indicators were obtained for different methods of falsification. Simultaneously, studies of natural wines and their composition were conducted, leading to the formation of a database of authentic samples and the determination of the ranges of variation for their indicators [12-13]. The deviation of values in falsified wines from those in natural wines confirms the fact of falsification (Table 2).

Thus, a change in the content of one indicator leads to a shift in the equilibrium of the wine system as a whole. For example, in the case of dilution of wine with water, there will be a decrease in ethyl alcohol, phenolic substances, organic acids, and there will be an increase in the concentration of sodium cations and chloride anions. After comparing the obtained values of the indicators with the ranges we have established and recommended, a decision is made - whether the wine corresponds to natural or not. If the values of the indicators are within the ranges - the wine is natural.

Table 2. Values of classification indicators of wines

Indicator	Limiting value
Content of phenolic substances (red wines)	not less than 1200 g/l
Optical density D_{620}	not less than 0.07
Ratio of monomeric anthocyanins in the total content of phenolic substances (red wines)	2-30 %
Ratio of aliphatic, aromatic and terpene alcohols in the sum of all aroma- producing substances	no more than 60 %
Presence of volatile components of non-grape origin	unacceptable
Glucose-fructose index in wines with introduction of concentrated grape must	no more than 1.01
Glucose-fructose index in semi-dry, semi-sweet and sweet wines with endogenic sugars	0.01-0.99
Content of disaccharides	no more than 0.7 g/l
Mass concentration of glycerol: White wines Red wines	 5.0-10.2 g/l 6.3-12.5 g/l
Glycerol factor: White wines Red wines	 5.5-10.3 6.3-12.2
Content of glycerol in fortified wines	1.3-7.0

Generalization of the results makes it possible to create a system based on the relationship of classifying indicators and their derivative parameters with types of wine adulteration, and to establish methods of estimation (Table 3).

The cross-validation procedure was carried out to evaluate the classification accuracy. Samples were separated into training and test sets in a ratio 2:1. The values of classifying indicators of the samples for each class were taken as basic data (750 samples of authentic wines, 250 – of counterfeits). As a test set, we composed a matrix of 500 samples (335 and 165, respectively). For the data of the training set we established a range of allowed indicator values of authentic wines. For the data of the test set, the identification accuracy was checked using the classification system. We cross-checked the resulting system, since the test set classification error of 6% corresponded to the classification accuracy of the training set.

Table 3. Classification system for detection of adulterated wines

Classifying indicators	**Parameters**	**Index of counterfeit**	**Method**
Profile of sugars	Content of disaccharides in total	1-4	[12]
	Glucose-fructose index		
Profile of organic acids	Content of particular acid in the complex of organic acids	3, 5-6	[8, 13]
	Test on presence of *DL*-tartaric acid		
Aromatic profile	Content of higher and aromatic alcohols in the complex of aroma-producing substances	1, 7, 13-15	[11, 13]
Composition of phenolic substances	Content of monomeric anthocyanins in the total	1,7-9, 12-13	[14]
	Folin & Ciocalteu reagent		[11, 14]
	Test on presence ofcoloring agents		
Cation-anion composition	Ratio of the components	10-12	[11]
Content of glycerol	Glycerol factor	11, 13	[13]
	Synthetic glycerol impurities		[11]
Physicochemical characteristics	pH	10, 12-13	[4, 15]
	Buffer capacity		
	Chromatic characteristics		[14]

The problem of wine identification is relevant for wine-producing countries of the global community. Its solution is constrained by a small list of indicators of regulatory documents and leads to distribution of counterfeit products on the wine market. Currently, there is no unified test or "black box method" to establish definitely the authenticity of wine samples.

The identification is carried out in accordance with numerous indicators [3, 6-9], allowing to identify one specific type of adulteration, while there is no methodological approach based on the system of mutually complementary criteria. The instrumental support of these developments assumes using of complex and expensive set of instruments and equipment, often out of reach of wine producers and regulatory authorities.

In this article we introduce a novel system of complex diagnostics of wines to identify a wide range of prohibited additives and a number of technological methods. The proposed system, in comparison with existing regulatory documents and methods, assumes an integrated approach to the evaluation of wines in order to identify their authenticity. The qualification system of indicators, combined in 7 uniform groups, allows to detect with high accuracy 15 types of wine adulteration. Using of each new criterion increases the reliability of identification. System elements can be used to detect several counterfeit types. System approbation shows that the ratio of counterfeit products in representative sampling averages 30 % with year-to-year variation from 25 to 40 %. The system is open for seeding and spreading. Methods for analyzing classification indicators are straightforward and accessible for industrial laboratories of various levels.

4 Conclusion

A system for the detection of prohibited technological methods and additives during the identification of wines, including classifying indicators and their derivatives, methods of analysis, types of adulteration, has been developed. With high accuracy (6 %) the system allows for the identification of 15 types of counterfeits and the differentiation of authentic and adulterated wine samples. he use of this scientific development ensures the protection of the economic interests of countries, wine producers, and consumer health. Further research will aim to expand the range of detected counterfeits and to replenish the system with new classifying indicators and methods of analysis.

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