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Effects of high pressure processing on polyphenoloxidase enzyme activity of grape musts

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The effects of high pressure processing (300, 600, 900 MPa for 2, 6 and 10 min) on the activity of polyphenoloxidase (PPO) enzymes were evaluated in a synthetic must to which an enzymatic extract obtained from grapes had been added, and in a white grapes must. Despite the fact that the results vary as a function of the medium studied, it could be seen that the PPO typical of the musts could only be partly inactivated by high pressure treatment (300–600 MPa). On the other hand, at higher pressures (900 MPa), PPO enzymatic residual activity was clearly lower (1 to 16%) in both the synthetic and the white must. © 1997 Elsevier Science Ltd

INTRODUCTION

In grape juice and musts, the polyphenoloxidases (PPO) activity causes colour and turbidity modifications (Macheix *et al.*, 1991) which can damage the stability and the organoleptic characteristics, so the polyphenol oxidation reactions in musts are generally controlled using SO_2 (Schopfer & Aerny, 1985; Macheix *et al.*, 1990). Sulphites could be detrimental to human health so, today, many studies are carried out to reduce SO_2 levels in foods and beverages (Galassi & Mancini, 1985; Müller-Späth, 1990; F.A.O., 1992). High pressure processing (HPP) is an innovative technique proposed for the microbiological stabilization of foods instead of heat treatment (Dall'Aglio, 1993). Many authors have described a variation in the activity of the PPO in pears, potatoes and apples after high pressure treatment (Asaka & Hayashi, 1991; Knorr, 1993; Hoover, 1993; Gomes & Ledward, 1996). In this work, we have studied the effects of different HPP on polyphenoloxidases activity in a synthetic grape must and in a white grape must.

MATERIALS AND METHODS

Musts

The trials were made using both a white grape must and a synthetic must. The white must was obtained by pressing 600 kg of white sound Trebbiano grapes with a horizontal press. The free-run juice was clarified at 4°C in a 500-litre stainless steel tank for 48 h and then kept in a 10-litre stainless steel tank under N_2 at 4°C until the HPP treatment. The white must had a reduced sugar content of 183 g litre^{-1} and a pH of 3.22. The synthetic must was prepared by adding 10 g of tartaric acid, 6 g of malic acid, 200 g of glucose and 200 g of fructose to 2 litres of distilled water. It was then buffered to a pH of 2.9 with 2M NaOH, and added with 400 ml of raw enzymatic extract made from 1 kg of white Trebbiano grapes, as proposed by Kidron *et al.* (1978). Synthetic must was kept in a 10-litre stainless steel tank under N_2 at 4°C until the HPP treatment.

High pressure processing

The synthetic must and the model system were put into 100-ml PET vials and hermetically closed under vacuum by thermal sealing without headspace.

All the high pressure treatments were carried out using an Asea Brown Boveri (ABB) QFP6 pilot hydrostatic press installed at the 'Stazione Sperimentale per le Conserve', Parma, Italy. The white must was subjected to a treatment of 600 and 900 MPa for 10 min. The synthetic must underwent treatments of 300, 600 and 900 MPa for 2, 6, and 10 min. Under our conditions, we have observed a temperature increase of about 3°C × 100 Mpa^{-1} in the samples during processing (Rovere *et al.*, 1993).

Each treatment was in triplicate with samples at an initial temperature of 4°C and water in the pressure

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vessel at 15°C. For each treatment, three samples were subjected to the same procedure, but without the HPP treatment, and were taken as reference samples (controls). After the high pressure treatment, the samples and their respective controls were immediately refrigerated at 4°C and analysed after 12 h.

Determination of the PPO activity

A 20% (w/v) DOPA (3,4-dihydroxy-L-phenylalanine) solution in phosphate buffer at pH 6.00 as a substrate was used (Guerzoni *et al.*, 1977). 100 μl of sample was added in 1.9 ml of DOPA solution and optical density at 480 nm was measured every 15 s for 5 min using a 1-cm glass cuvette and a Jasco UVIDEC 430. Polyphenoloxidase activity was calculated as the increase of optical density per min (milliunit of absorbance per min — mUAbs $\times$ min^{-1}). Before the HPP treatments, the PPO activity in white must was 0.36 mUAbs $\times$ min^{-1} and the PPO activity in synthetic must was 12.3 mUAbs $\times$ min^{-1}.

RESULTS AND DISCUSSION

In the synthetic musts, the treatments at 300 and 600 MPa lowered (on average) 10% of the PPO activity with respect to that of the controls (Table 1). The inhibitions in the PPO activity at these two pressure levels were not significantly different and no interactions were observed between pressure and time. On the other hand, at a pressure of 900 MPa there was greater inhibition of the PPO activity, even for a short period of treatment (2 min) and significant interaction between pressure and time. In particular, after a treatment of 900 MPa for 10 min, the residual PPO activity was less than 1% that of the control.

These results were partially confirmed by the musts obtained from the white grapes. In fact, in this case the residual PPO activity after an HPP treatment of 600 and 900 MPa for 10 min was 61.6% ($\pm$Std. Dev. = 4.1) and 15.9% ($\pm$Std. Dev. = 2.45), respectively. The difference between these results and those of the synthetic must could be due to the different characteristics of the medium (pH, initial PPO activity, presence of colloid components) which influence the effect of the HPP treatment, as already reported by other authors (Ogawa *et al.*, 1990; Nicoli *et al.*, 1993; Eshtiaghi & Knorr, 1993; Rovere, 1995).

Table 1. Per cent residual PPO activity in synthetic musts after high pressure processing

	2 min	6 min	10 min
300 MPa	[a]87.2 (5.80)[b]	88.3 (6.05)	89.2 (10.9)
600 MPa	90.6 (5.66)	90.7 (5.60)	92.1 (4.67)
900 MPa	20.7 (1.45)	3.8 (1.04)	0.9 (0.76)

[a]Mean of three replications.
[b]Standard deviation.

These preliminary results, however, demonstrate that the PPO typical of grapes can also be inactivated by hyperbaric treatment. The inactivation is limited at pressures between 300 and 600 MPa, which some authors claim to be sufficient for the microbial stabilisation of the musts (Moio *et al.*, 1994; Lonvaud-Funel *et al.*, 1994; Delfini *et al.*, 1995). At 900 MPa, low levels of PPO enzymatic activity are reached in both the synthetic must and the white must. It can be hypothesised that, in order to reach the complete inactivation of the PPO in musts, instead of using pressures higher than 600 MPa, it would be necessary to use HPP treatment together with a mild thermal treatment (40 to 50°C), as indicated for other enzymes (Knorr, 1993; Rovere *et al.*, 1993).

High pressure technology can, however, be considered an interesting technique for the control of PPO activity in musts and grape juices, thus sparing chemical additives, such as sulphur dioxide.

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