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Research Paper

Membrane Processing of Grape Must for Control of the Alcohol Content in Fermented Beverages

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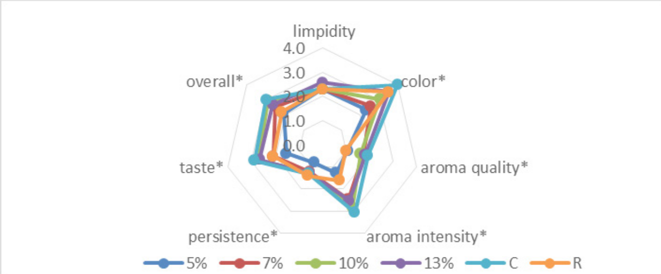
Highlights

- Grape must was concentrated by RO, during 55 minutes, from 23.7 ºBx to 28.5 ºBx.
- The average permeate flux was 2.12 L.m^{-2}h^{-1}.
- The must reconstitution was carried out to obtain fermented beverages with an alcohol volume content of 5%, 7%, 10% and 13% (v/v).
- Beverages with low alcohol content had a lower content of polyphenols compounds which influenced the sensory evaluation.

Graphical abstract



Abstract

The great demand of beverages, both alcohol-free and with low alcohol content, is a great challenge for the production of beverages with controlled alcohol content through the use of sustainable enological practices. The present work addresses this challenge with the processing of grape must by reverse osmosis (RO) for must reconstitution with different sugar contents prior to the alcoholic fermentation. The original must came from grapes grown in Quinta do Quinto, in Santarém, collected after destemming and mechanic crushing, and preserved in a refrigerated chamber at -1.6 °C until processing by RO. The RO processing was carried out in Escola Superior Agrária de Santarém, with a pilot plant equipped with RO spiral wound modules, M38RO, from Alfa Laval, Denmark. The total membrane permeation area is 15 m^2. The work pressure was 55 bar. The original must had 23.7 ºBrix, a density of 1108 g.L^{-1}, 15.2% (v/v) of probable alcohol, and a conductivity of 2.01 mS.cm^{-1}. The must reconstitution was carried out, by mixing the concentrated grape must with the vegetal water produced by RO (permeate) to obtain beverages with a nominal alcohol content of 5%, 7%, 10% and 13% (v/v). The fermentation average temperature was between 18.2 and 19.7 °C, and the final density rounded about 993 g.cm^{-3}. The beverages were analysed by different parameters, including total polyphenols, total anthocyanins, colour intensity and hue, the coordinates CIELab, alcohol content, total acidity, volatile acidity, pH, free SO_2 and total SO_2. The attributes of the beverage, corresponding to the visual appearance, aroma and taste senses, as well as the overall judgment were evaluated by the tasters. The proposed method can produce beverages with controlled low alcohol content. The decrease of the alcohol content led to lower content of polyphenols compounds which influenced the sensory evaluation.

1. Introduction

The increase in the concentration of sugar in grapes and grape musts is often associated with climate change [1]. The decreasing alcohol consumption is a worldwide trend and lower consumption rates are associated with positive health benefits. This fact presents a great challenge in the production of fermented beverages with controlled content of alcohol, using sustainable practices.

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For removing ethanol from beverages, heat or membrane-based processes can be employed. A summary of the main technologies utilised to reduce alcohol levels in beverage is presented in the literature [2, 3]. The traditional methods of dealcoholisation such as distillation [4], vacuum distillation [5] and spinning cone column [6, 7] are mainly based on phase change, which may affect the aroma compounds. These techniques are giving place to alternative membrane processes, namely evaporative perstraction [8-14], pervaporation [15, 16], reverse osmosis (RO) and nanofiltration (NF) [17-28]. Innovative membrane based processes can follow a different approach of reduction of sugar in grape musts prior to alcoholic fermentation. As far as reduction of sugar in grape musts, the literature describes different processes combining UF/NF [20, 29-31], NF/NF [32, 33] and NF/RO [34] and Memstar process, which comprises NF units and a membrane contactor (Liquid-Cel®) [22, 35].

Some studies [2, 36-38] investigated the importance of partial dealcoholisation by membrane processes on beverage phenolic compounds, chromatic characteristics, volatile composition and sensory attributes. However, no data has reported on the effect of partial must reconstitution on the phenolic, chromatics and sensorial characteristics of the fermented beverages.

The aim of this project was the optimization of reverse osmosis (RO) for grape must processing and the subsequent use of the RO streams for the reconstitution of musts with lower sugar contents that will allow the production of fermented beverages with controlled lower alcohol content.

2. Material and methods

2.1. Musts

Grapes from Tinta Roriz, Syrah and Alicante Bouschet were harvested at Quinta do Quinto, in Santarém (Tejo Region, Portugal). The grapes were destemmed and crushed into a stainless-steel vessel. The original must was preserved in a refrigerated chamber at -1.6°C until processing by RO.

Reverse osmosis was performed by circulating the grape must through the membrane modules under a pressure gradient to separate the permeate, depleted of sugar, and the retentate which contains higher concentration of sugar. After separation, different formulations were performed. Retentate and permeate streams were mixed in specific proportions to obtain a must that would contain less sugar than the untreated must, and would allow the production of fermented beverages with controlled lower alcohol content, but with pleasant sensory characteristics. For such, a must reconstitution was carried out to obtain fermented beverages with an alcohol volume content of 5%, 7%, 10% and 13% $(^{v}/_{v})$, in duplicate, in 4-L tanks. The formulations were as follows: 5% $(^{v}/_{v})$ (1.75 L of retentate + 3.25 L of permeate), 7% $(^{v}/_{v})$ (2.45 L of retentate + 2.55 L of permeate), 10% $(^{v}/_{v})$ (3.5 L of retentate + 1.5 L of permeate), 13% $(^{v}/_{v})$ (4.55 L of retentate + 0.45 L of permeate). The musts were inoculated with commercial activated *Saccharomyces cerevisiae* preparation.

Untreated must (control must) and retentate were fermented, too. The alcoholic fermentation occurred during a period of ten days at 18-20 °C. When completed, the beverages were racked, bottled and analysed. The initial characteristics of the grape must are shown in Table 1.

Table 1
Initial characteristics of the grape musts.

Lots	Density	Temperature	pH
Control (untreated must)	1108±0.58	18±0	3.11±0.01
5%	1035±0.58	18±0	3.11±0.01
7%	1046±1.00	18±0	3.15±0.02
10%	1070±1.20	18±0	3.27±0.06
13%	1089±1.08	18±0	3.30±0.03
Retentate	1120±0.00	18±0	3.33+0.00

2.2. Experimental procedures

The reverse osmosis processing was carried out in the technological laboratory of the Escola Superior Agrária de Santarém. A 160 L volume of grape must was concentrated, in batch mode with an RO unit from Alpha Laval, with two sanitary spiral-wound modules RO99-3838/30 placed in series. The total active membrane area was 15 m^2 (see Figure 1).

The RO test was performed with an average transmembrane pressure of 55 bar and a feed flow rate of 5500 L/h. The temperature of the grape must increases from 4.5 ºC to 25 ºC during the experiment. A volume of 60 L of permeate and 222 L of retentate were collected during the experiment. A fraction of the retentate remained inside the RO unit and was lost during the cleaning procedure. The retentate was a sugar-rich stream and the permeate was a sugar-depleted stream.

Before and after the RO test, the membranes were cleaned with Ultrasil 11 (Henkel Ecolab) at pH 11 for about 30 minutes.

Fig. 1. Reverse osmosis unit with spiral wound modules (Alpha Laval).

2.3. Analysis of conventional oenological parameters, colour and chromatic characteristics

Alcohol content (v/v%), density, pH, titratable acidity, volatile acidity and chromatic characteristics (L*, a* and b*), as well as free and total SO_2 were determined in accordance with the official OIV methods [39].

The total polyphenol index (TPI) was measured by the absorbance at 280 nm [40], the total anthocyanins determination was done according to Ribéreau-Gayon and Stonestreet [41]. The colour intensity (absorbance at 420 nm, 520 nm and 620 nm) and tonality (A_{420} / A_{520}) were evaluated too. UV/Vis spectrophotometric methods were performed using a Perkin Elmer-LAMBDA 25 UV/Vis spectrophotometer.

2.4. Sensory analysis

The sensory analysis was performed by a trained panel consisting of 5 panelists, with extensive tasting experience. The samples were stored in appropriate light and temperature conditions. Samples were presented to the panel in tasting glasses. The selected attributes were: visual (limpidity, color), aroma (intensity, quality), taste (intensity, quality) and overall judgment. Panelists were asked to rate all attributes on a 7-point scale.

3. Results and discussion

The RO-based grape must concentration was carried out from an initial sugar concentration of 23.7º Brix to a final sugar concentration of 28.5 ºBrix in the conditions specified in Table 2.

Table 2
RO grape must characteristics.

Average Transmembrane pressure	55 bar
Initial temperature	4.4 ºC
Final temperature	24.7ºC
Grape must feed volume	300L
Concentrate volume	222L
Permeate volume	60L
Feed sugar concentration	23.7ºBx
Retentate sugar concentration	28.5ºBx
Feed conductivity	1712 $\mu S.cm^{-1}$
Retentate conductivity	1840 $\mu S.cm^{-1}$

The permeate flux decline during the time interval of 55 minutes for the operation of concentration is shown in Figure 2 and the physical-chemical characteristics are shown in Table 3. Permeate is a vegetal water, poor in sugars and rich in salts.

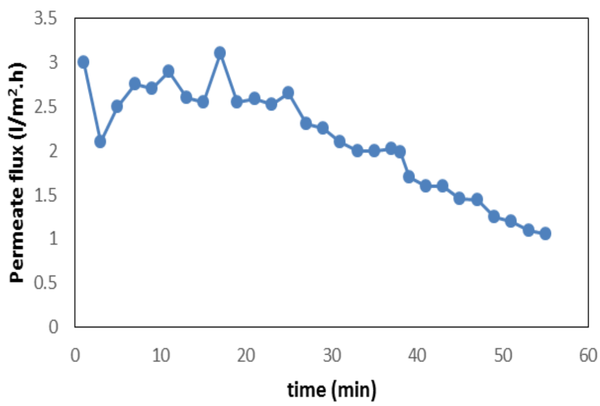

Fig. 2. Permeate flux versus time.

After the RO test, the permeate and retentate were mixed in specific proportions to obtain a must that contains less sugar than the untreated beverage (control) and an alcohol volume content of 5%, 7%, 10% and 13% (v/v). These must samples were fermented, as were the control must and the retentate. The beverages analysis were made and the results of oenological parameters of beverages are depicted in Table 4. Manipulating the initial sugar content prior to fermentation led to beverages whose final alcohol content after fermentation was what had been expected. All formulated beverages had an alcohol degree lower than the control beverage. The retentate alcohol content (15.7%) was higher than that of the control beverage, as expected due to the sugar retention.

It's very important to analyze the main characteristics of the obtained fermented beverages. The titratable acidity increased significantly ($p<0,05$) with the increasing proportion of retentate, since the retentate concentrates the acids. However, no significant differences were observed between the beverage with 13% of alcohol content and the control, and also in comparison with the retentate beverage. The increase of the pH with the rise in alcohol content is not uniform.

Regarding the volatile acidity (VA), no significant differences ($p>0,05$) were found between beverages with 5%, 7%, 10% and 13% alcohol content, although the control beverage still has significantly higher VA, probably because of the occurrence of some microbiological contamination due to its sugar content.

Table 3
Characterization of permeate over time.

Time (minutes)	Sugar (°Bx)	pH	Condutivity (μS.cm^{-1})
10	0.7	2.62	152.7
20	0.7	3.07	195.6
30	0.6	2.84	230.0
40	0.5	2.87	329.0
50	0.4	2.65	372.0

The residual sugar at the end of the fermentation is lower in the beverages with less alcohol content, probably due to the increased efficiency of the yeast during the fermentation. At the end of the fermentation, the SO_2 free and the total level were very low, as expected. Since it is consumed during fermentation, it is always necessary to add SO_2 at the end of the fermentation. The phenolic compounds have higher molecular weight than sugars, so they are highly retained. They are mainly related to the colour of the beverage.

The total polyphenol index (TPI) of the beverage with 5% of alcohol content was significantly lower than that of the ones with a higher alcohol content, even the one with 13% alcohol content had a significantly lower TPI than the control. This phenomenon may be attributed to membrane adsorption of polyphenols, since the control that is no submitted to RO has higher TPI value. The same results were reported in the literature [33], beverages with a decreased alcohol content revealed lower concentrations of phenolic compounds than in the control beverage.

Reverse osmosis affects the concentration of polyphenols and also the parameters related to it, such as the colour [36]. As could be observed in Table 4, the total anthocyanin content was significantly lower in manipulated beverages than in the control beverage. The beverages with 5% and 13% alcohol content presented 68% and 50.2% anthocyanins content loss, respectively, and when compared with the control beverage.

Regarding the colour of the beverages, the intensity increased significantly with the alcohol content, probably due to the concentration of phenolic compounds and, namely anthocyanins. These results are in accordance with those reported by Salgado et al. [36] and Garcia-Martin et al. [32].

The hue of the control beverage was significantly lower than the one of the other beverages. The brightness (L*) decreased significantly with alcohol content. In better words, the beverage with 5% was the brightest, while the colorimetric parameters a* (red) and b* (yellow) had an important decrease with the increase in alcohol. This darkening of the 13% beverage, as well as the increase in red color (has a higher a* value) and the higher yellowness (with a higher b* value) were mainly attributed to the beverage matrix, namely the polyphenolics. These results are in agreement with the values found for colour intensity and tonality.

Table 4
Beverage quality parameters.

	beverage's nominal alcohol concentration					
	control	**5%**	**7%**	**10%**	**13%**	**retentate***
Alcohol (%)	15.2e ±0.07	5.4a ±0.05	7.5b ±0.05	10.7c ±0.05	13.8d ±0.14	15.7
Titratable acidity (g/L tartaric acid)	6.6c ±0.20	3.7a ±0.30	3.9a ±0.10	5.2b ±0.1	6.3c ±0.1	6.6
Volatil acidity (g/L acetic acid)	0.72b ±0.03	0.44a ±0.05	0.36a ±0.03	0.37a ±0.05	0.44a ±0.09	0.92
pH	3.31ab ±0.00	3.27a ±0.03	3.35bc ±0.01	3.39c ±0.01	3.34bc ±0.00	3.38
Glucose + fructose (g/L)	3.2c ±0.06	0.2a ±0.49	0.5a ±0.17	1.3b ±0.11	2.6c±0.21	2.3
SO_2 F (mg/L)	0.0a ±0.0	0.0a ±0.0	0.27a ±0.18	3.2b ±1.3	4.3b ±0.8	1.6
SO_2 T (mg/L)	15.5a +3.0	9.9a ±1.0	10.13a ±2.1	12.3a +1.6	15.5a ±2.7	4.8
TPI	26.88e ±0.86	9.60a ±0,69	12.82b ±0.06	18.32c ±0.35	22.02d ±1.72	25.75
Total Anthocyanins (mg/L malvidin-3-gluc.)	141.02b ±12.38	45.08a ±5.00	50.48a +3.26	61.49a±15.94	70.26a ±2.22	48.24
Intensity	36.99d ±0.11	13.14a ±0.06	18.96b ±0.07	26.96c ±0.14	38.26d ±0.06	38.95
Tonality	0.594a ±0.00	0.686b ±0.01	0.700b ±0.01	0.691b ±0.03	0.690b ±0.00	0.786
L*	40.68a ±0.58	63.90d ±1.60	54.97c ±0.76	46.60b ±1.43	38.70a ±0.38	39.40
a*	65.35d ±0.33	40.31a ±1.23	48.49b ±0.94	57.23c ±1.53	63.05d ±0.21	59.53
b*	33.81d ±0.59	14.19a ±0.58	18.94b ±0.76	26.81c ±1.42	34.99d ±0.52	41.29

Mean value ± standard deviation of two replicates, except to retentate; different letters in a line (for each parameter) indicate significant differences (Tukey Test, $p<0.05$). TPI – total polyphenols index, * no replicates

Some authors demonstrated that the beverage matrix, mainly alcohol content may have a significantly impact on the sensory attributes [43-46].

Spider diagram obtained from average values of visual, olfactory and gustative attributes from sensory analysis of beverages with 5%, 7%, 10%, 13% alcohol content, control and retentate beverages are reported at Figure 3. Significant differences (p < 0,05) in sensory profiles of reduced alcohol beverages were perceived by the panelists, as compared with the original beverage, apart from the limpidity. The greatest variations were presented to the beverage with low alcohol content, the panelists reported that beverages with low alcohol content had lower colour, decreased aroma intensity, increased perception of some defects such as oxidation and hydrogen sulphite, and very short persistence, as well. This is in agreement with some authors [33, 44, 45] who have reported that great differences in the alcohol range, greater than 2%, produce noticeable sensory changes. The original beverage was considered to be the most balanced.

These results are not only related to the alcohol content, but also to the chemical composition of the beverages in particular of the phenolic composition. Some studies [43, 46] have found that reduction of beverage alcohol content can result in a significant decrease in the perception of beverage quality by the consumers.

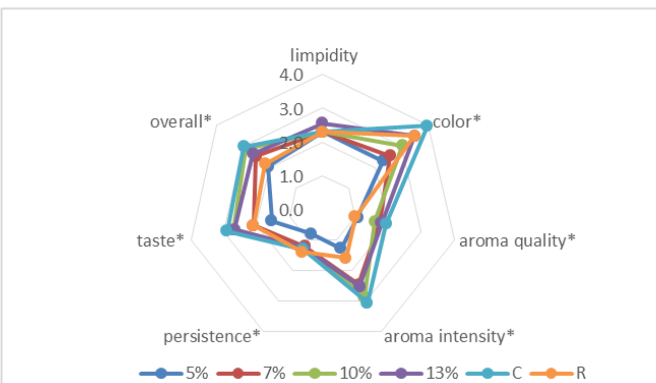

Fig. 3. Sensory evaluation of beverages with different alcohol content, control and retentate beverages. *indicates significance at $p < 0.05$.

4. Conclusions

The negative effects of alcohol on health as well as the reduction of calorie intake has led to the development of technology to reduce the alcohol content in fermented beverages in recent years.

This study provides a first report on the effect of reduced alcohol content by reconstituted grape musts, mixing permeate and retentate in different proportions, instead of the application of membrane technology in beverage, with the aim of preserving the sensorial characteristics. This study demonstrated that beverage´s chemical composition and sensory attributes can be modified significantly. The two beverages with the highest permeate proportions (with low alcohol content) have a lower content of total polyphenols, anthocyanins, and colour intensity, which influences the sensory evaluation. Due to the obtained results, it will be necessary to continue the studies in order to optimize the process with less influence in the composition of the beverages.

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