

Full Length Research Paper

Amplification of allyl isothiocyanate in red cabbage using high hydrostatic pressure treatment

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Red cabbage contains sinigrin which is hydrolyzed to form allyl isothiocyanate (AITC) by myrosinase. AITC has antimicrobial, anticancer, and cytoprotective activities. Here, high hydrostatic pressure (HHP) treatment (100 to 400 MPa) and subsequent incubation (20 to 80°C) were employed to amplify AITC content in red cabbage. The highest quantity of AITC was 39.6 µmol/kg fresh weight from a HHP treatment of 400 MPa followed by standing at 40°C and 6.4 times higher than that from HHP-untreated control. Cell membrane disruption by HHP treatment was assumed to be the most plausible reason for the high AITC yield and was confirmed by biological impedance measurement.

Key words: Allyl isothiocyanate, biological impedance, high hydrostatic pressure, red cabbage.

INTRODUCTION

Brassica vegetables possess cancer chemopreventive potency due to their unique compounds stored in the form of glucosinolates (GLSs) (Hayes et al., 2008). In intact vegetables, GLSs are stably localized in vacuoles but rapidly hydrolyzed when they contact myrosinase (thioglucoside glucohydrolase EC 3.2.3.1); an enzyme located in vacuole-like structures of special myrosin cells (Jones et al., 2006). Hydrolysis products include isothiocyanates (ITCs), thiocyanates, nitriles, epithionitriles, hydroxynitriles, and oxazolidine-2-thiones (Fahey et al., 2001; Jones et al., 2006). Among them, ITCs are regarded as cancer chemopreventive agents (Zhang et al., 1992; Munday and Munday, 2004). To generate health-beneficial ITCs, cell membrane disruption such as cutting, chopping, grinding and chewing is required to bring myrosinase into contact with GLSs. However, during most conventional cooking, heat is applied to the vegetables before tissue disruption for blanching or pasteurization, resulting in the inactivation of myrosinase and no generation of beneficial ITCs.

According to Van Eylen et al. (2009), high hydrostatic pressure (HHP) treatment, a fast growing nonthermal technology, is a promising approach to accumulate more ITCs in Brassica vegetables prior to heat treatment. That study showed that treating broccoli with high pressure (100 to 500 MPa) at moderate temperatures (20 to 40°C), promotes the conversion of GLSs into ITCs (Van Eylen et al., 2009). Measuring the electrical properties of cells, tissues and organs is sometimes very useful for obtaining physiological information. A biological impedance analyzer system measures the dielectric properties of a medium as a function of frequency and is based on the interaction of an external electric field with the inherent electric dipole moment of the material being measured (Angersbach et al., 1999; Hartmann et al., 2006). Compared to other physiological characterization techniques, impedance measurement is simple and readily applicable (Wu et al., 2008) and has been widely used to estimate the physiological state of various biological tissues including cell membrane integrity.

Red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra* DC.) has been mostly consumed as salad, coleslaw and beverage. It has cancer chemopreventive properties (Uhl et al., 2004) and contains glucoraphanin, sinigrin and glucoiberin as major GLSs (Meyer and Adam, 2008). Among them, sinigrin is transformed into allyl isothio

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Table 1. Effect of high hydrostatic pressure treatment (HHP) and incubation temperature on allyl isothiocyanate yield ($\mu\text{mol kg}^{-1}$).

Pressure (MPa)	Temperature			
	20°C	40°C	60°C	80°C
HHP-untreated	7.00±1.29 ^{aA}	6.19±0.69 ^{aA}	6.00±0.34 ^{aA}	6.19±0.44 ^{aA}
100	10.77±0.44 ^{bA}	12.93±0.79 ^{bB}	10.95±0.55 ^{bA}	9.74±1.39 ^{bA}
200	19.11±2.14 ^{cA}	23.54±1.54 ^{cB}	15.78±1.13 ^{cC}	11.80±0.57 ^{bD}
300	26.94±1.83 ^{dAB}	30.91±4.19 ^{dB}	23.29±1.19 ^{dA}	20.41±2.08 ^{cA}
400	37.42±2.10 ^{eC}	39.57±1.82 ^{eC}	32.54±2.06 ^{eB}	21.99±1.78 ^{cA}

Values are expressed as mean \pm standard deviation, $n = 3$. Means with different superscripts in the same column (a-e) and row (A-D) are significant differences at $P < 0.05$.

cyanate (AITC), which has antimicrobial, anticancer and cytoprotective activities (Zhang, 2010). To date, the increase of ITCs after harvest has been studied mainly in broccoli. In this study, HHP treatment was applied to red cabbage to amplify the health-beneficial ITCs, especially AITC.

Different pressures and incubation temperatures were evaluated for the generation of AITC in red cabbage. Finally, cell membrane disruption by HHP treatment was traced with impedance measurements.

MATERIALS AND METHODS

Preparation and treatment of red cabbage

Red cabbage was purchased from local markets (Gangneung, Korea) and stored at 4°C until further use. After removing the outer leaves, 50 g of red cabbage was cut into pieces of approximately $7 \times 7 \text{ cm}^2$ and packed in a heat-sealed vacuum bag. The sample bags were placed in a high-pressure laboratory-scale vessel with a filling volume of 2.5 L (Autoclave Engineering Systems Inc., Columbus, OH, USA).

HHP treatment was performed at four different pressures (100, 200, 300, and 400 MPa) for 10 min, not including the pressure buildup and release time.

The temperature of the vessel was maintained at 25°C using an external water circulator (RW-1025G, Lab Companion, Seoul, Korea). After HHP treatment, the vacuumed sample bags were incubated at four different temperatures (20, 40, 60, and 80°C) for 1 h to induce AITC formation by myrosinase.

Allyl isothiocyanate (AITC) analysis

For the analysis of AITC, 50 g of HHP-treated sample was extracted with 100 mL of n-hexane. The amount of AITC in the extract was determined according to the modified method of Zhang et al. (2010) using a gas chromatography-mass spectrometry system (6890 GC connected to 5973i MSD MS, Agilent Technologies Inc., Palo Alto, CA, USA), equipped with a flame ionization detector and a HP-wax capillary column (30 m \times 0.32 mm i.d., 0.5- μm film thickness, Agilent Technologies). The oven temperature was programmed from 50 to 250°C at a rate of 15°C/min with initial and final holding times of 2 and 5 min, respectively. The injection port and detector temperatures were 240 and 250°C, respectively. The flow rate of the carrier gas (He) was 2 mL/min, and a split ratio of 10:1 was used.

Biological impedance measurement

Biological impedance analyzer was used to determine the extent of cell membrane disruption induced by the HHP treatment. The biological impedance (z) for intact and HHP-treated red cabbage was determined with a specially assembled impedance spectrometer. The system consisted of a high-resolution digitizer (PXI5124, National Instruments, Austin, TX, USA), an arbitrary waveform generator (PXI5422, National Instruments), programming tools (Labview, National Instruments) and a probe fixture. Input voltages were of equal amplitude (typically between 1 and 5 V peak to peak) and measurements were obtained between 10^4 and 10^7 Hz. The impedance was measured on a piece of sample held between parallel plate disk electrodes with a diameter of 10.0 mm. The distance between the electrodes was 5.0 mm.

Statistics

The mean and standard deviations were calculated from data obtained from triplicate trials. The data were analyzed by Student's t-test or Student-Newman-Keuls test using GraphPad Prism 4 as statistical software. A $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Cell membrane disruption of red cabbage led to a mixture of GLSs and myrosinase, which were segregated by the membrane and consequently induced the formation of ITCs including AITC. In this study, HHP treatment was employed as physical disruption process because it has the advantage of creating membrane leakage without a change in physical appearance.

The effect of HHP treatment and incubation temperature on AITC yield is shown in Table 1. A small amount of AITC, averaging 6.2 $\mu\text{mol/kg}$ fresh weight, was present in the HHP-untreated sample. HHP treatment of red cabbage increased AITC yield with an increase in applied pressure, indicating that pressure up to at least 400 MPa could efficiently disrupt the cell membranes of red cabbage. The most effective incubation temperature after HHP treatment was 40°C. The AITC yields at 60 and 80°C were lower than those at 20°C. This tendency was observed for each HHP treatment at different pressures.

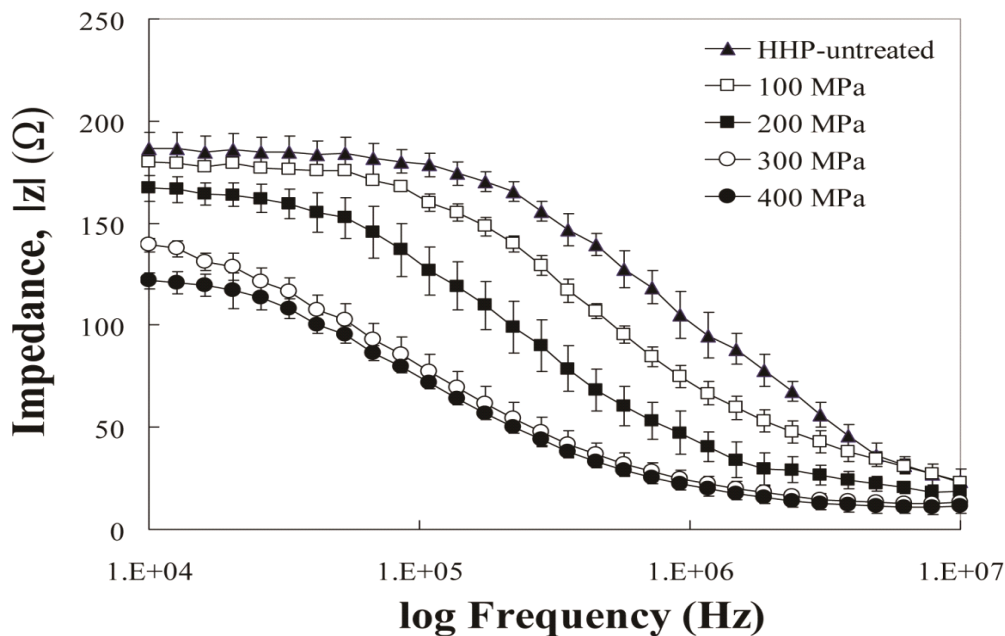


Figure 1. Impedance value of high hydrostatic pressure (HHP)-treated and untreated red cabbage as a function of frequency.

This result may be closely related to the thermal stability of endogenous myrosinase in red cabbage because broccoli myrosinase is stable until the temperature reaches 45°C but its activity decreases by about 80% after a 10 min treatment at 60°C (Van Eylen et al., 2008). Overall, the highest quantity of AITC was 39.6 $\mu\text{mol/kg}$ fresh weight from the 400 MPa HHP treatment followed by standing at 40°C, corresponding to 6.4 times higher than that from HHP-untreated controls.

Isothiocyanate formation by treatment of HHP and temperature has been reported in broccoli (Van Eylen et al., 2009). The effect was consistent with our research in terms of amplification of isothiocyanate by HHP and temperature. The results here were similar to our previous study relating to the amplification of sulforaphane content in red cabbage as well. The highest quantity of sulforaphane was 99.7 $\mu\text{mol/kg}$ fresh weight from the 400 MPa HHP treatment followed by standing at 60°C. HHP treatment and incubation temperature were also effective to the improvement of sulforaphane content (Koo et al., 2011).

To evaluate the degree of cell membrane disruption by HHP treatment, impedance measurements were conducted in HHP-treated red cabbage and presented as a plot of impedance magnitude ($|z|$) versus frequency (Figure 1). The shapes of the impedance spectra of HHP-treated samples were basically similar to those of untreated HHP samples in all frequency ranges (10^4 to 10^7 Hz). However, the impedance value had a tendency to decrease at each frequency when the applied pressure increased from 0 to 400 MPa. Because the intact cells of red cabbage are insulated by cell membranes, the

untreated sample impeded the current flow generated by an outer electric field. Therefore, lower impedance values at higher pressures suggested that disruption of the cell membranes in HHP-treated red cabbage occurred in proportion to the applied pressure and facilitated the intercellular transport of materials such as myrosinase and sinigrin required to produce AITC.

In conclusion, we analyzed the effect of HHP treatment and subsequent incubation temperature on AITC yield in red cabbage. Our findings suggest that the highest quantity of AITC was obtained under HHP treatment conditions of 400 MPa followed by standing at 40°C. Cell membrane disruption by HHP treatment was assumed to be the most plausible reason for the high yield of AITC, which was confirmed by biological impedance measurements.

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REFERENCES

- Angersbach A, Heinz V, Knorr D (1999). Electrophysiological model of intact and processed plant tissues: cell disintegration criteria. *Biotechnol. Progr.*, 15: 753-762.
- Fahey JW, Zalcmann AT, Talalay P (2001). The chemical diversity and

- distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, 26: 5-51.
- Hartmann C, Mathmann K, Delgado A (2006). Mechanical stresses in cellular structures under high hydrostatic pressure. *Innov. Food Sci. Emerg.*, 7: 1-12.
- Hayes JD, Kelleher MO, Eggleston IM (2008). The cancer chemopreventive actions of phytochemicals derived from glucosinolates. *Eur. J. Nutr.*, 47(Suppl 2): 73-88.
- Jones R, Faragher J, Winkler S (2006). A review of the influence of postharvest treatments on quality and glucosinolate content in broccoli (*Brassica oleracea* var. *italica*) heads. *Postharvest Biol. Technol.*, 41: 1-8.
- Koo SY, Cha KH, Song DG, Chung D, Pan CH (2011). Amplification of sulforaphane content in red cabbage by pressure and temperature treatments. *J. Korean Soc. Appl. Biol. Chem.*, 54: 183-187.
- Meyer M, Adam ST (2008). Comparison of glucosinolate levels in commercial broccoli and red cabbage from conventional and ecological farming. *Eur. Food Res. Technol.*, 226: 1429-1437.
- Munday R, Munday C (2004). Induction of phase II detoxification enzymes in rats by plant-derived isothiocyanates: comparison of allyl isothiocyanate with sulforaphane and related compounds. *J. Agric. Food Chem.*, 52: 1867-1871.
- Uhl M, Kassie F, Rabot S, Grasl-Kraupp B, Chakraborty A, Laky B, Kundi M, Knasmüller S (2004). Effect of common *Brassica* vegetables (Brussels sprouts and red cabbage) on the development of preneoplastic lesions induced by 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in liver and colon of Fischer 344 rats. *J. Chromatogr. B*, 802: 225-230.
- Van ED, Oey I, Hendrix M, Van LA (2008). Effects of pressure/temperature treatments on stability and activity of endogenous broccoli (*Brassica oleraceae* L. cv *italica*) myrosinase and on cell permeability. *J. Food Eng.*, 89: 178-186.
- Van ED, Bellostas N, Strobel BW, Oey I, Hendrix M, Van LA, Sorensen H, Sorensen JC (2009). Influence of pressure/temperature treatments on glucosinolate conversion in broccoli (*Brassica oleraceae* L. cv *italica*) head. *Food Chem.*, 112: 646-653.
- Wu L, Ogawa Y, Tagawa A (2008). Electrical impedance spectroscopy analysis of eggplant pulp and effects of drying and freezing-thawing treatments on its impedance characteristics. *J. Food Eng.*, 87: 274-280.
- Zhang Y (2010). Allyl isothiocyanate as a cancer chemopreventive phytochemical. *Mol. Nutr. Food Res.*, 54: 127-135.
- Zhang YS, Talalay P, Cho CG, Posner GH (1992). A major inducer of anticarcinogenic protective enzymes from broccoli— isolation and elucidation of structure. *Proc. Natl. Acad. Sci. U.S.A.*, 89: 2399-2403.
- Zhang ZQ, Kim WT, Park YC, Chung D (2010). Thermodynamics of partitioning of allyl isothiocyanate in oil/air, oil/water, and octanol/water systems. *J. Food Eng.*, 96: 628-633.