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Original Research Article

Effect of Combination Treatment of High Pressure Processing and Anti-browning Agents on Discoloration of Fresh-cut Burdock (*Arctium lappa* L.)

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ABSTRACT

Recently, high hydrostatic pressure processing (HPP) has been noted since the advantage of both non-thermal and non-chemical treatments with small degradation of flavor and texture. In this study, the effect of HPP and anti-browning agents on the discoloration of fresh-cut burdock was investigated. Treated fresh-cut burdock samples were stored at 6°C for 3 days and browning level was evaluated every day as indicated by L*, and h° values. The results showed that there were no significant difference in L* and h° values between 1 MPa and 80 MPa. The L * and h ° values in the combination treatment of HPP and 5% citric acid were higher than those of HPP with 1% citric acid. The results infer that a combination of HPP and citric acid was more effective for anti-discoloration than non-treated, or treated with only HPP.

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INTRODUCTION

The consumption of semi-cooked vegetables has been increasing due to its convenience and availability. However, some of these vegetables are susceptible to the discoloration, such as browning and yellowing as a result of polyphenoloxidase (PPO) reacting activity. Burdock roots (Gobo, *Arctium lappa* L.) are easily turned brown rapidly after peeling and cutting, therefore addition of anti-browning agents such as citric acid or ascorbic acid, and a blanching treatment have been used for inhibiting the discoloration in order to keep their market value. In general, it takes long time for inhibiting process of produce discoloration by soaking treatment using anti-browning solution with high concentration. Further improvements of browning inhibition process and the reduction of chemicals are extremely required by the effective arrangement of alternative technologies. High hydrostatic pressure (HHP) treatment has been reported to inactivate the activity of PPO *in vitro* study (Gomes and Ledward, 1996). Although HHP is a typical non-thermal/chemical technology and does not have any negative influences on the important quality attributes of fresh produces such as flavor, taste, color, nutrition etc., however, the reports on application of HHP as an anti-browning treatment in burdock root is limited.

In this study, the effect of HPP and anti-browning agents on the discoloration and PPO activity of fresh-cut burdock was investigated. Therefore, the changes of browning degree and polyphenol oxidase (PPO) of burdock after HHP treatment and HHP combined with browning inhibitor were examined.

MATERIALAND METHODS

Plant materials and sample preparation

Burdock samples used in this study were 'Yanagawa risou' harvested from Kagoshima and Miyazaki prefecture, and obtained from Kagoshima Organic Farmer's Association. Soil and debris on burdock surface were washed away by tap water, and rinsed by deionized water. Burdock samples with more than 15mm diameter were cut into 4 cm length by sterile knife.

High pressure processing

The prepared fresh-cut burdocks were packaged in polyethylene bag with 100mL of 1% (pH2.34) or 5% (pH1.93) citric acid solution as an anti-browning agent, and tightly sealed without air. Distilled water was used as a control treatment. HHP treatments were performed by commercial pressurization apparatus (Marugoto Ekisu-2L, Toyo Koatsu Inc., Hiroshima, Japan) as shown in Figure 1. Prepared sample pouch was put into vessel with distilled water. Pressure levels were 1 or 80MPa and the treatment time and temperature was 30 min and 20°C, respectively. The rates of pressurization/depressurization to/from 80MPa were approximately 5 min. After pressurization, burdock samples were removed from polyethylene bag and excessive solution was removed. Treated samples were in packaged another plastic bag, and storage test was conducted in refrigerator at 6°C for up to 3 days. Quality evaluation described below was conducted.

Color measurement of surface of burdock

Color of burdock sample was measured by color meter (NF333, Nippon Denshoku Industries Co. Ltd., Tokyo). The 5mm edge of burdock sample was cut off, and the color of cortex part of the cross sectioned surface was measured. The obtained L* (brightness) and h° (hue) values were used as the indicator of discoloration during storage.

Measurement of PPO activity

PPO activity was measured by the methodology of Yoshida and Hashimoto (2014) with some modification.

Epidermis of burdock samples immediately after HHP treatment and 3 days in storage at 6°C were removed by kitchen peeler. A 2.5 g of sample was homogenized by blade mixer with 150mL of 0.1M phosphate buffer (pH6.8) and 1.0 g of polyvinyl-pyrrolidone at 0°C for 1 min. Homogenized sample were transferred to 50mL plastic conical tube, and centrifuged at 10,000×g, 4°C for 15min. The obtained supernatant filtrated through a filter paper was used for the following PPO activity evaluation.

Fig. 1 High pressure processing apparatus (Toyo Koatsu Inc.)

As a reaction substrate, 10mM of chlorogenic acid (Wako Pure Chemical Co, Saitama, Japan) dissolved in 0.1M phosphate buffer (pH6.8) was used in this study. A 0.1 mL of crude enzyme solution was mixed with 4 mL of chlorogenic acid solution, and the periodical absorbance of mixed solution at 420nm was measured by self-record spectrophotometer (U-2900, Hitachi High-Tech Science Co., Tokyo, Japan) up to 5min. PPO activity as expressed unit (U) was defined as a 0.0001 increase in the optical absorbance during 1 min per 1 mL of crude solution. All PPO activity assay was performed below 25°C. A crude solution inactivated at 85°C for 15 min was used as a control sample. Both solutions of crude enzyme extract and chlorogenic acid were incubated at 30°C for 10 min before reacting procedure.

RESULTS AND DISCUSSION

Effect of combination of HHP treatment with anti-browning agents on the surface colour of cut burdock

Table 1 shows the L* and h° of cut burdock treated by HHP with distilled water and stored up to 3 days. After HHP treatment, both 1 and 80MPa, L* value (brightness) of burdock samples were lower than that of control. The measured h° values of cut burdock after 3 days in storage were decreased comparing with the day 0 sample. It is obvious that brightness reduction of cut burdock was accelerated by HHP treatment with distilled water due to the penetration of water molecule into both of cell intercellular spaces. The obtained L* values of HHP treated cut burdock were smaller than the control sample, however, browning phenomena resulted from oxidation reaction were not observed.

Table 1 Effect of HHP treatments on the surface color of cut burdock

	L*		h°	
	just after treatment	3 days	just after treatment	3 days
control	54.56 a	55.36 a	84.77 a	69.21 a
1MPa	33.27 b	35.06 ab	91.17 ab	58.91 ab
80MPa	30.71 b	32.63 b	98.62 b	53.58 b

Values have same letter within storage days for each color parameter are not significantly different at 5% level by Tukey Kramer test (n=5).

Table 2 Effect of combination of HHP treatments with citric acid on the L* value of cut burdock

	1%CA		5%CA	
treatment	just after treatment	3 days	just after treatment	3 days
control	54.56 a	55.36 a	54.56 a	55.36 a
soaking	57.73 b	58.15 a	58.30 b	58.94 a
1MPa	40.33 c	37.08 b	42.58 c	45.00 b
80MPa	34.68 d	35.22 b	39.46 c	45.58 b

Values have same letter within storage days for each color parameter are not significantly different at 5% level by Tukey Kramer test (n=5).

Table 3 Effect of combination of HHP treatments with citric acid on the h° value of cut burdock

	1%CA		5%CA	
	just after treatment	3 days	just after treatment	3 days
control	84.78 a	69.22 a	84.77 a	69.22 a
soaking	89.03 c	70.94 a	88.88 a	81.54 b
1MPa	99.56 bc	71.09 a	112.21 b	88.68 b
80MPa	104.79 b	79.14 b	103.52 b	97.61 c

Values have same letter within storage days for each color parameter are not significantly different at 5% level by Tukey Kramer test (n=5).

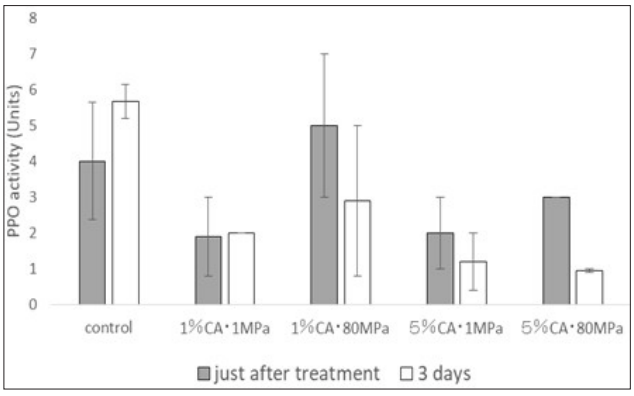

Fig. 2 Changes in polyphenoloxidase (PPO) activity of cut burdock treated with high pressure treatment (1MPa, 80MPa) and citric acid solution (1%, 5%). Error bars represent SE of the means (n=3)

The combination with citric acid were investigated and the results of surface color related to the change of L* and h° are shown in Table 2 and 3, respectively. Comparing with the control sample, both L* and h° values were lower significantly, and lost the quality by HHP at 1 and 80 MPa treatments with 1 and 5% of citric acid solutions. After 3 days in storage, combination with 5% citric acid solution could maintain h° values rather than 1% solution. It seemed that higher concentration of citric acid had a potential to inhibit the surface discoloration.

Murata and Homma (1998) reported that citric acid affected as a chelating agents on PPO activity, and it might be possible to inhibit the discoloration of burdock surface in this study. In addition, the HHP treatment under acidic condition might be important for the inhibition of discoloration since PPO activity of burdock could be suppressed strongly under lower pH condition (<4.0) as indicated by Nakabayashi (1968).

Effect of combination of HHP treatment with anti-browning agents on the PPO activity of fresh-cut burdock

Figure 2 shows the PPO activity of fresh-cut burdock treated by 1 and 80 MPa HHP combined with 1 and 5 % of citric acid solution. Comparing with un-treated sample (control), 5% of citric acid solution inhibit the PPO activity at both 1 and 80MPa of HHP treatment before/after storage at 6°C. With the concentration of 1%, 80MPa HHP treatment did not reduce the PPO activity before storage test. Cell wall damage by higher levels of HHP treatment could enhance the contacting efficiency between substrate and PPO, and might result in the acceleration of PPO activity of cut burdock with 1%CA. On the other hand, PPO activity was not enhanced at 80MPa with 5%CA since higher concentration of CA could act as an inhibitor against PPO activity.

It is well known that PPO activity, relating to the tissue browning, is inhibited by treatment with some anti-browning agents such as citric acid, or ascorbic acid. However, the reduction of the amount of such anti-browning agents has been a big concern for the better taste and environmental aspect.

CONCLUSION

In this study, the combining effect of high hydrostatic pressure (HHP) and citric acid (CA) were investigated for the inhibition of discoloration (browning) of burdock surface. Treatment with HHP had a potential to reduce the PPO activity comparing with control samples, however, treatment with the lower concentration of CA with higher level of HHP did not have any inhibiting effect on the discoloration which might be resulted from the substrate-enzyme contact due to cell breakage during HHP process. Further experiments are needed to clarify the optimum treatment condition of HHP level/temperature and the concentration of anti-browning agents.

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