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ORIGINAL ARTICLE

Kinetics of argy wormwood (*Artemisia argyi*) leaf peroxidase and chlorophyll content changes due to thermal and thermosonication treatment

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Abstract The effects of different thermal and thermosonication blanching treatments on the inactivation of peroxidase (POD) and the retention of total chlorophyll in argy wormwood leaves were studied. Inactivation of POD followed a biphasic first-order model under thermal blanching treatments below 90 °C while a first-order model at 90 °C. In contrast, for thermosonication treatments, the inactivation kinetics of POD fit a first-order model well for all the temperatures tested. Thermosonication treatment was found to inactivate POD faster and retain more of the total chlorophyll than thermal blanching treatment. A thermosonication protocol using ultrasonic intensity of 11.94 W/cm^2 at 85 °C for 60 s was found to be the most suitable protocol for blanching the argy wormwood leaves. This protocol inactivated 92. 7 % POD while retaining 96.7 % of total chlorophyll.

Keywords Thermosonication · Peroxidase · Inactivation kinetics · Chlorophyll · Argy wormwood

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Introduction

Argy wormwood (*Artemisia argyi*), a perennial herb, belongs to feverfew family. It widely grows in Europe, North America, Asia, and South Africa. Argy wormwood is a healthy green plant rich in amino acids, vitamins, minerals and bioactive components such as coumarins, phenolics, steroids, and terpenoid compounds (Brisibe et al. 2009). Due to these nutritional values, it is commonly used as an ingredient for Chinese cuisine. However, the green color of argy wormwood leaves easily deteriorated in a short shelf-life of approximately 7 days even under chilling storage (4 °C). Therefore, research on the maintenance of green color of argy wormwood leaves in order to extend their shelf-life is necessary.

Peroxidase (POD) is considered to play an important role in off-flavors and off-colors of raw and un-blanched frozen vegetables (López et al. 1994). Walker (1964) suggested that POD is an important player in chlorophyll degradation. Kampis and others (1984) also proposed that POD is responsible for color changes of frozen green vegetables during long storage at −18 °C. The mechanism of POD is based on the formation of enzyme-hydrogen donor complexes. POD oxidizes the phenolic compounds containing hydrogen peroxide, thus forming phenoxy radical. The phenoxy radical then oxidizes chlorophyll and its derivatives to colorless low molecular weight compounds (Yamauchi et al. 2004).

Therefore, inactivation of POD increased the shelf-life of vegetables during frozen storage and it is often used as an index for blanching adequacy (Bahçeci et al. 2005).

Freezing does not prevent green color deterioration as enzymes remain active even at sub-zero temperatures. Blanching has been considered one of the most important pretreatment technologies needed to inactivate enzymes and stabilize the color of processed fruit and vegetable. However, if blanching is not well carried out, it can cause undesirable changes in the properties of fruits and vegetables such as unexpected loss of nutritious components (organic acid, sugars, minerals and vitamins), or loss of color (Gupta et al. 2011; Sobukola et al. 2008) and flavor (Korus 2011; Song et al. 2003; Volden et al. 2009). Therefore, it is necessary to develop a novel blanching treatment that results in only minimal quality loss in processed foods. One of the novel and promising methods is ultrasound assisted blanching which has attracted considerable interests due to its benefits on food processing and has been successfully applied for the inactivation of enzymes in foods. Ultrasound when coupled with heat (thermosonication) has been reported to inactivate enzymes such as peroxidase (Ercan and Soysal 2011), polyphenoloxidase (Cheng et al. 2007), pectinmethylesterase and polygalacturonase (Gamboa-Santos et al. 2012; Terefe et al. 2009). The thermosonication treatment thus resulted in a better quality of foods compared to conventional thermal blanching, for example, better color maintenance of watercress (Cruz et al. 2007), and improved quality of tomato juice (Wu et al. 2008), strawberry (Cao et al. 2010), watermelon juice (Rawson et al. 2011) and watercress (Cruz et al. 2011). The ultrasound-assisted thermal blanching or thermosonication offers several advantages including: (1) reduced treatment time compared with single thermal blanching method; (2) higher throughput and lower energy requirement; (3) similar or longer shelf-life as thermal only blanching but maintaining food quality parameters as natural status.

The effects of thermosonication on the inactivation of POD and chlorophyll content in argy wormwood leaves have not been reported. Therefore, the main objective of this work was to investigate the blanching effect of thermosonication on POD inactivation by comparing the effects of thermal blanching treatments. Changes in chlorophyll content after different thermosonication and thermal treatments were also measured in order to assess the effects of these treatments on green color as an index of product quality.

Materials and methods

Sample preparation

Fresh mature argy wormwood leaves were procured from a local farm in Wuxi city, Jiangsu province, China. Edible tender leaves were randomly selected by farmers in the area of about 100 m^2. The leaves were harvested early in the morning and transported to the laboratory within 2 h. Bruised leaves were discarded. The leaves were then washed with clean tap water, drained thoroughly and the ones having uniform color were selected and subsequently stored at 4 °C. These leaves were used within 24 h.

Processing

Thermal treatment

Thermal treatment was according to the method proposed by Cruz et al. (2008) with some modifications. Thermal treatments were carried out with five temperatures (70, 75, 80, 85 and 90 °C) and different times of exposure (0, 15, 30, 45, 60, 90, 120, 150 and 180 s). Each sample (approximately 3 g argy wormwood leaves) was blanched in individual beakers (250 mL) with 90 mL of distilled water, in a circulating water bath (HH-1, Aohua Istrument Inc., Changzhou, China). To stop the blanching treatments, the beakers were immediately cooled in an ice water bath. The time–temperature data for each run was monitored using a digital thermocouple (±0.1 °C accuracy). The water on the leaf surface was removed before further analysis. Each blanching treatment was repeated three times.

Thermosonication treatment

The leaf samples were subject to thermosonication at the same sample-water ratio as described above. The method of thermosonication was performed according to Tiwari et al. (2009) with some modifications. The ultrasound treatment was carried out at 20 kHz frequency on an ultrasound processor (JY98-IIIDT, Xinzhi Biology Inc., Ningbo, China) with 20 mm probe. The treatments were performed at 7.96, 11.94 and 15.92 W/cm^2 with a nominal energy conversion efficiency of 40 %, 60 % and 80 %, respectively. The ultrasound probe was immersed 25 mm in depth with respect to the liquid surface. Samples were removed at different time intervals (0, 15, 30, 45, 60, 75, 90, 120, 150 and 180 s) and cooled in ice water bath as in the case of thermal treatment. In order to avoid too much heat generated by ultrasound which would raise the temperature of the water (average 2 °C/min, for 100 mL water), two methods were used to make sure the temperature of thermosonication maintained at 70, 75, 80, 85 and 90 °C during 180 s: (1) samples were ultrasonic treated with pulse durations of 5 s on and 10 s off; (2) samples were placed in a 150 mL jacketed vessel through which water at 68, 73, 78, 83 and 88 °C circulated at 0.5 L/min.

Sample characterization

POD assay

The leaf of argy wormwood has a slender shape (length=3± 0.5 cm, width=1±0.2 cm). In order to extract POD, one leaf was shredded into 8 pieces. Samples were then mixed with chilled sodium phosphate buffer solution (0.1 mol/L, pH 6.5, 4 °C) at a w/v ratio of 1:5 (g sample/mL buffer). Each sample-buffer mixture was homogenized in a Waring blender (AT320, Kenwood Co., London, U.K.) for 3 min. The homogenate was filtered through several layers of gossamer fabric and centrifuged at 10,000 g at 4 °C for 10 min. The filtrate was used as the enzyme source.

POD was assayed according to the method proposed by Agüero et al. (2008) with some modifications. POD substrate solution was a mixture of 10 mL guaiacol (1 mL/100 mL), 1 mL hydrogen peroxide (0.3 mL/100 mL), and 10 mL sodium phosphate buffer (0.1 mol/L; pH 6.5). The substrate solution was mixed for 10 s using a Fisher vortex homogenizer (Model 58, Fisher Scientific Co., Springfield, N.J., U.S.A.). Substrate solution (3.48 mL) was added to the enzyme extract (0.12 mL), mixed and vortexed for 10 s. Subsequently, the absorbance at 470 nm (A_{470} nm) was determined using a UV-2006 spectrophotometer (Unico Instrument Co. Ltd., Shanghai, China) at room temperature. The POD activity unit was defined as a 0.001 change in absorbance per minute. Enzymatic activity was expressed as activity units/g fresh tissue. Each blanching treatment condition was analyzed three times.

Chlorophyll content determination

The chlorophyll concentration was determined as described by Weemaes et al. (1999). Argy wormwood leaf samples (2.5 g) were shredded into pieces, mixed with 25 mL of 80 % acetone solution, and homogenized with a Waring Blender at high speed for 3 min. The homogenate was centrifuged at 10,000 g for 5 min at 4 °C. The pellet was washed with 20 mL 80 % acetone solution and centrifuged until it was decolorized. The supernatant was filtered and then chlorophyll content was measured using a spectrophotometer (UV-2006, Unico Instrument Co. Ltd., Shanghai, China). Total chlorophyll concentration was calculated using Eq. (1) given below.

$$\text{Chlorophyll}(\text{mg/L}) = 18.80A_{662} + 34.02A_{645} \quad (1)$$

Where A_{660} and A_{645} are absorbance at 662 and 645 nm, respectively. The results of chlorophyll concentration (mg/L) was converted to μg chlorophyll per g of argy wormwood leaves. Each blanching treatment condition was analyzed three times.

Modeling of inactivation kinetics

Inactivation kinetics of enzymes can be represented by a first-order reaction model (Gonçalves et al. 2007), as shown in Eq. (2).

$$C/C_0 = e^{-k(T)t} \quad (2)$$

Where, C is the active POD at time t, C_0 the measured active POD at time zero, t the thermal treatment time (s) and $k_{(T)}$ is the rate constant (s^{-1}).

If POD inactivation does not follow the first-order kinetics, the inactivation can be separated as heat labile and heat resistant phases according to the procedure outlined by Ling and Lund (1978). But using this approach, with two inactivation rate constants, inactivation kinetics of enzymes follow a biphasic first-order model (Ling and Lund 1978). The model can be expressed using Eq. (3), given below.

$$C/C_0 = f_{L,0}e^{-kL(T)t} + f_{R,0}e^{-kR(T)t} \quad (3)$$

Where $f_{L,0}$ and $k_{L(T)}$ are the initial fraction and the inactivation rate constant of heat labile isoenzyme, respectively. Similarly, $f_{R,0}$ and $k_{R(T)}$ are those of the heat-resistant fraction.

The temperature dependence of the inactivation rate constant is usually expressed by Arrhenius equation (Zheng and Lu 2011), given by Eq. (4).

$$\ln K_{(T)} = -Ea/RT + C \quad (4)$$

Where, E_a is the activation energy (kJ/mol) required for the reaction to occur, R is the universal gas constant (8.315 J/mol·K) and T is the absolute temperature (K).

Statistical analysis

The kinetic parameters were determined directly from experimental data by performing least square method using Origin Pro 8.6.0 software (OriginLab Co., USA). All experimental data were analyzed using ANOVA and Duncan's multiple range tests with SPSS 15.0 statistical package. Data with significance level 95 % ($P<0.05$) were considered significant.

Results and discussion

Effect of thermal treatment on POD activity

Residual activity of POD in argy wormwood leaves as a function of thermal blanching time for five different processing temperatures is presented in Fig. 1. In general, POD was inactivated rapidly during the first 45 s of thermal treatment. However, after that, inactivation became several

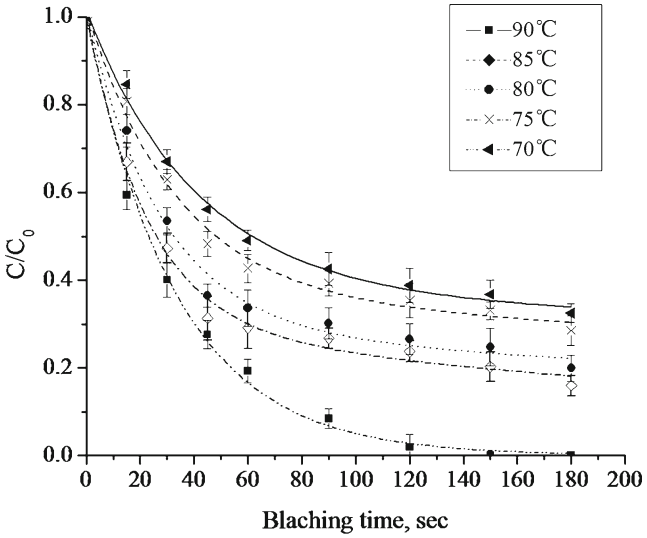

Fig. 1 Changes in residual peroxidase (POD) activities in argy wormwood leaves during blanching at different blanching temperatures. n= 3. C/C_0: the ratio of residual peroxidase activities and initial peroxidase activities in argy wormwood leaves

orders of magnitude slower except in the case of 90 °C. These results suggested a biphasic first-order model, which was first proposed by Ling and Lund (1978) due to the fact that the POD consists of two isoenzyme groups, thermal stable and thermal unstable. The heat-labile fraction of POD is easily inactivated while the heat-resistant fraction cannot be inactivated completely below 90 °C. In the current research, the average absolute error for POD between the model predicted and experimental was 4.5 % with a range varying from 2.5 % to 6.4 % and a coefficient R^2 around 0.99. These results agreed with other reports on the inactivation of POD native to different vegetables, such as broccolis, green asparagus, carrots, watercress and zucchini (Cruz et al. 2006; Morales-Blancas et al. 2002).

For samples blanched at 90 °C, POD inactivation kinetics was found to follow the first-order kinetics (5.1 % error, R^2= 0.99, Fig. 1). The observation of first order kinetics is due to the fact that the inactivation of heat-labile fraction of POD is too rapid to be detected and its effect on the inactivation kinetics is negligible. The current results indicated that the inactivation of POD followed a biphasic model at low blanching temperature and followed a first order model at high temperature. These results were consistent with other findings on enzymes. Agüero et al. (2008) researched the effect of thermal treatment on the inactivation of POD in butternut squash. They observed a biphasic inactivation of POD at low temperature (below 70 °C) and a first order inactivation at high temperature (above 70 °C). Vercet et al. (2001) also found that inactivation of trypsin by heat treatments at low temperatures (50–75 °C) showed biphasic behavior, but at high temperatures (110–120 °C), it showed first order kinetics.

Parameters of POD thermal inactivation kinetics

The parameters of thermal inactivation kinetics of POD are presented in Table 1. As can be seen from the table, the values of inactivation rate constants increased with increased temperatures. Interestingly, the inactivation rate constants for heat-labile fraction (K_L) are several times higher than those for heat-resistant fraction (K_R). For instance, K_L was approximately 30 and 20 times of K_R at 70 °C and 80 °C, respectively. This can be explained by the fast decrease in enzyme activity in heat labile phase during the first 45 s of thermal blanching treatment (Fig. 1). The activation energy of heat-labile (E_{aL}) and heat-resistant fractions (E_{aR}) was 40.4 KJ/mol (R^2=0.98) and 94.8 KJ/mol (R^2=0.98), respectively. Thus, E_{aL} of POD in argy wormwood leaves was lower than E_{aR}. These results agreed with some vegetables, such as green bean (Agüero et al. 2008) and carrot (Ercan and Soysal 2005) but contradict some other vegetables that E_{aL} is higher than E_{aR}, such as butternut squash, asparagus, and horseradish (Ling and Lund 1978). This divergence of different inactivation kinetic parameters might be caused by experimental condition. As shown previously, the inactivation kinetic parameters were influenced by the time–temperature relationship and the methodology applied (Arabshahi and Lund 1985). In conclusion, experimental conditions such as the initial enzyme concentration, ionic strength of the substrate and pH of the buffer solutions also affected the final parameters of enzyme kinetics (Saraiva et al. 1996).

Effect of thermosonication treatment on POD activity

Effects of thermosonication blanching treatments on the inactivation of POD are shown in Fig. 2. Different levels of ultrasonic intensity and temperature were applied. Compared with thermal blanching treatment (Fig. 1), thermosonication treatment inactivated POD more effectively. For example, at 90 °C, treatment time of 150 s was required to fully inactivate POD by thermal only blanching treatment while only 90 s was required when thermosonication was used at 11.94 W/cm^2 intensity level. Although POD was not completely inactivated at temperatures below 90 °C by thermal treatment alone, it was completely inactivated by thermosonication treatment. For example, at 70, 75, 80 and 85 °C, POD was totally inactivated at 150 s, 150 s, 120 s and 90 s, respectively, with sonication intensity of 11.94 W/cm^2. These data indicated an enhanced effect of thermosonication on enzyme inactivation, which has been reported before. Cruz et al. (2006) reported that at high temperatures (82.5 to 90 °C), thermosonication had a synergistic effect on watercress peroxidase inactivation. Especially, at 90 °C, the processing time of thermosonication treatment was 13 times lower than the time of normal thermal treatment (Cruz et al. 2011). In addition, thermosonication

Table 1 Kinetic parameters of thermal inactivation of peroxidase (POD) in argy wormwood leaves

Parameters	Temperature (°C)				
	70	75	80	85	90
$K_L \times 10^4$ (s^{-1})	244.1±11.31	299.4±13.24	358.0±12.41	445.0±11.62	298.8±10.30
$K_R \times 10^4$ (s^{-1})	6.5±1.42	10.7±1.30	14.9±0.64	27.4±2.10	–
$f_{L,0}$	0.643±0.016	0.651±0.021	0.726±0.014	0.712±0.016	–
$f_{R,0}$	0.357±0.017	0.349±0.019	0.274±0.015	0.288±0.016	–
R^2	0.99	0.99	0.98	0.99	0.99
E_{aL} (KJ/mol)	40.4±3.54				–
R^2	0.98				–
E_{aR} (KJ/mol)	94.8±8.70				–
R^2	0.98				–

treatment (72 °C and 20 kHz) also increased the inactivation rate of pectinmethylesterase in orange juice 25 times (Raso and Barbosa-Cánovas 2003).

The inactivation of enzymes by ultrasound treatment is mainly attributed to the mechanical and chemical effects of cavitation, which is the formation, growth, and implosion of bubbles (Raviyan et al. 2005). Collapse of bubbles was accompanied by extremely localized increase in temperature (5,000 K) and pressure (50 MPa) on a micro-scale (Suslick 1989; Vercet et al. 1999). In addition, the stable cavitating bubbles interacting with the acoustic field generated strong micro-streaming and high shear. Under extreme conditions, ultrasound could cause the breakdown of hydrogen bonding and Van der Waals interactions in the polypeptide chains of enzymes, leading to the modification of their secondary and tertiary structures, thus the loss of biological activity of enzymes (Zhong et al. 2004). On the other hand, thermosonication treatments enhanced chemical reactions involving hydroxyl and hydrogen free radicals, which were formed by the decomposition of water inside the oscillating bubbles. The free radicals formed may react with some amino acid residues that participate in enzyme stability, substrate binding or catalytic function, with a consequent change in biological activity (Barteri et al. 2004; Grintsevich et al. 2001).

Kinetic parameters of thermosonication inactivation of POD

A first-order inactivation kinetics model was found to adequately represent the experimental inactivation data of POD during thermosonication treatment for all the temperatures tested. The values predicted by the model and the experiments were reasonably close, which had a relative error between 2.3 % and 7.5 % and the coefficient of R^2 varing around 0.97–0.99. The average relative error was 4.9 %. Previous research also reported that POD inactivation exhibited first-order kinetics when thermosonication was used (Cruz et al. 2006; Terefe et al. 2009). As temperatures increased, the inactivation rate constants were significantly increased (Table 2). However, at 90 °C, the inactivation rate constants did not increase significantly. This phenomenon indicates that the effect of ultrasound in inactivating POD of argy wormwood leaves decreased at high temperature, especially in the vicinity of 90 °C. This might be attributed to the increased water vapor pressure at high temperatures, which acted as a cushion resulting in less violent collapse (Terefe et al. 2009) and cavitation of bubbles less violent. Furthermore, increased temperatures lowered the viscosity and the rate of hydroxyl radical production (López et al. 1998). The decreased efficiency of ultrasound due to high temperature has been observed in the previous studies. For example, in the study of Raviyan et al. (2005), the increase of inactivation rate of tomato pectinmethylesterase decreased to 84-fold at 72 °C compared to 374-fold at 61 °C. Terefe et al. (2009) showed that the increase in the inactivation rate of pectinmethylesterase in tomato decreased to 1.5 times at 75 °C compared to 6 times at 60 °C. Yaldagard et al. (2008) also reported that the ultrasound inactivation efficiency on alpha-amylase decreased at high temperature around 90 °C. Therefore, in order to improve the efficiency of thermosonication treatments, appropriate temperatures must be carefully selected.

Effect of blanching treatments on chlorophyll content

Chlorophyll content is an important parameter determining the final quality of blanched vegetables such as argy wormwood leaves as it is highly susceptible to degradation during processing, resulting in color changes in preservation. Total chlorophyll content in the fresh argy wormwood leaves was 778.12 μg/g. This is higher than many vegetables, such as broccoli (21 μg/g), green bean (75 μg/g), lettuce (245 μg/g) and parsley (632 μg/g) (Bohn et al. 2004). Therefore, argy wormwood leaf is a very good source of chlorophyll.

The effects of thermal only blanching and thermosonication blanching on total chlorophyll content of argy wormwood

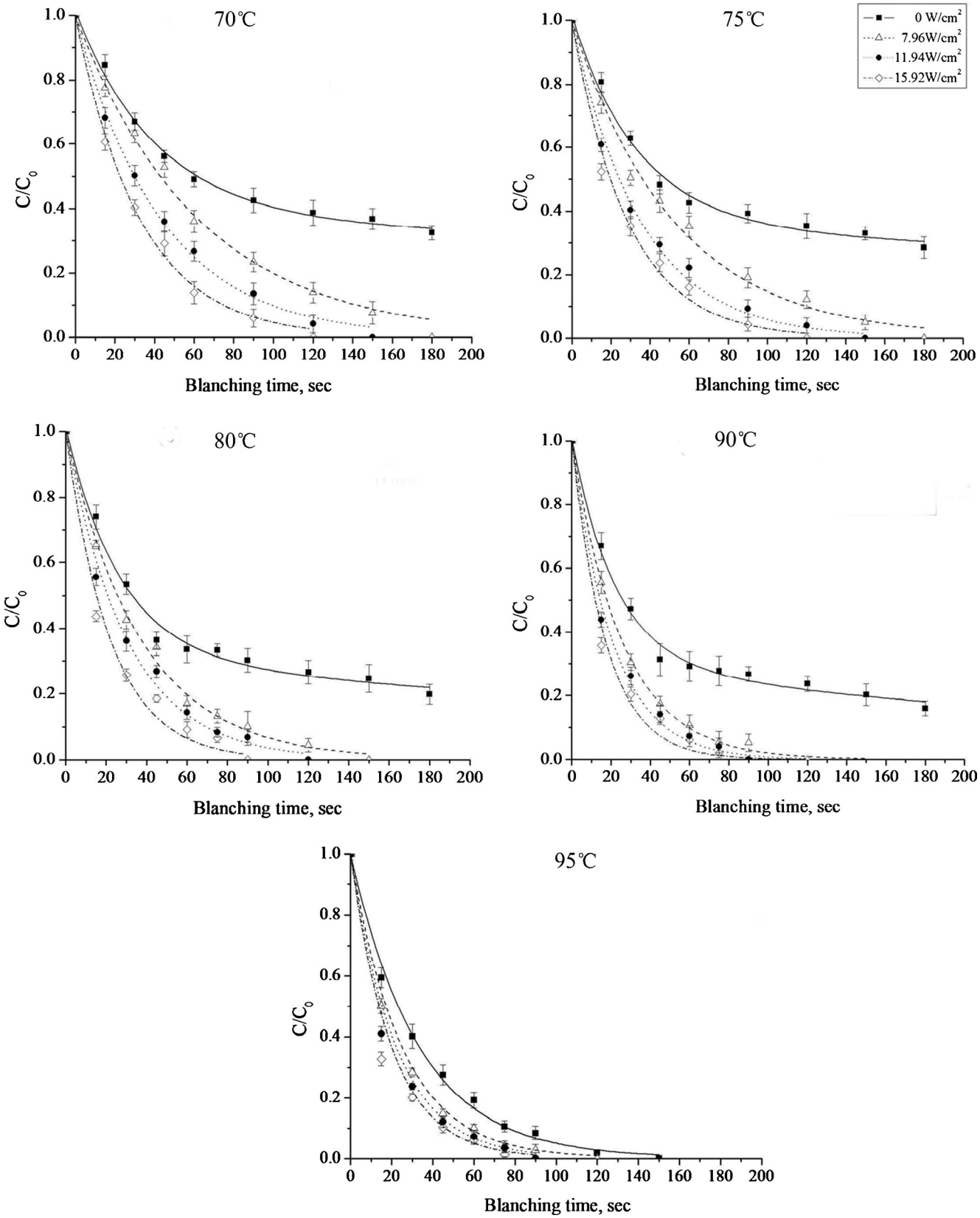

Fig. 2 Changes in residual peroxidase (POD) activities in argy wormwood leaves during blanching at different temperatures under different thermosonication treatments. n=3. C/C_0: the ratio of residual peroxidase activities and initial peroxidase activities in argy wormwood leaves

Table 2 Reaction rate constants (k) and inactivation energy (E_a) of peroxidase (POD) in argy wormwood leaves by thermosonication treatment

Thermosonication treatment	$k \times 10^4$ (s^{-1})					E_a (KJ/mol)
	70 °C	75 °C	80 °C	85 °C	90 °C	
7.96 W/cm^2	16.0±1.24^a	19.1±1.27^b	27.0±1.63^c	38.7±3.85^d	41.5±2.38^d	63.1±4.62
11.94 W/cm^2	23.1±2.82^a	28.3±2.95^b	33.2±2.67^c	47.7±1.40^d	48.6±3.86^d	47.9±2.63
15.92 W/cm^2	30.5±2.44^a	35.0±1.80^b	45.4±1.02^c	47.9±2.65^d	51.9±5.49^d	42.2±3.90

Results are mean ± standard deviation (n=3)

The values for the same ultrasonic intensity that have different letters in the same row are significantly different (P< 0.05)

leaves are shown in Table 3. Table 3 also compared the effect of thermal and thermosonic blanching on POD inactivation. In general, thermosonication treatment required less processing time to achieve a similar or higher POD inactivation rate compared with traditional thermal method. When thermal treatment was carried out at 85 °C for 180 s, only 84.1 % of the POD was inactivated. However, only 60 s of thermosonication treatment with ultrasonic intensity of 7.96, 11.94 and 15.92 W/cm^2 resulted in corresponding POD inactivation of 90.2 %, 92.7 % and 93.9 %, respectively. The less processing time of thermosonication treatment led to a higher retention of chlorophyll content. For example, when the samples were thermosonicated with ultrasonic intensity of 7.96, 11.94, 15.92 W/cm^2, respectively at 85 °C for 60 s, only 3.3 %, 3.3 % and 3.6 % chlorophyll was lost and more than 90 % POD was inactivated. For optimum quality retention of vegetables during storage, it is recommended that a reduction of 90 % of the POD activity has to be achieved during blanching (Bahçeci et al. 2005). In the case of argy wormwood leaves, we recommend that the thermosonication treatment (11.94 W/cm^2) be carried out at 85 °C for 60 s after comparing the results in Table 3. This protocol decreased 92.7 % of POD

Table 3 Effects of thermal and thermosonication blanching on chlorophyll content of argy wormwood leaves

Blanching method	Blanching conditions	Time (s)	POD inactivation (%)	Chlorophyll content (μg/g)	Chlorophyll loss (%)
No treatment	–	–	0	778.1±5.24^f	0
Thermal treatment	70 °C	180	67.5±2.15^i	738.1±7.16^{cd}	5.1±0.92^{cd}
	75 °C	180	71.5±3.39^i	728.4±6.30^{bc}	6.4±0.15^{bc}
	80 °C	180	80.2±3.03^{gh}	719.6±5.29^b	7.5±0.68^b
	85 °C	180	84.1±2.30^{fg}	709.0±6.69^a	8.9±0.86^a
	90 °C	120	91.6±2.24^{bcde}	722.8±5.45^b	7.1±0.70^b
Thermosonic treatment	70 °C, 7.96 W/cm^2	90	76.6±3.01^h	757.1±6.22^e	2.7±0.80^e
	70 °C, 11.94 W/cm^2	90	86.5±3.39^{ef}	755.7±6.30^e	2.9±0.81^e
	70 °C,15.92 W/cm^2	90	94.0±2.82^{bcd}	749.7±6.77^{ed}	3.7±0.87^{ed}
	75 °C, 7.96 W/cm^2	90	80.9±3.17^{gh}	752.0±6.15^e	3.4±0.79^e
	75 °C, 11.94 W/cm^2	90	90.6±2.81^{bcde}	751.1±3.89^e	3.5±0.50^e
	75 °C, 15.92 W/cm^2	90	95.6±2.18^{abc}	748.3±7.39^{ed}	3.8±0.95^{ed}
	80 °C, 7.96 W/cm^2	90	90.0±4.66^{de}	746.8±5.84^{ed}	4.0±0.75^{ed}
	80 °C, 11.94 W/cm^2	90	93.2±2.39^{bcd}	745.6±6.07^{ed}	4.2±0.78^{ed}
	80 °C, 15.92 W/cm^2	90	100.0±0.00^a	744.9±6.61^{ed}	4.3±0.85^{ed}
	85 °C, 7.96 W/cm^2	60	90.2±3.16^{cde}	752.7±6.07^e	3.3±0.78^e
	85 °C, 11.94 W/cm^2	60	92.7±3.23^{bcd}	752.1±4.82^e	3.3±0.62^e
	85 °C, 15.92 W/cm^2	60	93.9±1.97^{bcd}	750.1±8.40^e	3.6±1.08^{ed}
	90 °C, 7.96 W/cm^2	60	94.0±3.27^{bcd}	749.9±6.38^{ed}	3.6±0.82^{ed}
	90 °C, 11.94 W/cm^2	60	95.8±2.13^{ab}	748.1±7.55^{ed}	3.9±0.97^{ed}
	90 °C, 15.92 W/cm^2	60	96.0±2.45^{ab}	748.9±4.67^{ed}	3.8±0.60^{ed}

Results are mean ± standard deviation (n=3)

The values within a column that have different letters in the same column are significantly different (P< 0.05)

activity while retaining 96.7 % (3.3 % loss) of chlorophyll content. These results agreed with those reported by Cruz et al. (2011), where watercress thermosonication at 92 °C for 2 s and 86 °C for 30 s (20 KHz, 125 W) was chosen as the optimal blanching conditions. Under these optimal conditions, POD inactivation was more than 90 % and vitamin C retention was maximized, thus green color can be maintained the best. Further work is needed to elucidate the mechanisms of POD inactivation and degradation of chlorophyll by thermosonication treatment.

Conclusions

In argy wormwood leaves, POD enzyme was found to consist of heat-labile and heat-resistant fractions. A biphasic inactivation kinetics model was found to adequately represent POD inactivation by thermal blanching treatment at 70–85 °C. A model of first-order kinetics was found to represent the inactivation data of 90 °C. In contrast, POD inactivation by thermosonication treatment fit a model of first-order kinetics well. Thermosonication treatment inactivated POD enzyme more rapidly compared to thermal blanching treatment. In addition, an optimized thermosonication protocol using ultrasonic intensity of 11.94 W/cm^2 at 85 °C for 60 s was proposed to be the most suitable protocol for blanching argy wormwood leaves. This protocol inactivated 92.7 % POD while retaining 96.7 % of total chlorophyll.

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References

Agüero MV, Ansorena MR, Roura SI, del Valle CE (2008) Thermal inactivation of peroxidase during blanching of butternut squash. Lebensm Wiss Technol 41(3):401–407

Arabshahi A, Lund DB (1985) Considerations in calculating kinetic parameters from experimental data. J Food Proc Eng 7(4):239–251

Bahçeci KS, Serpen A, Gökmen V, Acar J (2005) Study of lipoxigenase and peroxidase as indicator enzymes in green beans: change of enzyme activity, ascorbic acid and chlorophylls during frozen storage. J Food Eng 66(2):187–192

Barteri M, Diociaiuti M, Pala A, Rotella S (2004) Low frequency ultrasound induces aggregation of porcine furnarase by free radicals production. Biophys Chem 111:35–42

Bohn T, Walczyk T, Leisibach S, Hurrell RF (2004) Chlorophyll-bound magnesium in commonly consumed vegetables and fruits: relevance to magnesium nutrition. J Food Sci 69:347–350

Brisibe EA, Umoren UE, Brisibe F, Magalhães PM, Ferreira JFS, Luthria D, Wu XL, Prior RL (2009) Nutritional characterisation and antioxidant capacity of different tissues of *Artemisia annua* L. Food Chem 115(4):1240–1246

Cao SF, Hu ZC, Pang B, Wang HO, Xie HX, Wu F (2010) Effect of ultrasound treatment on fruit decay and quality maintenance in strawberry after harvest. Food Control 21(4):529–532

Cheng LH, Soh CY, Liew SC, Teh FF (2007) Effects of sonication and carbonation on guava juice quality. Food Chem 104(4):1396–1401

Cruz RMS, Vieira MC, Silva CLM (2006) Effect of heat and thermosonication treatments on peroxidase inactivation kinetics in watercress (*Nasturtium officinale*). J Food Eng 72(1):8–15

Cruz RMS, Vieira MC, Silva CLM (2007) Modeling kinetics of watercress (*Nasturtium officinale*) color changes due to heat and thermosonication treatments. Innov Food Sci Emerg Technol 8(2):244–252

Cruz RMS, Vieira MC, Silva CLM (2008) Effect of heat and thermosonication treatments on watercress (*Nasturtium officinale*) vitamin C degradation kinetics. Innov Food Sci Emerg Technol 9:483–488

Cruz RMS, Vieira MC, Fonseca SC, Silva CLM (2011) Impact of thermal blanching and thermosonication treatments on watercress (*Nasturtium officinale*) quality: thermosonication process optimisation and microstructure evaluation. Food Bioprocess Technol 4(7):1197–1204

Ercan SŞ, Soysal Ç (2005) Kinetics and inactivation of carrot peroxidase by heat treatment. J Food Eng 68(3):349–356

Ercan SŞ, Soysal Ç (2011) Effect of ultrasound and temperature on tomato peroxidase. Ultrason Sonochem 18(2):689–695

Gamboa-Santos J, Montilla A, Soria AC, Villamiel M (2012) Effects of conventional and ultrasound blanching on enzyme inactivation and carbohydrate content of carrots. Eur Food Res Technol 234(6):1071–1079

Gonçalves EM, Pinheiro J, Abreu M, Brandão TRS, Silva CLM (2007) Modelling the kinetics of peroxidase inactivation, colour and texture changes of pumpkin (*Cucurbita maxima L.*) during blanching. J Food Eng 81:693–701

Grintsevich EE, Adzerikho IE, Mrochek AG, Metelitza DI (2001) Polydisulfides of substituted phenols as effective protectors of peroxidase against inactivation by ultrasonic cavitation. Biochemistry (Moscow) 66:740–746

Gupta RK, Kumar P, Sharma A, Patil RT (2011) Color kinetics of aonla shreds with amalgamated blanching during drying. Int J Food Prop 14(6):1232–1240

Kampis A, Bartucz-Kovács O, Hoschke A, Vámos-Vigyázo L (1984) Changes in peroxidase activity of broccoli during processing and frozen storage. Lebensm Wiss Technol 17:293–295

Korus A (2011) Effect of preliminary processing, method of drying and storage temperature on the level of antioxidants in kale (*Brassica oleracea L. var. acephala)* leaves. LWT Food Sci Technol 44:1711–1716

Ling AC, Lund DB (1978) Determining kinetic parameters for thermal inactivation of heat-resistant and heat-labile isozymes from thermal destruction curves. J Food Sci 43(4):1307–1310

López P, Sala FJ, Fuente JL, Condon S, Raso J, Burgos J (1994) Inactivation of peroxidase, lipoxygenase, and polyphenol oxidase by manothermosonication. J Agric Food Chem 42(2):252–256

López P, Vercet A, Sanchez AC, Burgos J (1998) Inactivation of tomato pectic enzymes by manothermosonication. Eur Food Res Technol 207(3):249–252

Morales-Blancas EF, Chandia VE, Cisneros-Zevallos L (2002) Thermal inactivation kinetics of peroxidase and lipoxygenase from broccoli, green asparagus and carrots. J Food Sci 67(1):146–154

Raso J, Barbosa-Cánovas GV (2003) Nonthermal preservation of foods using combined processing techniques. Crit Rev Food Sci Nutr 43(3):265–285

Raviyan P, Zhang Z, Feng H (2005) Ultrasonication for tomato pectinmethylesterase inactivation: effect of cavitation

intensity and temperature on inactivation. J Food Eng 70:189–196

Rawson A, Tiwari BK, Patras A, Brunton N, Brennan C, Cullen PJ, O'Donnell C (2011) Effect of thermosonication on bioactive compounds in watermelon juice. Food Res Int 44(5):1168–1173

Saraiva J, Oliveira JC, Lemos A, Hendrickx M (1996) Analysis of the kinetic patterns of horseradish peroxidase thermal inactivation in sodium phosphate buffer solutions of different ionic strength. Int J Food Sci Technol 31(3):223–231

Sobukola OP, Awonorin SO, Sanni LO, Bamiro FO (2008) Optimization of blanching conditions prior to deep fat frying of yam slices. Int J Food Prop 11(2):379–391

Song JY, An GH, Kim CJ (2003) Color, texture, nutrient contents, and sensory values of vegetable soybeans (*Glycine max (L.) Merrill*) as affected by blanching. Food Chem 83:69–74

Suslick KS (1989) The chemical effects of ultrasound. Sci Am 260(2):80–86

Terefe NS, Gamage M, Vilkhu K, Simons L, Mawson R, Versteeg C (2009) The kinetics of inactivation of pectinmethylesterase and polygalacturonase in tomato juice by thermosonication. Food Chem 117(1):20–27

Tiwari BK, Donnell CPÓ, Cullen PJ (2009) Effect of sonication on retention of anthocyanins in blackberry juice. J Food Eng 93(2):166–171

Vercet A, Lopez P, Burgos J (1999) Inactivation of heat-resistant pectinmethylesterase from orange by manothermosonication. J Agric Food Chem 47(2):432–437

Vercet A, Burgosa J, Crelier S, Lopez-Buesa P (2001) Inactivation of proteases and lipases by ultrasound. Innov Food Sci Emerg Technol 2(2):139–150

Volden J, Borge GIA, Hansen M, Wicklund T, Bengtsson GB (2009) Processing (blanching, boiling, steaming) effects on the content of glucosinolates and antioxidant-related parameters in cauliflower (*Brassica oleracea L. ssp. botrytis*). LWT Food Sci Technol 42:63–73

Walker GC (1964) Color determination in frozen French beans (*Phaseolus vulgaris*). J Food Sci 29:383–388

Weemaes CA, Ooms V, Van Loey AM, Hendrickx ME (1999) Kinetics of chlorophyll degradation and color loss in heated broccoli juice. J Agric Food Chem 47:2404–2409

Wu J, Gamage TV, Vilkhu KS, Simons LK, Mawson R (2008) Effect of thermosonication on quality improvement of tomato juice. Innov Food Sci Emerg Technol 9(2):186–195

Yaldagard M, Mortazavi SA, Tabatabaie F (2008) The effect of ultrasound in combination with thermal treatment on the germinated barley's alpha-amylase activity. Korean J Chem Eng 25(3):517–523

Yamauchi N, Funamoto Y, Shigyo M (2004) Peroxidase-mediated chlorophyll degradation in horticultural crops. Phytochem Rev 3:221–228

Zheng H, Lu HF (2011) Effect of microwave pretreatment on the kinetics of ascorbic acid degradation and peroxidase inactivation in different parts of green asparagus (*Asparagus officinalis L.*) during water blanching. Food Chem 128:1087–1093

Zhong MT, Ming XW, Su PW, Ju QK (2004) Effects of ultrasound and additives on the function and structure of trypsin. Ultrason Sonochem 11:399–404