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The effects of high hydrostatic pressure on β-glucosidase, peroxidase and polyphenoloxidase in red raspberry (*Rubus idaeus*) and strawberry (*Fragaria × ananassa*)

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Abstract

The effects of high hydrostatic pressure on 3 important enzymes involved in flavour and colour bioformation, namely β-glucosidase, peroxidase and polyphenoloxidase, in red raspberry (*Rubus idaeus*) and strawberry (*Fragaria × ananassa*) were studied. Fruit samples were pressurised under 400, 600 and 800 MPa for 5, 10 and 15 min at a temperature controlled between 18 and 22 °C. After application of pressure, the enzymatic activities (A) were measured and compared to the initial activities of the samples (A_0). The effect of high pressure is presented as a function of the ratio A/A_0 over pressurising time. The inactivation of the enzymes is linked to the stability of anthocyanins in both fruits.

Keywords: Raspberry; Strawberry; High hydrostatic pressure; β-Glucosidase; Peroxidase and polyphenoloxidase

1. Introduction

Raspberries (*Rubus idaeus*) belong to the same family as strawberries (*Rosaceae*) and are known for their exquisite flavour. They are widely used to prepare juices, liqueurs, sorbets, ice cream, concentrates and lip colourings (cosmetics) (Latrasse, 1991). Strawberry (*Fragaria × ananassa*) is a very sensitive and expensive fruit with an extremely short shelf-life (Perez, Olias, Olias, & Sanz, 1998).

Apart from micro-organisms, enzymes, namely peroxidase (POD) and polyphenoloxidase (PPO) are involved in the fast deterioration of both fruits (Robinson & Eskin, 1991). Water blanching is the most common method for inactivating vegetable enzymes (Fellows, 2000). It causes the denaturation and therefore inactivation of the enzymes but also causes destruction of thermosensitive nutrients and is rarely used for soft fruits.

Given the increasing use of high hydrostatic pressure (HHP) in producing new food products (Gimenez, Kajda, Margomenou, Piggott, & Zabetakis, 2001), we studied and here report the effects of HHP on the activities of three enzymes, namely β-glucosidase, peroxidase (POD) and polyphenoloxidase (PPO), that affect the biosynthesis and deterioration of both flavour and colour molecules in these berries.

2. Materials and methods

2.1. Solvents and enzyme extraction solution

All solvents and chemicals used were of highest available purity and purchased from Sigma, Gillingham, U.K. The enzyme extraction solution was a 0.2 M sodium phosphate buffer (pH 6.5) containing 4% (w/v) PVPP and 1% (v/v) Triton X-100.

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2.2. High pressure treatment

The fruits were subjected to three different high pressure treatments (HPT): 400, 600 and 800 MPa for 5, or 10 min at a temperature of 18–22 °C as described previously (Gimenez et al., 2001).

2.3. Enzyme assays

After the high pressure treatment, 10 g of the fruit sample were mixed with 20 ml of the enzyme extraction solution; this mixture was homogenised under ice-cooled conditions for 3 min. The homogenate was centrifuged at 7500*g* for 15 min. The supernatant was collected and analysed for enzymatic activities.

β-Glucosidase was assayed as described previously (Orruño, 2001).

For the POD assay, 25 μl of the enzyme extract were mixed with 2.7 ml of 0.05M sodium phosphate buffer (pH 6.5), 0.2 ml of 1% (w/v) *p*-phenylenediamine (H-donor) and 0.1 ml of 1.5% (w/v) hydrogen peroxide (oxidant). The blank was prepared in the same way but adding double distilled water instead of fruit extract. The measurement of *p*-phenylenediamine oxidation was carried out immediately after the extract was added, using a Cecil 2041 double beam spectrophotometer at 485 nm at ambient temperature. Readings were taken every 30 s in the first 3 min and then at 1 min intervals for up to 15 min. The blank was measured at the same time as the sample.

For the PPO assay, 75 μl of the enzyme extract were mixed with 3.0 ml of 0.07 M catechol (*o*-diphenol) in 0.05 M sodium phosphate buffer (pH 6.5) solution. The blank was prepared in the same way but adding double distilled water instead of fruit extract. Readings (at 420 nm) were taken every 30 s in the first 3 min and then at 1 min intervals for up to 15 min. The blank was measured at the same time as the sample.

All analyses were carried out in triplicate and the results expressed as mean values.

3. Results

After the HPT, the enzymatic activities of the fruit samples (A) were measured and compared to the initial activities of these samples (A_0). The ratios A/A_0 were calculated for each HPT and these results were depicted as a function of high pressure and pressurising time.

3.1. Effect of HPT on the enzyme β-glucosidase

The effect of HPT on the enzyme β-glucosidase in red raspberries and strawberries was studied and the results are shown in the Figs. 1 and 2, respectively.

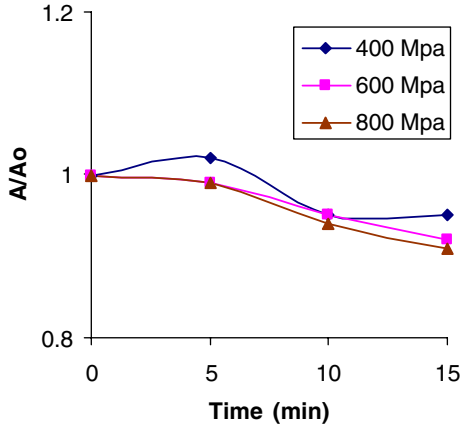

Fig. 1. The effect of HPT on the enzyme β-glucosidase in red raspberries.

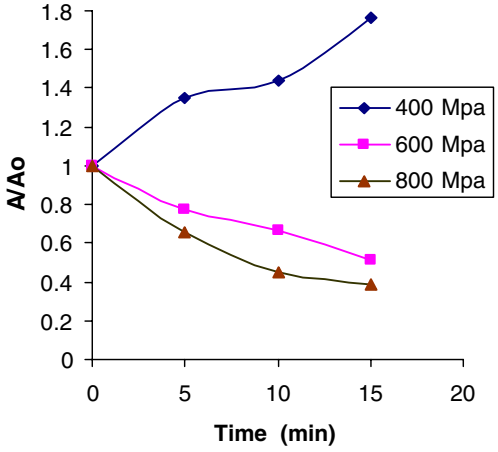

Fig. 2. The effect of HPT on the enzyme β-glucosidase in strawberries.

In red raspberries, the β-glucosidase activity was relatively small and rather steady in all cases including the control. However, there was a slight decrease (about 10%) after the HPT of 600 and 800 MPa for 15 min. In contrast, the HPT of 400 MPa resulted in a small activation of the enzyme (2%) when treated for 5 min and small inactivation (5%) when treated for 10 and 15 min. Total inactivation of the enzyme was not achieved in any case.

In strawberries, the β-glucosidase activity was increased after all the 400 MPa HPT. Interestingly, the highest increase of activity (76%), at 400 MPa, was observed when the pressure was applied for 15 min. These results are in good agreement with previous work of our group in strawberries (Zabetakis, Koulentianos, Orruño, & Boyes, 2000a, 2000b). The HPT of 600 and 800 MPa were quite efficient for reducing the activity of β-glucosidase and, after 15 min of HPT, the reduction of the activities were 49% and 61%, respectively.

3.2. Effect of HPT on the enzyme peroxidase

The effect of HPT on the POD enzyme in strawberries was studied and the results are shown in Fig. 3. Similarly to β-glucosidase, the effect of 400 MPa on the activity was activating by 13% and 1% when applied for 5 and 10 min, respectively. Only when applied for 15 min, was a 5% enzymatic inactivation achieved. The pressures of 600 and 800 MPa were more efficient in inactivating the enzyme. Inactivations in the range of 11–35% were observed when the most cost-effective conditions were 600 MPa for 15 min.

No POD activity was detected in red raspberries, either fresh or after the HPT.

3.3. Effect of HPT on the enzyme polyphenoloxidase

The effect of HPT on the PPO enzyme in red raspberries and strawberries was studied and the results are shown in the Figs. 4 and 5, respectively.

In red raspberries, PPO was activated when the fruits were subjected to an HPT of 400 and 800 MPa for 5 min, by 15% and 8%, respectively, and PPO was inactivated (in the range of 3–29%) when treated for 10 and 15 min. In fact, a significant inactivation by 29% was observed when the fruits were subjected to a HPT of 800 MPa for 15 min. In contrast, the HPT of 600 MPa resulted in a dramatic activation of the enzyme when applied for 5 or 10 min (54% and 41%, respectively) whereas the 15 min experiment showed a decrease of the activity by 1%.

In strawberries, all HPT extensively reduced the enzymatic activity (ranging from 69% to complete inactivation). The most cost effective conditions for inactivating PPO in strawberries were either 600 MPa for 15 min or 800 MPa for 10 min.

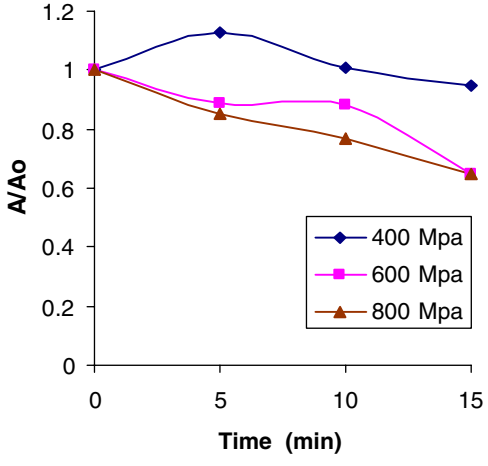

Fig. 3. The effect of HPT on the enzyme peroxidase in strawberries.

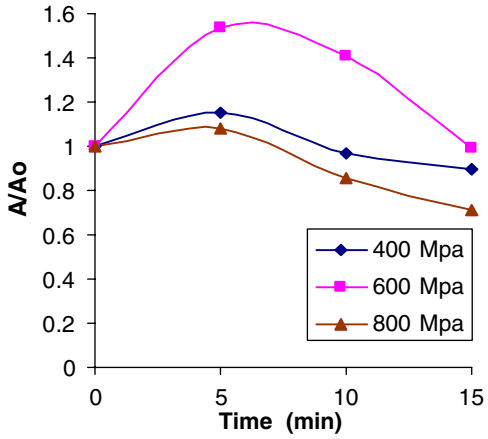

Fig. 4. The effect of HPT on the enzyme polyphenoloxidase in red raspberries.

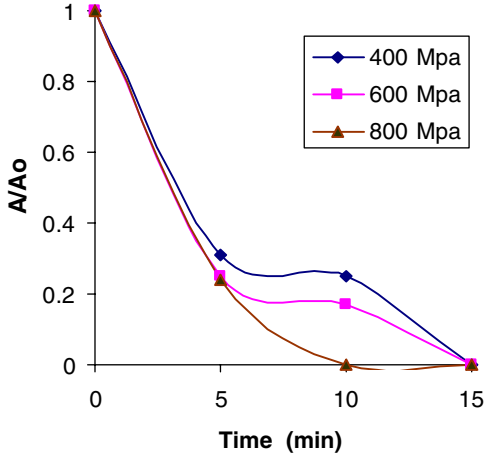

Fig. 5. The effect of HPT on the enzyme polyphenoloxidase in strawberries.

4. Discussion

In both fruits and after all HPT, β-glucosidase was not extensively inactivated but this should be regarded as a positive result. This enzyme is involved in the liberation of volatile aglycones from non-volatile glucosides (Zabetakis, Gramshaw, & Robinson, 1999). Therefore, it is important to maintain this enzymatic activity in the fruit for the subsequent flavour release.

In strawberries, the maximum POD inactivation (35%) was achieved after an HPT at either 600 MPa or 800 MPa for 15 min. This result is in good agreement with previous work in strawberries where an inactivation of 25% was achieved after an HPT of 15 min at 230 MPa (Cano, Hernandez, & De Ancos, 1997)

In red raspberries, the enzyme PPO was not inactivated after all HPT and this result could be linked to the stability of the anthocyanins present in this fruit. Red raspberries contain mainly cyanidin-3-glucoside and

cyanidin-3-sophoroside. Both anthocyanins were less stable when the fruits were subjected to 400 MPa for 15 min, the only exception being of cyanidin-3-glucoside when stored at 30 °C (Suthanthangjai, Kajda, & Zabetakis,). This result on the stability of anthocyanins can now be linked to the remaining PPO activity in the fruit after HPT. As shown in Fig. 4, PPO was not inactivated at any pressure and this residual activity could be the major reason for reduced colour stability.

An analogous link of the PPO activity and the stability of anthocyanins in strawberries can be envisaged. It has been shown that pelargonidin-3-glucoside and pelargonidin-3-rutinoside were most stable when a pressure of 800 MPa for 15 min was applied (Zabetakis et al., 2000a, 2000b). In our present study, we showed that PPO is completely inactivated after 15 min at 800 MPa and this inactivation contributes vitally to the stability of the two pelargonidins.

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