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Correlation of Wine's Main Components' Concentration with the Density of Model Aqueous Solutions and Wine Samples

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Abstract: Density is an important physical property, affecting wine mouthfeel, while it can also be used for monitoring alcoholic fermentation in winemaking. Aim of this study was to elucidate the correlation of ethanol, glucose/fructose, glycerol and tartaric acid on the density of model aqueous solutions and Greek wine samples. Various model aqueous solutions were prepared and density was measured at 20 ˚C. Density of dry white and red wine samples was also measured. A linear regression analysis was performed and theoretical fermentation monitoring curves by density measurement were obtained. The resulting models presented a coefficient of determination over 97.3%. Tartaric acid was found to increase density the most, followed by glucose and glycerol, whereas ethanol decreased density. The knowledge of the correlation of the concentration of each wine component with density may be beneficial to quantitative analysis of wine and to optimizing wine mouthfeel.
Keywords: Wine density; Ethanol; Glycerol; Glucose; Tartaric acid.

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1. Introduction

Density is an important physical property of all liquids, defined as the mass of a substance per unit of volume. Density along with viscosity and surface tension are rheological properties that affect the mouthfeel of beverages, thus influencing consumer preference [1-4]. The perception of texture in wines, due to the complexity conferred by its components, is described by several terms, including viscosity, density, astringency, body and unctuosity [5]. Many studies refer to wine mouthfeel, some aiming to determine how main wine components affect body perception and others focus on instrumental techniques for the characterization of this sensory perception [6-8].

To our knowledge, there are few studies that correlate the wine density with wine's components and most of them refer to the perceived density. Nurgel and Pickering evaluated the contribution of glycerol, ethanol, and sugar to perceived density for model wine solutions, finding that sugar had the greatest influence on perceived density, while ethanol had a moderate effect and glycerol's contribution was nominal [9,10]. Another study on Greek red, white and sweet wines revealed that dry extract and sugar content increases density. Statistical analysis on the density of the two red wines NAOUSA and NEMEA showed that there was no statistically significant difference in mean density between the two red wines or between the two white wines SANTORINI and MANTINEIA [11]. However, there was a statistically significant difference in the mean density between the two white wines and the two red wines studied. The two red wines had higher density due to the increased dry extract. Furthermore, as it is reasonable, sweets wines' density MAVRODAFNI and SAMOS was considerably higher due to the high sugar content.

Measurements of Brazilian red wines' density instrumentally, showed that density was mainly influenced by the wine alcohol content, viscosity was closely linked to the dry extract, whereas reducing sugar content did not present any correlation with the physical properties [12]. In the same study, wine density slightly decreased at higher temperatures. Also, both alcohol and density in wine found to be important for testing the quality of red and white wine, based on an analysis of classification techniques in data mining for determining quality of wine product [13].

Several researchers have tried to understand the role of glycerol in wine sensory perception. At the levels at which glycerol is found in wine, its primary contribution to the sensory properties is sweetness [14]. Volk and Kahler presented an analytical expression to accurately calculate the density of aqueous glycerol solutions and showed a linear correlation between density and glycerol in a range of concentration 0-40% w/w [15]. Moreover, according to Laguna et al, when studding the wine texture through instrumental measurements, saliva seems to be a key factor [5]. In their study, density measurements were performed on model-wine samples mixed with human saliva and in the presence of glycerol density was found to increase.

Apart from wine mouthfeel, density has also a key role in wine industry, as it provides information for fermentation kinetics and can determine an abnormal wine fermentation. The measurement of density is made usually manually during fermentation, several times a day, and its changing data is correlated with the metabolism of sugars by the yeast, which results in the production of ethanol and carbon dioxide. In order to contribute to the improvement of the wine fermentation process, a variety of alternative procedures have been used for monitoring the grape must fermentation, including density measurements [16-20]. Cao y Paz and coauthors suggested the use of a sensor placed inside the must tank, based in plastic optical fiber probes for the continuous monitoring of density during fermentation [17]. In another study, an AlN-based piezoelectric microresonator, serving as a density sensor, was used for automated on-line measurement [18]. In order to detect early an abnormal fermentation, the use of Support Vector Machine (SVM) as a tool was suggested and density was one of the best individual chemical markers for prediction [19]. Recently, Nelson et al studied the use of pressure transducers to measure density throughout wine fermentation [20].

Wine is complicated mixture, consisting of many substances. Generally, studding a solution of n components, density, as a linear combination of other physical parameters, can be given by the expression:

$$d = a + b_1x_1 + b_2x_2 + b_3x_3 + \ldots + b_nx_n,$$

where, a = is the constant that corresponds to the density of the solvent, b_1, b_2, b_3, …, b_n are coefficients of partial dependence, and x_1, x_2, x_3, …, x_n are the components of the solution.

Density of an aqueous solution is changed with the addition of a solute, depending on the physical state of the solute. The dissolution of solid solute in water always increases the density, but the dissolution of liquid solute in water increases the density, only if its density is greater than the density of water. However, if the density of the liquid solute is less than the density of water, then the density of the solution decreases. If we consider wine as an aqueous solution of ethanol, glycerin, acids and sugar, we observe that ethanol decreases the density of the solution while all the other components increase it.

As density is an important factor in wine mouthfeel and in fermentation process, aim of this study was to investigate the effect of wine's main components, ethanol, glucose/fructose (sugars), glycerol and tartaric acid on the density of aqueous solutions and wines, and to elucidate the correlation of each component's concentration with density, as previously done by our group for other rheological properties [21, 22]. Understanding these factors may be beneficial to quantitative analysis of wine and to optimizing mouthfeel.

2. Materials and methods

2.1 Materials

One hundred twenty-one (121) model aqueous solutions of ethanol, tartaric acid, glucose/fructose and glycerol were prepared by adding ethanol (absolute), tartaric acid (analytical), glucose/fructose (analytical) and glycerol (99.9%, w/w) to distilled water. Ethanol, tartaric acid, glucose/fructose, and glycerol were purchased from Chembiotin, Penta, Chembiotin and Sigma-Aldrich, respectively. Dry commercial Greek white and red wines of the varieties RODITIS and AGIORGITIKO, produced by various wineries, were purchased from a local store.

2.2 Methods

Model aqueous solutions with a different concentration of each component were prepared (data not shown). Thirteen (13) white wine and eleven (11) red wine samples were prepared, altering the concentration of their main components by adding specific amounts of ethanol, tartaric acid, glucose or glycerol (data not shown).

A digital densimeter (Anton Paar DMA 35 Basic) was used to measure density (d) at 20 ºC and each measurement was taken three times. The quantitative analysis of chemical ingredients of wines was done with classical methods. Alcohol content of wines was determined by distillation [23], glycerol was quantified by HPLC and by enzymatic analysis, the total acidity by titration (neutralization) and the reducing sugars using the Luff method. The experimental results were analyzed with the Minitab Statistical Software. The statistical significance of density was evaluated by analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test with $P < 0.05$.

3. Results and discussion

3.1 Model aqueous solutions

The effect of the major components of wine on density was studied in model aqueous solutions of one, two, three or four components.

3.1.1 One component model aqueous solution

Model aqueous solutions containing only one component, ethanol (Eth), glucose/fructose (Gluc), glycerol (Glyc) or tartaric acid (Tart), were prepared and the density was measured.

Ethanol and glycerol are liquids, whereas glucose and tartaric acid are solids and as a result when dissolved in water, the total amount of water changes differently. In order to compare the interactions of each component with water, the amount of water molecules must be the same in each case, as well as the amount of each solute. For this reason, concentration of each component is expressed also as molality, m (mol/Kg H_2O). In this way, the effect on density of every 1 mol/kg H_2O increase of each component concentration becomes more obvious.

The equations that emerged from the regression analysis, in each case, are shown in Table 1. In each case p-values was under 0.001.

Table 1. Equations emerged from the regression analysis of one component model aqueous solutions

Component	**Equation**	**Eq. No**	**R^2(adj)**
Ethanol (Eth)	d_{20} = 0.9976 – 0.001237 Eth (%vol)	1a	99.68%
	d_{20} = 0.9963 – 0.005663 Eth (m)	1b	99.75%
Glucose (Gluc)	d_{20} = 0.9977 + 0.000392 Gluc (g/L)	2a	99.98%
	d_{20} = 0.9980 + 0.06646 Gluc (m)	2b	99.97%
Glycerol (Glyc)	d_{20} = 0.9979 + 0.000222 Glyc (g/L)	3a	99.92%
	d_{20} = 0.9982 + 0.01874 Glyc (m)	3b	99.86%
Tartartic acid (Tart)	d_{20} = 0.9980 + 0.000471 Tart (g/L)	4a	99.75%
	d_{20} = 0.9980 + 0.06967 Tart (m)	4b	99.72%

According to the above equations, for every 1 mol/kg H_2O increase of ethanol concentration, density reduces by 0.005663. It must be noted that this linear reduction applies to aqueous solutions and ethanol concentrations of up to 45%vol, as for higher concentrations ethanol acts as a solvent. This reduction is due to the fact that ethanol is a liquid with lower density than water ($d_{ethanol}$= 0.7895 g/mL and d_{water}= 0.9979 g/mL at 20 ºC) and appropriate calculations lead to the conclusion that the addition of 1mL of ethanol in water reduces total volume by 0.08mL ($V_{total} = V_{eth} + V_{water} - 0.08$). Studies have reported that while ethanol produces significant changes in density and especially in viscosity, when it is measured instrumentally, it did not have an effect on wine body perception [6].

Glycerol is also a liquid, but with a higher density than water (d_{glyc}= 1.261 g/mL at 20 ºC). According to above equations, for every 1 mol/kg H_2O glycerol increase, density increases by 0.01874. This also applies to aqueous solutions and for glycerol concentrations of up to 45%w/w, as it has also been shown by [15]. For higher concentrations, glycerol acts as a solvent and water as a solute. As above, appropriate calculations lead to the conclusion that an 1g glycerol addition in water reduces total volume by 0.013mL ($V_{total} = V_{glyc} + V_{water} -0.013$).

On the other hand, glucose and tartaric acid are both solids and according to equations, both increase density when added in water and this linear increase applies to all aqueous solutions. Particularly, for every 1 mol/kg H_2O glucose, density increases by 0.06646 and for every 1 mol/kg H_2O tartaric acid, density increases by 0.06967.

Tartaric acid is a solid with high polarity, same as glucose, but its molecules occupy less volume in the solution, compared to glucose molecules. This means that more molecules of tartaric acid may fit in 1mL water, than glucose in the same water volume, affecting the final density of the solution. Both tartaric acid and glucose, when added in water, increase the solution volume. Particularly, proper calculations from all of the one-component equations lead to the conclusion that 1 g glucose increases volume by 0.65 mL, whereas tartaric acid by 0.5mL.

In conclusion, tartaric acid has the highest effect in density, whereas glycerol has the least effect. The linear dependence of the concentration of each component with density resulting from equations 1b-4b is shown in Figure 1.

3.1.2 Two component model aqueous solution

The equations that emerged from the regression analysis of the two component model aqueous solutions are shown in Table 2. In each case p-value was under 0.001. Response surface plots are shown in Figure 2.

3.1.3 Three and four component model aqueous solution

The equations that emerged from the regression analysis of the three component model aqueous solutions are shown in Table 3. In each case p-values was under 0.001.

Each solute itself has its own coefficient of correlation with density, which changes in the presence of other solutes. The ANOVA analysis of the correlation coefficients in the presence of other substances enables us to ascertain whether the change in the values of the coefficient due to the presence of other solutes is statistically significant or not. The one-way ANOVA analysis (Figure 3) of the partial correlation coefficients of each component versus the number of components of the aqueous solutions, showed no statistically significant difference between groups ($\alpha = 0.5$) for ethanol ($F = 0.19$, $p = 0.895$), glucose ($F = 0.22$, $p = 0.876$), glycerol (F =

0.21, p = 0.882) and tartaric acid (F = 1.0, p = 0.480). No statistically significant difference was also found by Tukey Pairwise Comparisons (Figure 4).

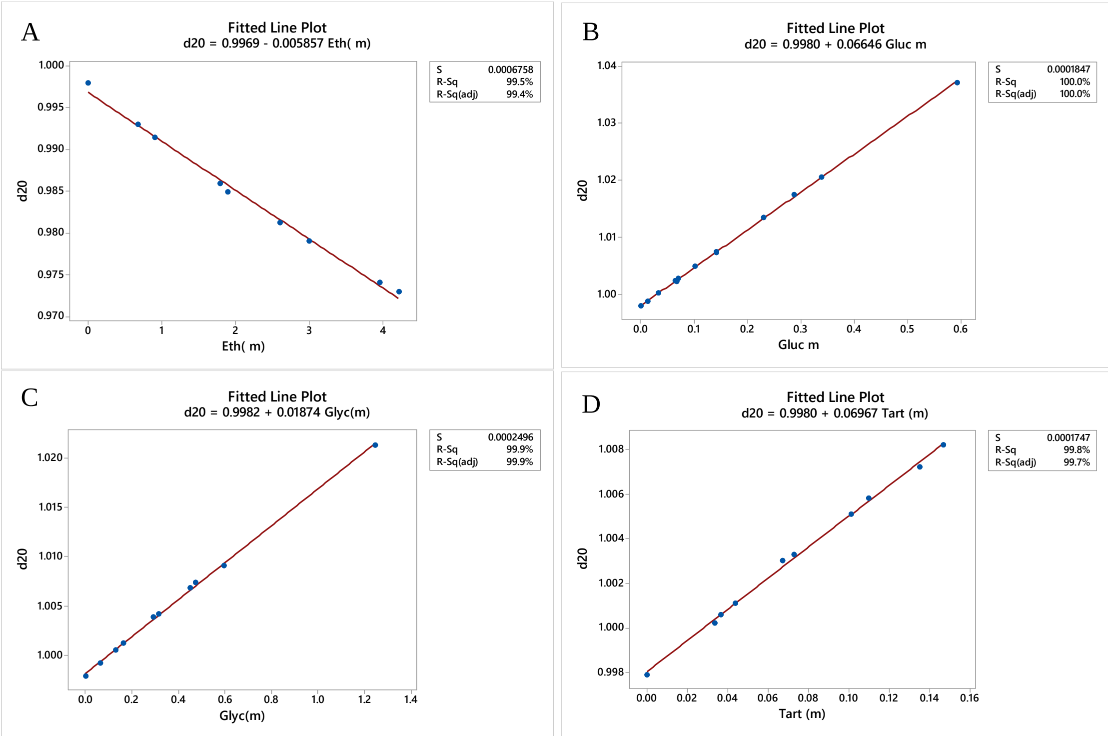

Figure 1. Effect of ethanol (A), glucose (B), glycerol (C) and tartaric acid (D) concentration (m) on the density of aqueous solutions of each component, at 20 °C.

Table 2. Equations emerged from the regression analysis of the two component model aqueous solutions

Components	Equation	Eq. No	R^2(adj)
Eth-Gluc	d_{20} = 0.9953 - 0.001232 Eth (%vol) + 0.000423 Gluc (g/L)	5a	90.73%
	d_{20} = 0.9948 - 0.006079 Eth (m) + 0.0675 Gluc (m)	5b	90.88%
Eth-Glyc	d_{20} = 0.9977 - 0.001289 Eth (%vol) + 0.000258 Glyc (g/L)	6a	99.73%
	d_{20} = 0.9974 - 0.006056 Eth (m) + 0.01846 Glyc (m)	6b	97.42%
Eth-Tart	d_{20} = 0.9978 - 0.001268 Eth (%vol) + 0.000397 Tart (g/L)	7a	99.95%
	d_{20} = 0.9974 - 0.005523 Eth (m) + 0.0350 Tart (m)	7b	99.05%
Gluc-Glyc	d_{20} = 0.9980 + 0.000387 Gluc (g/L) + 0.000218 Glyc (g/L)	8a	99.96%
	d_{20}= 0.9981 + 0.06834 Gluc (m) + 0.01698 Glyc (m)	8b	99.67%
Gluc-Tart	d_{20} = 0.9979 + 0.000156 Gluc (g/L) + 0.000457 Tart (g/L)	9a	99.97%
	d_{20}= 0.9980 + 0.02708 Gluc (m) + 0.06704 Tart (m)	9b	99.92%
Glyc-Tart	d_{20} = 0.9980 + 0.000233 Glyc (g/L) + 0.000437 Tart (g/L)	10a	99.84%
	d_{20} = 0.9981 + 0.02073 Glyc (m) + 0.06436 Tart (m)	10b	99.77%

3.2 Wine samples

Density of samples of dry white and red wines was measured and the equations of density that resulted from the regression analysis of the experimental data are shown in Table 4.

Although equations 15a, R1 and W1 refer to the same chemical components in aqueous solutions, red and white wine samples, respectively, they cannot be compared due to the fact that wine contains many other chemical substances in small concentrations that are not included in the aqueous solutions. The differences in the coefficients of tartaric acid are due to the fact that wine contains many other organic acids expressed as tartaric acid that alter its density. Thus, there cannot be a safe conclusion of how each principal component of wine affects its density.

Nevertheless, the knowledge of the factors affecting density of wine has an important practical value for the estimation of the fermentation kinetics. The quantitative correlation of wine components to the density can be a useful tool in predicting the density during the fermentation process of wines.

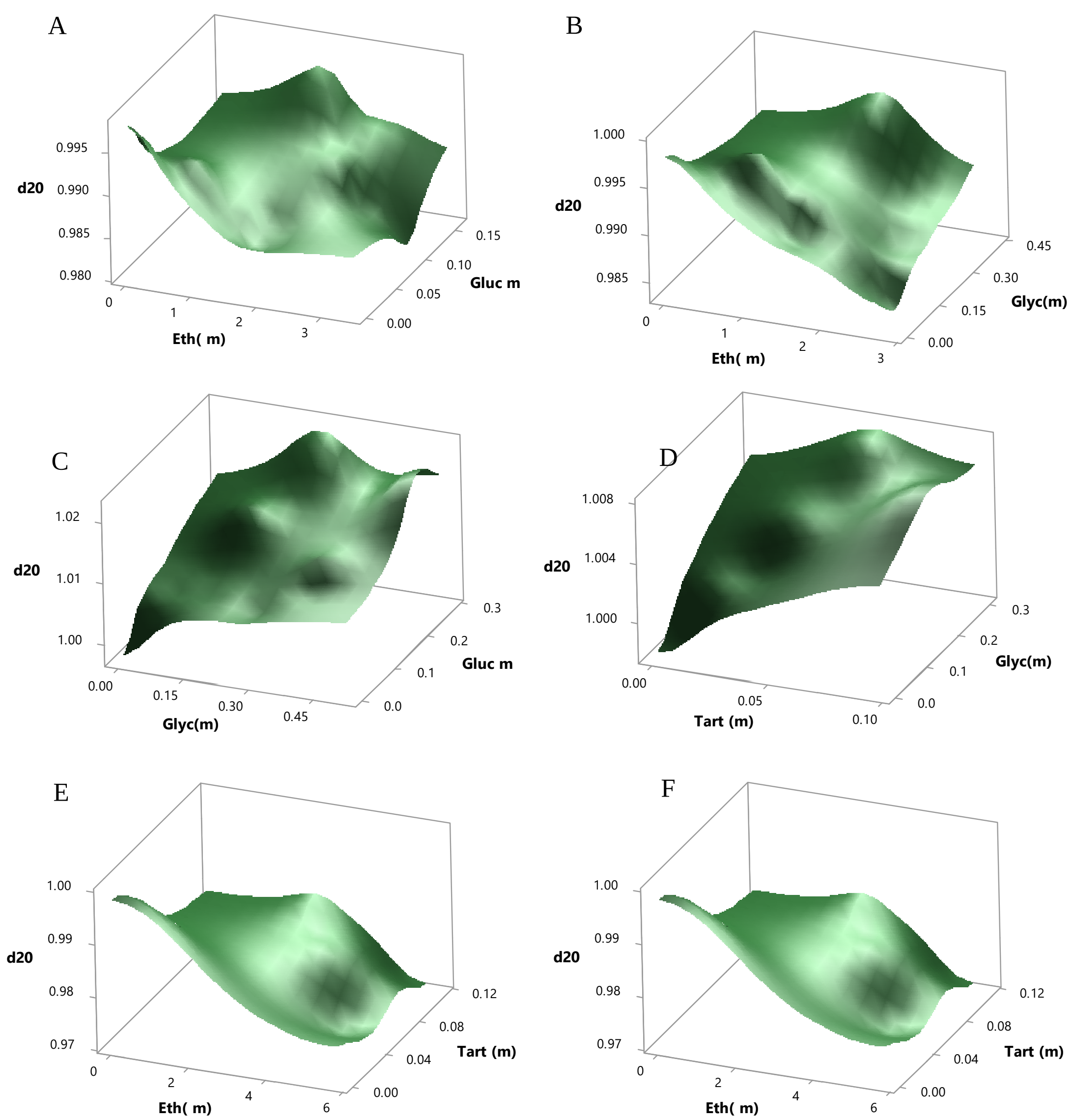

Figure 2. Response surface plots of density (d20) and A: Glu(m)-Eth(m), B: Gly(m)-Eth(m), C: Glu(m)-Gly(m), D: Gly(m)-Tart(m), E: Tart(m)-Eth(m) and F: Gluc(m)-Tart(m)

Table 3. Equations emerged from the regression analysis of the three and four component model aqueous solutions

Compo-nents	Equation	Eq. No	R^2(adj)
Eth-Gluc-Glyc	d_{20} = 0.9968 - 0.001325 Eth (%vol)+ 0.000384 Gluc (g/L) +0.000335 Glyc (g/L)	11a	97.24%
	d_{20} = 0.9954 - 0.006060 Eth(m) + 0.0578 Gluc(m) + 0.02529 Glyc(m)	11b	93.75%
Eth-Gluc-Tart	d_{20} = 0.9973 - 0.001264 Eth(%vol) + 0.000384 Gluc(g/L) + 0.000388 Tart (g/L)	12a	99.63%
	d_{20} = 0.9970 - 0.005802 Eth (m) + 0.0598 Gluc (m) + 0.0385 Tart (m)	12b	98.70%
Eth-Glyc-Tart	d_{20} = 0.9964 - 0.001145 Eth (%vol) +0.000155 Glyc (g/L) + 0.000404 Tart (g/L)	13a	97.78%
	d_{20} = 0.9960 - 0.005215 Eth (m) + 0.01369 Glyc (m) + 0.0396 Tart (m)	13b	97.13%
Gluc-Glyc-Tart	d_{20} = 0.9978 + 0.000399 Gluc (g/L) + 0.000218 Glyc (g/L) + 0.000443 Tart (g/L)	14a	99.75%
	d_{20} = 0.9978 + 0.06931 Gluc (m) + 0.01916 Glyc (m) + 0.06534 Tart (m)	14b	99.8%
Eth-Gluc-Glyc-Tart	d_{20} = 0.9981 - 0.001196 Eth (%vol) + 0.000413 Gluc (g/L) + 0.000212 Glyc (g/L) + 0.000296 Tart (g/L)	15a	98.69%,
	d_{20} = 0.9978 - 0.005636 Eth (m) + 0.06670 Gluc (m) + 0.01543 Glyc (m) + 0.03152 Tart (m)	15b	98.69%,

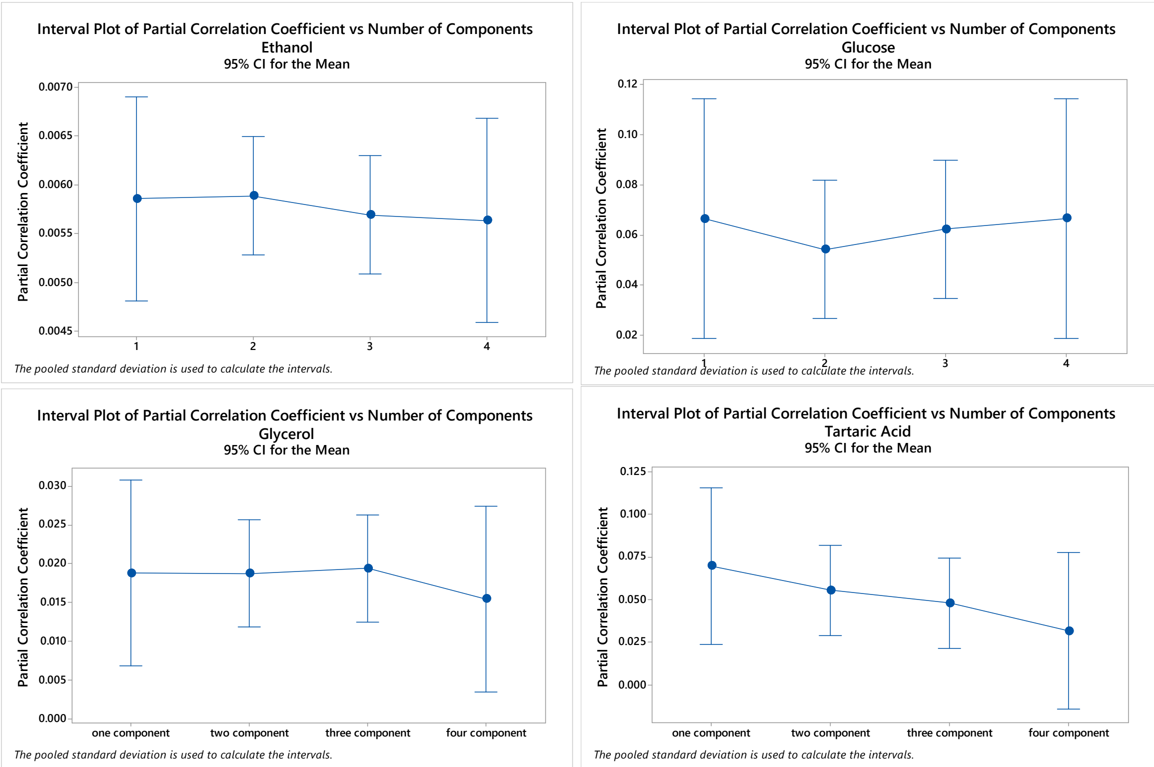

Figure 3. One-way ANOVA analysis of the partial correlation coefficients of each component versus the number of components of the aqueous solutions

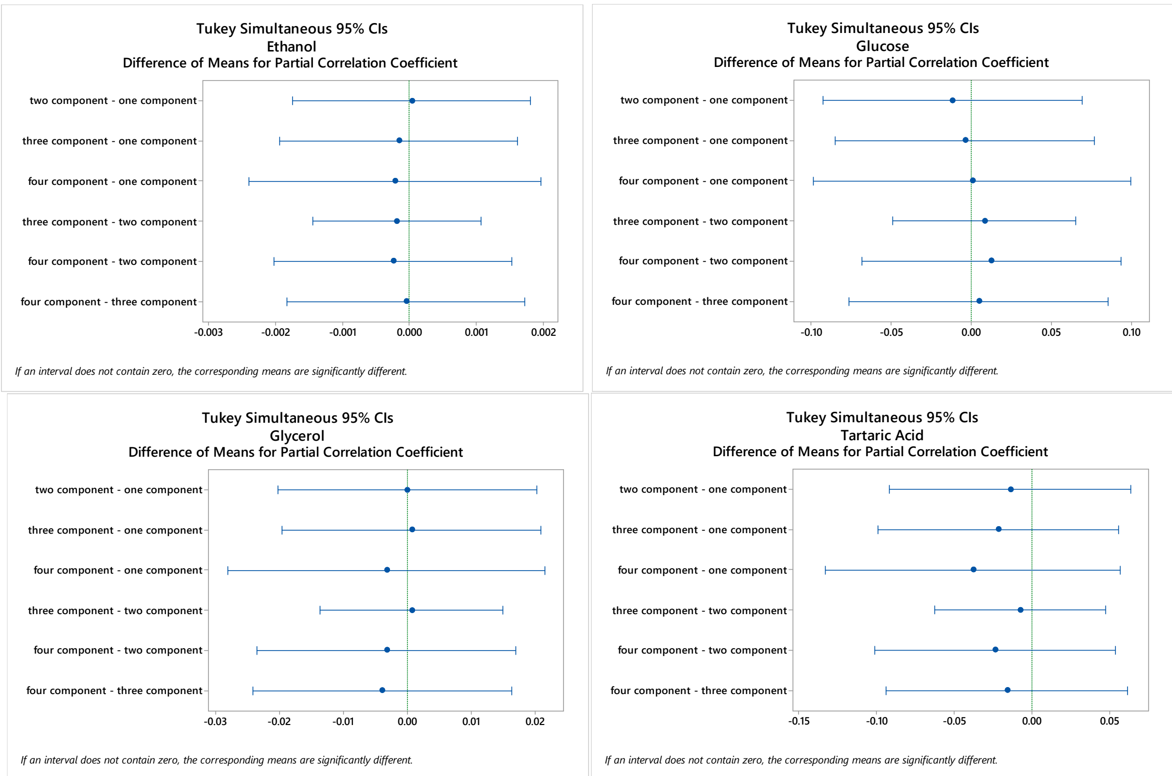

Figure 4. Tukey's HSD post hoc test. Difference of means for the partial correlation coefficient.

During fermentation, where glucose is converted to ethanol and CO_2, which –as a gas is removed- the mass is significantly reduced, resulting in a decrease in density. The decrease in density continues up to low sugar concentrations and then stabilizes before the completion of the fermentation. Fermentation kinetics can be monitored by continuous density measurement. The comparison of the measured density during the fermentation process with the values of the theoretical curve calculated based on equations 15a and R1 for a hypothetical must, provides useful information about the evolution of the fermentation (Figure 5).

Table 4. Equations emerged from the regression analysis of wine samples.

Wine Sample	Equation	Eq. No	R^2(adj)
Red	d_{20} = 0.9986 - 0.000943 *Eth* (%vol) + 0.000429 *Gluc* (g/L) + 0.000259 *Glyc* (g/L) + 0.000542 *TotAc* (g/L)	R1	99.53%,
	d_{20} = 0.9986 - 0.005503 *Eth* (mol/L) + 0.07730 *Gluc* (mol/L) + 0.023823 *Glyc* (mol/L) + 0.08113 *TotAc* (mol/L)	R2	99.53%,
White	d_{20} = 0.9981 - 0.000943 *Eth* (%vol) + 0.000429 *Gluc* (g/L) + 0.000259 *Glyc* (g/L) + 0.000535 *TotAc* (g/L)	W1	99.98%,
	d_{20} = 0.9981 - 0.005506 *Eth* (mol/L) + 0.077274 *Gluc* (mol/L) + 0.023868 *Glyc* (mol/L) + 0.08037 *TotAc* (mol/L)	W2	99.98%,

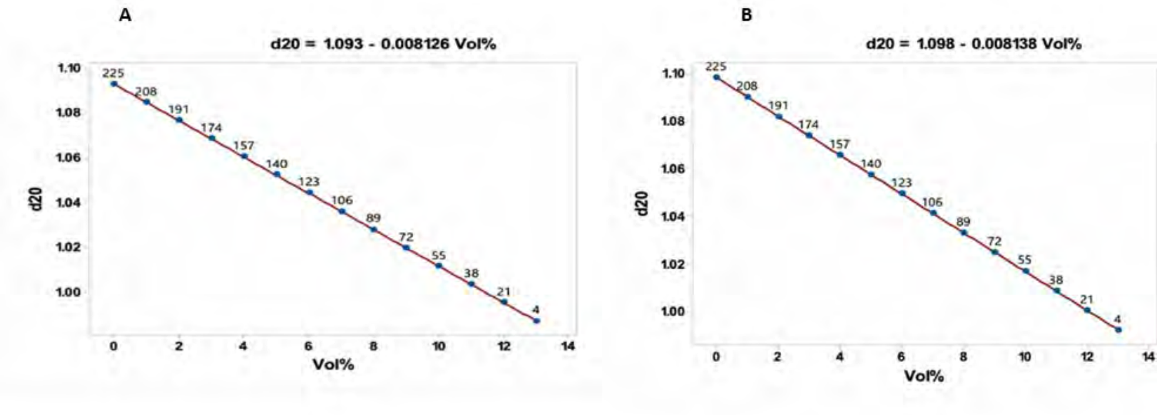

Figure 5. The theoretical fermentation monitoring curves by density measurement, based on (A) model aqueous solution and (B) dry white wine samples.

4. Conclusions

We measured the density of wine samples and model aqueous solutions consisting of ethanol, tartaric acid, glucose/fructose and glycerol. The different physical condition of the dissolved substances (solid or liquid) affects the volume and concentration of the final solution. It is known that, ethanol and glycerol are liquids with lower and higher density than water, respectively, whereas glucose and tartaric acid are solids. Each of these components affects the final volume and concentration of the solution, when added in water, in a different way. Tartaric acid was found to produce the greatest increase in the density, followed by glucose and glycerol, while ethanol decreased density. The resulting regression models for both aqueous solutions and wine samples had a coefficient of determination (R squared) over 97.3%.

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