Determination and identification of organic acids in wine samples. Problems and challenges.

Alicia Robles[a], Magdalena Fabjanowicz[b]*, Tomasz Chmiel, Justyna Płotka-Wasylka[b]

[a] *Department of Chemistry, Bromatology, Faculty of Exact and Natural Sciences, Mar del Plata National University, 3350 Funes Street, 7600, Mar del Plata, Buenos Aires, Argentina*

[b] *Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, 11/12 Narutowicza Street, 80-233 Gdańsk, Poland*

**corresponding author: magdalenafabjanowicz@gmail.com*

Abstract

For long time, organic acids were underestimated. However, during last two decades there is an increasing interest of natural compounds having antioxidant, antimicrobial and anti-inflammatory properties thus organic acids are very preferable. Wine stands as one of the sources of organic acids since they are responsible for its organoleptic and aestethic character. Nevertheless, it is important to not exceed acceptable level of acidity at particular stage of vinification process. Therefore its determination and quantification is of high importance. Given study gathers data regarding current knowledge with respect to organic acids, focusing on their occurrence in different types of food including wines, their properties and effects on the human body, potential correlations between organic acids and other components of wine. Moreover, the comparison of analytical techniques used for the organic acids determination and challenges, considering their process and green assessment is provided.

Key Words organic acids, wine, chromatography, Eco-Scale, GAPI index

1. Introduction

Proper nutrition plays an important role in the development of many diseases, especially those related to cardiovascular disorders and cancer, which are associated with oxidative stress. Broadening the knowledge regarding food having health beneficial properties stands for the crucial issue of scientific contribution in recent years. The most widely studied are antioxidants - the group of compounds, which occurs naturally in plant materials, animal tissues and microorganisms. Fruits, vegetables, cereals, grains, oilseeds, and teas are important sources of plant-derived antioxidants in a human diet. A variety of antioxidant constituents present in such resources have been characterized and quantified, including vitamins E and C, polyphenols, carotenoids, antioxidant peptides and enzymes [1], as well as some organic acids. Organic acids were always considered to have weak antioxidant power and were usually discarded in the extraction process. Therefore, they have been neglected for a long time and their pharmacological actions have not been sufficiently studied [2].

Regarding the bioactive capacity of organic acids, little is known about their beneficial effects on human health. Moreover, there is lack of knowledge regarding the beneficial effects of the consumption of foods rich in these compounds, with the exception of ascorbic acid, which has a high antioxidant power [3]. However, there are more and more studies examining the organic acids characteristics, searching for positive effects of given compounds on the human body. It is commonly known, that benzoic and salicylic acids exhibit antibacterial activity, hydroxycinnamic acids and their derivatives anti-inflammatory, gallic acid is an antimutagenic, anticarcinogenic and anti-inflammatory agent [4]. What is more succinic acid, acetic acid, citric acid, lactic acid, malic acid, glutamic acid and their salts promote the absorption of iron [5]. In addition, Nagai *et al.* (2010) demonstrated that, oral administration of citric acid improvements ketosis and protects against the development of diabetic in an animal model of type 1 diabetes [6]. Likewise, Marunaka (2018) revealed that intake of weak organic acids is found to ameliorates the insulin resistance by elevating the lowered interstitial fluid pH in diabetes mellitus in humans [7]. Moreover, citric and malic acids have significant protective effects on the myocardium and act on ischemic lesions, according to a study by Tang *et al*. (2013) [2], where the addition of these compounds in the diet of patients was the great importance.

Although organic acids have been used to offset with pathogens in food for many years, there is an evident need to assess and improve their continued effectiveness and sustainability. Organic acids play a principal role in maintaining the quality and nutritional value of food. These compounds can be added as acidulants or stabilizers (e.g., citric, ascorbic, benzoic, fumaric and malic acids). In the case of insufficient sterilization and/or microbial contamination during storage, sugar fermentation results in the formation of volatile acids (C_2–C_{12}) and impairs the quality of some products [8]. Additionally, as natural components, organic acids contribute to the organoleptic (flavor, color and aroma) and healthy properties (antioxidant and antimicrobial activity) of food. They are present in a natural way in all types of food, like different fruits and vegetable, mushrooms and beverages like juice, coffee, tea and wine [9, 12-20].

The quantitative determination of organic acids in these types of samples is of high interest of many industrial and research institutes. For example, it can be used in the quality control of wine as an indicator of deterioration due to storage or aging (because the classes and content of organic acids give a characteristic taste to wine) or even to determine authenticity and to ensure that food can be safely consumed [8].

Organic acids are important for the wine stability. Therefore, their analysis in wines is required for quality control as well as to check the evolution of acidity during the different stages of winemaking (starting from the grapes juices, continuing to the alcoholic fermentation and wine stabilization processes), since important changes in wine would be detected by alterations in the acid content.

Acidity is one of the most important elements regarding the wine making process. There are different acids, in free or compound state, some derivatives of the natural grape or organic (malic, tartaric and citric acids) and others (succinic, acetic and lactic acids) that arise from the different processes of fermentation. Fermentations of a wine contribute to the transformation, disappearance or appearance of the different acids.

Concerns and potential risks regarding the use of synthetic chemical antimicrobials and antioxidants have renewed the interests of consumers using natural and safe alternatives [16]. Therefore, it is important to monitor the content of organic acids in wine samples, not only from the food (mainly wine) quality control point of view, but also due to their beneficial properties to human health. The milestone in the field of development of knowledge of organic acids characteristics is presented on the Figure 1.

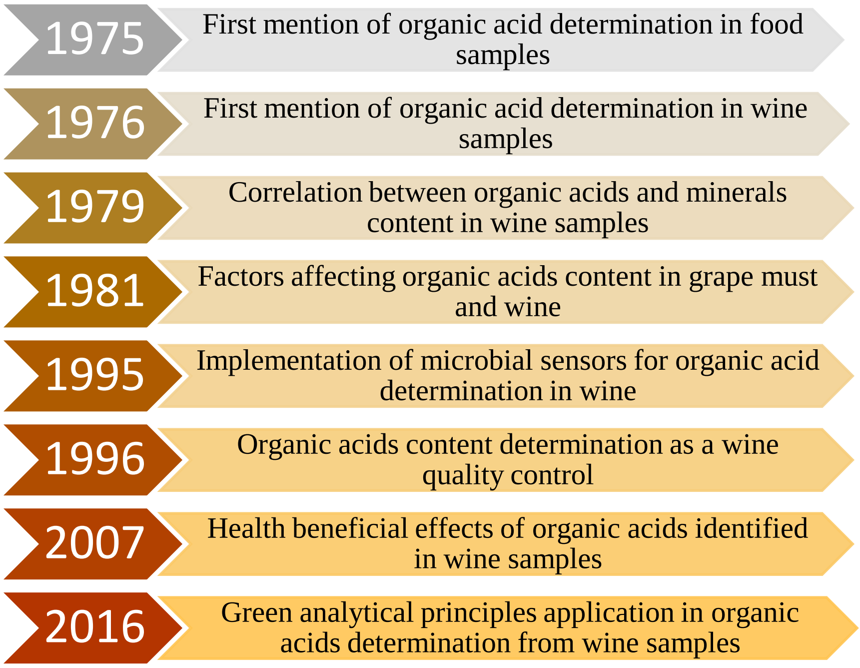

Figure 1 Milestone in the field of development of knowledge regarding organic acids [21-28].

The scope of a given review is to present the current knowledge regarding organic acids (sources, properties, the absorption and bioavailability) and to summarize the concentration of given compounds in different types of wines. In addition, the concentration of organic acids from wine is compared to the concentration of following acids in different types of food.

Due to the trace concentrations and physicochemical characteristics of organic acids, an overview of the analytical methodologies, cleanup, pre-concentration technique, a comparison between the type of derivatization agents and environmental assessment with the use of GAPI tool and Eco-Scale has been made. The review is based on the literature data from last two decades and it stands for the critical assessment of what has been done by the researchers during given period of time. What is more, the study is focused on the main issues of organic acid determination in different types of samples characterized by complex matrices composition that should be broaden and further investigated. In order to find data necessary for the review the Web of Science, Mendeley and Scopus were used with the application of such keywords as: organic acid determination, green analytical chemistry (GAC) and terms related with all the aspects covered under the GAC.

2. Organic acids in wine

Organic acids play various functions in food. They act as a *buffer* in a process where pH changes with temperature. Additionally, they fulfill the function of *antioxidants*, where they prevent against the oxidation of lipids, including some pigments and naturally flavored oils. Moreover, certain organic acids work as *synergists*, improving the ability of antioxidants to control free radicals, at the same time also helping to control free radicals in the human body system when these are consumed as a component of the nutrition diet. They also have antibiotic qualities, therefore, work as *preservatives*. In addition, organic acids form water-soluble chelates with metal ions, thus, they work as *sequestrants, or chelating agents* [29]. In accordance to wine, they are strongly connected to the aroma and taste of wine. In order to obtain wine, which will satisfy the customer preferences in terms of organoleptic and aesthetic character the balance between sugar and acid content needs to be maintained. The main organic acids found in wine are: oxalic, tartaric, formic, malic, acetic, citric. fumaric, succinic, gallic and lactic acids. They come from two sources:

- directly from grapes (over 90% of organic acids found in grapes are dedicated to malic and tartaric acids);
- as a result of vinification process (due to combined metabolism of yeast and microorganisms).

Table 1 present a specific characteristic of a selected organic acids, which are found in wine samples.

Table 1 Characteristics of a selected organic acids based on data available in the PubChem database [30].

Organic acids chemical formula	**Molecular weight [g mol^{-1}]**	**Density [g cm^{-3}]**	**Melting point [°C]**	**Dissociation Constant [pKa]**
Oxalic acid $C_2H_2O_4$ or $(COOH)_2$	90.034	1.90	189.5	4.40
Tartaric acid $C_4H_6O_6$	150.086	1.699	168	3.07
Formic acid CH_2O_2 or HCOOH	46.025	1.220	8.3	3.75
Malic acid	134.087	1.601	127-132	3.51; 5.03

$C_4H_6O_5$				
Acetic acid $C_2H_4O_2$ or CH_3COOH	60.052	1.0446	16.6	4.76
Citric acid $C_6H_8O_7$	192.123	1.54	153	2.79
Succinic acid $C_4H_6O_4$	118.088	1.572	188	4.21
Fumaric acid $C_4H_4O_4$	116.072	1.635	287	4.54
Gallic acid $C_7H_6O_5$	170.12	1.69	258-265	4.40
Lactic acid $C_3H_6O_3$ or $CH_3CHOHCOOH$	90.078	1.2	16.8	3.86

All of the organic acids gathered in the Table 1 are of low molecular weight, varying between 46.025 of formic acid and 192.123 g mol^{-1} of citric acid. They creates a group of strong acids with a pKa values oscillating between 2.79 for citric acid and 5.03 for malic acid.

They can be further subdivided into two groups:

- *carboxylic acids*: lactic, citric, formic, acetic, fumaric, succinic, tartaric, oxalic, malic acids;
- *phenolic acids*: gallic acid [30].

Knowing the diverse food sources that provide these compounds to the diet becomes a very important factor.

2.1. Sources and metabolism of organic acids

Regardless of the literature describing the metabolic effects of phenolic compounds and their absorption in the human body, scarce data are available on organic acids and their absorption from diet.

Marukana (2018) described a possible mechanism of absorption of organic acids in humans. Different values of pKa of organic acids, which are less than the physiological pH value, 7.4, of blood and the interstitial fluid (acetic acid (pKa = 4.76), propionic acid (pKa = 4.88), citric acid (pKa = 3.13, 4.76 and 6.40)), were considered.

In the intestine, only the ionized forms of these organic acids can be absorbed [7] under physiological acidic conditions. However, only a percentage of organic acids intake would be absorbed in the human body.

At intracellular level, organic acids participate in the Tricarboxylic Acid Cycle. It involves a series of redox reactions that provide energy to the cell. Steps in the cycle involve ionized forms of organic acids (Acetyl CoA - Citrate – Isocitrate – α-ketoglutarate – Succinyl CoA – Succinate – Fumarate – Malate – Oxaloacetate). Therefore, different types of food, being a source of some of these organic acids would collaborate with this cycle.

Organic acids are abundant in the diet. Moreover, there is an evidence of their antimicrobials and antioxidants function [29]. Knowledge regarding the bioavailability of organic acids in the diet would be helpful in identification of those that can be considered healthy. Fruits like peach, berries, orange, and grapes contain, mainly, citric, malic, fumaric, tartaric and ascorbic acids.

Wines might be also considered as an important source of organic acids. Although, they contribute mainly to the flavor, color and aroma; their presence could also suggest healthy properties [27, 31]. The summary of selected organic acids concentration in different types of foods and wines is presented in Table 2 and Table 3.

Table 2. Concentrations of Organic acids in different natural foods.

Food	FA	CA	MA	TA	Reference
Peach Fruit	10.9 mg/100g	5206.9 mg/100g	8704 mg/100g	--	[9]
Apple Fruit	--	2.7 – 9.6 mg/100g	130 – 201 mg/100g	--	[10]
Orange Fruit		692 – 1515 mg/100g	18 – 39 mg/100g	44 – 72 mg/100g	
Lemon Juice		5192 – 7696 mg/100ml	355 – 1105 mg/100ml	3.9 – 9.4 mg/100ml	
Sweet cherry (*Prunus avium L.*)	0.57 – 7.56 mg/kg	0.11 – 0.54 mg/kg	3.53 – 8.12 mg/kg	--	[11]
Table grapes	--	Tr – 1.03 g/L	0.38 – 29.9 g/L	1.28 -7.45 g/L	[12]
Quince (*Cydonia Oblonga Miller*) seeds	1.8 – 6.2 mg/kg	93.2 – 213 mg/kg	281 – 383 mg/kg	--	[32]
Turkish white grapes and grape juices	--	0.065 g/L	2.43 g/L	5.98 g/L	[33]
25 Wild or Cultivated Berry Species	7.2 – 130.9 mg/kg	0.9 – 20.0 g/kg	0.6 – 30.3 g/kg	0.02 – 3.38 g/kg	[34]
European elderberry (*Sambucus nigra L.*)	0.17 g/kg	3.50 g/kg	1.10 g/kg	--	[35]

FA: Fumaric Acid, CA: Citric Acid, MA: Malic Acid, TA: Tartaric Acid, Tr: Traces

Table 3. Organic acids content in different types of wine.

Wine		TA	MA	SA	AA	LA	Reference
Brazilian Red Wine		0.6 – 5.6 g/L	0.06 – 1.56 g/L	--	0.15 – 0.28 g/L	1.2 – 3.4 g/L	[17]
Pinot Noir Red Wine (six different countries)		1092 – 1937 mg/L	n.d. – 332 mg/L	341 – 830 mg/L	479 – 652 mg/L	1131 – 3418 mg/L	[18]
Spanish Red Wine		1483 mg/L	547 mg/L	556 mg/L	359 mg/L	2108 mg/L	[36]
Spanish Wine	**White**	3092.5 mg/L	1968 mg/L	409 mg/L	209.5 mg/L	625 mg/L	[37]
	Rose	3159 mg/L	171 mg/L	212 mg/L	215 mg/L	762.5 mg/L	
	Red	2705 mg/L	389.5 mg/L	226.5 mg/L	478.5 mg/L	836.5 mg/L	
Spanish Wine	**White Red**	-- --	1300 mg/kg 14 mg/kg	365 mg/kg 62.5 mg/kg	137.5 mg/kg 175 mg/kg	540 mg/kg 1300 mg/kg	[8]
Brazilian Wine	**White Red**	1048 – 1362 mg/L 1023 – 2212 mg/L	1117 – 2627 mg/L 2153 – 2243 mg/L	83 – 631 mg/L 66 – 700 mg/L	224 – 924 mg/L 118 –1003 mg/L	61 – 3933 mg/L 35 – 7306 mg/L	[38]
German Wine	**White Red**	7.03 mM 12.6 mM	5.86 mM 4.34 mM	4.63 mM 6.05 mM	6.12 mM 6.95 mM	5.22 mM 1.90 mM	[20]
Czech Republic wine	**White Red**	4.4 – 17.4 mM 10.2 – 12.4 mM	12.4 – 46.4 mM n.d. – 0.9 mM	1.1 – 7.6 mM 4.6 – 6.7 mM	1.7 – 27.9 mM 7.4 – 11.6 mM	1.4 – 27.4 mM 23.2 – 42.3 mM	[39]

TA: Tartaric Acid, MA: Malic Acid, SA: Succinic Acid, AA: Acetic Acid, LA: Lactic Acid. N.d.: no detectable

The fact that organic acids are natural, and have antioxidant activity act on favor and place them beforehand the synthetic antioxidants. This characteristic makes them attractive for commercial food processors, and fulfills continuous consumer demand for natural ingredients. In addition, the antioxidant property is also important to know how these compounds work in the human body.

2.2. Main effects in the human body

Primary and secondary plant metabolites have critical roles in the health of humans and could be nutritionally important.

Fruits and vegetables stands for different sources of organic acids from which ascorbic acid is the most known. Among its metabolic functions, the most important one is its ability to protect the immune system and to reduce the harshness of allergic reactions. In addition, ascorbic acid helps to repel infections [3]. On the other hand, if the intake of ascorbic acid is not sufficient, a lot of illness can be observed, like anemia, scurvy, infections, and muscle degeneration, etc. [3]. Orange and lemon juices are significant sources of ascorbic acid and it can be also found in wine made from oranges [14].

Strong antioxidant activities also has been suggested to gallic acid. It provides efficient oxidative damage protection caused by reactive species in biological systems. Likewise, this triphenolic compound has demonstrated to be the antioxidant responsible for the efficient antiradical and anticancer properties in plant extracts [40].

It is commonly known that iron is an essential mineral for human growing. In the diet, not always enough iron is absorbed although the availability of it is wide. Bortz et al. (2006) [5] reported organic acid of short chain (succinic acid, acetic acid, citric acid, lactic acid, malic acid, glutamic acid and their salts) are useful as second supporters of iron absorption.

Knowing wines as a source of organic acids, Daglia *et al.* (2007) [27] evaluated in marketable red and white wines, the oral *streptococci* and *S. pyogenes* antibacterial action. These bacteria are responsible for caries development and for pharyngitis, respectively. Authors assigned to succinic, malic, lactic, tartaric, citric, and acetic acid as compounds responsible for antibacterial activities [27]. In addition, Boban *et al.* (2010) [31] examined the antimicrobial action of different wine components compared to intact wine. Listed authors applied separately intact wine, wine stripped of phenols, dealcoholized wine, ethanol and low pH and also examined them in combination. Based on their results, they concluded that the non-phenolic constituents of wine were responsible for antimicrobial activity according to the reported by Daglia et al. (2007)[27] and other authors [41]. Moreover, Boban *et al.* (2010) also confirmed the non-phenolic constituents of wine, ethanol and low pH present a synergistic antimicrobial activity.

The different benefits of organic acids in the human body have been described. Anyway, evaluating effectiveness of organic acids for specific applications requires more understanding general and specific knowledge about processes, such as winemaking or food preservation.

3. Effects of winemaking technologies on organic acids content

During the ripening of the grapes, sugar and flavonoid are accumulated, the content of organic acids are modified, and the concentration of volatile substances can fluctuate [42]. The composition of a wine, from a chemistry point of view, is a crucial aspect for the wine industry. Individual acids and oenological variables are as important as the sugar-acid balance in the wine; therefore, to evaluate in details all compounds in wine is necessary.

Total acidity is the most important parameter in wine; however, the organic acids represent significant constituents and should be regularly determined in wines. They act as main determinants of quality of grape varieties. Grapes contain mainly malic, citric and tartaric acids. Whereas succinic and acetic appear as a consequence from the fermentation process. These compounds influence the quality and character of the wine. They are key components since they reflect the process of wine production, where the grape variety, the yeast strain, the containers used for fermentation and storage are included [43]. The organoleptic quality of wines and their aesthetic character are perceived due to the acidity in the wine, which affects different levels of the winemaking process [44].

In the literature, the main information found was about the phenolic content and its changes in the vinification processes [45, 46]. However, little was found about organic acids content and their changes by effects of the winemaking process.

Generally, the total acidity of the wine increases from 1 to 2 g dm^{-3}, during alcoholic fermentation as a result of the production of malic, acetic, succinic and lactic acids. However, significant variations can be observed due to the contribution of specific yeast strains [44].

In a method, carried out in Sauvignon Blanc wines, which included four different wine-making procedures (traditional white vinification, skin cryomaceration, vinification in a reductive environment, and a combination of the last two procedures) [47], was reported a slightly variation of malic acid content resulted from the vinification procedure. Whereas that tartaric acid concentration was strongly reduced during cryomaceration process performance. Citric acid concentration was 0.62 g dm^{-3} in wines that included a cryomaceration production, with respect to 0.42 g dm^{-3} obtained by means of traditional or reductive vinification. It can suggest that microorganism activity was inhibited due to low temperature on the process. In addition, the highest amounts of lactic acid and the lowest contents of acetic acid were reported in wines exposed to a reductive vinification including a 24-h skin cryomaceration, whereas those obtained only by cryomaceration process showed the opposite relationship [47].

Mena *et al*. (2012) reported changes in the organic acids profile in pomegranate juices and wine after winemaking process. Citric acid concentration did not indicate changes in juices and wines varietals, remaining almost constant in the different stages of wine production. However, malic acid showed important variations during the vinification process and a considerable increase was noted for acetic acid, but no deviations took place for tartaric acid [48].

Likewise, the addition of the mannoproteins like an oenological agent showed to affect the concentrations of organic acids in rosé wines [49]. Changes in the malic and succinic acids concentrations were observed, which increased considerably with the treatment applied and throughout the biological aging. However, the tartaric acid levels decreased in the wines put to the control. In conclusion, Sartor *et al*. (2018) has demonstrated the influence of mannoproteins in the concentration of organic acids and in the sensory quality of rosé sparkling wines [49].

On the other hand, different strains influence in the organic acid composition of wines and, Chidi *et al*. (2015) [50] found that changes to the predominant fermentation conditions, including changes to the pH, fermentation temperature, initial sugar concentration and aeration are responsible of the organic acids variations.

In addition, it is important to note that organic acids can be used as food by many yeasts. Therefore, knowing and understanding the transport of organic acids used by yeasts would be important as well as understanding the mechanism by which they could control intracellular pH and contribute to intracellular charge balance [51].

Finally, it should not be forgotten that climate change is influencing more and more deeply the composition of the grape, and consequently affects winemaking, microbiology and wine chemistry. Advanced harvest times and temperatures, increased grape sugar concentrations that lead to high levels of alcohol in wine, less acidity and modification of varietal flavor compounds are among the most important effects related to climate change. Higher production of acetic acid, as fermentation co-products is a response to stress in yeast due to an increase in the concentration of sugars. The increased risk of deterioration and organoleptic degradation are the main consequences of the effects of climate change on the production of grapes and wines [52].

4. Correlations between organic acids and other components of wine

The composition of wine is characterized, mainly, by content of complex mixture of compounds at varietal concentrations. The so-called primary metabolites include sugars, organic acids and amino acids, while the secondary metabolites are represented by flavonoids, hydroxycinnamates, hydroxybenzoates and anthocyanins [53]. The structure of a given compounds are varying during the winemaking process, especially of organic acids and polyphenols what is shown on Figure 2.

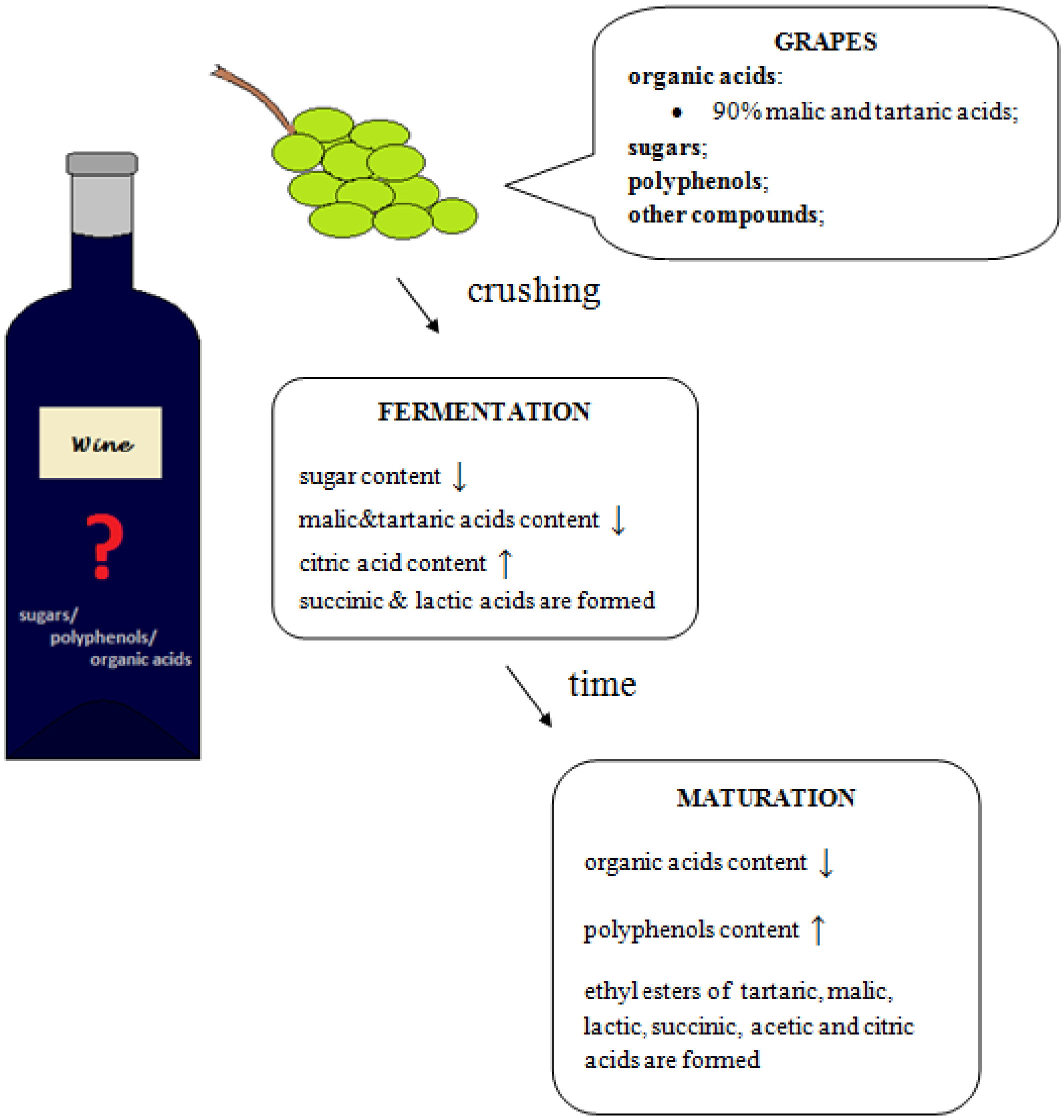

Figure 2 Schematic representation in the structural changes within the main wine component.

Organic acids content in must and wine influence in the balance of the flavor as well as in the chemical stability, pH, and thus in the quality of wine [54]. Their correlation with other compounds present in wines have allowed not only to make a classification of different varietals, but also to know the best ratio for a good quality of them. In addition, adulterations detection can result in their analysis.

Each compound has its function in the flavor of the wine [53]. Amino acids influence the aromas during the maturation process; sugars are consumed by yeasts during fermentation, except in specific sweet wines; organic acids are of great importance because they influence organoleptic properties, collaborate in the control of microbiological growth, and are a critical parameter in the stabilization of wine. Finally, phenolic compounds are associated with appearance, taste, mouth-feel, and antimicrobial activity [53].

Fermentation coproducts, such as acetic acid and biogenic amines, are considered undesirable substances. They are produced during malolactic fermentation where, in addition to the transformation of malic acid into lactic acid, many other substances can be metabolized, such as sugars, citric acid and amino acids [55]. Therefore, the formation of undesirable amines is associated to the decrease of malic acid concentrations in the fermentation process. The factors that influence the type of co-products that can be found in wine include differences in the winemaking process (such as the genera and strains of lactic acid bacteria formed), the time and conditions of storage and the quality of the raw material [56].

Astringency is an important characteristic of red wine quality [57]. The perception of astringency is thought to be due to the interaction of the tannins present in the wine with salivary proteins. The concentration of organic acids, sugar, available acetaldehyde concentration, viscosity and the presence of other compounds that interact with tannins can also affect it. Other compounds may be residual yeast proteins or polysaccharides present in the grape [57].

In addition, Gawel *et al*. (2007) reported correlative approaches between the acidity and phenolic and pigment compositions with the in-mouth textural profiles. These would help to clarify possible relationships between red wine components and the mouthfeel of full-bodied red wines. Greater organic acid concentrations combined with larger acidity contribute to the chalky characteristics of red wine [58].

Enzymatic or chemical esterification of organic acids and alcohols leads to the formation of esters, and in wines, the most common are ethyl esters. Ethyl esters of the main organic acids in wine (tartaric, malic, lactic, succinic, acetic, and citric acids) are formed in all wines during aging and contribute to the improvement of wine aroma [59]. This means organic acids concentration decreases with the progressive aging of wines.

5. Analytical challenges of organic acids determination in wine samples

Wines present a diverse group of low and high-molecular-weight compounds including amino acids, organic acids, polyphenols and carbohydrates, which makes the analysis a highly challenging task (Figure 3). Additionally, each wine has its own characteristic, understood by its own, specific compounds compilation. This is due to the surroundings in which grapes are grown. It was investigated, that environmental conditions, plays a crucial role in terms of particular compounds concentrations in fruits. Grapes coming from the cool climate, where the vegetation temperature is less or equal to 15°C, are richer in acids and poorer in the sugar content than grapes coming from favorable climatic conditions [60]. Given variations of compounds concentration among different types of wines may influence the possible interaction between volatile and nonvolatile compounds according to different mechanisms. Thus, the comparison of the organic acids content between varied wine sample should not be based only on statistical data, but should cover the sample preparation and analysis method as well.

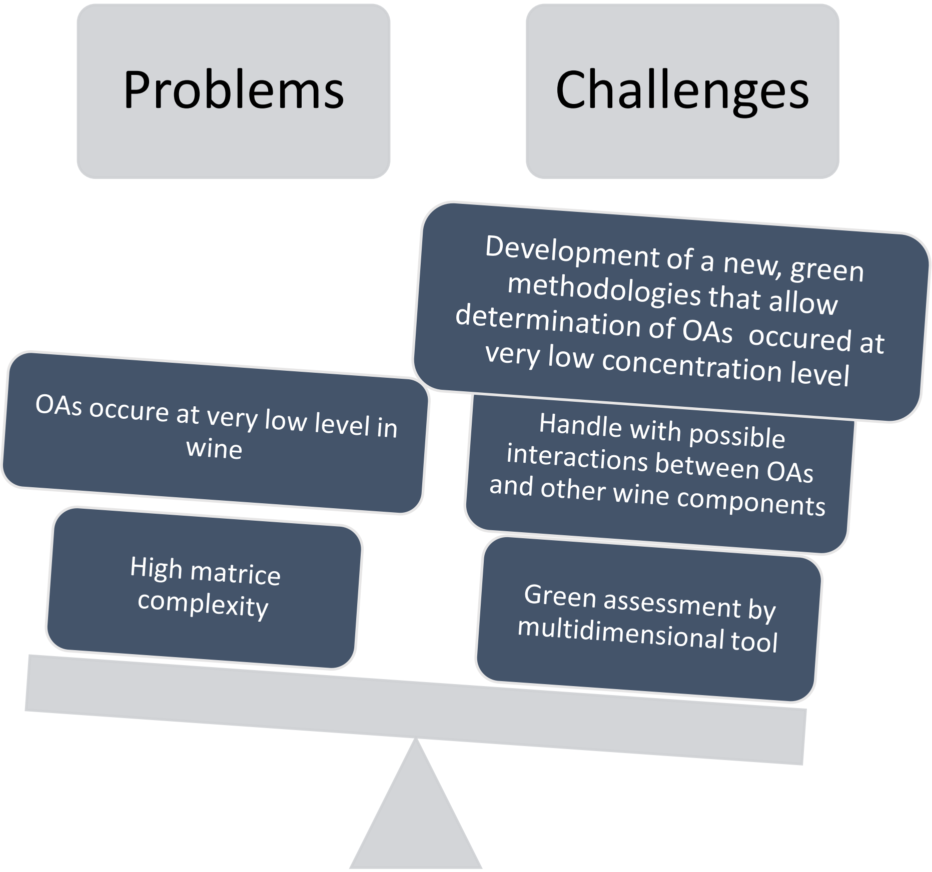

Figure 3 Schematic representation of main analytical problems and challenges in terms of wine analysis with respect to organic acids.

The recent rapid development of a range of analytical techniques, including Capillary Electrophoresis (CE), High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), coupled to Mass Spectrometry (MS) and Infrared (IR) spectroscopy, could allow separation, detection, characterization and quantification of such metabolites [61]. Several methods have been developed for identifying and quantifying organic acids in wines. Figure 4 shows the most widely applied technique used for organic acids determination. The most favorable technique applied by many researchers was HPLC, presented in around 50% of published cases.

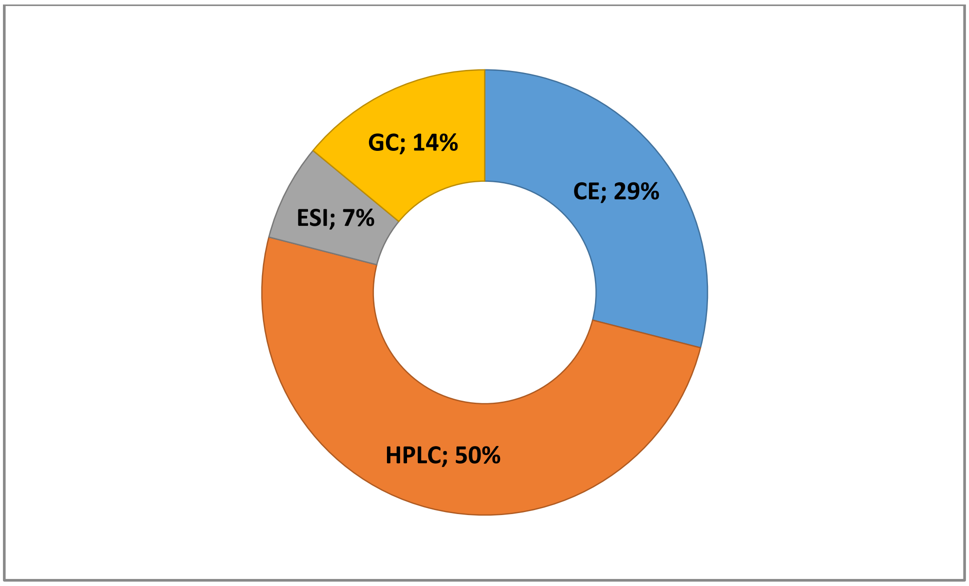

Figure 4 Percentage share of main methods used for determination and quantification of organic acids from wine matrixes [Data base: Web of Science, Mendeley, Scopus].

Additionally, The International Organization of Vine and Wine (OIV, Paris, France) controlled the recommended methods for organic acid analysis. The listed standard methods are reviewed and adjusted annually [39].

5.1. Sample preparation technique

Sample preparation is a critical part of every analytical procedures. The increasing demand to determine compounds at low concentrations in complex matrices requires a preliminary step. Isolate or enrich the analytes of interest before the use a detection and quantification technique, helps to obtain good sensitivity and low detection limits [62].

Among the common techniques applied for OAs determination in wines, no sample pre-treatment, samples with only dilution and filtration as well as complex treatment procedures were applied and reported in the last twenty years.

The only technique where there was no need for sample pre-treatment was direct infusion electrospray ionization mass spectrometry [63]. Dilution and filtration process with different types of filters (HA, PTFE, Nylon and PP) have been reported before the CE[18], [36]–[38], FT-IR[64] and HPLC analysis [14, 17, 65-66] inclusively.

Among the more complex treatment procedures, Castellari *et al.* (2000) [67] tested and compared direct injection and sample clean-up with a SAX cartridge previous HPLC. SAX performance reported good results; however, the direct injection of diluted wine presented the best quality parameters (precision and accuracy) of analysis. Other authors have combined clean-up with polyvinylpyrrolidone, followed by SAX cartridge filtration before HPLC analysis [68] or filtration with PTFE and column for phenolic compounds removal, followed by vortex and centrifugation [54], also in advance HPLC determination. Cunha *et al.* (2002) [69] used cation exchange columns (Dowex 50W-X8) preceding derivatization process and HPLC analysis. In two cases, authors used samples freeze-dried before extraction methods; Dopico-García *et al.* (2007) [70] before SPE-HPLC-UV and Zhang *et al.* (2018) [71] in advance the derivatization process and GC-MS. Finally, some authors have used on-line pre-treatment samples procedures, like Kritsunankul *et al.* (2009) [19] with a flow injection on-line dialysis coupled to HPLC and Jurado Sanchez *et al.* (2011) [8] with a continuous module for the preconcentration/clean-up before GC-MS analysis.

5.2. Derivatization process

When it comes to deal with highly complex matrices, it is encourage to use the derivatization process in order to improve the parameters of separation such as, volatility, thermal stability, resolution as well as detection parameters when gas chromatography is used; sensitivity when high performance liquid chromatography is applied and charge loading to the specific component when electrophoresis is used [72].

However, with the growth of greener ideas, less and less derivatization processes are used. There is a general tendency to find an appropriate techniques to determine the analytes of interest without resorting to these processes that usually use large organic molecules as derivatizing agents. In the consulted literature, only Cunha *et al.* (2002) [69] used O-(4-nitrobenzyl)-N,N’-diisopropylisourea (NBDI) as derivatizing agent before HPLC development and, Zhang *et al.* (2018) [71] used MSTFA [N-methyl-N-(trimethylsilyl) trifluoroacetamide] before GC-MS analysis.

5.3. Analytical instrumentations

The content of organic acids in wines must be controlled for the check of the maturation of the grapes and the development of the acidity, mainly in the processes of fermentation and aging. The development of analytical techniques that allow the monitoring of organic acids in different stages of the winemaking process represents an analytical challenge. Up till now, several options have been reported from enzymatic biosensors to sophisticated chromatographic techniques. Information on analytical methodologies developed for organic acids determination in wine samples are presented in Table 3.

5.3.1. Enzymatic biosensors

Organic acids detection by enzymatic methods are mostly based on the reaction of an enzyme, formation of complex, that is translated into an electrical signal [39]. In other words it is a combination of immobilized form of biological component with a transducer, which monitors the complexes formation [73]. The use of biosensor can help in food quality control process. They can find application in the food composition determination in terms of:

- sugars and organic acids determination as well as to follow their maturation process;
- polyphenols and fatty acids identification together with their rancidification process control;
- alcohol, amino acids determination.

Moreover, they help in the biogenic amines content assessment which can be used as a freshness marker of different type of food [74].

In addition, various biosensors for the determination of individual organic acids were developed and tested for the wine analysis. Zeravik *et al*. (2016) [39] presented a very good review of biosensor developments for these analytes. For specific organic acids, diverse publications have been reported. Molinero-Abad *et al.* (2014) have developed a specific malic acid sensor applied to wines, using a screen-printed carbon electrodes modified with gold nanoparticles, tetrathiafulvalene and malate quinone oxidoreductase enzyme [75]. In wines and ciders, Loaiza *et al.* (2015) [76] have developed a lactate biosensor using platinum nanoparticles supported on graphitized carbon nanofibers surface. Although, lots of researchers is publishing their work with the use of biosensors showing their advantages like good results of reproducibility: RSD on the level of 4,9%, n=10, good sensitivity, and relevant limit of detection achieving 6.9 µM, only few systems are commercially available [61, 74]. This is the result of the restrictions related with the use of biosensors like: limited lifespan of biological components they are made of, difficulties of handling and mass production.

Since enzymatic biosensors may be excellent alternative for the traditional analytical techniques as chromatography, it is worth to invest time for their development to develop inexpensive, reliable system able to work under realistic conditions. Only when following criteria will be satisfied given technique could play a prominent role in the food quality control [74].

5.3.2. Capillary electrophoresis

Capillary electrophoresis (CE) is commonly applied technique for the organic acids determination in wine samples [36, 38, 61]. Among the main characteristic of CE, good resolution, automation, simplicity, high speed (more rapid separation than LC), low consumption of chemicals and reduced sample preparation have been highlighted. Thanks to easy sample preparation and possibility of thermal instable compounds separation it stands beforehand gas chromatography. CE requires different sample preparation (direct injection or SPE) and can operate in varietal modes, like capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), micellar electrokinetic chromatography (MECK), electrokinetic chromatography (EKC) as well as non-aqueous capillary electrophoresis (NACE) what enables separation of a wide range of analytes from small inorganic ions to large proteins. To improve the separation efficiency the capillary length, buffer ionic strength, pH and viscosity should be adjusted. Castineira *et al*. (2002) [36] indicated that only dilution 1:40 and filtration was necessary prior to the final analysis. They used alternatively the phosphate-carrier electrolyte with direct UV determination for five organic acids determination: tartaric, malic, succinic, acetic and lactic acids. The results were very satisfied, achieving high resolution and reproducibility in single and fast analysis.

However, there are several reports indicating that CE presents two main disadvantages such as robustness and sensitivity [77]. Since organic acids in wine samples appear at low concentrations, the pre-concentration technique as liquid liquid extraction (LLE) or solid phase extraction (SPE) might be used to solve the problem of sensitivity. Moreover, the on-line pre-concentration can be used which has big advantages as increase of sensitivity without loss in separation efficiency [78]. From the practical point of view, the volume of sample injected, narrow capillary diameter in ultraviolet detector and interfaces with MS detector are typical issues that affect the analyte bands [77]. Since, due to the contamination of the ion source it cannot be combined directly with the MS.

5.3.3. Spectroscopy Techniques

The combination of chemometric software with spectroscopy techniques has provided an analytical tool of high interest, suitable for the control of processes, such as the manufacture of wines [64]. Fourier - transform infrared spectroscopy (FT-IR) with partial least squares (PLS) was used by Regmi *et al.* (2012) to establish a calibration model for tartaric, malic, lactic, succinic, citric and acetic acids in wines, vinegars and spirits. The big advantage of given technique is easy sample pre-treatment, since only filtration was performed prior the analysis in order to remove particles or degassing when it came to cope with the samples containing CO_2. On the other hand, recovery experiments were performed in sparkling wine samples with different amounts of organic acids (tartaric, malic, lactic, acetic and citric) by Moreira and Santos (2005) [79]. However, the authors informed that percentages for IR analysis of the individual organic acid concentrations were 61, 73, 44, and 11%, for tartaric, malic, lactic, and citric acids, respectively. There was the relationship noticed, in which acids appearing in higher concentration ranges like tartaric (1,16-2,07 g dm^{-3}) and malic (1,87 – 1,98 g dm^{-3}) had better recoveries than acids of lower concentration ranges as citric (0,24 – 0,29 g dm^{-3}) or lactic acid (0,55 – 1,28 g dm^{-3}). It can be explained by the spectroscopic interferences existing between organic acids, which results in final, lower accuracy. Challenges related with the FT-IR analyses are based on the organic acids similarities within IR spectra. Since they have in common stretching of:

- acids C=O;
- acid and alcohol C – O;
- aliphatic compounds C – H;
- as well as bending from acid and alcohol O – H;

what strongly disturb the separation and significantly influence the recoveries results of each individual acid [79]. What is more, ethanol and water dominate the absorption in the mid-IR region. Since FT-IR results interpretation are based on the calibration statistics, it is necessary to point out difficulties of wine samples analysis by given methods due to high matrices variability. When it comes to deal with the calibration curves large number of samples needs to be involved in the processing in order to cope with outlier samples and related dissimilarities. Moreover, given technique is not suitable for concentrations lower than 0,2 g dm^{-3} [80].

5.3.4. Liquid Chromatography

The most widely employed technique used for the organic acid determination has been HPLC in its several modes like reversed phase liquid chromatography (RPLC), flow injection analysis high performance liquid chromatography (FID-HPLC), ion exchange high performance liquid chromatography (IE-HPLC). They are usually coupled with one of the several different detection technique like UV diode array detection (DAD), reflection index detection (RIP) or UV photodiode array (PDA). RIP detection is used a universal one. However, huge limitations of given technique is low sensitivity and temperature dependence, what place him behind UV and DAD detection [81].

Kelebek *et al*. (2009) determined citric, malic and ascorbic acids in orange juice and orange wine using HPLC-DAD [14]. Also, Monteiro Coelho *et al.* (2018) showed a simultaneous determination of sugars and organic acids in wines and grape juices with HPLC using RID and DAD [17]. UV detection is the most common detection system coupled to HPLC. Kritsunankul *et al.* (2009) determined six organic acids (tartaric, malic, lactic, acetic, citric and succinic acids) [19] at 210 nm. Likewise, Cunha *et al.* (2002) detected the same groups of OAs at 265 nm [69] and Zotou *et al.* (2004) at 230 nm [68].

As it has been commented previously, many complex matrices require previous treatments to the chromatographic determination of organic acids. To avoid this problem, Ohira *et al.* (2014) reported an electrodialytic transfer of organic acids from wines to ultrapure water, using cellulose membranes modified with N,N-dimethylaminoethyl methacrylate before ion exclusion chromatography and direct UV detection [20].

5.3.5. Gas Chromatography

Finally, gas chromatography mainly with the mass spectrometer as a detection technique (GC – MS) stands for an attractive alternative to determine volatile compounds. Among the main characteristics, simplicity, separation efficiency and excellent sensitivity and selectivity were highlighted. Long chain fatty acids present in wine have been determined by this technique. However, other acids should be derivatized to be converted into stable derivates appropriate for GC determination. To avoid the derivatization process of organic acids, some authors have successfully employed capillary GC columns coated with polar stationary phases such as polyethylene glycol or nitroterephthalic acid modified polyethylene glycol [82, 83]. Moreover, Jurado Sanchez *et al.* (2011) developed a method based on continuous solid-phase extraction and GC–MS for the direct determination of 29 organic acids [8], which avoided the derivatization process.

Recently, very good results were obtained in organic acids determination using GC-MS with derivatization process, having LOD even between 0.14 - 667.9 μg/L and recovery of almost 127% [84-85]. Other reported methods were applied in different matrix samples using N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) or N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) as derivatization agents and performed diverse microextraction techniques [86-87] which consumed less time and used green solvents. Few reports were done in wine samples [71, 88], which were efficient and highly reproducible, with the analysis time sometimes shorter the LC methods achieving better LOD and LOQ results. Therefore, it creates an open door to apply this technique for the following kind of samples.

Table 4. Information of analytical methodologies development for Organic Acid determination in wine.

Separation techniques	Sample preparation	Derivatization: type of derivatizing agent	LOD/LOQ	Recovery	RSD	Detection	Organic acids determined	Time of analysis	Ref
CE	1:40 dilution. Filtration: Millipore 0.45 μm HA	No	LOD: 0.015 – 0.054 mg/L LOQ: 0.050 – 0.178 mg/L	90.0 – 102%	0.40 – 0.96 %	UV	Tartaric acid, Malic acid, Succinic acid, Acetic acid, Lactic acid.	6 min	[36]
CZE	1:200 dilution. Filtration: 0.5 μm PTFE	No	LOD: 0.05 – 0.38 mg/L LOQ: 0.29 – 1.31 mg/L	92.7 – 105.8%	≤ 3.69%	UV	Tartaric acid, Malic acid, Succinic acid, Citric acid, Acetic acid, Lactic acid.	3 min	[37]
CE	1:5 dilution. Filtration: Millipore 0.45 μm	No	LOD: 0.64 – 1.02 mg/L LOQ: 2.12 – 5.15 mg/L	95 – 102 %	≤ 5 %	UV	Tartaric acid, Malic acid, Succinic acid, Citric acid, Acetic acid, Lactic acid.	5.5 min	[38]
CE	1:50 dilution	No	LOD: 1 mg/L LOQ: 2 mg/L	--	5.6 – 10.5 %	PDA-UV/Vis	Tartaric acid, Malic acid, Succinic acid, Citric acid, Acetic acid, Lactic acid.	8 min	[18]
FID-HPLC	On-line dialysis	No	LOD: 135 - 213 mg/L LOQ: -	84 – 104 %	0.1 – 5.4 %	UV	Tartaric acid, Malic acid, Succinic acid, Citric acid, Acetic acid, Lactic acid.	8 min	[19]

HPLC	SAX cartridge	No	LOD: 1 – 12 mg/L LOQ: -	93 – 101 %	0.65 – 2.12 %	UV/RI	Tartaric acid, Malic acid, Fumaric acid, Succinic acid, Citric acid, Acetic acid, Lactic acid.	35 min	[67]
HPLC	Strong cation-exchange resin (Dowex 50W-X8)	Yes: O-(4-nitrobenzyl)-N,N’-diisopropylisourea (NBDI)	LOD: 0.005 – 0.05 g/L LOQ: 0.008 – 0.199 g/L	94.9 – 118.9 %	≤ 2.9 %	UV	Tartaric acid, Malic acid, Succinic acid, Citric acid, Acetic acid, Lactic acid.	30 min	[69]
IE-HPLC	1:10 dilution. Filtration: 0.22 µm	No	LOD: 0.01 – 1.02 mg/L LOQ: -	-	1.09 – 4.74 %	UV/RID	Tartaric acid, Malic acid, Succinic acid, Fumaric acid, Shikimic acid, Citric acid, Acetic acid, Lactic acid.	25 min	[65]
HPLC	1:2 dilution. Filtration: 0.45 µm nylon	No	LOD: 0.003 – 0.098 g/L LOQ: 0.008 – 0.199 g/L	76 – 106 %	0.1 – 1.4 %	RID/DAD	Tartaric acid, Malic acid, Citric acid, Acetic acid, Lactic acid.	20 min	[17]
RP-HPLC	Polyvinyl-Pyrrolidone and SAX cartridge	No	LOD: 0.001 – 0.044 g/L LOQ: 0.008 – 0.199 g/L	78 – 106.8 %	0.2 – 3.3 %	UV	Tartaric acid, Malic acid, Succinic acid, Citric acid, Acetic acid, Lactic acid.	12 min	[68]

HPLC	Filtration: 0.45 µm PP	No	LOD: 0.11 – 0.30 g/L LOQ: -	-	-	ATR-FTIR	Tartaric acid, Malic acid, Citric acid, Acetic acid, Lactic acid.	20 min	[66]
ESI	No	No	LOD: 0.278 – 0.711 µg/mL LOQ: 0.843 – 2.157 µg/mL	70 – 120 %	≤ 5 %	MS	Malic acid, Tartaric acid, Citric acid.	5 min	[63]
GC	SPE: LiChrolut EN–Supelclean ENVI-18 (1:1)	No	LOD: 0.2 – 2.0 µg/kg LOQ: -	93 – 98 %	≤ 6.2%	MS	Tartaric acid, Malic acid, Succinic acid, Citric acid, Acetic acid, Lactic acid.	30 min	[8]
GC	Lyophilisation	Yes: MSTFA [N-methyl-N-(trimethylsilyl) trifluoroacetamide]	LOD: 0.14 – 667.9 µg/L LOQ: 0.53 – 1001.8 µg/L	76.7 – 126.9 %	≤ 5 %	MS	Tartaric acid, Malic acid, Succinic acid, Citric acid, Oxalic acid, Pyruvic acid, Lactic acid.	30 min	[71]

CE: Capillary Electrophoresis; CZE: Capillary Zone Electrophoresis; FID-HPLC: Flow Injection Analysis – High Performance Liquid Chromatography; IE: Ionic Exchange; RP: Reverse Phase; ESI: ElectroSpray Injection; GC: Gas Chromatography; UV/Vis: Ultraviolet – Visible Detection; PDA: PhotoDiode Array; RID: Reflexion Index Detection; DAD: Diode Array Detection; ATR-FTIR: Attenuated Total Reflectance – Fourier Transform Infrared Spectroscopy; MS: Mass Spectrometry.

LOD: Limit of Detection; LOQ: Limit of Quantification

5.4. Green assessment of selected analytical procedures applied for determination of organic acids in wine samples

“Greenness” tends to be one of the most important factor in terms of new analytical methodology development. There is much attention put on the reduction of side effects of analytical practices regarding both operators and environment. Even if the emissions from the analytical laboratories are low they are highly dispersed what significantly impact their monitoring and control. Green Analytical Chemistry (GAC) helps to deal with a given problem. According to GAC, twelve principle has been stated, which are focused on renewable resources usage, sample preparation and miniaturization of extraction techniques, manage analytical laboratories in a sustainable way in terms of energy and costs. Very often it is difficult to meet the compromise between GAC principle application and high values of analytical chemistry characteristic factors like for example accuracy, sensitivity, selectivity or precision. Another obstacle to overcome is to find the appropriate tool to assess green character of analytical methodology. There are several methods available. To evaluate the selected methodology for the organic acids determination in wine sample the following tools were used: Analytical Eco-Scale and GAPI index [89].

Analytical Eco-Scale is widely applied tool to assess green character of laboratory practices in terms of many aspects related to environmental impact. It is based on Penalty Points (PPs), which are assigned to each part and are subtracted from the base of 100. The more PPs are left, greener a given procedure is. However, there is one limitation due to the lack of information regarding hazards related with the solvents, reagents, etc [90].

Basing on the penalty points calculated for each procedure (Table 5) one can notice that the highest score is achieved by procedure 1 (94 PPs) basing on water capillary ion analyzer. This would signalized that following technique is the greenest in terms of being environmental friendly. While just behind it, procedure 3 and procedure 5 are placed. Both gathered 87 PPs. Procedure 3 used ESI-TQD-MS while procedure 5 is based on capillary electrophoresis. The least green having only 77 PPs is dedicated to procedure 2 where HPLC-UV was used.

In order to verify the best from three procedures which gathered the highest amount of penalty points the GAPI tool was applied. GAPI index is a new tool, presented in 2018, which stands for the graphical representation in the form of pictogram. Each part of pictogram represents different step of analytical procedure. There are three – stages color scale used to assess the green character of each part. Green means low environmental impact, yellow - medium and red - high respectively [90].

Figure 5 is gathering pictograms of GAPI index for all discussed procedures used for the organic acids determination. Here, three the best procedures which has been chosen by the Eco-Scale can be compared in terms of the related hazard. It is easily visible that procedure 5 has the lowest and the least hazardous solvent and reagent used, what act on its favor, placing given analytical practice before procedure 1 (best in Eco-Scale assessment). While the worst results from those three considered, procedure 5 obtained.

Table 5. Calculated PPs for some evaluated analytical procedures for Organic Acid determination in wine.

Procedure 1 [37]		**Procedure 2** [69]		**Procedure 3** [63]		**Procedure 4** [8]	
Reagents	PPs	Reagents	PPs	Reagents	PPs	**Reagents**	PPs
NaH_2PO_4	0	benzylmalonic acid (IS)	1	Methanol	3	Methanol	6
Na_2HPO_4	0	O-(4-nitrobenzyl)-N,N'-diisopropylisourea	2	Water	0	HCl	2
Tetradecyltrimethyl ammonium hydroxide	2	Dioxane	6	Ammonium hydroxide	6	2-tert-butyl-4-methylphenol (IS)	4
$CaCl_2$	0	Acetonitrile	6				
Water	0	Water	0				
	Σ 2		Σ 15		Σ 9		Σ 12
Instruments	**PPs**	**Instruments**	**PPs**	**Instruments**	**PPs**	**Instruments**	**PPs**
Transport	1	Transport	1	Transport	1	Transport	1
Water Capillary Ion Analizer	2	Heater	2	ESI-TQD-MS	2	Extraction	1
Occupational hazard	0	HPLC-UV	2	Occupational hazard	0	GC-MS	2
Waste	1	Occupational hazard	0	Waste	1	Occupational hazard	0
		Waste	3			Waste	2
	Σ 4		Σ 8		Σ 4		Σ 6
Total PPs : 6		Total PPs: 23		Total PPs: 13		Total PPs: 18	
Score : 94		Score : 77		Score : 87		Score : 82	

Procedure 5 [18]		**Procedure 6** [19]		**Procedure 7** [65]		**Procedure 8** [71]	
Reagents	PPs	Reagents	PPs	Reagents	PPs	**Reagents**	PPs
2,3-pyrazine dicarboxylic acid	3	Acetonitrile	6	n-Propyl alcohol	6	Pyridine	3
Cetyltrimethylammonium bromide	4	KH$_2$PO4	0	H$_3$PO4	2	Methoxyamine hydrochloride	7
Tricina	1	H$_3$PO4	2	Tetrahydrofuran	5	MSTFA N-methyl-N-(trimethylsilyl)trifluoroacetamide	3
BaCl$_2$	0	Water	0	Water	0	Ribitol (IS)	0
Urea	0					Water	0
NaOH 0,1M	1						
Water	0						
	Σ 9		Σ 8		Σ 13		Σ 13
Instruments	**PPs**	**Instruments**	**PPs**	**Instruments**	**PPs**	**Instruments**	**PPs**
Transport	1	Transport	1	Transport	1	Transport	1
Capillary Zone Electrophoresis	2	Cell - Dialysis	2	HPLC-UV	1	Lyophilization	1
Occupational hazard	0	FID-HPLC	1	Occupational hazard	0	GC-MS	2
Waste	1	Occupational hazard	0	Waste	1	Occupational hazard	0
		Waste	3			Waste	2
	Σ 4		Σ 7		Σ 3		Σ 6
Total PPs : 13		Total PPs: 15		Total PPs: 16		Total PPs: 19	
Score : 87		Score : 85		Score : 84		Score : 81	

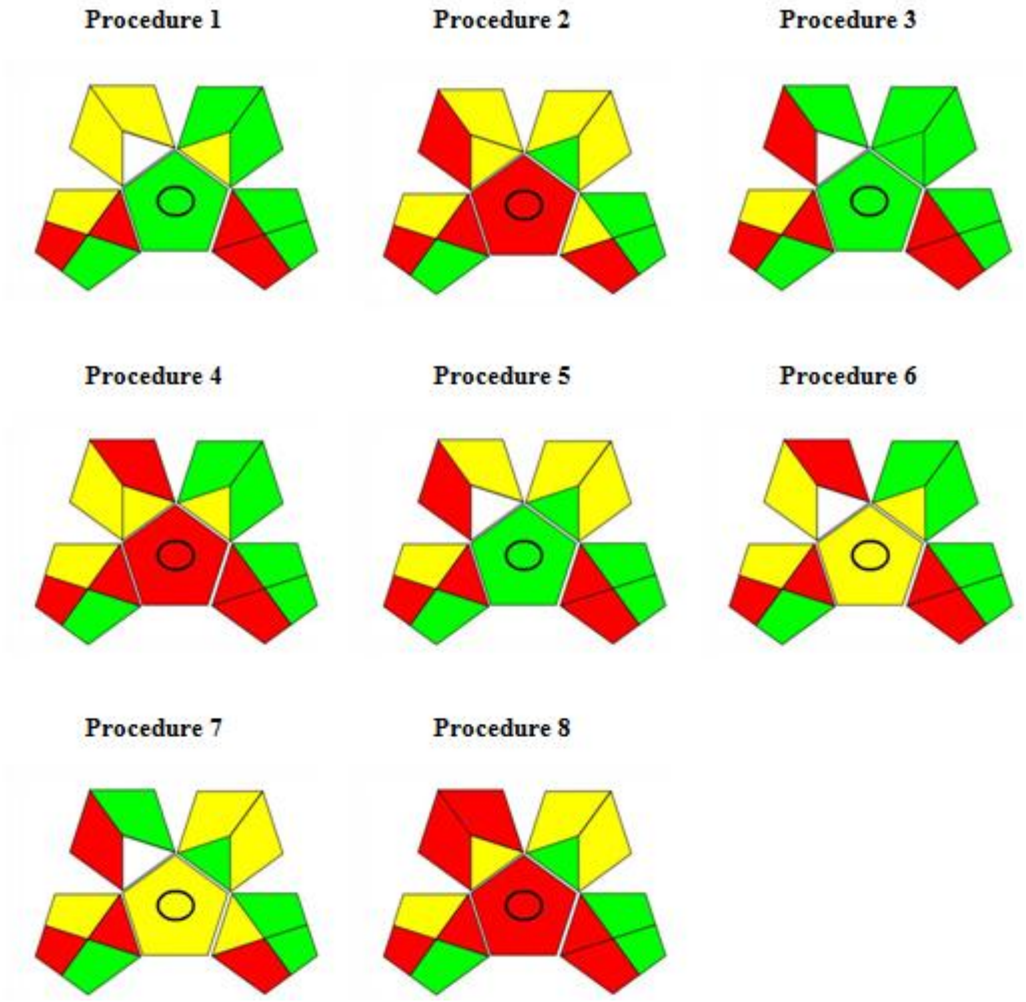

Figure 5 Assessment of the green profile of evaluated procedures applied for organic acids determination in wine samples using GAPI tool.

6. Conclusions

Over last twenty years there is an increasing interest in organic acids as the compounds having beneficial properties on the human health. Due to the strong consumer incentives for the natural ingredients having among the others antioxidant and antimicrobial properties organic acids seem to be crucial. There are several studies performed discussing their sources, properties and benefits in terms of food preservatives as well as beneficial effects on the human body. Wine is considered as one of the sources of the following compounds. Since it is commonly known that wine acidity should be at acceptable level at different stages of vinification process. Moreover, to satisfy the specific characteristic of a given wine expressed by the strictly defined aroma and flavor. Taking into account all of those aspects, the determination and quantification of organic acids is of high importance. Nevertheless, matrix in which they occur is very complex what makes the analysis very difficult. Due to this fact lots of attention needs to be paid for sample preparation, which are compared and discussed together with separation and detection techniques basing on the parameters like: LOD/LOQ, recovery, RSD, number of analytes, sample throughputs and the time of analysis. Moreover, green character of the discussed techniques were assessed with the use of Eco-Scale and GAPI tool. Most of the analytical methods are based on the capillary electrophoresis or high performance liquid chromatography, little is known about the gas chromatography which seems to be relevant in terms of low limit of detection, precision and recovery, which may be a further step in future research.

Conflict of interest

Alicia Robles declares that she has no conflict of interest. Magdalena Fabjanowicz declares that she has no conflict of interest. Tomasz Chmiel declares that he has no conflict of interest. Justyna Płotka-Wasylka declares that she has no conflict of interest.

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