Analysis of volatile compounds in low alcoholic wines obtained by reverse osmosis of grape must

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Abstract. Climate changes, advanced viticultural practices and modern winemaking techniques led to the gradual production of wines with high alcohol concentrations. Although ethanol is indispensable for the aging, stability and organoleptic properties of wine, its negative psychological and physiological effects on human health cannot be overlooked. The aroma and taste of beverages are critical to their acceptance by consumers, and the primary determinants of aroma and taste are volatile compounds. The purpose of this study was to evaluate the variation of volatile compounds in low alcohol wines obtained by reverse osmosis of grape must, controlled blending of permeate and retentate and subsequent fermentation. Muscat Ottonel grape must (Copou-Iași wine center, Romania) was used for obtaining six experimental variants with alcohol concentrations between 3.50 and 8.50% vol. By gas chromatography (OIV-MA-AS315-27 method) in low alcohol wine headspace were identified and quantified nine volatile compounds, mainly esters and higher and inferior alcohols. Ethyl acetate was found in the highest concentration (22.88-34.24 mg L^{-1}), while acetone showed the lowest concentration in all variants. However, acetone concentration increased with the alcoholic strength of the samples (up to 0.81 mg L^{-1}). The results indicated that volatile composition of the low alcoholic wine obtained by reverse osmosis is very complex and varies with the change in alcoholic concentration; however, the synthesis of some volatile compounds can be favored in wines without the protection of high ethanol concentration.
Key Words: gas chromatography, grape must, headspace, reverse osmosis, volatile compounds.

Introduction. Wine is defined as the food product obtained exclusively by fermentation of grapes or grape must, with an alcoholic strength of minimum 8.5% (v/v). The alcoholic concentration of wines has increased progressively, a trend attributed to climate changes (a gradual increase in average air temperatures) and afterwards to new developed viticultural practices, improved plant material and modern vinification techniques. All these changes led primary to obtaining elevated sugar levels in grapes and furthermore to an increase in alcohol content of wines. Besides its negative psychological and physiological effects on human health, ethanol is indispensable for the aging, stability and organoleptic properties of wine (Ozturk & Anli 2014). Considering all these aspects, the production of wines with low ethanol concentration has become a great challenge for wine producers that have gained considerable attention over the past 20 years.

Membrane technologies applied before alcoholic fermentation have as purpose the reduction of grape must sugar. In principle, reverse osmosis is a filtration method that removes water from unfermented grape must (semi-permeable polymer membrane), used since 1970 (Schmitt & Christmann 2019). Water molecules are the smallest components of the grape must and can pass from the high concentration solution across the membrane to the lower concentration solution (at low temperatures). A transmembranar pressure of up to 90 atm removes water from the must through the membrane pores (permeate), while the aroma, sugar and tannin molecules are not filtered (retentate). Low alcohol wines are obtained from the blending in different proportions of the two phases resulting from the osmosis process (permeate and retentate) and subsequent fermentation. Beside the fact that previous experiments did not report a significant change in the concentrated must characteristics, one of the important problems of obtaining low-alcohol wines by membrane processes is the variation of volatile compounds concentrations. The type and concentration of the synthesized volatile substances are dependent on multiple factors, like the nitrogen concentration of must, fermentation temperature or yeast strain (Molina et al 2007).

The aroma and taste of wine are critical to their acceptance by consumers, and the primary determinants of aroma and taste are volatile compounds (Arellano et al 2012). There are several methods for determining volatile compounds concentration that does not require solvents for extraction. As principle, wine sample is heated to the desired temperature in a closed vial while the volatile compounds are vaporized, until equilibrium is reached between the sample and the free space. Volatile compounds are injected into the gas chromatograph (GC), this method being known as headspace extraction (Kolb & Ettre 1997). GC headspace analysis allows quick and low cost evaluation of the volatile compounds responsible for the odor of the low alcohol wines. As disadvantage, this procedure is restricted to samples where the volatile compounds to be evaluated are not retained in the sample matrix (Arellano et al 2012).

The purpose of this study was to evaluate the volatile composition in the headspace of low alcohol wines obtained by reverse osmosis of grape musts and to highlight the differences that can occur between wines with different ethanol concentrations.

Material and Method. In order to obtain the experimental variants, Muscat Ottonel variety grapes, harvested at full maturity from the Copou Iasi viticultural center, were crushed and destemmed in the industrial winemaking system. Grape must (1000 L) was clarified by flotation (Viazym Flot; Martin Viallate) until a value of turbidity <400 NTU (nephelometric turbidity units) and subjected to the reverse osmosis process using an automated Bucher Vaslin Flavy ML 2 reverse osmosis unit. The permeate used for subsequent dilutions was obtained by passing the grape must through the semipermeable membranes of the reverse osmosis unit at a pressure of 60 bar, a temperature of 17°C, at a flow rate of 850-890 L $hour^{-1}$. By calculation, osmosis water (permeate) was added to the retentate in certain proportions in order to obtain six musts with sugar concentrations between 60 and 145 g L^{-1}. After alcoholic fermentation were obtained six experimental wines with alcohol concentrations of 3.50, 4.50, 5.50, 6.50, 7.50 and 8.50% vol. The control sample was represented by the wine obtained through the classic winemaking technology (12.50% vol).

The main physico-chemical parameters of wines were analyzed acording to the Compendium of international methods of wine and must analysis (OIV 2019).

Volatile compounds were quantified using an Agilent 7890A gas chromatograph with a sensitive flame ionization detector (FID). The determination of volatile compounds was conducted according to the method OIV-MA-AS315-27: analysis of volatile compounds in wines by gas chromatography (OIV 2019). The method was adapted for headspace analysis.

Table 1

Peak integration and coefficients of determination for the calibration curves of pure volatile compounds (GC-FID)

Volatile compounds	*Peak integration (min.)*		*Coefficient of determination for calibration curves*
	Start time	*End time*	
Acetaldehyde	3.84	4.17	0.99733
Acetone	5.48	5.71	0.99832
Ethyl acetate	7.36	7.85	0.99801
Methanol	7.85	8.40	0.99896
Propan-1-ol	14.31	14.77	0.99793
2-methyl-propan-1-ol	17.46	17.93	0.99819
Isoamyl acetate	18.77	19.39	0.99551
Butan-1-ol	20.45	20.69	0.99651
Ethyl lactate	33.45	34.02	0.99129

Volatile compounds were quantified using a capillary column coated with a bonded polar phase. Each compound was identified and quantified based on calibration curves obtained previously using pure standard compound. Coefficients of determination of calibration curves used for the quantitative determination of volatile compound varied from 0.99129 to 0.99896 (Table 1).

Results and Discussion

Physico-chemical characteristics of low alcohol beverages. Although the minimum alcohol concentration of wine required by current legislation is 8.50 % vol., the sample obtained can be called low-alcohol wines or low-alcoholic beverages obtained by grape must fermentation. The alcoholic concentration of the wine samples was similar to the one designed by initial calculation (different retentate/permeate proportions). Ethanol content assessed by distillation was very close to the projected concentrations (Table 2).

Total acidity is represented by organic and inorganic acids, free or bound as salts. A suitable total acidity should be between 4.5 and 7.5 g tartaric acid L^{-1}, amplifying the freshness of the wine and the fruity notes. In the tested low-alcohol wines, due to the addition of permeate (water) the acidity of the wines was low, increasing with the upraise of the proportion of must and implicitly of the alcohol content of wines (2.81-3.48 g tartaric acid L^{-1}), further corrections being necessary to improve the acidity. Control wine, obtained by conventional must fermentation, showed a low acidity, specific to the analyzed variety (Muscat Ottonel).

The volatile acidity of the samples was very low (0.26-0.28 g acetic acid L^{-1}), in the same time, the concentrations of SO_2 used ensured the microbiological and antioxidant protection of the low alcohol wine samples. Concentration of reducing sugars was reduced in all samples (dry wines), the highest amounts of residual sugars being determined for the control wine, 0.70 g L^{-1}.

Excepting the alcoholic concentration, the physico-chemical parameters of the experimental wines were within the ranges of values provided by the current Romanian legislation.

Analysis of volatile compounds*.* The identified and quantified volatile compounds of low alcohol wines are presented in Table 2.

Acetaldehyde (or ethanal) is the most important volatile wine carbonyl compound and can be formed both biologically (through yeast activity) and chemically (by wine oxidation). In wine alcoholic fermentation acetaldehyde formed by decarboxylation of pyruvic acid cannot be converted entirely to ethyl alcohol. A small part of acetaldehyde participates in the formation of secondary chemical compounds, such as: glycerol, 2,3-butylene glycol, lactic, acetic, citromalic, propionic, succinic, fumaric acids or acetoin (Țârdea 2007). In the experimental low alcohol wine obtained by reverse osmosis of Muscat Ottonel grape must acetaldehyde concentration varied significantly, between 3.62 (V3-5.50) and 14.72 mg L^{-1} (V1-3.50). In wines affected by aerobic diseases acetaldehyde is the most important compound, formed in large amount (up to 200 mg L^{-1}) and may affect the wine aroma. High concentrations of acetaldehyde are usually associated with a negative impact on wine quality. At lower concentrations it can impart a fruity flavour to wine, which often occur in freshly fermented wine. At higher concentrations, from 100 to 120 mg L^{-1}, it can be pungent and is often associated with bruised apple, sherry, nuts and oxidation (Byrne & Howell 2017).

Ethyl acetate is the most important neutral ester formed in wine. Although the threshold for olfactory perception of ethyl acetate in wine is between 180-200 mg L^{-1}, in healthy wines ethyl acetate content ranges from 0.5 to 1.5 mg L^{-1} (Țârdea 2007). Ethyl acetate is formed both during alcoholic fermentation and during the storage/aging of wines. The esterification process is performed by the activity of yeasts and acetic bacteria (using esterases). Acetic bacteria have the highest esterogenic capacity. In all low alcohol wines analyzed ethyl acetate was found in the highest concentration (22.88-34.24 mg L^{-1}).

Table 2

Physico-chemical parameters of low alcoholic wine obtained by reverse osmosis

Parameter	*Low alcohol wine samples*						
	Control	*V1-3.50*	*V2-4.50*	*V3-5.50*	*V4-6.50*	*V5-7.50*	*V6-8.50*
Alcohol (% vol)	12.50	3.56	4.52	5.56	6.52	7.55	8,52
Total acidity (g $C_4H_6O_6$ L^{-1})	4.52	2.81	2.92	3.04	3.11	3.22	3.48
Volatile acidity (g $C_2H_4O_2$ L^{-1})	0.33	0.28	0.27	0.26	0.28	0.26	0.26
Free SO_2 (mg L^{-1})	12	18	16	14	19	17	16
Total SO_2 (mg L^{-1})	60	82	79	76	68	64	72
pH	3.39	3.29	3.24	3.24	3.22	3.25	3.28
Sugars (g L^{-1})	0.70	0.26	0.64	0.28	0.38	0.42	0.49
Density (g L^{-1})	0.9900	0.9968	0.9958	0.9950	0.9934	0.9928	0.9916
Non-reducing extract (g L^{-1})	16.70	4.90	5.90	7.40	6.40	8.50	8.50

Table 3

Volatile compounds identified and quantified in the Muscat Ottonel low alcohol wines headspace

Volatile compounds (mg L^{-1})	*Low alcohol wine samples*							*Mean*	±
	Control	*V1-3.50*	*V2-4.50*	*V3-5.50*	*V4-6.50*	*V5-7.50*	*V6-8.50*		
Acetaldehyde	10.64	14.39	14.72	3.26	4.05	5.33	5.15	8.22	4.93
Acetone	0.71	nd	nd	0.59	0.56	0.64	0.81	0.66	0.10
Ethyl acetate	35.60	31.54	30.47	22.88	34.24	32.4	30.41	31.08	4.09
Methanol	23.22	27.45	26.95	26.83	28.89	33.63	28.14	27.87	3.11
Propan-1-ol	19.04	7.19	nd	10.85	13.95	14.74	10.82	12.77	4.08
2-methyl-propan-1-ol	15.84	10.83	13.53	17.87	21.84	21.00	15.48	16.63	3.94
Isoamyl acetate	5.75	3.82	3.97	4.14	4.67	5.03	4.58	4.57	0.67
Butan-1-ol	1.02	nd	nd	0.86	0.93	0.93	0.88	0.92	0.06
Ethyl lactate	27.40	17.92	20.31	21.45	28.22	30.14	27.25	24.67	4.68

nd - not detected; ± - standard deviation (between experimental variants).

In Figure 1 is presented the GC-FID chromatogram of volatile compounds found in low-alcohol wines obtained by reverse osmosis of Muscat Ottonel grape must. As can be observed on the chromatogram ethyl acetate predominates in the analyzed wines.

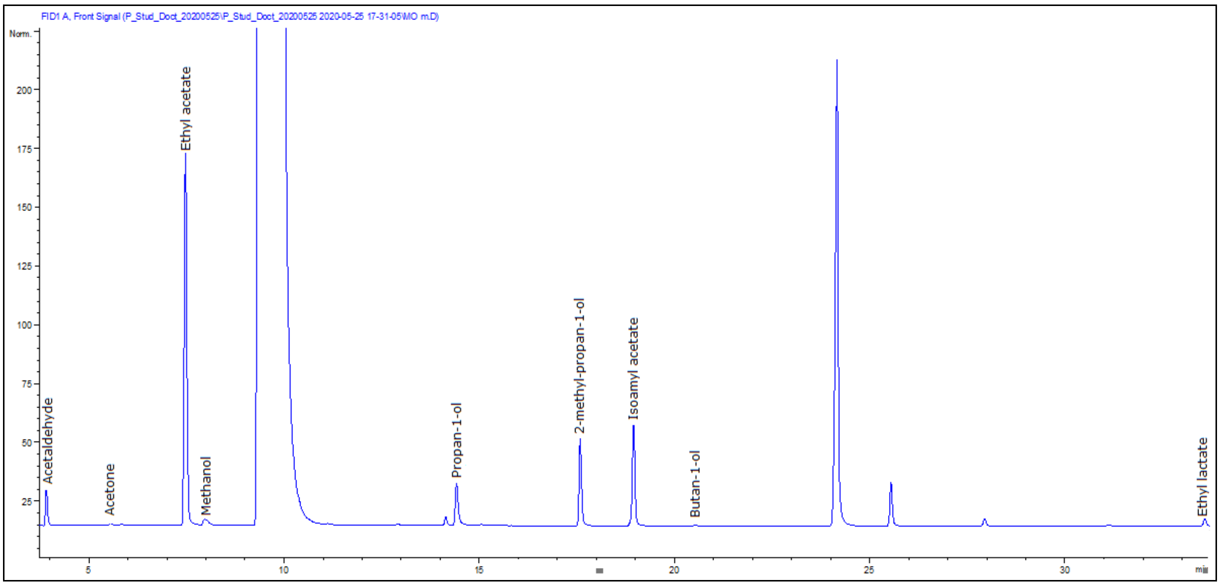

Figure 1. GC-FID chromatogram of volatile compounds found in low-alcohol wines obtained by reverse osmosis of Muscat Ottonel grape must.

Ethanol is indispensable for the stability and organoleptic properties of wine (Ozturk & Anli 2014). The lack of high concentrations of ethanol in wine can led to a microbiological instability and an increase in ethyl acetate and acetic acid concentrations. The contribution of acetic bacteria to the formation of ethyl acetate in wine is much greater than that of yeasts. Long contact of the wine with oxygen is the decisive factor.

Isoamyl acetate is usually derived from yeast metabolism during the alcoholic fermentation. This compound contributes to a banana-like note and gives complexity to white wines (Cotea et al 2021). Isoamyl acetate concentrations varied within small limits between samples (average value 4.57±0.67 mg L^{-1}) as well as butan-1-ol (average value 0.92±0.06 mg L^{-1}). 1-butanol (butan-1-ol), also known as n-butanol, is a primary alcohol that occurs naturally as a minor product in sugars fermentation and is present in most alcoholic beverages (Hazelwood et al 2008).

Isobutyl alcohol (2-methyl-propan-1-ol) is a volatile higher alcohol, which is formed during alcoholic fermentation by the nitrogenous metabolism of yeasts. It gives to wine the "vinous" character. In low-alcohol wines tested, isobutyl alcohol was found in higher concentrations in samples with higher ethanol concentrations, where the proportions of must were higher and the fermentation last longer (V3 - 17.87 mg L^{-1}; V4 - 21.84 mg L^{-1}; V5 - 21.00 mg L^{-1}). Isobutyl alcohol is ussually present in wines in concentration up to 43-54 mg L^{-1}, along with 1-propanol (7.5-20.5 mg L^{-1}) and hexanol (0.19-0.21 mg L^{-1}). Although they are present in large quantities, their olfactory impact on the aroma is weak, the olfactory perception threshold being very high (800 mg L^{-1} for 1-propanol and 200 mg L^{-1} for isobutyl alcohol) (Țârdea 2007).

In normal conditions, during alcoholic fermentation are formed neutral esters of lactic acid, such as ethyl lactate, which gives a pleasant aroma to the wine. In low alcohol wines ethyl lactate showed higher concentrations in comparison to the other compounds identified, also showing a low variation between samples (24.67±4.68 mg L^{-1}).

Methanol is an inferior alcohol also formed during alcoholic fermentation by demethylation of pectins by enzymes. Normally, it is present in white wine in a very small amount of only 150 mg L^{-1}. Methanol is produced before and during alcoholic fermentation from the enzymatic hydrolysis of fruit pectins. More methanol is produced when grape must is fermented on skins (maceration-fermentation). In our experiment, methanol concentrations varied in very small limits, with an average value of 27.87±3.11 mg L^{-1}. An essential factor for an optimal reverse osmosis process is the high clarity of the must. For this reason, the maceration of grapes has been avoided, although this process is generally recommended for aromatic grape varieties.

A possibility to form acetone in wine is the oxidation of citric acid (Țârdea 2007). Acetone was identified in the lowest concentration in all low alcohol wines, however, increasing with the alcoholic strength of the samples (up to 0.81 mg L^{-1}).

Conclusions. Volatile composition in headspace of low alcohol wines obtained by reverse osmosis of Muscat Ottonel grape must was complex, nine compounds being identified and quantified using gas chromatography with flame ionization detector (GC-FID). The identified compounds belonged mainly to the class of esters, higher and lower alcohols, most of them being normally synthesized during alcoholic fermentation. Ethyl acetate, compound specific to the processes of microbial degradation of wine, was found in the highest concentration, while acetone was present in the lowest concentrations in all experimental variants, however, increasing with the alcoholic strength of the samples. The results indicated that volatile composition of the low alcoholic beverages headspace is very complex and vary with the change in alcoholic concentration. Also, the synthesis of some negative volatile compounds can be favored in wines without the protection of high ethanol concentrations. Regarding the physico-chemical parameters of wines, the decrease of alcoholic concentration was correlated with a decrease in total acidity and non-reducing extract (due to the higher proportion of permeate), and an increase in wine density.

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