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Synergistic impact of sonication and high hydrostatic pressure on microbial and enzymatic inactivation of apple juice

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ABSTRACT

The combination of innovative, non-thermal technologies for the production of safe and quality fruit juices is a recent trend in food processing. The purpose of this study was to evaluate the effects of combined treatment of ultrasound (US) and high hydrostatic pressure (HHP) on enzymes (polyphenolase, peroxidase and pectinmethylesterase), microorganisms (total plate counts, yeasts and molds) and phenolic compounds (total phenols, flavonoids and flavonols) of apple juice. Moreover, its effects on ascorbic acid, antioxidant capacity and DPPH free radical scavenging activity, color values, pH, soluble solids and titratable acidity were investigated. Fresh apple juice was treated with US (25 kHz and 70% amplitude) at 20 °C for 60 min with subsequent HHP treatment at 250, 350 and 450 MPa for 10 min at room temperature. The results revealed that the combined US-HHP450 treatment caused highest inactivation of enzymes with complete inactivation of total plate counts, yeasts and molds. It also significantly improved the phenolic compounds, ascorbic acid, antioxidant capacity, DPPH free radical scavenging activity and color values. The present results suggest that the combination of US and HHP can act as a potential hurdle to produce safe and high quality apple juice with reduced enzymes and microbial activity and improved nutrition.

1. Introduction

Apple (*Malus domestica*) is the most widely consumed fruit in the world. It is available in the market round the year and utilized by all classes of people due to its high nutritional profile (Vinson, Su, Zubik, & Bose, 2001). The use of apple juice reduces the risk of many diseases by improving the antioxidant level of blood (He et al., 2011). There has been a growing interest of consuming apple juice owing to its high nutritious and medicinal importance. However, some quality defects appearing during the processing and storage of apple juice limit its scope in the market if they are not controlled effectively. Enzymatic browning is the major defect that causes discoloration of apple juice and deteriorates its quality. It is well known that polyphenol oxidase (PPO) and peroxidase (POD) are involved in the browning of fruit and vegetable juices by the oxidation of phenolic compounds and contributes to the quality loss (Chisari, Barbagallo, & Spagna, 2007). In addition, microorganisms (yeasts, molds and aciduric bacteria) cause spoilage of fruit juices (Doyle & Beuchat, 2007; Jay & Anderson, 2001). They are responsible for the development of off-flavors, odors and gas production. Since quality is of utmost importance to the customers, therefore, inactivation of these enzymes and microorganisms is desirable to resolve the quality issues arising during the processing and storage of apple juice.

It is well established that heat treatments ensure the microbial safety and improve the shelf life, but they also degrade the nutritional value of food products (Gómez, Welti-Chanes, & Alzamora, 2011). Consequently, researchers are now looking for the most promising and result-oriented novel techniques that can be used without causing any loss of the nutrients. In recent years, interest has been developed from thermal food preservation methods towards non-thermal technologies. Therefore, several non-thermal technologies have been introduced such as ultrasound (US), high hydrostatic pressure (HHP), high pressure homogenization (HPH) and pulse electric field (PEF). The combination of these non-

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thermal techniques can improve the quality and shelf life of fruit juices and has become a potential alternative to the conventional methods (Rupasinghe & Yu, 2012). This newly emerging trend, known as hurdle technology, requires an intelligent and fruitful combination of non-thermal technologies for the preservation of food with improved quality and safety thus, can be regarded as a milder treatment. Previously, US processing coupled with mild heat and pressure have been reported to increase the inactivation of enzymes and microorganism (Lopez et al., 1994; Sala, Burgos, Condón, Lopez, & Raso, 1995). Many reports are available on evaluating the effects of combined treatments of different novel techniques in a variety of food products (Demirdöven & Baysal, 2008; Park, Lee, & Park, 2002; Ross, Griffiths, Mittal, & Deeth, 2003; Rupasinghe & Yu, 2012). Improvement in the quality of carrot juice as a result of combined treatment of blanching and sonication has recently been reported by our research team (Jabbar et al., 2014). To our knowledge, there is no report available on investigating the effects of combined treatment of US and HHP on the quality of apple juice. Therefore, the clear objective of this research work was to evaluate the combined effects of US and HHP on enzymes (PPO, POD and pectinmethylesterase (PME)), microorganisms (total plate counts, yeasts and molds), total phenols, flavonoids, flavonols, ascorbic acid, pH, titratable acidity, soluble solids and color of apple juice. It is expected that the findings of the present research would be useful in resolving the quality issues of apple juice.

2. Materials and methods

2.1. Chemicals

Gallic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin Ciocalteu reagent was purchased from Fluka (Buchs, Switzerland). Catechin was purchased from Funakoshi Co., Ltd. (Tokyo, Japan). Quercetin was purchased from Kayon Biological Technology Co., Ltd, (Shanghai, China). HPLC grade methanol was purchased from Hanbon Science and Technology (Nanjing, China). Sodium nitrite, sodium carbonate, sodium hydroxide, sodium chloride, pectin, catechol, tartaric acid, molten agar and potato dextrose agar, aluminum trichloride ($AlCl_3$), ascorbic acid, diabasic potassium phosphate (K_2HPO_4), monobasic potassium phosphate (KH_2PO_4), hydrogen peroxide (H_2O_2) and pyrogallol were obtained from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China). All other chemicals used were of analytical grade.

2.2. Preparation of apple juice

Fresh apples (*M. domestica cv. Fuji*) were purchased from a local market of Nanjing, China. Samples of 15 fruits per treatment were randomly selected and washed to get rid of dust and dirt particles, and cut into four pieces after drying with paper towels. Stems, seeds and over-ripe portions were discarded manually using a stainless steel knife. The juice was then extracted by using electrical machine of MJ-M176P (Panasonic Manufacturing Berhad, Malaysia) and passed through a double layered sterilized muslin cloth to remove the impurities and coarse particles. The juice was then mixed and subjected to US and HHP treatments. All the treatments were performed in triplicate.

2.3. Treatment of apple juice by US

Apple juice was treated with US using a bath cleaner of SB-500 DTY (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China). Briefly, the juice sample of 80 mL was taken in a 100 mL jacketed vessel and treated at 25 kHz frequency and 70% amplitude for 60 min. The ultrasonic intensity measured by using HI 9063 thermocouple (Hanna Instruments Ltd., UK) was 2 W cm^{-2}. The temperature was maintained at 20 °C by circulating the cold water through a jacketed vessel. To avoid any interference of light with samples, all the treatments were applied in darkness. The US treated samples were packed in polyethylene terephthalate pouches (washed with 2:98 v/v H_2O_2 and pasteurized at 80 °C for 30 min) and subsequently subjected to HHP treatment. All the treatments were performed in triplicate.

2.4. HHP treatment of apple juice

HHP processing of US treated samples was done in an ultra-high pressure pilot plant (Model UHPF, 3.5 L capacity, capable of 800–1000 MPa, Baotou Hi-tech Food Machinery Ltd., China) with water as a pressure transmitting medium. The HHP unit had a temperature control unit. The juice samples were subjected to pressures 250, 350 or 450 MPa for 10 min at room temperature. The pressure-increase time and pressure-release time were not included in the treatment time of the samples reported in this study. Six pouches per treatment containing 80 mL of sample in each were introduced into the pressure vessel. After HHP treatment, three pouches were selected by simple random sampling and stored at 4 °C for analysis within 2 days. All the treatments were performed in triplicate. The fresh (untreated) apple juice was taken as control.

2.5. Determination of POD residual activity

POD activity was measured by the method of Kwak et al. (1995) using pyrogallol as the substrate. Briefly, the juice sample was centrifuged at 10,000 *g* for 10 min at 4 °C (Avanti J-E Centrifuge, Beckman Coulter, Inc., USA). The reaction mixture contained 2.2 mL of centrifuged sample, 0.32 mL of potassium phosphate buffer (100 mM, pH 6), 0.32 mL of pyrogallol (5% w/v) and 0.16 mL of H_2O_2 (0.15 M). Hydrogen peroxide was added to initiate the reaction and the increase in absorbance (Abs) at 420 nm was measured by a spectrophotometer (LabTech Bluestar-A UV spectrophotometer, China) after every 60 s for 3 min. The percent residual activity of PPO was calculated by using following equation:

$$\text{Residual activity}\ (\%) = 100 \times \text{Abs}_t/\text{Abs}_0 \quad (1)$$

where Abs_t is the Abs of the treated sample and Abs_0 is the Abs of the control sample.

2.6. Determination of PPO residual activity

PPO activity was determined by the method described by Augustin, Ghazali, and Hashim (1985). The sample was centrifuged as mentioned above, and the reaction mixture contained 1.5 mL of centrifuged sample, 0.5 mL of catechol (0.05 M) and 3.0 mL of potassium phosphate buffer (0.2 M, pH 6.8) making the total volume of 5.0 mL. Increase in Abs at 410 nm was measured after every 60 s for 10 min, starting from zero by using a spectrophotometer. The percent residual activity of PPO was measured by using the Equation (1).

2.7. Determination of PME residual activity

PME activity was determined by measuring free carboxyl groups produced as a result of action of enzyme on pectin. The reaction mixture contained 10.0 mL centrifuged sample and 40.0 mL pectin solution (1:99, w/v) in 0.15 mol L^{-1} NaCl. The pH of the reaction mixture was adjusted to 7.7 by adding 100 μL 0.05 mol L^{-1} NaOH and the time required to reach pH 7.7 was measured (50 ± 2 °C).