Advance Journal of Food Science and Technology 7(9): 681-684, 2015
DOI:10.19026/ajfst.7.1627
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: July 18, 2014 Accepted: August 26, 2014 Published: March 25, 2015

Research Article

Effects of Dense Phase-CO_2 Treatments on Microflora, Enzymes and Browning of Chinese Winter Jujube Juice

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Abstract: Chinese winter jujube juice was treated with Dense Phase Carbon Dioxide (DPCD) to inactivate microorganisms, Polyphenoloxidase (PPO) activity, Peroxidase (POD) activity and control browing. The effect of varying pressures (5 to 30 MPa) and different treatment time (0 to 50 min) on the amount of natural microorganisms, PPO activity, POD activity and browning degree were measured. After experiment, the inactivation of natural microorganisms, PPO activity and POD activity exposed to DPCD were significantly increased with increasing pressure. Treated jujube juice had lower browning degree than untreated jujube juice. The experimental results showed that the DPCD treatment can be regarded as a good pretreatment means for winter jujube juice processing.

Keywords: Chinese winter jujube, dense phase carbon dioxide, enzymatic browning, juice, microorganism

INTRODUCTION

Chinese winter jujube (*Zizyphus jujuba* Miller) is indigenous to China with a history of over 4000 years (Wang *et al*., 2009). It has been widely planted in China for its high nutritional value, good taste (Li *et al*., 2007; Zhu *et al*., 2009). There have been numerous studies focused on the preservation and processing of Chinese winter jujube. The cloudy Chinese winter jujube juice has a growing share in the current market due to its sensory and nutritional qualities, which meets the consumers' demand for fresh-like products with little or no degradation of nutritional and organoleptic properties. Non-thermal pasteurization of juices have been increased interests, such as high hydrostatic pressure (Chen *et al*., 2012), pulsed electric fields (Huang *et al*., 2014) and Dense Phase Carbon Dioxide (DPCD) (Chen *et al*., 2010).

DPCD has been reported to inactivate different microorganisms in liquid foods without exposing them to the adverse effects of heat which allows retain their fresh-like physical, nutritional and sensory properties (Yuk *et al*., 2010). It has also been proven effective in inactivating many undesirable enzymes including Polyphenoloxidas (PPO) and Peroxidase (POD) (Niu *et al*., 2010; Carmen *et al*., 2013). Although there have been reports on DPCD treatment to inactivate microorganisms and browning enzyme in many fruit juices, there is a lack of information on the inactivate microorganisms and browning enzyme of Chinese winter jujube juice with DPCD. The objectives of the present study were to investigate the feasibility of inactivating microorganisms, PPO and POD in Chinese winter jujube juice by DPCD treatment and to understand some quality attributes of Chinese winter jujube juice after DPCD treatment.

MATERIALS AND METHODS

Preparation of jujube juice: Chinese winter jujube were purchased from the local market in Zhengzhou (China) and stored in a cold warehouse at an average temperature of 4°C. It were washed and squeezed with a screw juice extractor (SC-100, Kexin Food Machinery Plant, Zhengzhou, China) and were then filtered with 4 layers of cheesecloth. Then the jujube juice was continuously centrifuged (Westfallia centrifuge, Germany) in 6000 r/min for 15 min. Supernatant was divided into different sterilized containers for experiment.

Processing of cloudy jujube juice using DPCD: The DPCD equipment was consisted of a CO_2 cylinder, a heat exchanger, a filiter, a pump, a seperator and a sample processor. For each treatment, aliquots of 5 mL of cloudy jujube juice in 10 mL of plastic test tubes (15 mm diameter) were placed in the treatment vessel. The sensitivity of microorganisms, PPO, POD and related qualities of cloudy jujube juice to treatment with DPCD were determined under varying pressures (5 to 30 MPa) and temperatures 25°C, for different treatment durations (0 to 50 min). All the experiments were repeated in triplicate.

Control treatment of jujube juice: Five milliliter of jujube juice in 10 mL of plastic test tubes were placed

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in the DPCD sample processor without pressurizing and treatment time as DPCD treatment.

Microbiological analysis: China national standard (GB4789.2-94) was used to calculate the amount of bacteria in the jujube juice. The results are expressed as $\log N/N_0$, where N represents the number of colonies in the treated sample and N_0 is the number in untreated sample.

PPO activity determination: PPO activity was measured by a spectrophotometric method (Baslar and Ertugay, 2013). Jujube juice samples were centrifugal at 12000 r/min, 4°C for 15 min. The supernatant liquid was used to determination of enzyme activity. Catechol was chosen as the substrate and 10 mmol/L catechol substrate solution was prepared with 0.1 mol/L phosphate buffer (pH 7.0). The assay was performed for all samples by adding 0.1 mL jujube juice into 2.9 mL substrate solution. Mixture was keep temperature at 30±0.1°C. The increase in absorbance at 398 nm was monitored at intervals of 0.1 sec with a spectrophotometer (Puxi Co. Ltd., Beijing, China). One unit of PPO activity was defined as an increase in the absorbance of 0.001 min^{-1} at 398 nm. The slope of the very first linear region of the reaction curve was used as the PPO activity. The Residual Activity (RA) of PPO was estimated using the following formula:

RA (%) = (PPO activity after treatment/PPO activity before treatment) ×100%

POD activity determination: POD activity was measured by a spectrophotometric method (Hirsch *et al*., 2008). Ten milliliter jujube juic samples were mixed with 1% polyvinylpolypyrrolidone. Mixtures were centrifugal at 10000 r/min, 4°C for 20 min. The supernatant liquid was used to determination of enzyme activity. The assay was performed for all samples by adding 2.7 mL 200 mmol/L phosphate buffer (pH 6.0), 0.05 mL 0.5% hydrogen peroxide, 0.2 mL 2% guaiacol and 0.05 mL juice supernatant liquid. Mixture was keep temperature at 20±0.1°C for 3 min. Then the increase in absorbance at 470 nm was monitored at intervals of 30 sec with a spectrophotometer (Puxi Co. Ltd., Beijing, China). One unit of POD activity was defined as an increase in the absorbance of 0.001 min^{-1} at 470 nm. The Residual Activity (RA) of POD was estimated using the following formula:

RA (%) = (POD activity after treatment/POD activity before treatment) ×100%

Determination of Browning Degree (BD): After DPCD treatment, jujube juice samples were kept at 4°C for 24 h to determination of BD. The BD of cloudy apple juice was analyzed using a spectrophotometric method. Jujube juice centrifuged with a refrigerated centrifuge a TGL-16G-A refrigeration centrifuge (Anting apparatus Co., Shanghai, China) at 10,000×g at 4°C for 30 min, then passed through a 0.45 μm cellulose nitrate membrane (Beijing Bomex Co., Beijing, China). The BD was determined by measuring the A (absorbance at 420 nm) value using a spectrophotometer (Puxi Co. Ltd., Beijing, China) at an ambient temperature (20±1°C) with a 1 cm pathlength cell.

Statistical analysis: All experiments were performed in triplicate. Data were Analysis of Variance (ANOVA) using the software Microcal Origin 7.5 (Microcal Software, Inc., Northampton, USA). Means of treatments were separated at the 5% significance level.

RESULTS AND DISCUSSION

Inactivation of natural microorganisms in jujube juice: To determine the effect of DPCD on inactivation of natural microorganisms, samples were exposed to DPCD at different pressure (5 to 30 MPa) and different treatment time (0 to 50 min) at 25°C. The reductions in natural microorganisms counts were determined and the results are expressed as log N/N_0 as shown in Fig. 1.

At certain temperature (25°C), the inactivation of natural microorganisms exposed to DPCD was significantly increased ($p<0.05$) with increasing pressure. The inactivation curves of microorganisms were divided into three stages, namely the lag, exponential death and stationary phases. Theoretically, pressure controls both the rate of solubilization of CO_2 and its solubility in a suspending medium. Thus, higher pressure enhances CO_2 solubilization to facilitate its contact with and penetration into cells. Furthermore, CO_2 dissolves in solutions can form carbonic acid to reduces the pH of the solutions. All these results can increase CO_2 into cells and inactivation of natural microorganisms.

Inactivation of PPO in cloudy jujube juice: To determine the effect of DPCD on inactivation of PPO in

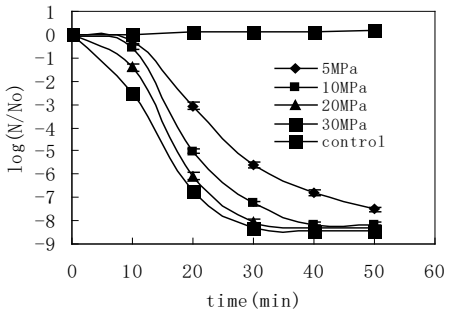

Fig. 1: Effect of DPCD on inactivation of natural microorganisms at various pressures

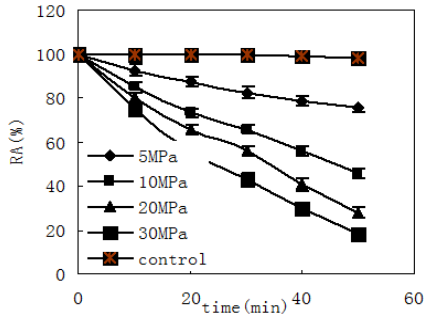

Fig. 2: Effect of DPCD on inactivation of PPO at various pressures

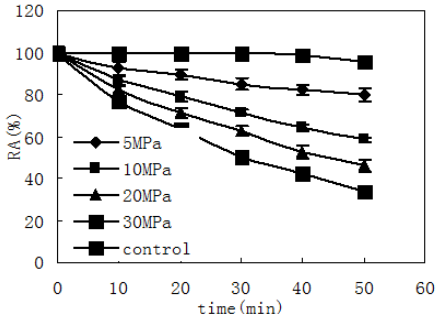

Fig. 3: Effect of DPCD on inactivation of POD at various pressures

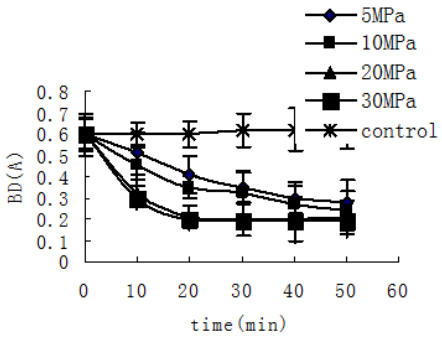

Fig. 4: Effect of DPCD on change of browning degree at various pressures

cloudy jujube juice, samples were exposed to DPCD at different pressure (5 to 30 MPa) and different treatment time (0 to 50 min) at 25°C. The residual activity of PPO were determined and the results are expressed as RA as shown in Fig. 2.

At certain temperature, the inactivation of PPO exposed to DPCD was significantly increased ($p<0.05$) with increasing pressure similar results were reported in previous investigations (Liu *et al*., 2010). The inactivation curves of PPO were fit for first-order kinetics equations. Theoretically, the changes in PPO activity may be attributed to changes in protein structure, enzyme stability and/or disruption of enzyme-substrate interactions under the conditions of DPCD.

Inactivation of POD in cloudy jujube juice: To determine the effect of DPCD on inactivation of POD in cloudy jujube juice, samples were exposed to DPCD at different pressure (5, 10, 20 and 30 MPa, respectively) and different treatment time (0, 10, 20, 30, 40 and 50 min, respectively) at 25°C. The residual activity of POD were determined and the results are expressed as RA as shown in Fig. 3.

At certain temperature, the inactivation of POD exposed to DPCD was significantly increased ($p<0.05$) with increasing pressure similar to PPO. The inactivation curves of POD were also fit for first-order kinetics equations. The changes in POD activity may also be attributed to changes in protein structure, enzyme stability and/or disruption of enzyme-substrate interactions under the conditions of DPCD as PPO.

Change of browning degree: To determine the effect of DPCD on turbidity of cloudy jujube juice, samples were exposed to DPCD at different pressure (5 to 30 MPa) and different treatment time (0 to 50 min) at 25°C. The change of browning degree as shown in Fig. 4.

At certain temperature, the browning degree exposed to DPCD was significantly decreased ($p<0.05$) compared to controls. PPO and POD were the key enzyme in juice browing reaction. These observations also confirmed that HPCD treatment effectively inhibited the PPO activity and POD activity to inhibite the enzymatic browning in jujube juice.

CONCLUSION

The inactivation of microorganisms by DPCD treatment in jujube juice had a positive correlation with pressure and holding time. The inactivation of PPO and POD activity by DPCD treatment in jujube juice also had a positive correlation with pressure and holding time. The inactivation of PPO and POD activity can inhibite the enzymatic browning in jujube juice. Therefore, HPCD was possibly regarded as a pretreatment means in winter jujube juice processing.

ACKNOWLEDGMENT

This study was financially supported by the national science and technology support of China (2012BAD36B07).

REFERENCES

Baslar, M. and M.F. Ertugay, 2013. The effect of ultrasound and photosonication treatment on polyphenoloxidase (PPO) activity, total phenolic component and colour of apple juice. Int. J. Food Sci. Tech., 48: 886-892.

Carmen, O., D. Trang and B. Murat, 2013. Combined high hydrostatic pressure and carbon dioxide inactivation of pectin methylesterase, polyphenol oxidase and peroxidase in feijoa puree. J. Supercrit. Fluid., 82: 56-62.

Chen, J.L., J. Zhang and L. Song, 2010. Changes in microorganism, enzyme, aroma of hami melon (*Cucumis melo* L.) juice treated with dense phase carbon dioxide and stored at 4 degrees C. Innov. Food Sci. Tech., 11: 623-629.

Chen, D., H.P. Xi and X.F. Guo, 2012. The effect of high hydrostatic pressure on the microbiological quality and safety of carrot juice during refrigerated storage. Food Microbiol., 30: 205-212.

Hirsch, A.R., K. Foerch and S. Neidhart, 2008. Effects of thermal treatments and storage on pectin methylesterase and peroxidase activity in freshly squeezed orange juice. J. Agr. Food Chem., 56(14): 5691-5699.

Huang, K., L. Yu and W. Wang, 2014. Comparing the pulsed electric field resistance of the microorganisms in grape juice: Application of the Weibull model. Food Control, 35: 241-251.

Li, J.W., L.P. Fan and S.D. Ding, 2007. Nutritional composition of five cultivars of Chinese jujube. Food Chem., 103: 454-460.

Liu, Y., C. Zhang and X.Y. Zhao, 2010. Inactivation of polyphenol oxidase from frozen red raspberry (*Rubus idaeus* L.) by high pressure carbon dioxide treatment. Int. J. Food Sci. Tech., 45(4): 800-806.

Niu, H., Z.H. Xu and Y.D. Fang, 2010. Comparative study on cloudy apple juice qualities from apple slices treated by high pressure carbon dioxide and mild heat. Innov. Food Sci. Emerg., 11: 91-97.

Wang, Y., T. Yu and Y. Li, 2009. Postharvest biocontrol of *Alternaria alternata* in Chinese winter jujube by *Rhodosporidium paludigenum*. J. Appl. Microbiol., 107(5): 1492-1498.

Yuk, H.G., D.J. Geveke and H.Q. Zhang, 2010. Efficacy of supercritical carbon dioxide for nonthermal inactivation of Escherichia coli K12 in apple cider. Int. J. Food Microbiol., 138: 91-99.

Zhu, S.H., L. Sun and J. Zhou, 2009. Effects of nitric oxide fumigation on phenolic metabolism of postharvest Chinese winter jujube (*Zizyphus jujuba* Mill. cv. Dongzao) in relation to fruit quality. LWT-Food Sci. Technol., 42: 1009-1014.