

## **Impact of High Hydrostatic Pressure Processing on Fruit Flesh Quality of Fruit Containing Carrot Juice**

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**Abstract.** The quality of fruit flesh containing beverage is highly affected by the inside flesh. Using high hydrostatic pressure process, dealing with three different flesh types (apple, water chestnut, pear) carrot juice, analysis of different pressure (300 MPa, 400 MPa and 500 MPa) of polyphenol oxidase and peroxidase activity and total phenol content, the trend of total antioxidant value, and its influence to the flesh browning. The results show that different pressure treatment for apple and water chestnut of polyphenol oxidase enzyme activity change is not significant, and pear enzyme activity is positively correlated with the pressure. Apple and pear peroxidase enzyme activity are suppressed by pressure. Total phenol content has varying degrees of decline by high hydrostatic pressure process. Total antioxidant value is generally a downward trend with pressure rise, and the pear flesh has a better resistance to adverse environment. High hydrostatic pressure processing is effective in anti-flesh browning.

**Keywords:** high hydrostatic pressure, diverse fruit flesh, anti-browning.

### **1. Introduction**

Fruit containing carrot juice has broad market prospects because of its unique flavor and taste. At present, carrot juice products adopt the traditional way of thermal processing. It is known that thermal processing induces significant changes in chemical composition, affecting the bioaccessibility and the concentration of nutrients and health-promoting compounds such as vitamin C, carotenoids, and polyphenols [1].

Ultra high Pressure technology, also known as the High Static Pressure technology, is an effective alternative to the traditional thermal technology, providing high pressure (100-1000 MPa) and certain temperature conditions (below 0 °C to 100 °C) in food packing for a certain time. In order to eliminate harmful pathogens and the microorganisms responsible for vegetative spoilage, and to inactivate enzymes, with minimal modifications in nutritional and sensory quality [2]. There is abundant research on fruit juice itself while the study on the flesh inside the juice is quite less. The quality of inner flesh will affect the overall quality of the product, and the flesh browning may affect the appearance of the product seriously. Polyphenol oxidase (PPO) is a major cause of enzymatic browning. Peroxidase (POD) can participate in catalyzing oxidation discoloration of phenolic, glutathione, and ascorbic acid. POD may also involve in browning and the oxidation of phenols and ascorbic acid. Author choose three kinds of common fruits with a certain shape (water chestnut, apple, pear) as flesh additives into carrot juice analyzing the influence of HHP treatment on flesh additives. Indicators as the following aspects: the polyphenol oxidase (PPO) and peroxidase (POD) activity, total phenol content and total antioxidant capacity. Research in HHP influence on flesh browning resistance and internal flesh quality changes. Research aim to support HHP technology used in flesh containing carrot juice industry.

### **2. Method and Materials**

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## **2.1. Material**

Fresh carrots, apples, water chestnuts, pears were purchased from a local farmers' market (Cangyuan Road, Shanghai). Regents were AR level as sodium dihydrogen phosphate, disodium hydrogen phosphate, PVPP, Triton - 100, catechol and guaiacol etc. Experimental water was ultrapure water.

## **2.2. Method**

### **2.2.1. Preparation for juice sample**

Choose fresh carrot for washing, peeling, cutting, crushing, squeezing and filtered with mesh sieve. The ratio of material to solvent (ultrapure water) is 1:2. Choose fresh apples, water chestnuts, pears to clean, peeled, cored and sliced, cut into cube with 8 mm diameter by a certain mould. The ratio of fruit flesh to carrot juice was 1:10, packed by vacuum heat sealing. Juice sample were treated with 300 MP, 400 MP and 500 MP pressure processing respectively, holding time was 10 min.

### **2.2.2. PPO enzyme activity determination method**

#### **2.2.2.1. Extraction of crude enzyme**

Grind fruit flesh (5 g) with a small amount of quartz sand and phosphate buffer (PBA 0.2 M pH = 7) in a mortar, then transfer into centrifuge tube to 15 mL. Add 4% PVPP, 1% (w/v) Triton- 100 (v/v), 50  $\mu$ L 1 M NaCl solution into supernatant after centrifuging, blend mixture up, take 1.5 mL compound to 14000 g centrifugal 30 min. Take the supernatant as the crude enzyme.

#### **2.2.2.2. Determination of enzyme activity**

Add 300  $\mu$ L 0.05 M PBA (pH = 5.8 0.07 M catechol) as substrate and 10  $\mu$ L crude enzyme into 96-well plates. 300  $\mu$ L catechol solution and 10  $\mu$ L ultrapure water compound as blank control. The enzyme activity was determined in ELIASA under 420 nm absorbance value for 15 min. Determination repeated to express the enzyme activity by average [3], [4].

### **2.2.3. POD enzyme activity determination method**

#### **2.2.3.1. The extraction of crude enzyme**

Same as the PPO enzyme extraction method.

#### **2.2.3.2. Determination of enzyme activity**

Add 300  $\mu$ L 0.05M PBA (pH = 5.8, 7.2 mM guaiacol, 11.8 mM hydrogen peroxide) as substrate and 10  $\mu$ L crude enzyme into 96-well plates. The enzyme activity was determined in ELIASA under 470 nm absorbance value for 10 min. Determination had to be repeated to express the enzyme activity by average [5], [6].

### **2.2.4. Determination of total phenol content**

#### **2.2.4.1. Sample preparation**

Select the processed juice sample and treat the inside flesh as follows: blanching→ cooling→ enucleating→ cutting→ squeezing→ filtered with mesh sieve→ subpackage.

#### **2.2.4.2. Total phenol standard curve plotting**

Weigh 50 mg gallic acid accurately, dilute with water to 50 mL. Transfer 100, 200, 300, 400, 500  $\mu$ L into volumetric flask respectively dilute with water to 10 mL to gain standard solution of 10, 20, 30, 40, 50 mg/L concentration. Transfer 1 mL above-mentioned different concentration standard solution to 10 mL volumetric flask, with 2.5 mL Folin Ciocalteu reagent, standing for 5 min after blending. Add 2 mL sodium carbonate solution (75 g/L), dilute with water. Water bath (30 °C, 2 h), determine 760 nm absorbance values and plot the standard curve.

#### **2.2.4.3. Total phenol content determination**

Mix 1 mL sample with 2.5 mL Folin -Ciocalteu reagent, stand for 5 min after blending. Add 2 mL sodium carbonate solution (75 g/L), dilute with water. Water bath (30 °C, 2 h), determine 760 nm absorbance values. Ultrapure water as the blank control. The total phenol content of samples expressed as gallic acid equivalent (GAE, mg GAE/mL) per milliliter juice [7].

### **2.2.5. Determination of total antioxidant capacity**

### 2.2.5.1. Sample preparation

Grind flesh sample 5 g with a small amount of quartz sand and PBA (0.2 M pH = 7) in a mortar. Transfer into centrifuge tube to 15 mL, centrifuging for 15 min under 4000 rpm condition. Take 1 mL supernatant as the sample to be tested.

### 2.2.5.2. Standard curve plotting

Weighing 27.8 mg  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  diluted with ultrapure water to 1 mL, of which concentration is 100 mM. Dilute 100 mM  $\text{FeSO}_4$  solution to 0.15, 0.3, 0.6, 0.9, 1.2 and 1.5 mM.  $\text{FeSO}_4$  solution should be fresh.

### 2.2.5.3. Determination of total antioxidant capacity

(1) Add 180  $\mu\text{L}$  FRAP working liquid to 96-well plates.

(2) Mix 5  $\mu\text{L}$  water with working liquid as blank control; Mix 5  $\mu\text{L}$  various concentration of FRAP solution with working liquid as standard solution; Mix 5  $\mu\text{L}$  sample with working liquid to be tested. Gently blend.

(3) 37 °C incubation for 3-5 min and determine absorbance value under 593 nm.

Calculate the total antioxidant capacity according to the standard curve.

### 2.2.6. Data analysis

Experimental index determination repeated for three times. Using GraphPad Prism 6 software for data processing and statistical analysis, significance test was carried out by Duncan multiple comparison method ( $P < 0.05$ ).

## 3. Results and Analysis

### 3.1. Influence of HHP Treatment on PPO Enzyme Activity

Enzyme activity is expressed by relative enzyme activity, namely  $A/A_0$  ( $A$  is the absorbance value before reaction,  $A_0$  is the absorbance value after reaction, %). Fig. 1 shows that apple flesh has the lowest relative activity, about 90%. Apples and water chestnut flesh PPO enzyme activity do not change significantly under the treatment of different pressure processing (300 MPa, 400 MPa and 500 MPa). Pear flesh is different from the former two fleshes for its relative enzyme activity increase as processing pressure increase, and rise to peak about 137% when 500 MPa. At present, research about the influence of HHP technology on juice inside flesh PPO enzyme activity is not much while the application of HHP technology to fruits and vegetables and their by-products is quite advanced. For PPO enzyme activity, thermal procession seizes significant inhibition of fruits and vegetables [8]. HHP processing is different from thermal processing for its impact is inapparent on PPO enzyme from flesh containing carrot juice. Guerrero-Beltran, Barbosa-Canovas [9] found that under over 500 MPa and 5 min holding time condition, the inhibition to PPO enzyme activity from peach flesh has significantly increased. Similar result also found in spinach [10]. Gonzalez-Cebrino [11] found plum flesh PPO enzyme have no significant change under 400 to 600 MPa pressure condition with various processing time (1-300 s). HHP processing has different influence on different fruits and vegetables' enzyme activity, which consistent with the results of the study.

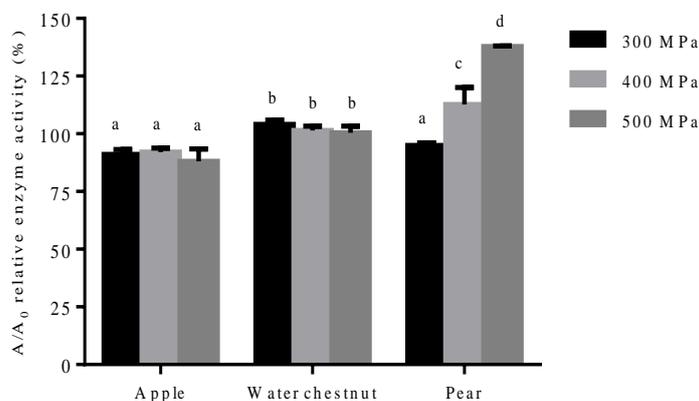


Fig. 1: HHP effects on PPO enzyme activity

Note: abcd from figure show the significances of different treatment groups

The reason PPO enzyme not significantly inhibited could be the high pressure result in cell membrane rupture and release of insoluble substance. Which cause the rise of relative enzymes, lead to the increase of enzyme activity determination. What's more, the release of cytosol component could activate enzyme potentially. Such activation would increase enzyme activity and neutralize the HHP procession inhibition to enzyme, lead to the observed result [12]. Hypotheses also suggest that HHP treatment could lead to generate more activated isoenzymes [9].

### 3.2. Influence of HHP Treatment on POD Enzyme Activity

Fig. 2 shows that apple flesh POD enzyme activity is significantly inhibited under three pressure condition (300 MPa, 400 MPa and 500 MPa), while pressure change cast a little influence on enzyme activity change, the greatest inhibition obtained under 300 MPa condition. Water chestnut POD enzyme activity has no significant change or significant inhibition because of pressure change. Low pressure (300 MPa) may promote water chestnut flesh enzyme activity, when pressure up to 400 MPa and 500 MPa, POD enzyme fell slightly but not dramatically. Pear flesh POD enzyme activity goes up and down as pressure rise, and reaches the peak about 400 MPa. This maybe because the effect of pressure inhibition to enzyme and pressure promotion to system enzyme generation has dynamic equilibrium, and the promotion effect come to peak about 400 MPa condition. Then as the pressure rise, pressure inhibition is predominant. Overall, the pressure for POD enzyme activity in the pear flesh is of inhibition. Woolf found avocado POD enzyme activity fell 50 % down under 400 -600 pressure condition with 3 -5 min processing time, which conform to the apple flesh POD enzyme change in this study. Other researches found that under the condition of high temperature HPP (600 MPa, 60 °C, 10 min), POD enzyme activity of strawberry fruit flesh with significant decrease of 16% to 37% [13].

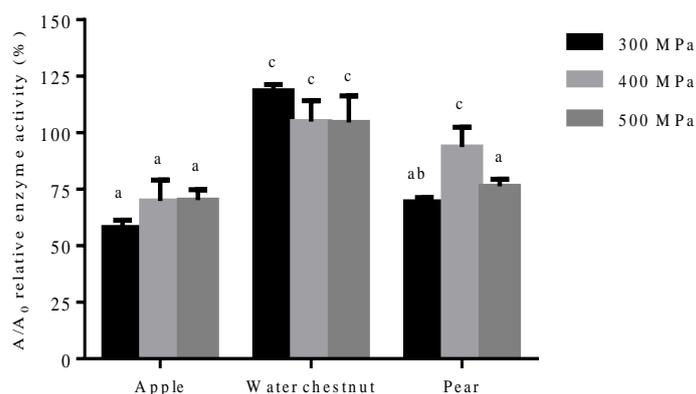


Fig. 2: HHP effects on POD enzyme activity

Note: abc from figure show the significances of different treatment groups

### 3.3. Influence of HHP Treatment on Total Antioxidant Capacity

Standard curve equation as follow:  $y = 0.3358x + 0.0174$  ( $R^2 = 0.9938$ ). As Fig. 3 shows, among such three kinds of experimental fruit flesh, pear flesh has no significant change, water chestnut flesh's antioxidant activity decrease under 400 MPa and 500 MPa, apple flesh antioxidant activity reduce under the condition of high pressure (500 MPa).

Antioxidant activity is a very important quality index on the trophic level for fruits and vegetables. McInerney, Seccafien, Stewart [7] found the influence of HHP treatment on water-soluble antioxidant activity was related to the types of fruits and vegetables. HHP treatment had no effect on water-soluble antioxidant activity in broccoli. And under lower pressure level (400 MPa) procession, carrot water-soluble antioxidant activity was slightly lower. However, mung beans' water soluble antioxidant activity increased under 400 MPa and 600 MPa pressure condition. Three kinds of experimental fruit antioxidant activity have different reaction to the change of pressure. Patras [14] found pressure processing for antioxidant activity substance, or promote, or have no obvious influence. Zhou [15] indicated that HHP treatment and traditional thermal treatment have a significant adverse impact on pumpkin components antioxidant activity, while HHP treatment do less adverse effect compared with traditional thermal treatment.

The lower of the antioxidant activity may be due to the decrease of flesh Vc and total phenol content. There are a lot of researches to support the above hypothesis and indicate that antioxidant activity and Vc, total phenol content has positive correlation effect [16], [17]. Therefore, the experimental results are also partly reflects the influence of HHP treatment on total phenol content and the stability of vitamin. Meanwhile, pear flesh antioxidant activity value has no significant change might due to high pressure, which results in the decomposition of natural substances in pear flesh and bioactive substances generation. Bioactive substances generation offset the antioxidative substance inhibition against high pressure. Besides, the flesh crushing degree and juice extraction rate during sample preparation step is also one of the factors that affect the results. Among three kinds of experimental fruit, apples and water chestnuts have weak pressure tolerance, which also indicate apples and water chestnuts are weak to adverse environment.

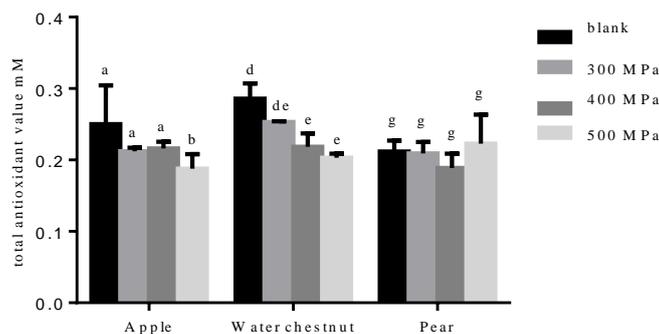


Fig. 3: HHP effects on total antioxidant value

Note: ab, de, g from figure show the significances of different treatment groups

### 3.4. Influence of HHP Treatment on Total Phenol Content

Standard curve equation as follow:  $y = 0.0081x + 0.0993 (R^2 = 0.9914)$ . Fig. 4 shows that treated group compared to untreated group, the total phenol content has a certain degree of decline. Apple flesh total phenol content went on as pressure rose. Water chestnut flesh phenol content went up and down as pressure rose, and came to maximum about 400 MPa, close to the untreated group content. Pear flesh total phenol content rises slightly as pressure rising, the trend goes gentle in the end and 400 MPa, 500 MPa total phenol content difference is not obvious. Apples and pears total phenol content rising trend is quite similar with Patras's [14] finding that pressure treatment can improve the total phenol content in vegetables. Increasing of total phenol content may because the rising pressure results in membrane penetration increasing and leaking more phenolic. Also because the high pressure environment lead to polyphenols polymer covalent bond destruction, polyphenols generate free of polyphenol compound monomer. Wang [16] also mentioned that HHP treated sample total phenol content rising is due to the high pressure and result in membrane penetration rising and bioactive substances releasing. In addition, because the products is fruit containing carrot juice products, fruit flesh is in carrot juice system, there may be part of the total phenol leaking into carrot juice, without being detected. So the actual total phenol content should be higher than the determined content.

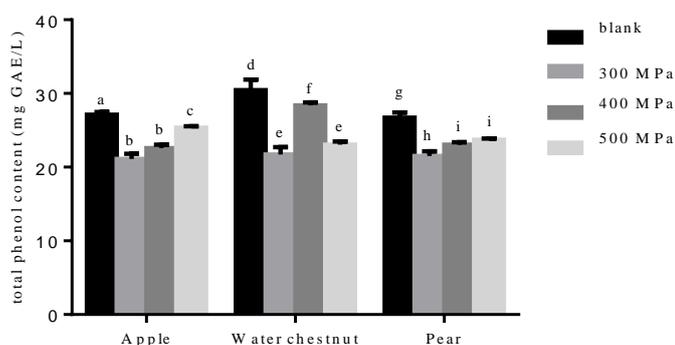


Fig. 4: HHP effects on total phenol content

Note: abc, def, ghi from figure show the significances of different treatment groups

## 4. Conclusion

Apple flesh PPO and POD enzyme decreased by HHP treatment while fall trend is not significant as pressure rising. The total phenol content increased as pressure rising. Such results may be because under the precondition of enzyme activity decrease, enzymes on the utilization rate of phenol reduced combined with the rising membrane penetration lead to release more phenolic. Therefore, HHP treatment is effective for inhibiting apple flesh browning.

Water chestnut flesh PPO enzyme is not sensitive to pressure, POD enzyme is promoted by low pressure environment. Total phenol content peaked under 400 MPa condition, which may be due to the PPO and POD enzyme synergy minimized under 400 MPa condition. High pressure could inhibit water chestnut flesh browning, and best under 400 MPa condition.

Pear flesh PPO enzyme activity rises as pressure rising, POD enzyme activity is inhibited under three pressure condition and peaked under 400 MPa condition. Total phenol content lowest under 300 MPa condition, increases slightly and basically the same under 400 MPa and 500 MPa condition. Probably pear flesh cell membrane permeability is easily affected by pressure change, which result in polyphenol content remains high under high PPO enzyme activity environment. 500 MPa condition, POD enzyme activity is low and PPO enzyme reach peak, polyphenol remain high level. Pressure processing is also helpful on pear flesh anti-browning.

## 5. Acknowledgements

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