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High pressure processing, thermal processing and freezing of 'Camarosa' strawberry for the inactivation of polyphenoloxidase and control of browning

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ABSTRACT

Polyphenoloxidase (PPO) is an enzyme present in strawberry (*Fragaria ananassa*) which causes undesirable fruit browning, especially if the fruit is cut in pieces or processed into puree. High Pressure Processing (HPP) is an emerging non-thermal food preservation technology with minimum impact on the food original sensory, functional and nutritional properties. As an alternative to conventional freezing/frozen storage of strawberry, the inactivation of PPO in 'Camarosa' strawberry puree by HPP (200 MPa and 600 MPa), thermal processing (29–71 °C) and their combination was attempted for 5 and 15 min processing times. The effect of conventional thermal processing and freezing/frozen storage (−18 °C and −70 °C) for 1 month on PPO was also investigated in puree and whole fruit. Room temperature processing at 600 MPa for 5 min and 15 min resulted in 35% and 82% PPO inactivation, respectively. A 5 min process of 600 MPa in combination with mild heat (40–60 °C) resulted in 9–65% residual activity as opposed to 44–100% with exclusively thermal processing. 200 MPa had minor effect on PPO. Thermal preservation of puree than whole fruit is preferable, whereas frozen storage of whole fruit than puree is better for avoiding strawberry browning. 'Camarosa' strawberry PPO is less resistant to pressure inactivation and more appropriate for HPP technology than 'Elsanta', 'Aroma' and 'Festival' strawberry cultivars.

1. Introduction

Strawberry (*Fragaria ananassa*) is a much appreciated summer fruit with a unique taste and flavour. It is also a good source of vitamin C and other antioxidants (Patras, Brunton, Da Pieve, & Butler, 2009), being available worldwide as a whole fruit '*in natura*' and also as a puree/juice/concentrate to be incorporated in nectar, ice cream, yoghurt, baby food and confectionary. 2010 worldwide production was 4.4 million tonnes (FAO, 2012). Some of the most common cultivars of strawberry are 'Camarosa', 'Ventana', 'Aroma', 'Elsanta', and 'Selva'. 'Camarosa', the variety used in this study, presents a number of advantages over other cultivars: grows for the longest period in Summer, presents good quality in terms of colour and taste, is less prone to rotten roots and produces a larger berry. Fresh strawberries are very perishable and can only be stored for approximately one week at 4 °C, presenting a very short post-harvest life. The short life is mainly due to the occurrence of grey mould infection on the strawberry surface by *Botrytis cinerea* (Bristow, McNicol, & Williamson, 1986; Castro, Gonçalves, Teixeira, & Vicentes, 2002; Williamson, Tudzynski, Tudzynski, & Van Kan, 2007). Therefore, the production of processed and durable products from strawberry such as puree, overcomes this short duration, thus avoiding fruit losses. Processed puree and concentrate can be used in the food industry as an ingredient to produce other food products. However, undesirable changes in the phenolic compounds cause browning of the cut fruit/puree during processing or storage (Lopez-Serrano & Barcelo, 2002). This is due to the activity of a specific endogenous enzyme called polyphenoloxidase (PPO), when the fruit tissues are exposed to oxygen. PPO has become the focus of this study because it is more resistant to pressure than other strawberry enzymes such as peroxidase (Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010). Fruits' PPO catalyzes the degradation of phenolic fruit constituents (o-diphenol oxidizes to o-quinones) in the presence of oxygen. The resulting o-quinone will subsequently polymerize with other o-quinone, protein or amino acids producing undesirable brown compounds (Fujita et al., 1995; Golangoldhirsh, Whitaker, & Kahn, 1984; Vámos-Vigyázó, 1981) and off taste/flavour in the strawberry products (Tomas-Barberan & Espin, 2001). This reaction could be inhibited or stopped either

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chemically by adding antioxidants such as vitamin C, or physically by freezing. Thermal denaturation at 70 °C or High Pressure Processing between 300 and 1000 MPa (Weemaes, Ludikhuyze, & van denHendrickx, 1998) can fully inactivate the enzyme.

Research and commercial developments in non-thermal processing technologies such as High Pressure Processing (HPP) (Tewari & Juneja, 2007) have been carried out because colour, flavour and nutrients of foods are almost not affected by HPP (Nguyen, Rastogi, & Balasubramaniam, 2007; Patras et al., 2009), and a number of food products have been commercialized. The process inactivates vegetative bacteria, yeast and moulds, however it is more difficult to inactivate spoilage bacterial spores and enzymes (IFT, 2000). Past attempts to use HPP or HPP combined with thermal processing, for PPO inactivation, were carried out with strawberries (Cano, Hernandez, & DeAncos, 1997; Cao et al., 2011; Garcia-Palazon, Suthanthangjai, Kajda, & Zabetakis, 2004; Terefe, Matthies, Simons, & Versteeg, 2009, 2010), strawberry extract (Dalmadi, Rapeanu, Van Loey, Smout, & Hendrickx, 2006) peaches (Guerrero-Beltran, Barbosa-Canovas, & Swanson, 2004), lychee (Phunchaisri & Apichartsrangkoon, 2005), apples (Bayindirli, Alpas, Bozoglu, & Hizal, 2006; Buckow, Weiss, & Knorr, 2009; Valdramidis et al., 2009), muscadine grapes (Del Pozo-Insfran, Del Follo-Martinez, Talcott, & Brenes, 2007) and avocados (Jacobo-Velázquez & Hernández-Brenes, 2010). All of these works focussing on pressures around 200–900 MPa and temperatures between 20 °C and 90 °C. PPO was partially inactivated in most works and the residual activity was recorded to be in the range of 5–90%, with the lowest recorded in 'Elsanta' strawberry PPO in phosphate buffer treated for 15 min at 800 MPa-25 °C, and the highest recorded in whole lychee treated for 20 min at 600 MPa-60 °C (Phunchaisri & Apichartsrangkoon, 2005). The following strawberry varieties were processed by HPP or HPP-thermal to investigate the effect of process on PPO: 'Pajaro' strawberry puree (Cano et al., 1997); 'Festival' strawberry halves (Terefe et al., 2009); 'Aroma' and 'Festival' strawberry purees (Terefe et al., 2010); 'Tongzi I' strawberry pulp (Cao et al., 2011).

The main objectives of this research were to HPP 'Camarosa' strawberry puree and investigate the inactivation of PPO, and also compare the PPO inactivation when using whole fruit vs. pureed strawberry for conventional preservation of the fruit by thermal processing and frozen storage. The specific objectives were to study: i) The effect of High Pressure Processing of strawberry puree at room temperature for 5 and 15 min on PPO activity; ii) The combined effect of pressure and mild temperatures processing of the puree on PPO activity; iii) The effect thermal processing of strawberry puree and whole fruit on PPO activity; iv) The effect of freezing and frozen storage of strawberry puree and whole fruit for 30 days at −18 °C and −70 °C, on PPO activity.

2. Materials and methods

The impact of the process on polyphenoloxidase (PPO) activity was assessed by the Residual Activity (Activity/Activity$_0$) × 100, where Activity$_0$ and Activity referred to the non-processed and processed fruit puree samples, respectively. The percentage enzyme residual activity (% RA) results were expressed as average ± standard deviation of replicate strawberry samples processed/stored under the same conditions.

2.1. Preparation of strawberry samples for processing

Ripe strawberries (*F. ananassa*, cv Camarosa) were bought from a local farm (Phil Greig Strawberry Farm) at Kumeu, New Zealand (puree pH is 3.31 ± 0.15 and soluble solids is 9.30 ± 0.10°Brix). Depending on the experiment, puree or whole strawberries were packed in food grade retort pouches (Cas-Pak, New Zealand) composed of polyester coated with silicon oxide, laminated to nylon and laminated to cast polypropylene (PET-SIOX(12)//ON(15)//RCPP(70)). These bags can withstand temperatures up to 130 °C, being suitable for thermal processing and high pressure applications. The low oxygen transmission rate (<2 cc/m^2/day) was suitable for freezing and frozen storage experiments. For HPP and thermal experiments 40 g of strawberry puree was packed in pouches of 150 mm × 105 mm and 1 mm thick. Two replicates of packed strawberry samples were processed for each processing condition (pressure, temperature, time) and the average enzyme residual activity ± standard deviation was calculated.

2.2. High Pressure Processing (HPP) of strawberry puree

Pressures of 200 MPa and 600 MPa for 5 min and 15 min at room temperature were tested. All HPP was carried out using the Avure 2L-700 HPP Laboratory Food Processing System (Serial No. 101130, USA) using distilled water as the pressure medium in the treatment chamber. The HPP chamber was equipped with a thermocouple to register the temperature during the HPP cycle.

2.3. Combined HPP-thermal processing of strawberry puree and thermal experiments

The effect of high pressure (200 MPa and 600 MPa) combined with mild temperature (50–71 °C) of strawberry puree on PPO activity was investigated for a 5 min treatment. The pressure–temperature–time processing conditions refer to the constant pressure phase of the HPP cycle. The initial total pressure increase took less than 2 min. The adiabatic heating for room temperature processes resulted in an average processing temperature of ca. 30 °C during the constant pressure phase of HPP. Initial temperature settings of 40 °C, 50 °C and 60 °C for 200 MPa and 600 MPa resulted in the average processing temperature of 50 °C, 58 °C and 65 °C and 57 °C, 62 °C and 71 °C, respectively, during the holding pressure phase. At the end of the constant pressure stage, the release of the pressure caused an instantaneous decompression. Then, the samples were immediately cooled in ice-water bath before the enzyme extraction.

The thermal inactivation of PPO in the absence of high pressure of packed strawberry puree was performed at 40 °C, 50 °C, 55 °C, 60 °C and 70 °C for 6.5 min (≈1.5 min puree centre come up time to the bath temperature, plus 5 min processing time) using a thermostatic water bath. The same amount of whole strawberries packed in the same pouch dimension was also thermally treated (40 °C, 50 °C), to investigate if there is any difference in PPO inactivation when using whole fruit rather than puree. The retort pouches with the whole fruit were introduced into the water bath, taken out at the pre-specified time intervals (5 min and 10 min), and cooled immediately with ice-cooled water before enzyme extraction. The result of PPO inactivation in whole strawberry was compared with puree.

2.4. Freezing and frozen storage of puree and whole fruit

The effect of freezing and frozen storage on PPO activity in puree and in the whole fruit was performed at −18 °C and −70 °C. Same harvest batch of whole strawberries (ca. 150 g each bag) and puree (about 20 g) were packed. Throughout a total storage period of 30 days, two replicates of frozen strawberry samples were taken, for assessing PPO enzyme activity at each storage time period.