Use of a solution of organic acids, a byproduct of the Rectified Concentrated Must production process, for the acidification of wines

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Abstract. The production cycle of Rectified Concentrated Must (RCM) includes a deionization phase performed with ion exchange resins, that are regenerated after each deionization cycle with strong mineral acids or strong bases. The resulting waste water is particularly rich in cations and anions removed from the must, and various processes for the recovery of tartaric acid are normally performed. More recently, new techniques have been developed for the recovery of all organic acids of the must as concentrated solutions of organic acids (SGA: Solutions of Grapes organic Acids). The work was aimed at determining the acidic profile and some specifics of SGA for a possible use as wine acidifiers. Moreover, some trials were carried out to define the correct dose of SGA to be added to wines in order to cause a given decrease of pH, equivalent to that obtained with H_2T. As a result, a method was proposed for the calculation of the dose of SGA needed to determine a given decrease of wine pH. The recovery and valorization of SGA from the RCM processing cycle can represent a virtuous example of biorefinery, within the framework of the circular and green economy promoted by EU.

1. Introduction

In the production of Rectified Concentrated Must (RCM), the concentration process of the sugar fraction is preceded by a deionization or rectification phase, during which the grape must passes through ion exchange resins, both anionic and cationic, that retain mineral salts, organic acids and polyphenolic substances present in it. After each deionization cycle, cationic resins are regenerated with strong mineral acids (hydrochloric and sulfuric), while anionic resins with strong bases (sodium hydroxide), in order to elute cations and anions and restore their capacity to exchange. The waste water from the regeneration of the resins is therefore particularly rich in cations and anions removed from the must. Various processes for the recovery of tartaric acid alone from anionic resins are generally carried out, and more recently new techniques for the recovery of all organic acids of the must have been studied: concentrated solutions of organic acids, hereinafter referred to as SGA (Solutions of Grapes organic Acids), have been obtained.

The recovery and valorization of SGA from the RCM processing cycle potentially represents a virtuous example of biorefinery, within the framework of the circular and green economy promoted by EU (program for a zero-waste Europe: COM (2014) 398) through the implementation of interventions aimed at radically changing the value chains, from product design to market and business models, from methods to transform waste into resources, to consumers habits.

In order to verify the suitability of SGA for the acidification of musts and wines, a preliminary study was carried out to determine the acidic profile and some specifics of the solutions. Moreover, a method for the calculation of the doses of SGA needed to determine a given decrease of wine pH was proposed and applied with a red wine.

2. Materials and methods

The organic acids of SGA were determined by HPLC, according to the method proposed by [1], after 100-fold dilution with distilled water.

The HPLC method proposed by [2] was used to evaluate the oxalic acid content. The undiluted sample was passed through a C18 cartridge and then injected. The analysis of the oxalic acid content was carried out for the SGA lot 361 (7 repetitions). The LOD (Limit Of Detection) and LOQ (Limit Of Quantification) values of oxalic acid were 1.6 and 5.3 mg/L, respectively [3].

Among contaminating toxins, aflatoxins B1, B2, G1, G2 were determined with an internal method based on HPLC techniques, and ochratoxin A according to OIV-MA-AS315-10: R2011 method.

Regarding microorganisms, the total count was carried out at $30\,°C$ (UNI EN ISO 4833-1: 2013 standard), the presence of total coliforms according to ISO 4832:2006 standard, Salmonella according to ISTISAN Report 96–35 and ISO 6579:2017 standard, and yeasts and mildews according to ISO 21527-2:2008 standards.

Table 1. Average composition of three different lots of SGA.

	lot A			lot B			lot C		
	mean	*SD*	*CV%*	*mean*	*SD*	*CV%*	*mean*	*SD*	*CV%*
Tartaric acid (g/L)	116.65	3.41	2.93	99.88	1.23	1.23	116.27	3.17	2.72
Malic acid (g/L)	97.43	0.70	0.71	92.63	1.08	1.17	90.92	0.26	0.29
Scikimic acid (g/L)	0.85	0.01	0.79	0.81	0.02	2.51	0.81	0.02	2.11
Lactic acid (g/L)	28.84	0.51	1.76	28.47	0.74	2.60	15.00	0.65	4.32
Citric acid (g/L)	traces			traces			traces		
Succinic acid (g/L)	traces			traces			traces		
density (°Brix)	30.25			30.25			29.25		

The possible release of styrene, deriving from the use of cationic resins for the production of RCM, was evaluated with an internal method based on GC/MS/SIM in SPME techniques.

The iron content was determined with the OIV-MA-AS322-05 R2009 method. Arsenic, mercury, lead, sulfates and chlorides were analyzed with ICP-MS techniques.

3. Results

Table 1 reports, as an example, the average content in organic acids of three different lots of SGA with a density of about 30 °Brix.

SGA are mixtures containing the main grapes organic acids, in particular tartaric, malic and lactic acids. The three lots described in Table 1 came from the processing of the same batch of RCM, and have been subjected to different purification processes, in particular the removal of iron and the decoloring of the solutions. This can explain the good homogeneity of acidic composition that was observed between the lots, that under other conditions was not observed (data not reported). In fact, normally the grapes have a variability of acidic composition linked to the cultivar, to the micro and macro-climatic conditions (vintage), to the geographic origin and to the ripeness degree of the grapes. This is a characteristic of foods and beverages with biological origin, such as wine and co-products/by-products of the wine industry, which consequently also concerns this product.

During the production process, the concentration of the solution may allow to increase the titre in organic acids; however, the producers verified the risk of precipitates appearing for densities higher than 37 °Brix. Therefore, the density of SGA should be indicatively included between 30 and 37 °Brix.

The specifications reported in the OIV Oenological Codex [4] concerning L-tartaric acid, the main acid authorized for the acidification of musts and wines, determine limits for the content of oxalic acid, which must not exceed 100 mg/kg of product. The average content of oxalic acid in SGA was 7.99 ± 0.53 (standard deviation) mg/L of SGA, corresponding to 74.3 mg/kg of tartaric acid in SGA and therefore lower than the OIV limit.

Actually, since SGA are a mixture of organic acids, the oxalic acid content should be referred to the sum of these acids and therefore its content could result lower.

Based on the LOQ values (5.3 mg/L), the method we used for the analysis of oxalic acid [2] allows to detect the exceeding of the limits for the oxalic acid

Table 2. Average composition after cold stabilization of the red wine used for the acidification trials.

	mean	st. dev.
Alcol %	13.05	0
Total extract (g/L)	31.3	0
pH	3.83	0.01
Titratable acidity (g/L)	5.59	0.05
Titratable acidity (meq/L)	74.5	0.71
Tartaric acid (g/L)	2.72	0.12
Malic acid (g/L)	0	0
Scikimic acid (g/L)	0.034	0.001
Lactic acid (g/L)	1.46	0.04
Citric acid (g/L)	0.17	0.01

content (100 mg/kg of tartaric acid) for SGA with a tartaric acid content higher than 53 g/L, without concentrating the sample.

The other analyses mainly focused on product specifications, as regards the presence of contaminants and metals. The presence of aflatoxins and ochratoxin A was not detected; coliforms, salmonellae, yeasts and mildews were absent; styrene, a constituent of the resins used in the rectification of the musts for the production of RCM, was not detected.
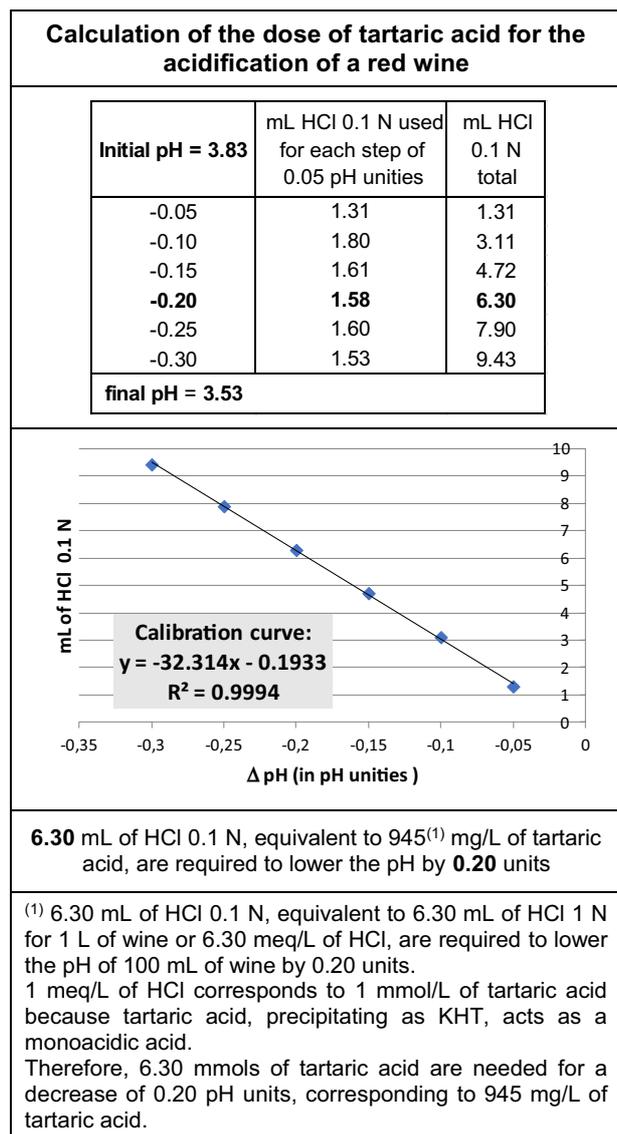
The content of chlorides, sulfates, iron, arsenic, mercury and lead (in mg/Kg of organic acids) of some SGA lots was compared to the limits set in [4], and expressed in mg/kg of product (99.5% of tartaric acid).

The contents of arsenic, mercury, lead were well below the limits, while in some tested lots of SGA the sulphate and chloride contents were above the limits (1 g/kg of product for both of them). All SGA had iron concentrations well above the limits set for H_2T (lower than 10 mg/Kg), and such as to require deferrization procedures. The iron concentrations, even after the iron removal treatments, were in any case higher than the limits set for tartaric acid.

Calculation of the doses of tartaric acid and SGA to determine a given decrease of pH.

The physicochemical composition of the wine used for the acidification trials is reported in Table 2.

The dose of tartaric acid (H_2T) was calculated according to [5]: 100 mL of cold-stabilized wine (-4 °C for 6 days) were acidified at given pH intervals of 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 units with progressive additions of a strong acid (HCl N/10).

Calculation of the dose of tartaric acid for the acidification of a red wine

Initial pH = 3.83	mL HCl 0.1 N used for each step of 0.05 pH unities	mL HCl 0.1 N total
-0.05	1.31	1.31
-0.10	1.80	3.11
-0.15	1.61	4.72
-0.20	**1.58**	**6.30**
-0.25	1.60	7.90
-0.30	1.53	9.43
final pH = 3.53		

Calibration curve:
y = -32.314x - 0.1933
R² = 0.9994

6.30 mL of HCl 0.1 N, equivalent to 945[1] mg/L of tartaric acid, are required to lower the pH by **0.20** units

[1] 6.30 mL of HCl 0.1 N, equivalent to 6.30 mL of HCl 1 N for 1 L of wine or 6.30 meq/L of HCl, are required to lower the pH of 100 mL of wine by 0.20 units.
1 meq/L of HCl corresponds to 1 mmol/L of tartaric acid because tartaric acid, precipitating as KHT, acts as a monoacidic acid.
Therefore, 6.30 mmols of tartaric acid are needed for a decrease of 0.20 pH units, corresponding to 945 mg/L of tartaric acid.

Scheme 1. Method for the calculation of the doses of H$_2$T needed to determine a given decrease of wine pH.

Table 3. Results of the acidification trials with different percentages of SGA.

	SGA 60%	SGA 70%	SGA 80%
pH	3.73	3.73	3.73
Titratable acidity (g/L)	5.77	5.85	5.92
Titratable acidity (meq/L)	77	78	79

Calculation of the dose of tartaric acid for the acidification of a red wine treated with organic acids

	mL HCl 0.1 N used for each step of 0.05 pH unities			mL HCl 0.1 N total		
	Sol. 60%	Sol. 70%	Sol. 80%	Sol. 60%	Sol. 70%	Sol. 80%
Initial pH	3.73	3.73	3.73	3.73	3.73	3.73
-0.05	1.73	1.68	1.65	1.73	1.68	1.65
-0.10	**1.74**	**1.75**	**1.65**	**3.47**	**3.43**	**3.30**
-0.15	1.70	1.70	1.65	5.17	5.13	4.95
-0.20	1.63	1.65	1.67	6.80	6.78	6.62
-0.25	1.71	1.71	1.62	8.51	8.49	8.24
-0.30	1.58	1.54	1.60	10.09	10.03	9.84
final pH	3.43	3.43	3.43	3.43	3.43	3.43

For a ΔpH = 0.10 :

	Sol. 60%	Sol. 70%	Sol. 80%
mL HCl 0.1 N	3.47	3.43	3.30
mmols/L H$_2$T	3.47	3.43	3.30
mg/L H$_2$T	520.5	514.5	495.0

y = -0.1317x + 599.25
R² = 0.9146

Scheme 2. Method for the calculation of the doses of SGA needed to determine a given decrease of wine pH.

Scheme 1 shows the results of the acidification trials carried out with the red wine and the calculation of the dose of H$_2$T to cause a reduction of 0.20 pH units.
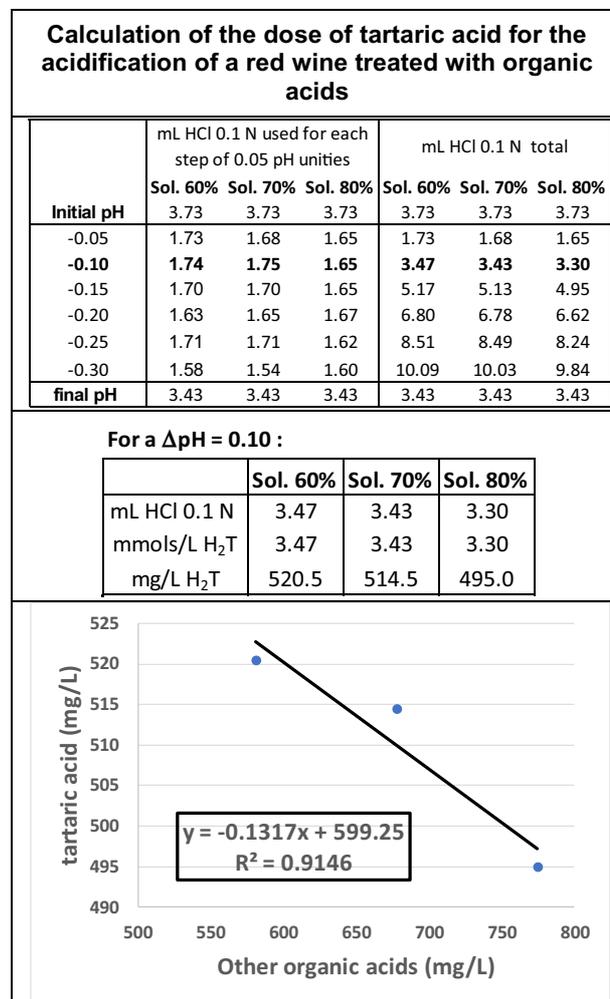
The dose of H$_2$T to be added to the wine was 945 mg/L. The same amount of H$_2$T was contained in 12.6 mL/L of SGA, but the contribution of this concentration of SGA to the wine was excessive since SGA are mixtures of different organic acids of the grapes. Some trials were therefore carried out to define the correct dose of SGA to be added in order to cause a decrease of pH equivalent to that obtained with H$_2$T, and a calculation procedure for the use of SGA was developed.

Among the organic acids of SGA, only H$_2$T acts in the wine as a strong inorganic acid with its first acidic function. The addition of H$_2$T causes, after cold stabilization, the precipitation of potassium bitartrate (KHT) and the loss of potassium. The same behavior is not observed with the other organic acids (malic, lactic and citric acids) that in the wine form soluble salts.

These differences in behavior were therefore taken into account for the calculation of the dose of SGA, and the

following procedure was followed: three 100 mL aliquots of cold-stabilized red wine were prepared. Each aliquot was treated with a model solution of organic acids (without H$_2$T) at the same concentration ratios they had in SGA, but the actual quantity of organic acids added to each trial was that present in a fraction of the SGA (60, 70 and 80% of 12.6 mL/L):

Thesis SGA 60%: wine treated with a quantity equal to the content of organic acids present in 60% of SGA;

Thesis SGA 70%: wine treated with a quantity equal to the content of organic acids present in 70% of SGA;

Thesis SGA 80%: wine treated with a quantity equal to the content of organic acids present in 80% of SGA.

The addition of organic acids caused a decrease of pH similar for the three theses and equal to 0.10 pH units (Table 3). Since the objective was to obtain a reduction of 0.20 pH units, it was necessary to determine the quantity of H$_2$T to be added as SGA in order to cause the further

Table 4. Results of the acidification trials with H2T and SGA.

	T	H$_2$T	SGA	sig.
pH	3.86 b	3.71 a	3.70 a	***
Titratable acidity (g/L)	4.87 a	5.47 b	6.30 c	***
Titratable acidity (meq/L)	65 a	73 b	84 c	***
Tartaric acid (g/L)	2.13	2.35	2.34	n.s.
Malic acid (g/L)	0 a	0 a	0.52 b	***
Scikimic acid (g/L)	0.036	0.035	0.040	n.s.
Lactic acid (g/L)	1.31	1.26	1.27	n.s.
Citric acid (g/L)	0.21 a	0.25 a	0.51 b	*
Succinic acid (g/L)	0.37 a	0.38 a	0.54 b	*

reduction of 0.10 pH units. The 3 theses were therefore acidified at given pH intervals of 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 units with progressive additions of a strong acid (HCl N/10).

The quantity of H$_2$T to be added to the SGA 60%, SGA 70% and SGA 80% theses to obtain a further reduction of 0.10 pH units was respectively 520.5, 514.5 and 495.0 mg/L (Scheme 2). The regression line was then calculated between the concentrations of organic acids without H$_2$T (X) and the H$_2$T values calculated after titration with HCl (Y) to cause a reduction of 0.20 pH units.

In the studied SGA the concentration ratio between H$_2$T and the other organic acids was 1 : 1. The relationship between the concentration in organic acids (without H$_2$T) and that of H$_2$T in SGA was described by the line Y = X. Among all the combinations able to cause a reduction of 0.20 pH units (regression line in Scheme 2) we chose the one that satisfied this condition (Y = X). The quantity of SGA to be added to the wine was therefore 7.1 mL/L (corresponding to an intake of about 530 mg/L of H$_2$T).

The additions of H$_2$T and SGA were carried out after 3 months from the analytical checks and the stability tests described above, then the wines were cold stabilized for about 15 days, filtered and bottled.

Table 4 reports the acidic profile of the H$_2$T and SGA theses compared to the control wine (without additions), and the ANOVA and Tukey's test results. The actual reduction of pH was around 0.15 units for both theses

(H$_2$T and SGA). Conversely, titratable acidity increased after acidification and resulted significantly higher for the SGA thesis than for H$_2$T. The H$_2$T content of the acidified theses was slightly higher than that of the control, but without statistical significance. Regarding the other organic acids, significant differences were found between SGA and the other two theses for the content of malic, citric and succinic acids: in particular, the SGA wine had a malic acid content of 0.5 g/L, while in the other two theses malic acid was absent.

4. Conclusions

The work was aimed at determining the acidic profile and some specifics of the concentrated solutions of organic acids (SGA), byproducts of the rectified concentrated must (RCM) production process, for a possible use as wine acidifiers.

From a regulatory point of view, in order to achieve these objectives, the evaluation of the potentialities of this agri-food byproduct may need to provide different criteria than those applied for tartaric acid, regarding in particular the maximum limits for the iron content, still maintaining the guarantee of compliance with the safety requirements of the final product.

In the course of the work, a method was proposed for the calculation of the doses of SGA needed to determine a given decrease of wine pH.

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