

1 **Determination and identification of organic acids in wine samples.**

2 **Problems and challenges.**

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10 **Abstract**

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

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

24 **1. Introduction**

25 Proper nutrition plays an important role in the development of many diseases,
26 especially those related to cardiovascular disorders and cancer, which are associated
27 with oxidative stress. Broadening the knowledge regarding food having health
28 beneficial properties stands for the crucial issue of scientific contribution in recent
29 years. The most widely studied are antioxidants - the group of compounds, which
30 occurs naturally in plant materials, animal tissues and microorganisms. Fruits,

31 vegetables, cereals, grains, oilseeds, and teas are important sources of plant-derived
32 antioxidants in a human diet. A variety of antioxidant constituents present in such
33 resources have been characterized and quantified, including vitamins E and C,
34 polyphenols, carotenoids, antioxidant peptides and enzymes [1], as well as some
35 organic acids. Organic acids were always considered to have weak antioxidant power
36 and were usually discarded in the extraction process. Therefore, they have been
37 neglected for a long time and their pharmacological actions have not been sufficiently
38 studied [2].

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

57 Although organic acids have been used to offset with pathogens in food for
58 many years, there is an evident need to assess and improve their continued effectiveness
59 and sustainability. Organic acids play a principal role in maintaining the quality and
60 nutritional value of food. These compounds can be added as acidulants or stabilizers
61 (e.g., citric, ascorbic, benzoic, fumaric and malic acids). In the case of insufficient
62 sterilization and/or microbial contamination during storage, sugar fermentation results
63 in the formation of volatile acids (C₂–C₁₂) and impairs the quality of some products [8].
64 Additionally, as natural components, organic acids contribute to the organoleptic



65 (flavor, color and aroma) and healthy properties (antioxidant and antimicrobial activity)
66 of food. They are present in a natural way in all types of food, like different fruits and
67 vegetable, mushrooms and beverages like juice, coffee, tea and wine [9, 12-20].

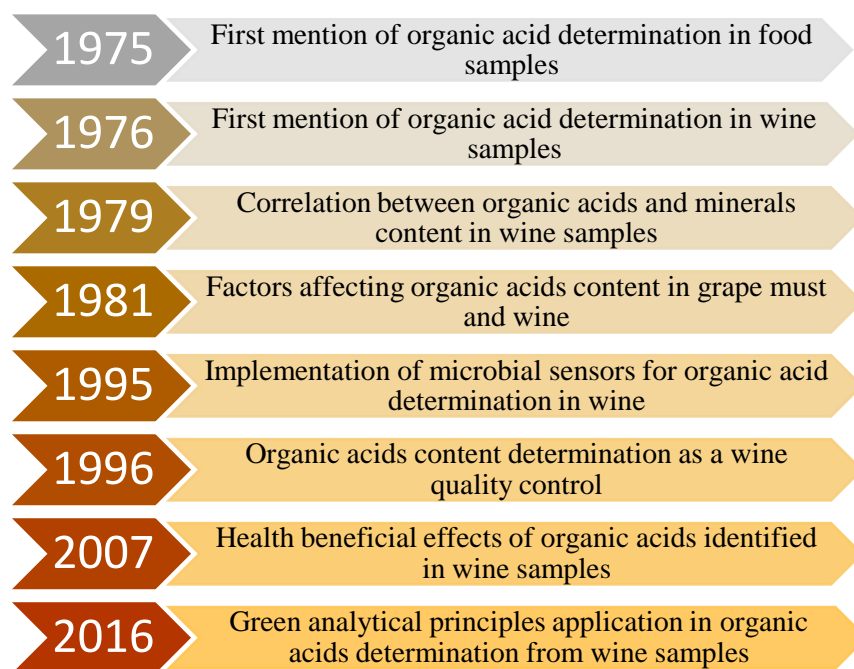
68 The quantitative determination of organic acids in these types of samples is of
69 high interest of many industrial and research institutes. For example, it can be used in
70 the quality control of wine as an indicator of deterioration due to storage or aging
71 (because the classes and content of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

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

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

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





91

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

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

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

111

112 **2. Organic acids in wine**



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

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

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

133 **Table 1 Characteristics of a selected organic acids based on data available in the**
 134 **PubChem database [30].**
 135

Organic acids chemical formula	Molecular weight [g mol ⁻¹]	Density [g cm ⁻³]	Melting point [°C]	Dissociation Constant [pKa]
Oxalic acid C ₂ H ₂ O ₄ or (COOH) ₂	90.034	1.90	189.5	4.40
Tartaric acid C ₄ H ₆ O ₆	150.086	1.699	168	3.07
Formic acid CH ₂ O 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
$C_6H_8O_7$				
Citric acid	192.123	1.54	153	2.79
$C_4H_6O_4$				
Succinic acid	118.088	1.572	188	4.21
$C_4H_4O_4$				
Fumaric acid	116.072	1.635	287	4.54
$C_7H_6O_5$				
Gallic acid	170.12	1.69	258-265	4.40
$C_3H_6O_3$				
Lactic acid or $CH_3CHOHCOOH$	90.078	1.2	16.8	3.86

136

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

141 They can be further subdivided into two groups:

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

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

147 **2.1. Sources and metabolism of organic acids**

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

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

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

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

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

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

173



174 **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	--	
Orange Fruit	--	692 – 1515 mg/100g	18 – 39 mg/100g	44 – 72 mg/100g	[10]
Lemon Juice		5192 – 7696 mg/100ml	355 – 1105 mg/100ml	3.9 – 9.4 mg/100ml	
Sweet cherry (<i>Prunus avium L.</i>)	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 (<i>Cydonia Oblonga Miller</i>) 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 (<i>Sambucus nigra L.</i>)	0.17 g/kg	3.50 g/kg	1.10 g/kg	--	[35]

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

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177

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179



180 **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	365 mg/kg	137.5 mg/kg	540 mg/kg	[8]
		--	14 mg/kg	62.5 mg/kg	175 mg/kg	1300 mg/kg	
Brazilian Wine	White Red	1048 – 1362 mg/L	1117 – 2627 mg/L	83 – 631 mg/L	224 – 924 mg/L	61 – 3933 mg/L	[38]
		1023 – 2212 mg/L	2153 – 2243 mg/L	66 – 700 mg/L	118 – 1003 mg/L	35 – 7306 mg/L	
German Wine	White Red	7.03 mM	5.86 mM	4.63 mM	6.12 mM	5.22 mM	[20]
		12.6 mM	4.34 mM	6.05 mM	6.95 mM	1.90 mM	
Czech Republic wine	White Red	4.4 – 17.4 mM	12.4 – 46.4 mM	1.1 – 7.6 mM	1.7 – 27.9 mM	1.4 – 27.4 mM	[39]
		10.2 – 12.4 mM	n.d. – 0.9 mM	4.6 – 6.7 mM	7.4 – 11.6 mM	23.2 – 42.3 mM	

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

182



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

188

189 **2.2. Main effects in the human body**

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

192 Fruits and vegetables stands for different sources of organic acids from which
193 ascorbic acid is the most known. Among its metabolic functions, the most important one
194 is its ability to protect the immune system and to reduce the harshness of allergic
195 reactions. In addition, ascorbic acid helps to repel infections [3]. On the other hand, if
196 the intake of ascorbic acid is not sufficient, a lot of illness can be observed, like anemia,
197 scurvy, infections, and muscle degeneration, etc. [3]. Orange and lemon juices are
198 significant sources of ascorbic acid and it can be also found in wine made from oranges
199 [14].

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

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

209 Knowing wines as a source of organic acids, Daglia *et al.* (2007) [27] evaluated
210 in marketable red and white wines, the oral *streptococci* and *S. pyogenes* antibacterial
211 action. These bacteria are responsible for caries development and for pharyngitis,
212 respectively. Authors assigned to succinic, malic, lactic, tartaric, citric, and acetic acid
213 as compounds responsible for antibacterial activities [27]. In addition, Boban *et al.*
214 (2010) [31] examined the antimicrobial action of different wine components compared
215 to intact wine. Listed authors applied separately intact wine, wine stripped of phenols,
216 dealcoholized wine, ethanol and low pH and also examined them in combination. Based



217 on their results, they concluded that the non-phenolic constituents of wine were
218 responsible for antimicrobial activity according to the reported by Daglia et al.
219 (2007)[27] and other authors [41]. Moreover, Boban *et al.* (2010) also confirmed the
220 non-phenolic constituents of wine, ethanol and low pH present a synergistic
221 antimicrobial activity.

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

226

227 **3. Effects of winemaking technologies on organic acids content**

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

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

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

247 Generally, the total acidity of the wine increases from 1 to 2 g dm⁻³, during
248 alcoholic fermentation as a result of the production of malic, acetic, succinic and lactic



249 acids. However, significant variations can be observed due to the contribution of
250 specific yeast strains [44].

251 In a method, carried out in Sauvignon Blanc wines, which included four
252 different wine-making procedures (traditional white vinification, skin cryomaceration,
253 vinification in a reductive environment, and a combination of the last two procedures)
254 [47], was reported a slightly variation of malic acid content resulted from the
255 vinification procedure. Whereas that tartaric acid concentration was strongly reduced
256 during cryomaceration process performance. Citric acid concentration was 0.62 g dm^{-3}
257 in wines that included a cryomaceration production, with respect to 0.42 g dm^{-3}
258 obtained by means of traditional or reductive vinification. It can suggest that
259 microorganism activity was inhibited due to low temperature on the process. In
260 addition, the highest amounts of lactic acid and the lowest contents of acetic acid were
261 reported in wines exposed to a reductive vinification including a 24-h skin
262 cryomaceration, whereas those obtained only by cryomaceration process showed the
263 opposite relationship [47].

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

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

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

281 In addition, it is important to note that organic acids can be used as food by
282 many yeasts. Therefore, knowing and understanding the transport of organic acids used



283 by yeasts would be important as well as understanding the mechanism by which they
284 could control intracellular pH and contribute to intracellular charge balance [51].

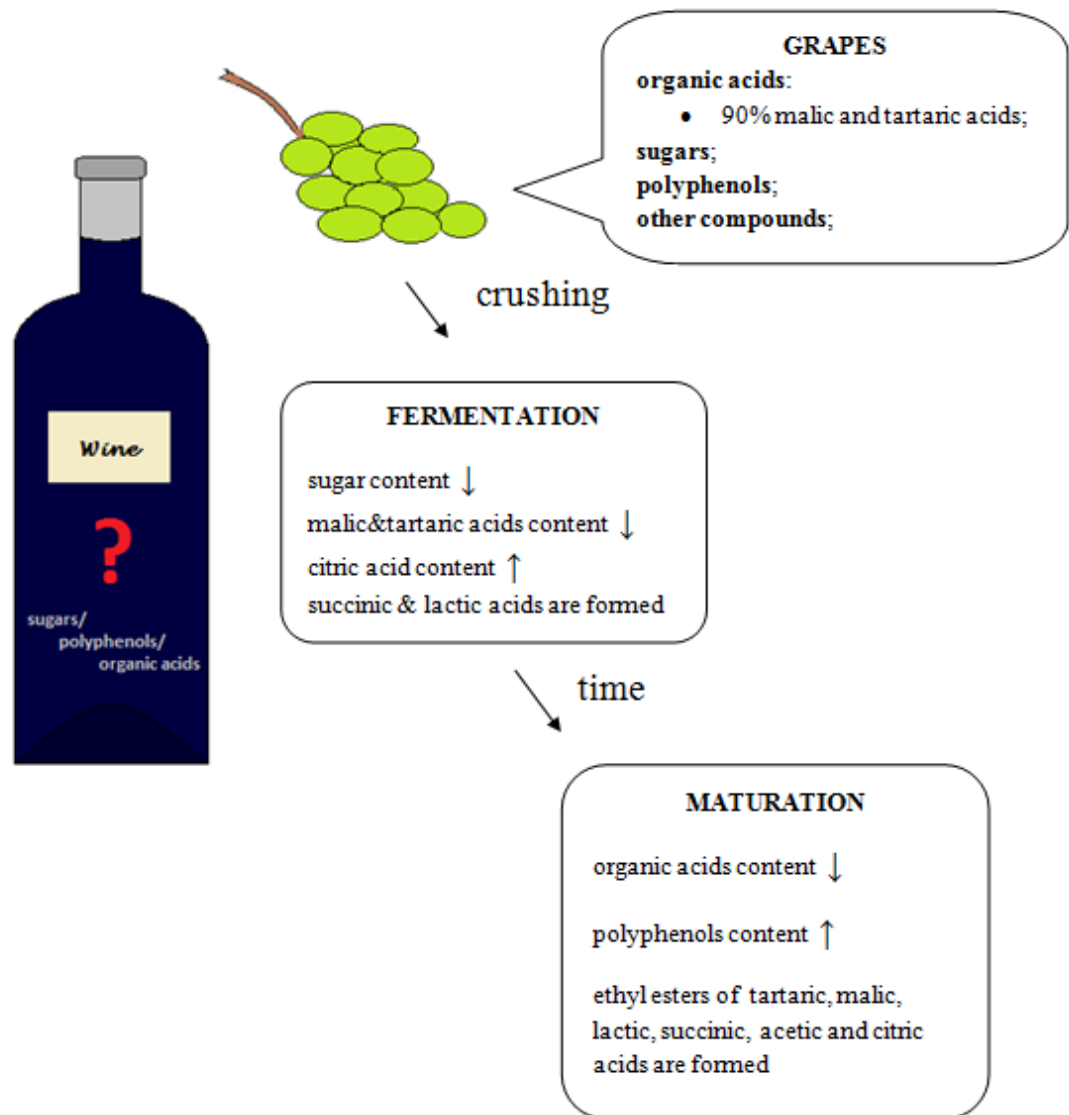
285 Finally, it should not be forgotten that climate change is influencing more and
286 more deeply the composition of the grape, and consequently affects winemaking,
287 microbiology and wine chemistry. Advanced harvest times and temperatures, increased
288 grape sugar concentrations that lead to high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

294

295 **4. Correlations between organic acids and other components of wine**

296 The composition of wine is characterized, mainly, by content of complex
297 mixture of compounds at varietal concentrations. The so-called primary metabolites
298 include sugars, organic acids and amino acids, while the secondary metabolites are
299 represented by flavonoids, hydroxycinnamates, hydroxybenzoates and anthocyanins
300 [53]. The structure of a given compounds are varying during the winemaking process,
301 especially of organic acids and polyphenols what is shown on Figure 2.





302

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

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

310 Each compound has its function in the flavor of the wine [53]. Amino acids
 311 influence the aromas during the maturation process; sugars are consumed by yeasts
 312 during fermentation, except in specific sweet wines; organic acids are of great
 313 importance because they influence organoleptic properties, collaborate in the control of
 314 microbiological growth, and are a critical parameter in the stabilization of wine. Finally,

315 phenolic compounds are associated with appearance, taste, mouth-feel, and
316 antimicrobial activity [53].

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

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

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

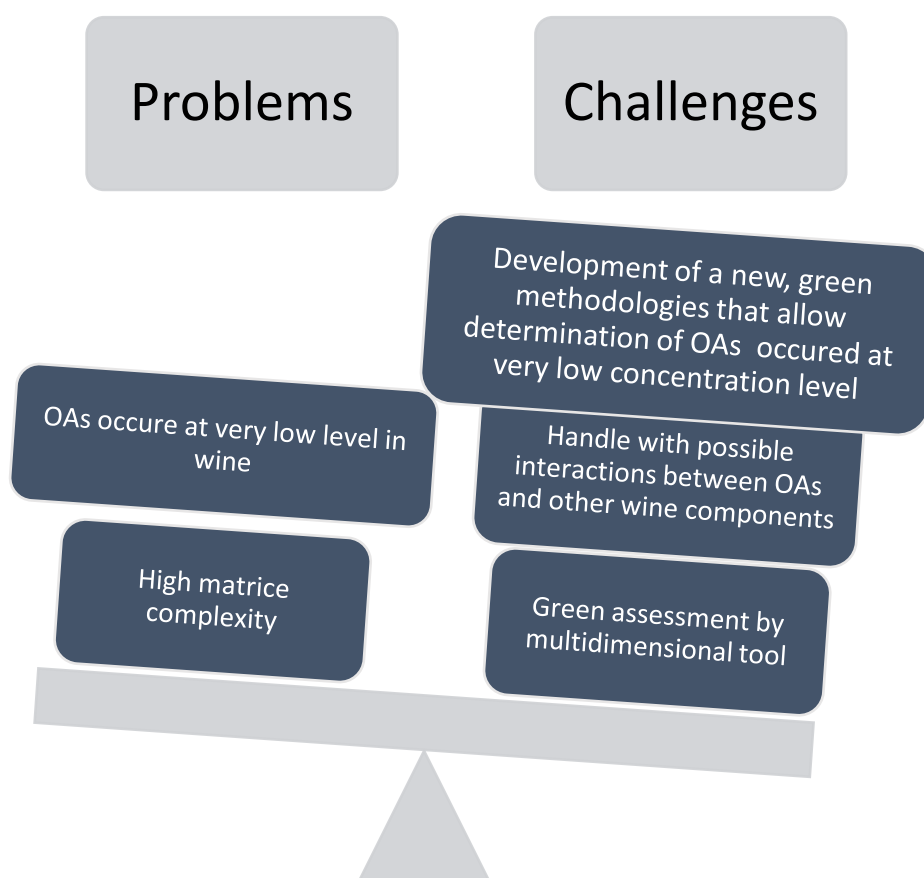
337 Enzymatic or chemical esterification of organic acids and alcohols leads to the
338 formation of esters, and in wines, the most common are ethyl esters. Ethyl esters of the
339 main organic acids in wine (tartaric, malic, lactic, succinic, acetic, and citric acids) are
340 formed in all wines during aging and contribute to the improvement of wine aroma [59].
341 This means organic acids concentration decreases with the progressive aging of wines.
342

343 **5. Analytical challenges of organic acids determination in wine samples**

344 Wines present a diverse group of low and high-molecular-weight compounds
345 including amino acids, organic acids, polyphenols and carbohydrates, which makes the
346 analysis a highly challenging task (Figure 3). Additionally, each wine has its own
347 characteristic, understood by its own, specific compounds compilation. This is due to
348 the surroundings in which grapes are grown. It was investigated, that environmental



349 conditions, plays a crucial role in terms of particular compounds concentrations in
350 fruits. Grapes coming from the cool climate, where the vegetation temperature is less or
351 equal to 15°C, are richer in acids and poorer in the sugar content than grapes coming
352 from favorable climatic conditions [60]. Given variations of compounds concentration
353 among different types of wines may influence the possible interaction between volatile
354 and nonvolatile compounds according to different mechanisms. Thus, the comparison of
355 the organic acids content between varied wine sample should not be based only on
356 statistical data, but should cover the sample preparation and analysis method as well.
357



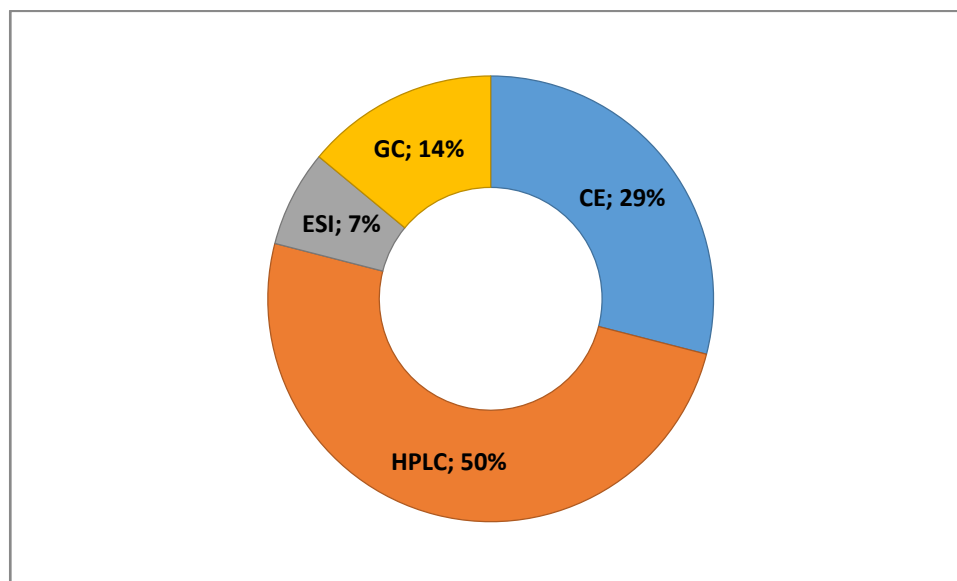
358

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

361 The recent rapid development of a range of analytical techniques, including
362 Capillary Electrophoresis (CE), High Performance Liquid Chromatography (HPLC),
363 Gas Chromatography (GC), coupled to Mass Spectrometry (MS) and Infrared (IR)
364 spectroscopy, could allow separation, detection, characterization and quantification of
365 such metabolites [61]. Several methods have been developed for identifying and
366 quantifying organic acids in wines. Figure 4 shows the most widely applied technique

367 used for organic acids determination. The most favorable technique applied by many
368 researchers was HPLC, presented in around 50% of published cases.

369



370

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

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

377

378 **5.1. Sample preparation technique**

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

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

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



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

406

407 **5.2. Derivatization process**

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

413 However, with the growth of greener ideas, less and less derivatization processes
414 are used. There is a general tendency to find an appropriate techniques to determine the
415 analytes of interest without resorting to these processes that usually use large organic
416 molecules as derivatizing agents. In the consulted literature, only Cunha *et al.* (2002)
417 [69] used O-(4-nitrobenzyl)-N,N'-diisopropylisourea (NBDI) as derivatizing agent
418 before HPLC development and, Zhang *et al.* (2018) [71] used MSTFA [N-methyl-N-
419 (trimethylsilyl) trifluoroacetamide] before GC-MS analysis.

420 **5.3. Analytical instrumentations**

421 The content of organic acids in wines must be controlled for the check of the
422 maturation of the grapes and the development of the acidity, mainly in the processes of



423 fermentation and aging. The development of analytical techniques that allow the
424 monitoring of organic acids in different stages of the winemaking process represents an
425 analytical challenge. Up till now, several options have been reported from enzymatic
426 biosensors to sophisticated chromatographic techniques. Information on analytical
427 methodologies developed for organic acids determination in wine samples are presented
428 in Table 3.

429 **5.3.1. Enzymatic biosensors**

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

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

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

443 In addition, various biosensors for the determination of individual organic acids
444 were developed and tested for the wine analysis. Zeravik *et al.* (2016) [39] presented a
445 very good review of biosensor developments for these analytes. For specific organic
446 acids, diverse publications have been reported. Molinero-Abad *et al.* (2014) have
447 developed a specific malic acid sensor applied to wines, using a screen-printed carbon
448 electrodes modified with gold nanoparticles, tetrathiafulvalene and malate quinone
449 oxidoreductase enzyme [75]. In wines and ciders, Loaiza *et al.* (2015) [76] have
450 developed a lactate biosensor using platinum nanoparticles supported on graphitized
451 carbon nanofibers surface. Although, lots of researchers is publishing their work with
452 the use of biosensors showing their advantages like good results of reproducibility: RSD
453 on the level of 4,9%, n=10, good sensitivity, and relevant limit of detection achieving
454 6.9 μM , only few systems are commercially available [61, 74]. This is the result of the

455 restrictions related with the use of biosensors like: limited lifespan of biological
456 components they are made of, difficulties of handling and mass production.

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

462

463 **5.3.2. Capillary electrophoresis**

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

482 However, there are several reports indicating that CE presents two main
483 disadvantages such as robustness and sensitivity [77]. Since organic acids in wine
484 samples appear at low concentrations, the pre-concentration technique as liquid liquid
485 extraction (LLE) or solid phase extraction (SPE) might be used to solve the problem of
486 sensitivity. Moreover, the on-line pre-concentration can be used which has big
487 advantages as increase of sensitivity without loss in separation efficiency [78]. From

488 the practical point of view, the volume of sample injected, narrow capillary diameter in
489 ultraviolet detector and interfaces with MS detector are typical issues that affect the
490 analyte bands [77]. Since, due to the contamination of the ion source it cannot be
491 combined directly with the MS.

492

493 5.3.3. Spectroscopy Techniques

494 The combination of chemometric software with spectroscopy techniques has
495 provided an analytical tool of high interest, suitable for the control of processes, such as
496 the manufacture of wines [64]. Fourier - transform infrared spectroscopy (FT-IR) with
497 partial least squares (PLS) was used by Regmi *et al.* (2012) to establish a calibration
498 model for tartaric, malic, lactic, succinic, citric and acetic acids in wines, vinegars and
499 spirits. The big advantage of given technique is easy sample pre-treatment, since only
500 filtration was performed prior the analysis in order to remove particles or degassing
501 when it came to cope with the samples containing CO₂. On the other hand, recovery
502 experiments were performed in sparkling wine samples with different amounts of
503 organic acids (tartaric, malic, lactic, acetic and citric) by Moreira and Santos (2005)
504 [79]. However, the authors informed that percentages for IR analysis of the individual
505 organic acid concentrations were 61, 73, 44, and 11%, for tartaric, malic, lactic, and
506 citric acids, respectively. There was the relationship noticed, in which acids appearing
507 in higher concentration ranges like tartaric (1,16-2,07 g dm⁻³) and malic (1,87 – 1,98 g
508 dm⁻³) had better recoveries than acids of lower concentration ranges as citric (0,24 –
509 0,29 g dm⁻³) or lactic acid (0,55 – 1,28 g dm⁻³). It can be explained by the
510 spectroscopic interferences existing between organic acids, which results in final, lower
511 accuracy. Challenges related with the FT-IR analyses are based on the organic acids
512 similarities within IR spectra. Since they have in common stretching of:

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

517 what strongly disturb the separation and significantly influence the recoveries results of
518 each individual acid [79]. What is more, ethanol and water dominate the absorption in
519 the mid-IR region. Since FT-IR results interpretation are based on the calibration

520 statistics, it is necessary to point out difficulties of wine samples analysis by given
521 methods due to high matrices variability. When it comes to deal with the calibration
522 curves large number of samples needs to be involved in the processing in order to cope
523 with outlier samples and related dissimilarities. Moreover, given technique is not
524 suitable for concentrations lower than $0,2 \text{ g dm}^{-3}$ [80].

525 **5.3.4. Liquid Chromatography**

526 The most widely employed technique used for the organic acid determination
527 has been HPLC in its several modes like reversed phase liquid chromatography (RPLC),
528 flow injection analysis high performance liquid chromatography (FID-HPLC), ion
529 exchange high performance liquid chromatography (IE-HPLC). They are usually
530 coupled with one of the several different detection technique like UV diode array
531 detection (DAD), reflection index detection (RIP) or UV photodiode array (PDA). RIP
532 detection is used a universal one. However, huge limitations of given technique is low
533 sensitivity and temperature dependence, what place him behind UV and DAD detection
534 [81].

535 Kelebek *et al.* (2009) determined citric, malic and ascorbic acids in orange juice
536 and orange wine using HPLC-DAD [14]. Also, Monteiro Coelho *et al.* (2018) showed a
537 simultaneous determination of sugars and organic acids in wines and grape juices with
538 HPLC using RID and DAD [17]. UV detection is the most common detection system
539 coupled to HPLC. Kritsunankul *et al.* (2009) determined six organic acids (tartaric,
540 malic, lactic, acetic, citric and succinic acids) [19] at 210 nm. Likewise, Cunha *et al.*
541 (2002) detected the same groups of OAs at 265 nm [69] and Zotou *et al.* (2004) at 230
542 nm [68].

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

549

550 **5.3.5. Gas Chromatography**

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

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



573 **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]

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

579

580

581



582 **5.4. Green assessment of selected analytical procedures applied for determination**
583 **of organic acids in wine samples**

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

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

605 Basing on the penalty points calculated for each procedure (Table 5) one can
606 notice that the highest score is achieved by procedure 1 (94 PPs) basing on water
607 capillary ion analyzer. This would signalized that following technique is the greenest in
608 terms of being environmental friendly. While just behind it, procedure 3 and procedure
609 5 are placed. Both gathered 87 PPs. Procedure 3 used ESI-TQD-MS while procedure 5
610 is based on capillary electrophoresis. The least green having only 77 PPs is dedicated to
611 procedure 2 where HPLC-UV was used.

612 In order to verify the best from three procedures which gathered the highest
613 amount of penalty points the GAPI tool was applied. GAPI index is a new tool,
614 presented in 2018, which stands for the graphical representation in the form of

615 pictogram. Each part of pictogram represents different step of analytical procedure.
616 There are three – stages color scale used to assess the green character of each part.
617 Green means low environmental impact, yellow - medium and red - high respectively
618 [90].

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

626

627



628 **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 ₂ PO ₄	0	benzylmalonic acid (IS)	1	Methanol	3	Methanol	6
Na ₂ HPO 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 Analyzer	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 PO ₄	0	H ₃ PO ₄	2	Methoxyamine hydrochloride	7
Tricina	1	H ₃ PO ₄	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	

629

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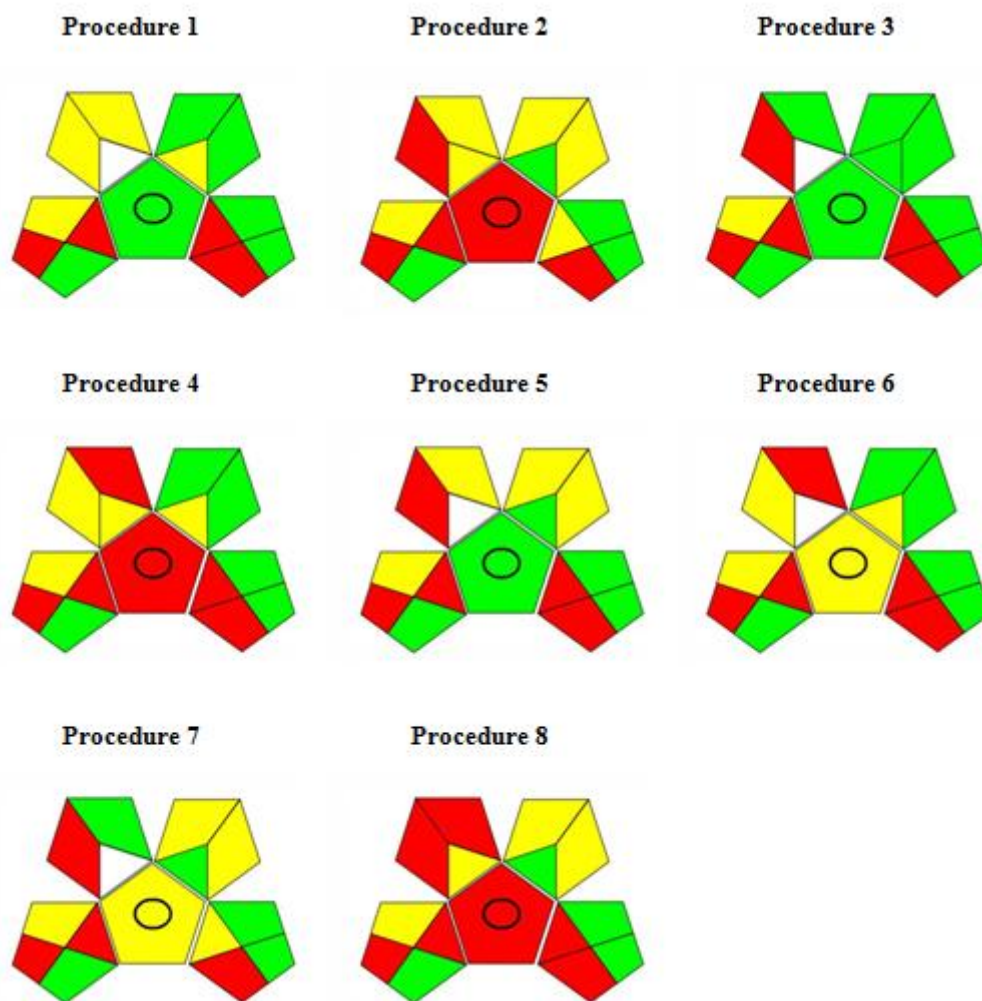
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639 **Figure 5 Assessment of the green profile of evaluated procedures applied for**
 640 **organic acids determination in wine samples using GAPI tool.**

641

642 **6. Conclusions**

643 Over last twenty years there is an increasing interest in organic acids as the
 644 compounds having beneficial properties on the human health. Due to the strong
 645 consumer incentives for the natural ingredients having among the others antioxidant and
 646 antimicrobial properties organic acids seem to be crucial. There are several studies
 647 performed discussing their sources, properties and benefits in terms of food
 648 preservatives as well as beneficial effects on the human body. Wine is considered as one
 649 of the sources of the following compounds. Since it is commonly known that wine
 650 acidity should be at acceptable level at different stages of vinification process.
 651 Moreover, to satisfy the specific characteristic of a given wine expressed by the strictly

652 defined aroma and flavor. Taking into account all of those aspects, the determination
653 and quantification of organic acids is of high importance. Nevertheless, matrix in which
654 they occur is very complex what makes the analysis very difficult. Due to this fact lots
655 of attention needs to be paid for sample preparation, which are compared and discussed
656 together with separation and detection techniques basing on the parameters like:
657 LOD/LOQ, recovery, RSD, number of analytes, sample throughputs and the time of
658 analysis. Moreover, green character of the discussed techniques were assessed with the
659 use of Eco-Scale and GAPI tool. Most of the analytical methods are based on the
660 capillary electrophoresis or high performance liquid chromatography, little is known
661 about the gas chromatography which seems to be relevant in terms of low limit of
662 detection, precision and recovery, which may be a further step in future research.

663 **Conflict of interest**

664 Alicia Robles declares that she has no conflict of interest. Magdalena Fabjanowicz
665 declares that she has no conflict of interest. Tomasz Chmiel declares that he has no
666 conflict of interest. Justyna Płotka-Wasyłka declares that she has no conflict of interest.

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668

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