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The effect of high hydrostatic pressure on strawberry flavour compounds

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Abstract

The effects of high hydrostatic pressure on strawberry flavour compounds and the enzyme β-glucosidase were investigated. Strawberry (*Fragaria* × *ananassa*, cv. Elsanta) samples were submitted to high pressure ranging from 200 to 800 MPa. After the high pressure treatment, the samples were kept for 24 h at three different storage temperatures (4, 20 and 30°C) and, thereafter, the flavour was extracted by simultaneous steam distillation-solvent extraction using dichloromethane. Analysis was carried out by GC–MS and HPLC. The analytes of this study are three acids (namely butanoic acid, 2-methyl-butanoic acid and hexanoic acid), one ketone (2,4,6-heptatrione) and three furanones (5-hexyl-dihydro-*3H*-furan-2-one, 2,5-dimethyl-4-hydroxy-*2H*-furan-3-one and 2,5-dimethyl-4-hydroxy-*2H*-furan-3-one-glucoside). The highest flavour stability was observed when samples were treated with lower pressures and they were stored at 4 and 30° C. The enzyme β-glucosidase was found to be activated when pressures of 200 and 400 MPa were applied but inactivated considerably in the 600 and 800 MPa treatments.

1. Introduction

High hydrostatic pressure (HHP) has been extensively applied for food preservation over the last decade (Gould, 1995; Watanabe, Arai, Kumeno & Honma, 1991). In a similar way to high temperature, HHP inactivates vegetative micro organisms, spores and enzymes and, as such, it increases the shelf-life of foods. Peaches and pears remained commercially sterile for at least five years after high pressure processing (Gould, 1995).

Despite the commercial applications of HHP, there is only a limited number of studies on the effect of HHP on flavour compounds in fruits. The overall flavour profiles of strawberry jams made by heat treatment or HHP were compared (Kimura, Ida, Yosida, Ohki, Fukumoto & Sakui, 1994) and HHP jam apparently has a much richer flavour than the heated one. Strawberry flavour is one of the most complicated fruit flavours in nature; it has ca. 350 compounds (Zabetakis & Holden, 1997). Despite the numerous food products where strawberry flavour compounds are responsible for the final flavour (e.g. jams, yoghurts, desserts), there are very limited studies on the effect of HHP on specific flavour compounds of strawberry (Lambert, Demazeau, Largeteau & Bouvier, 1999). In this study, some changes in the aromatic volatile composition of strawberry after HHP are reported. The effects of a wide range of HHP treatments (200–800 MPa) and three different storage temperatures (4, 20 and 30°C) on specific flavour components in strawberry are determined.

β-Glucosidases (β-glucoside glucohydrolase, EC 3.2.1.21) catalyse the hydrolysis of aryl and alkyl β-D-glucosides. In plants, β-glucosidases are involved in different key metabolic events, the release of flavour volatiles being the most important in terms of flavour bioformation in fruits (Hosel, 1981). The effect of HHP on the activity of β-glucosidase in crude strawberry extracts is also reported here.

2. Materials and methods

2.1. Materials

All solvents used were of HPLC grade and purchased from Fluka, Gillingham, UK. The reagents and filter

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papers used were purchased from BDH, Lutterworth, UK.

2.2. Samples and sample preparation

Strawberry plants (*Fragaria* × *ananassa*, cv. Elsanta) were grown in the experimental greenhouses (University of Leeds) and red ripe fruits were collected from these plants. The fruits were quick-frozen and stored at −25°C until HHP and analysis. Before analysis, fruits were allowed to thaw at room temperature for 1 h, and were then vacuum-sealed in polyethylene bags. These fruit samples were then subjected to HHP of 200, 400, 600 and 800 MPa for 15 min at a temperature of 18–22°C. After this HHP treatment, samples were stored at either refrigerator (4°C), room (20°C) or incubator temperature (30°C). A blank experiment was carried out where no HHP treatment was applied.

2.3. Flavour analysis and enzymatic assay

The flavour of the HHP treated samples was analysed using the Likens–Nickerson extraction method and GC–MS for identification and quantification. Strawberry samples, after the HHP treatment, were macerated in a Sorball mixer and the resultant fruit juice was extracted with dichloromethane using a Likens–Nickerson extraction apparatus for 1 h. The combined organic phase was concentrated to 10 ml using a flash rotary evaporator. The organic phase concentrate was then filtered through a Whatman phase-separator filter to remove any remaining water. The filtrate was further concentrated to 500 μl using a Kuderna–Danish evaporation concentrator. The analysis of 2,5-dimethyl-4-hydroxy-*2H*-furan-3-one (DMHF) and 2,5-dimethyl-4-hydroxy-*2H*-furan-3-one-glucoside (DMHF-glucoside) was performed by HPLC-UV as previously described (Zabetakis & Holden, 1996). The enzyme β-glucosidase was assayed as described by Orruño, Owusu Apenten and Zabetakis (2000).

2.4. Apparatus

Gas chromatographic (GC) analysis was carried out using a Carlo Erba 4200GC coupled to a Kratos MS80 RFA mass spectrometer with a source temperature of 180°C. A BP20 capillary column (25 × 0.32 × 0.5 μm film thickness; SGE, UK) was held at 40°C for 2 min and programmed from 40 to 220°C at 5°C min^{-1} using helium as a carrier gas at 2 ml min^{-1}. Injection of the sample (0.2 μl) was made onto the column. Mass spectra were recorded with an ion source energy of 70 eV. The identification of the analytes was based on comparing retention times and mass spectra to standard compounds obtained from Sigma, Poole, UK. The identification of the novel furanone was based on mass spectra data available in the mass spectra database.

2.5. Quantification

For quantification, hepten-3-ol, *n*-nonoic acid and methyl valerate were added to the fruit juice before extraction. Standard solutions of each internal standard in the range of 5–500 μg ml^{-1} were made and used to obtain calibration curves for each one. These curves were used to quantify the internal standards in the HHP samples. The amounts of acids were related to *n*-nonoic acid, ketones and the furanone to methyl valerate and aldehydes to hepten-3-ol. All analyses were carried out in duplicate and injected at least twice. The results are expressed as mean values.

3. Results and discussion

In this study, the effects of HHP on three acids, one alcohol, one ketone and three furanones that are flavour components of strawberry are reported. It is noteworthy that no ester has been identified following the HHP treatments, even though esters are reported as important flavour compounds in strawberry (Latrasse, 1991). This fact should be correlated to the high acid levels identified, which account for 50% of the total amount of volatiles identified. Given the sensitivity of strawberry volatiles to degradation, it could be suggested that esters were hydrolysed to the respective acids and alcohols during the HHP treatment. A hydrolytic effect of HHP has indeed been suggested (Tauscher, 1995) and, on this basis, the absence of esters should be accounted. The hydrolysis of butanoate and hexanoate esters was confirmed by the increased levels of butanoic and hexanoic acids in our samples as opposed to the reported literature values (Mussinan & Walradt, 1975).

In order to assess the effect of HHP on the flavour molecules at each storage temperature, the results are arranged and shown for each compound individually. All the results presented in the following figures were obtained after keeping the HHP samples for one day at the respective temperature [i.e. refrigerator (4°C), room (20°C) or incubator temperature (30°C)]. All the analyses were carried out in duplicate and the concentration of volatiles is expressed in (mol g^{-1} of fruit).

3.1. Acids

Three acids were measured after the HHP treatments, namely butanoic acid, 2-methyl-butanoic acid and hexanoic acid.

The content of butanoic acid in the HHP samples is shown in Fig. 1. These data show a decrease in butanoic acid content as the pressure levels of HHP treatment are

increased. The same behaviour is observed for samples stored at 4 and 20°C. A decrease in the levels of butanoic acid in all HHP-treated samples, as opposed to the control, was observed; however, this decrease is not linear with high pressure increase. A profound decrease was observed for the samples stored at room temperature (20°C) pressurised at 400 MPa, and for samples stored at 4°C treated at 800 MPa. For samples under the same HHP treatment, it was observed that those stored at 4 and 30°C show higher butanoic acid contents than the respective samples at room temperature.

The levels of 2-methyl-butanoic acid in the HHP samples are presented in Fig. 2. From those values, it could be observed that HHP samples stored at room temperature show a decrease in acid content up to 400 MPa. However, the HHP treatment at 800 MPa resulted in full retention of this acid after one day of storage at room temperature (20°C). For samples stored at 4 and 30°C, a similar pattern to butanoic acid (Fig. 1) is observed but, again, the decrease in the acid levels is not linear with the increase of pressure. In the case of 2-methyl-butanoic acid, the best retention was observed when a HHP of 400 MPa was applied and the sample was stored at 4°C.

In the case of hexanoic acid (Fig. 3), similar observations can be drawn as in the case of the previous acids. One distinct difference however is that the levels of hexanoic acid are much higher than the levels of the two previously discussed acids. This can be explained on the basis of higher contents of hexanoate esters in strawberry. Hydrolysis of these esters during HHP treatment has probably caused the increased levels of hexanoic acid shown in Fig. 3. Similar to 2-methyl-butanoic acid, the best retention of hexanoic acid was observed when an HHP treatment of 400 MPa was applied.

3.2. Alcohol

The major alcohol identified in this work was 1,6,10-dodecatrien-3-ol and the levels of this alcohol at different HHP treatments and storage temperatures are given in Fig. 4. The samples stored at room temperature exhibit an increase in alcohol content in the HHP-treated samples, as opposed to the fresh one where no HHP

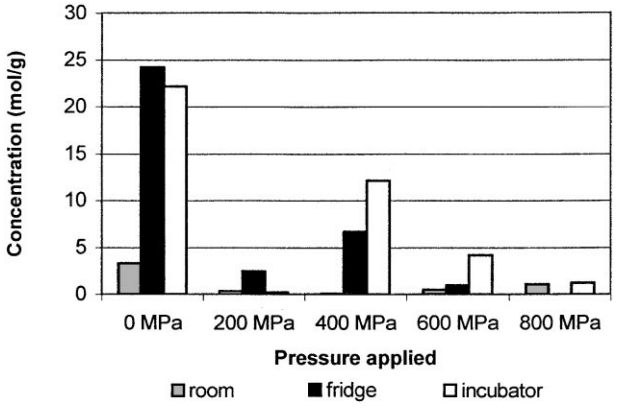

Fig. 1. Influence of high pressure and storage temperature on the levels of butanoic acid.

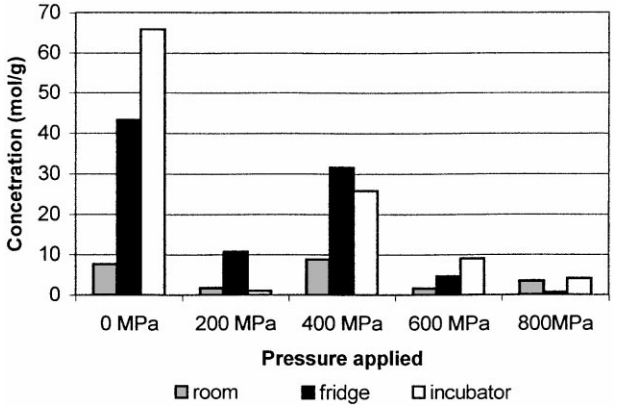

Fig. 3. Influence of high pressure and storage temperature on the levels of hexanoic acid.

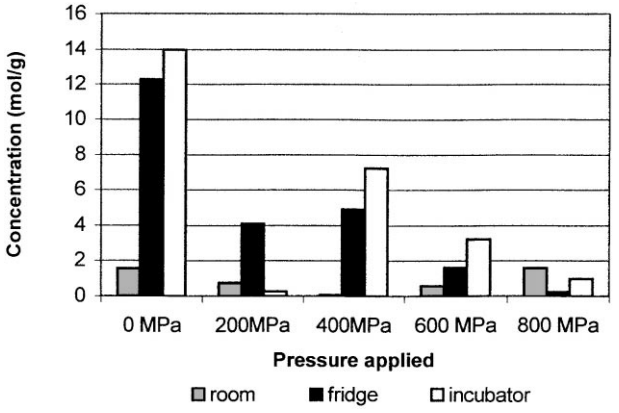

Fig. 2. Influence of high pressure and storage temperature on the levels of 2-methyl-butanoic acid.

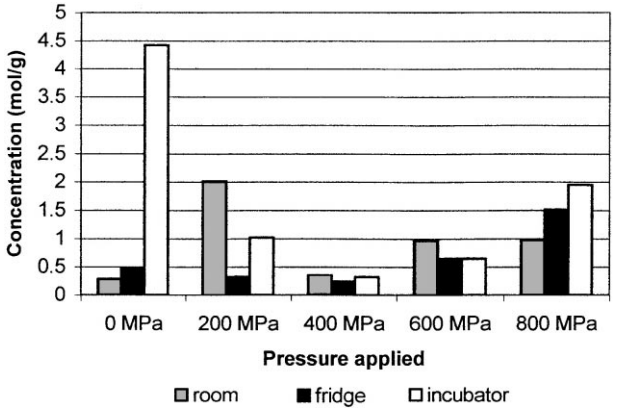

Fig. 4. Influence of high pressure and storage temperature on the levels of 1,6,10-dodecatrien-3-ol.

was applied. However, an opposite effect (i.e. a decrease in alcohol content) is observed for the samples stored at 4 and 30°C. The best retention of 1,6,10-dodecatrien-3-ol was observed when an HHP treatment of 800 MPa was applied and the sample was stored at 30°C.

3.3. Ketone

The dominant ketone identified after the HHP treatments was 2,4,6-heptanetrione. Its levels in the different samples are shown in Fig. 5. The application of HHP has caused a significant decrease in the levels of this ketone when samples were stored at 4°C. However, for 20 and 30°C, HHP treatments at 400 MPa resulted in a significant retention of this volatile after one day of treatment. The only differentiations from this trend are the HHP treatments of 600 and 800 MPa when samples were stored at 30°C (Fig. 5).

3.4. Furanones

A furanone was tentatively identified in the HHP samples by GC–MS. This compound was identified as 5-hexyl-dihydro-*3H*-furan-2-one and its levels in the different samples of this work are given in Fig. 6. In the blank sample, where no HHP was applied, higher levels of this furanone were observed when the sample was stored at 4°C. The notable difference in the levels of 5-hexyl-dihydro-*3H*-furan-2-one was observed for the samples treated at 600 MPa and stored at 30°C. It is suggested that the combination of this high pressure and the storage at the elevated temperature of 30°C resulted in the extensive formation of 5-hexyl-dihydro-*3H*-furan-2-one, possibly due to rearrangement of other furans that have been reported (Zabetakis & Holden, 1997) in strawberry such as 2,5-dimethyl-4-hydroxy-*2H*-furan-3-one and 2-furfural or 2-furancarboxylic acid. The formation of this furanone could also be due to the activation of some enzymatic systems, strawberry or microbial ones, or the rearrangement of other furans to this compound. This result is in good agreement with the formation of a furanone (3,4-dimethoxy-2-methyl-furan) after HHP of strawberry at 800 MPa that has recently been reported (Lambert et al., 1999). For storage temperatures of 20 and 30°C, all HHP treatments increased the levels of 5-hexyl-dihydro-*3H*-furan-2-one.

DMHF and DMHF-glucoside are two of the most important flavour compounds in strawberry (Zabetakis & Holden, 1997). In this paper, we report the effect of high pressure on these two furanones, DMHF in Fig. 7 and DMHF-glucoside in Fig. 8. The different high pressure treatments (with the exception of 200 MPa) do not dramatically alter the levels of the two furanones in the HHP-treated samples as opposed to the control (0 MPa). In the case of DMHF-glucoside (Fig. 8), the levels of this compound remain similar in all four different HHP treatments. This result suggests that HHP does not cause any destruction or formation of the glucoside. Given the higher stability of DMHF-glucoside as opposed to DMHF (Roscher, Schwab & Schreier, 1997), the data of Figs. 7 and 8 are consistent with the

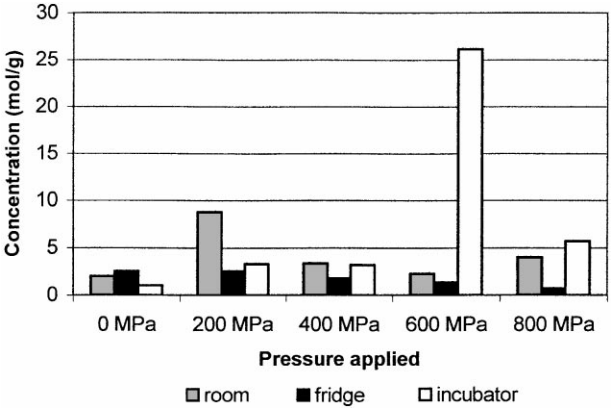

Fig. 6. Influence of high pressure and storage temperature on the levels of 5-hexyldihydro-*3H*-furan-2-one.

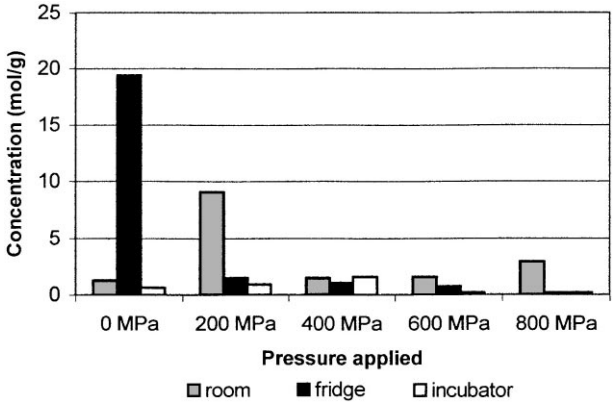

Fig. 5. Influence of high pressure and storage temperature on the levels of 2,4,6-heptanetrione.

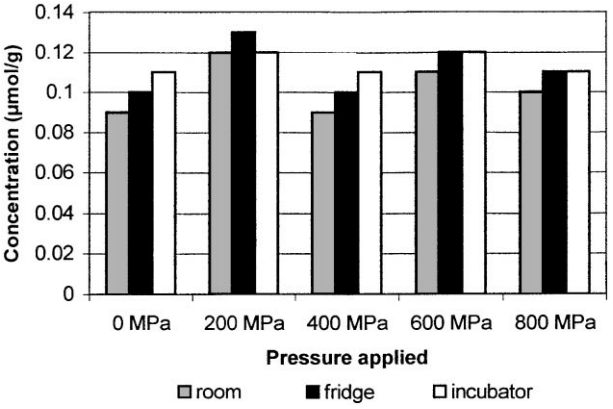

Fig. 7. Influence of high pressure and storage temperature on the levels of DMHF.

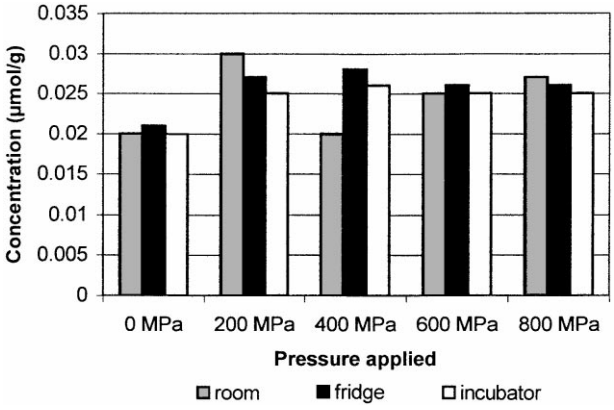

Fig. 8. Influence of high pressure and storage temperature on the levels of DMHF-glucoside.

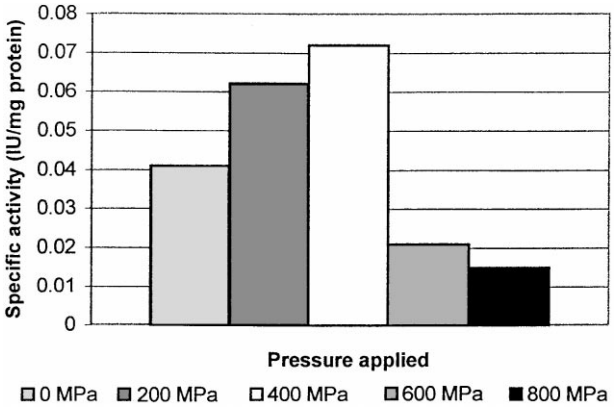

Fig. 9. Influence of high pressure on the activity of β-glucosidase in strawberry.

relative stability of the glucoside as opposed to the free aglycone.

The enzymatic activity of β-glucosidase in crude strawberry extracts was studied when the four different levels of HHP were applied to the fruit (Fig. 9). It was found that the specific enzymatic activity was increased by 50% when 200 MPa was applied and by about 70% when 400 MPa was applied as opposed to the activity measured for the control samples. At the higher pressures of 600 and 800 MPa, the enzymatic activity was reduced by 50 and about 65%, respectively, as opposed to the control value. It is suggested that the HHP is causing a conformational change of the enzyme that possibly results in a higher degree of exposure of the active site to the substrate. Subsequently, this conformational change could result in an increase in enzymatic activity.

In this study, the effect of HHP on specific flavour compounds was monitored for four different HHP treatments in conjunction with storage at three different temperatures. In the case of the acids above, the best flavour retention was observed at 400 MPa, whereas, for alcohol retention, the best pressure was 800 MPa. For ketone retention, the most effective pressures were found to be 200 and 800 MPa. It can be concluded that there is not a best HHP treatment for the optimum simultaneous retention of all flavour volatiles. Therefore, the choice of HHP should be carried out in relation to which type of flavour volatiles should be retained. Given that the strawberry matrix is quite complex in flavour terms, ongoing research is focusing on the effect of HHP on model systems containing mixtures of the above flavour compounds.

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