EFFECT OF HIGH PRESSURE ON MICROORGANISM´S GROWTH, ANTIOXIDANT CAPACITY, AND WINE COLOUR

Mickael da C. Santos[(1)], Ana Rodrigues[(2)], Carlos Rodrigues[(2)], Sónia Mendo[(3)], Jorge A. Saraiva[(1)], Cláudia Nunes[(1)], Manuel A. Coimbra[(1)]

[(1)]QOPNA, Department of Chemistry, University of Aveiro,
3810-193 Aveiro, Portugal
e-mail: mac@ua.pt
[(2)]Dão Sul –Sociedade Vitivinícola, S.A.,
3430-909 Carregal do Sal, Portugal
[(3)]CESAM, Department of Biology, University of Aveiro,
3810-193 Aveiro, Portugal

ABSTRACT

In this work, the effect of high hydrostatic pressure (HHP) treatment of wine, as substitute of SO_2, was evaluated by quantification of microbial load, antioxidant capacity, and colour. For this purpose, red wine of two consecutive harvests (2008 and 2009) was produced without the addition of SO_2. At the end of the alcoholic fermentation, the wine was pressurized at 350 MPa for 20 min at 20 ºC.
After nine months of storage, the wine submitted to HHP showed no growth of yeasts and bacteria. However, differences in colour and antioxidant activity were observed in pressurized wine from 2009 but not in pressurized wine from 2008, when compared to the wine with SO_2.
These results show that although HHP technology has already been proposed for wine pasteurization, more studies concerning its effect on wine, towards the use of HHP to reduce SO_2 in wine making, are still required.

Dans ce travail, l'effet du traitement avec pression hydrostatique élevée (PHE) au vin, comme substitut du SO_2, a été évalué par la quantification de la croissance microbienne, la capacité anti-oxydante et la couleur. Pour cet effet, un vin rouge de deux récoltes consécutives (2008 et 2009) a été produit sans addition de SO_2. À la fin de la fermentation alcoolique, le vin a été pressurisé à 350 MPa pendant 20 min à 20º C.
Le vin soumis à PHE n'a montré aucune croissance de levures et de bactéries après la pressurisation. Toutefois, il a été observé, après neuf mois de stockage, des différences de couleur et d'activité anti-oxydante dans le vin de 2009 pressurisé, mais pas dans le vin de 2008 pressurisé, par rapport au vin avec SO_2.
Ces résultats montrent que même si la technologie de PHE a déjà été proposée pour la pasteurisation du vin, d'autres études de son effet sur le vin sont nécessaires pour que cette technique puisse être utilisée comme un substitut du SO_2 dans la vinification.

INTRODUCTION

Sulphur dioxide (SO_2) is probably one of the most versatile and efficient additives used in winemaking due to its antiseptic and antioxidant properties. Moreover, sulphur dioxide reduces the rate of phenolic polymerisation and colour loss during wine aging. However, as sulphur dioxide has been related to allergic reactions in some consumers, wine producers seek to reduce its use (Bakker *et al.,* 1998).

During the last decade, the use of high hydrostatic pressure (HHP) for food preservation and processing and also for creating new types of food products has increased substantially. Foods commercially processed by HHP are submitted to pressures in the order of 400-600 MPa to destroy microorganisms and inactivate enzymes, with minimal effects on food quality characteristics (sensorial and nutritional) (Ramirez *et al.,* 2009). However, due to the complexity of food products, it is hard to generalize the effect of HHP processing in different foods.

The application of HHP in wine, using pressures between 300–500 MPa at room temperature, was shown inactivation of bacteria and yeasts with no changes in the wine organoleptic properties (Mok *et al.*, 2006).

These results suggest that HHP might be an alternative process for preservation of wine, which can lead to the production of a wine with reduced amounts of SO_2. However, the application of HHP in winemaking is still at an early stage of development and the effect on the physical-chemical characteristics of wine is still largely unknown, namely on colour, antioxidant activity, and phenolic compounds composition.

The objective of this work was to contribute for the evaluation of the feasibility of using HHP for an efficient conservation of wine with maintenance or improvement of its sensorial and nutritional characteristics. The results will contribute to evaluate the use of HHP to produce wines of superior and distinct quality with reduced amounts of SO_2.

MATERIAL AND METHODS

High pressure treatments

Red wine (*Vitis vinifera* L., Touriga Nacional variety) of two consecutive harvests (2008 and 2009) was produced without the addition of SO_2 and treated, at the end of alcoholic fermentation, with a hydrostatic press from Unipress Equipment, Model U33 (Warshaw, Poland), with a pressure vessel of 100 mL (35 mm diameter and 100 mm height). The wine samples from 2008 were pressurized in bags of 15 mL and the 2009 wine samples were pressurized in polyethylene bottles (36 mL), both at 350 MPa for 20 min at 20ºC. The wines were analyzed along nine months of storage.

Quantification of microbial load

In order to verify the microbiological stability of wines during the storage, microbiological tests were performed by inoculation on plates with specific media. Bacteria were grown on plate with Wallerstein differential agar (Fluka) and yeasts were grown on rose bengal chloramphenicol agar (Fluka). The incubation conditions for the enumeration were 48 h at 37 ºC for bacteria and 5 days at 25 ºC for yeasts.

Determination of colour and antioxidant activity

To measure the colour of wine, a spectrophotometric method was used that allows the calculation of tristimulus values and the coefficients required for trichromatic colour specification. In this method, the colour characteristics are expressed by colour intensity, which is given by the sum of the absorbance at 420, 520, and 620 nm, and

colour tonality, by the ratio of absorbance at 420 and 520 nm (Commission Regulation, 1990).
The antioxidant activity was determined by the ABTS method (Re *et al.,* 1999).

RESULTS AND DISCUSSION

Microorganism's growth

The inactivation of yeast in wine by HHP is shown in Table 1. At the beginning of storage the wine samples with 40 ppm of SO_2 presented the lowest amount of colony forming units (CFU) when compared to the untreated wine (neither SO_2 nor pressurization) for both harvests, due to the anti-microbial effect of the SO_2. All the wine samples submitted to HHP showed no growth of yeasts after the pressure treatments, contrary to the untreated wine and that with the addition of SO_2. These results showed the efficiency of high pressure technology on wine preservation for inactivation of yeasts.
No bacteria growth was detected in all wine samples, both at the beginning and after 9 months of storage.

Table 1 – Colony forming units, CFU/mL (Average ± standard deviation) in wine samples at the beginning and after 9 months of storage.

Wine		Beginning of storage		After 9 months of storage	
		Yeast	Bacteria	Yeast	Bacteria
		CFU/mL			
2008	Untreated	$5.0x10^5 \pm 2.8x10^4$	-	-	-
	Pressurized	-	-	-	-
	With 40ppm of SO_2	$1.6x10^3 \pm 3.5x10^2$	-	-	-
2009	Untreated	$2.1x10^4 \pm 3.3x10^3$	-	-	-
	Pressurized	-	-	-	-
	With 40ppm of SO_2	$1.1x10^4 \pm 7.8\ x10^2$	-	-	-

Wine colour

In Figures 1 and 2 it is observed that for both harvests, at the beginning of storage, all wine samples presented a similar level of colour intensity and tonality,.
Relatively to the colour tonality, after 9 months of storage, pressurized wine from 2008 and 2009 presented a slightly higher colour tonality, 31% and 35% respectively, when compared to the wine with SO_2. After storage, it can be observed that the pressurized wine from 2008 presented a decrease and the sample from 2009 showed an increase in colour tonality, in relation with the same wine at the beginning of storage (Fig. 1).
In Figure 2 it can be observed that the pressurized wine from 2008 presented a similar level of colour intensity when compared to the wine with SO_2 and to the wine at the beginning of storage. However, the wine from 2009 showed a decrease in colour intensity with the storage and comparatively to the wine with 40 ppm of SO_2.

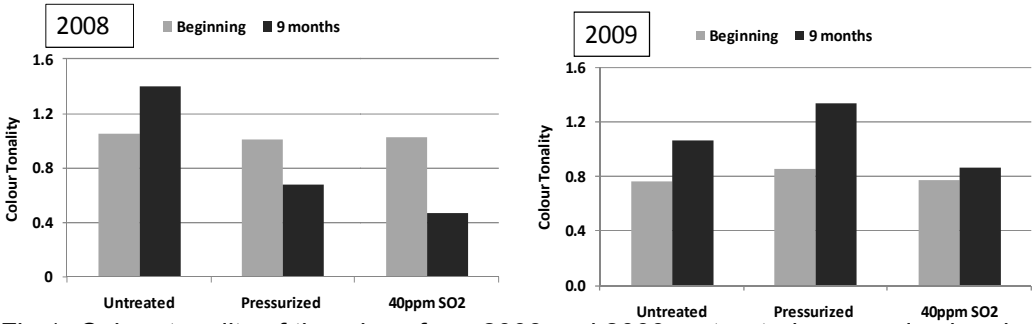

Fig.1- Colour tonality of the wines from 2008 and 2009: untreated, pressurized and with 40 ppm of SO_2.

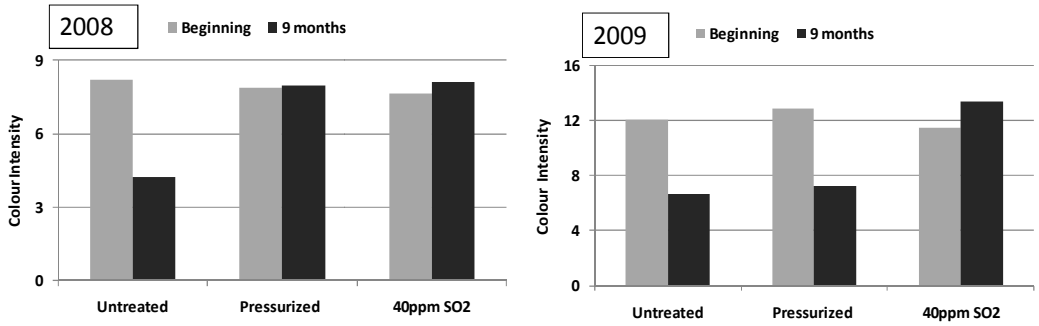

Fig.2- Colour intensity of the wines from 2008 and 2009: untreated, pressurized and with 40 ppm of SO_2.

Antioxidant activity

In Figure 3 it is visible a noticeable difference in the antioxidant activity for the pressurized wines of the two harvests. The pressurized wine from 2008 presented at the beginning of storage an antioxidant activity 31% and 48% higher than the wine with SO_2 and without treatment, respectively. After 9 months, this wine showed an antioxidant activity similar to the wine with SO_2 and 2-fold higher than the wine without treatment. However, the wine from 2009 treated by HHP showed a slight decrease (around 20%) in antioxidant activity at the beginning of storage and a more pronounced decrease (49%) after 9 months of storage comparing with wine with SO_2.

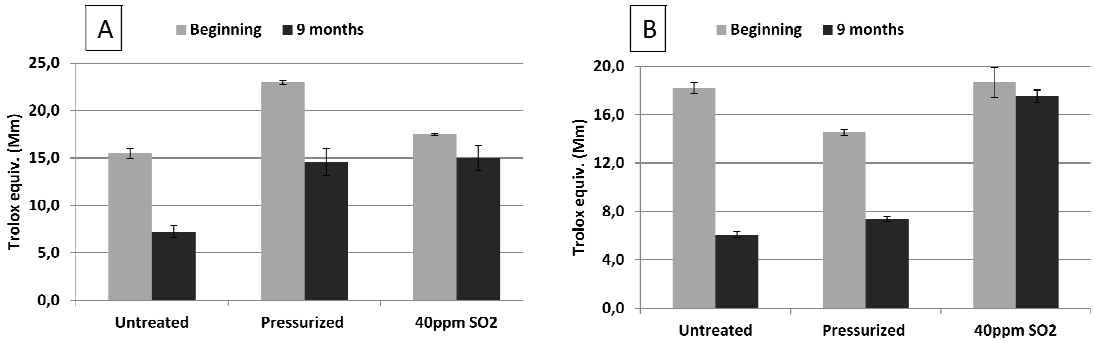

Fig.3- Antioxidant activity of wine samples from 2008 (A) and 2009 (B) at the beginning of storage and after 9 months

CONCLUSIONS

These results showed the efficiency of high pressure technology for wine preservation at the microbiological level. However, different behaviours were observed for the two years studied in the colour (Figs. 1 and 2) and antioxidant activity (Fig. 3) of the wine for the same storage time. These different behaviuors might be due to the use of different pressurization packaging. In 2009, the wine was pressurized in bags, while in 2008 the wine was pressurized in bottles. Then, an important point that should be taken with care during the high pressure processing is the type of package used to pressurize.

In conclusion, the use of HHP to pasteurize wine, as was proposed by previous works (Mok *et al.*, 2006), needs to be applied with care to minimize the impact on sensorial quality. More studies concerning the effect of HHP in wine, towards its use to reduce SO_2 in wine making, are still required to further evaluate the feasibility to preserve wine using this technology.

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ACNOWLEDGEMENTS

The authors thank the financial support of Projects QREN – SI I&DT Co-Promoção WineSulFree (nº 3462) and FCT - WineSulFree (PTDC/AGR-ALI/101251/2008), Research Unit 62/94 “Química Orgânica, Produtos Naturais e Agro-Alimentares”, and FCT for the grants (SFRH/BPD/46584/2008 and SFRH/BD/70066/ 2010).