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Thermal Inactivation of Myrosinase from White Mustard Seeds

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Abstract

Myrosinases (thioglucosidases) catalyze the hydrolysis of a class of compounds called glucosinolates, of which the aglycones show various biological functions. It is often necessary to minimize the loss of myrosinase activity during thermal processing of cruciferous vegetables. Myrosinase was isolated from a popular spice, white mustard (*Sinapis alba*), and its thermal inactivation kinetics was investigated. The enzyme was extracted from white mustard seeds and purified by a sequential processes of ammonium sulfate fractionation, Concanavalin A-Sepharose column chromatography, and gel permeation chromatography. At least three isozymes were revealed by Concanavalin A-Sepharose column chromatography. The purity of the major myrosinase was examined by native polyacrylamide gel electrophoresis and on-gel activity staining with methyl red. The molecular weight of the major enzyme was estimated to be 171 kDa. When the consecutive step model was used for the thermal inactivation of the major myrosinase, its inactivation energy was 44.388 kJ/mol for the early stage of destruction and 32.019 kJ/mol for the late stage of destruction. When the distinct two enzymes model was used, the inactivation energy was 77.772 kJ/mol for the labile enzyme and 95.145 kJ/mol for the stable enzyme. The thermal inactivation energies lie within energy range causing nutrient destruction on heating.

Key words: myrosinase isozyme, thermal inactivation, activity staining, mustard, biological function

Introduction

Myrosinase (EC 3.2.1.147, thioglucoside glucohydrolase) is the only known S-glycosidase that hydrolyzes S-glycosidic linkages of glucosinolates and β-D-glucosides containing sulfur, and belongs to glycoside hydrolase family 1 (GH1, O-glycosyl hydrolase) that hydrolyzes the glycosidic bond between carbohydrates, or between a carbohydrate and a non-carbohydrate moiety (Naumoff DG 2011). The enzyme exists as a dimer stabilized by a Zn^{2+} ion and is heavily glycosylated. The general acid/base glutamate at the active site of O-glycosidases is replaced by a glutamine residue in myrosinase (Burmeister et al. 1997).

Mustards are popular sources of myrosinases and are classified according to seed color (Divakaran & Babu 2016). Myrosinases of mustards have been the subject of investigation as a plant defense mechanism and for their use as spices, and are attracting more attention due to the biological function of their metabolites. Several forms of myrosinase seem to exist within one plant species: Three isozymes were detected in brown mustard (Shin et al. 1996) and four isozymes in mustard powder (unknown species) (Ohtsuru & Hata 1972). Information about the natural intact forms of myrosinase is insufficient. Enzyme proteins were mostly analyzed by polyacrylamide gel electrophoresis under denaturing condition, which does not give much information about intact forms of multimeric enzymes. It is desirable to run the gel under non-denaturing condition and locate catalytic protein bands on the gel for identification of multimeric enzymes.

Thermal processing is often essential for food quality and consumer satisfaction even though heating causes the destruction of nutrients and enzymes. Cooking methods affected on food quality of carrots (Kim et al. 2019). Thermokinetic data help to determine adequate heating methods and conditions. Kinetic and mathematical models explaining degradation reaction of nutrients by heat treatment were proposed and applied (Ling et al. 2015; Nambi et al. 2016). Degradation of heat-labile nutrients follows

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a zero－order or first-order reaction, which is relatively simple reaction compared with enzyme inactivation. Inactivation of enzyme by heating is expected to follow first-order reaction but such an ideal phenomenon is rare in reality. Several models have been proposed to explain the thermal inactivation kinetics of enzymes (Shalini et al. 2008). It seems that the inhibition kinetic model depends on the enzyme preparation. It is important to choose a right inhibition model since different models produce different kinetic data.

Plant myrosinases were reported to hydrolyze a few O-glycosides as well as S-glycosides (glucosinolates) (Durham & Poulton 1990) and aglycones produced from their hydrolysis have been known to display various biological functions (Halkier & Gershenzon 2006; Albena et al. 2012). In addition to plant defense activity against microorganisms or pests, their hydrolysis products such as sulforaphane (Mokhtari et al. 2018), indole-3-carbinol (Weng et al. 2008), phenylethyl isothiocyanate (Gupta et al. 2014) and allyl isothiocyanate (Zhang Y 2010) have been proven effective especially in preventing human cancer. Whether myrosinase enzyme remains active or is inactivated during thermal processing directly affects nutritional values of cruciferous vegetables. In this study, myrosinase was isolated from white mustard seeds, and its kinetics of thermal inactivation was investigated.

Materials and Methods

1. Preparation of myrosinases

Myrosinases were extracted from white mustard seeds and prepared following the procedure shown in Fig. 1.

1) Extraction of myrosinases

Dried white mustard (*Sinapis alba*) seeds produced in India were purchased from a local market and used as a source of myrosinase. The seeds were pulverized with a coffee grinder. To the seed powder was added extraction buffer (50 mM phosphate buffer, pH 6.5, 0.5 mM EDTA, 1.5 mM dithiothreitol) at a ratio of 1,000 mL per 50 g of powder. The extraction mixture was stirred for 15 min in a refrigerator. After centrifugation (12,000×g, Hanil, HA-50, Korea) at 4℃ for 30 min, clear supernatant was taken.

2) Ammonium sulfate fractionation

Ammonium sulfate was added to the supernatant for 50% saturation and left still for 1 h at 4℃ for protein precipitation. The precipitate was removed after centrifugation (12,000×g, 30 min) at 4℃, and clear supernatant was recovered. Additional ammonium sulfate was added to the supernatant for 85% saturation and left still for 12 h at 4℃ for protein precipitation. The resulting protein precipitate was obtained by centrifugation (12,000×g, 30 min) 4℃ and dissolved in a small volume of 50 mM phosphate buffer (pH 6.5).

3) Dialysis and lyophilization

To remove excess ammonium sulfate, the protein solution was dialyzed in cellulose tubing (MWCO 12 kDa, Sigma-Aldrich) against 50 mM phosphate buffer (pH 6.5) containing 0.01% sodium azide at room temperature for 2 days. The dialysate was centrifuged again (12,000×g, 30 min) at 4℃, and then only the clear supernatant was taken for the purification of myrosinases and lyophilized (Ilshin, TFD 5505, Korea).

4) Concanavalin A-Sepharose column chromatography

Concanavalin A-Sepharose affinity chromatography is useful for the purification of myrosinases (Bellostas et al. 2008; Mahn et al. 2014). A glass column was packed with Concanavalin A-Sepharose 4B resin (Sigma-Aldrich) to make a bed volume of

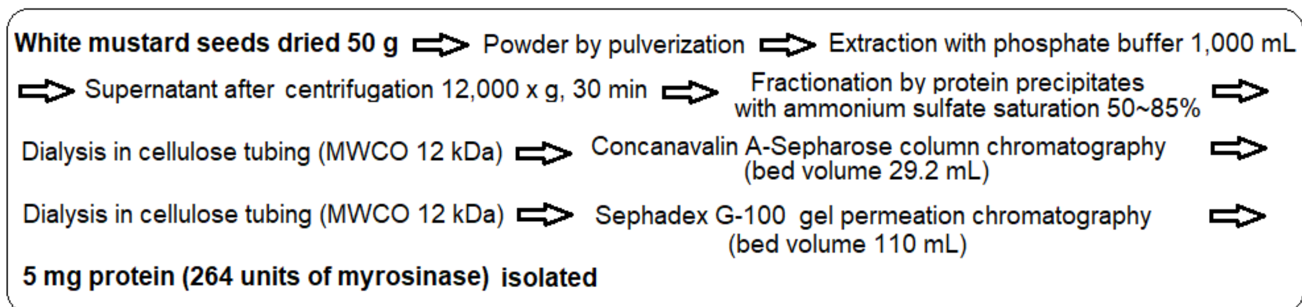

Fig. 1. Myrosinase preparation procedure.

29.2 mL. The column was flushed with five bed volumes of wash solution (1 M NaCl, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 5 mM $CaCl_2$), and then equilibrated with equilibration buffer (20 mM Tris buffer, pH 7.4, 0.5 M NaCl). The lyophilized enzyme preparation was dissolved in equilibration buffer at 75 mg/mL concentration and loaded on the Concanavalin A-Sepharose column. The unbound proteins were eluted in advance with two column volumes of equilibration buffer, and then the lectin-bound proteins were fractionated with 500 mM methyl-α-D-glucopyranoside. Each fraction of 5 mL was collected, and their protein concentration and myrosinase activity were determined.

5) Dialysis and lyophilization

The methyl-α-D-glucopyranoside in the active fractions was removed by dialysis in cellulose tubing (MWCO 12 kDa, Sigma-Aldrich) against 50 mM phosphate buffer (pH 6.5) containing 0.01% sodium azide at room temperature for 2 days. The dialysate was centrifuged again (12,000×g, 30 min) at 4℃, and then only the clear supernatant was taken and lyophilized (Ilshin, TFD 5505, Korea).

6) Gel permeation chromatography

The lyophilized enzyme preparation separated through a Concanavalin A-Sepharose column was re-dissolved in small amount of 50 mM phosphate buffer (pH 6.5) for further purification. This enzyme solution was loaded on a Sephadex G-100 column with a bed volume of 110 mL, which was previously equilibrated with 50 mM phosphate buffer (pH 6.5) containing 0.01% sodium azide. The effluent was collected in 3 mL fractions, and the protein concentration and myrosinase activity in each fraction were determined. Only those fractions showing myrosinase activity were combined together, lyophilized, and preserved for use in this work.

2. Polyacrylamide gel electrophoresis and staining

The enzyme proteins were analyzed on 5~20% gradient polyacrylamide gel (precast mini-slab gel, 90x83 mm, ATTO, Tokyo, Japan) based on the Orstein-Davis system (Davis BJ 1964; Ornstein 1964). Protein samples were mixed prior to running on gel at a 1:1 ratio with loading buffer (62.5 mM Tris-HCl, pH 6.8) containing 40% glycerol and 0.01% bromophenol blue. Tris-glycine buffer (25mM Tris, 192 mM glycine, pH 8.3) was used for gel running. Voltage was about 100 V at the beginning and about 200 V at the end of electrophoresis. The proteins on the gel were stained with Coomassie Brilliant Blue R-250 (Brunelle & Green 2014). Molecular weights of the protein were estimated from its relative mobility compared with bovine serum albumin (BSA) standards. The myrosinase activity on the gel was visualized by staining with methyl red as follows (Gonda et al. 2018): The polyacrylamide gel was washed with distilled water several times prior to staining. Then the gel was soaked in the staining solution containing 100 μg/mL methyl red, 6 mM sinigrin, 1 mM ascorbic acid and 1 mM Na_2HPO_4 (pH 7.5) at 37℃ for 10 min. Methyl red turned red due to protons (H^+) generated by the hydrolysis of sinigrin by myrosinase.

3. Myrosinase activity assay

The enzyme activity was determined using sinigrin (allylglucosinolate, 2-propenylglucosinolate) or 4-nitrophenyl-β-D-glucopyranoside (4-NPG) as a substrate as follows, unless mentioned otherwise. To 800 μL of 10 mM sinigrin (or 4-NPG) in 50 mM phosphate buffer (pH 6.5) were added 20 μL of 10 mM L-ascorbic acid and 100 μL of the enzyme solution, and then the total volume was adjusted to 1 mL by the addition of 50 mM phosphate buffer (pH 6.5). The reaction mixture was incubated at 37℃ for 30 min.

When sinigrin was used as a substrate, liberated glucose was measured using dinitrosalicylic acid (DNS) (Sumner JB 1924; Bhat et al. 2015). After incubation, 1 mL of DNS reagent was added to the reaction mixture and the test tube was immersed in boiling water for 5 min. The intensity of the red color that developed was measured with a microplate reader (DTX 800 Multimode Detector, Beckman Coulter, U.S.A) at 550 nm. One unit of myrosinase was defined as the amount of enzyme necessary for the liberation of 1 μmole glucose per minute by hydrolysis of sinigrin.

On the other hand, when 4-NPG was used as a substrate, liberated 4-nitrophenol was measured (Durham & Poulton 1990). Right after incubation, 100 μL of 1 M sodium carbonate (Na_2CO_3) was added to stop the enzyme reaction. The intensity of the yellow color was measured with a microplate reader (DTX 800 Multimode Detector, Beckman Coulter, USA) at 405 nm. One unit of the enzyme was defined as the amount of enzyme necessary for the liberation of 1 μmole 4-nitrophenol per minute by hydrolysis of 4-NPG.

4. Protein determination

Proteins were mostly determined according to Lowry et al.'s

spectrophotometric method (Lowry et al. 1951). Only the proteins in the fraction from column chromatography were quantitated by UV-absorption method (Layne E 1957), which measures the absorbance (A) of a protein sample at wavelengths of 260 nm and 280 nm. Bovine serum albumin (BSA) was used as a protein standard. The protein concentration was calculated using the following formula.

$$\text{Protein concentration (mg/mL)} = 1.55A_{280} - 0.76A_{260}$$

5. Thermokinetic study

The temperature dependence of the inactivation rate constant is well described by the Arrhenius equation, which predicts that a small increase in reaction temperature will produce a marked increase in the magnitude of the reaction-rate constant (k).

$$k = A\ \exp(-E_a/RT),$$

where E_a is activation energy, R is gas constant (8.314 $Jmol^{-1}K^{-1}$), T is absolute temperature (K) and A is pre-exponential factor.

To investigate the temperature dependence of enzyme stability, the enzyme solution was heated at temperatures between 50~70℃ at intervals of 10 degrees each for 5~30 min. Right after heating, the enzyme was cooled immediately and the residual activity was determined.

Thermokinetic parameters were obtained by fitting nonlinear models to the experimental data using Solver in Excel 2016 (Microsoft Corporation, Redmond, Washington, USA). The consecutive step model and distinct two enzymes model were tested for their applicability to the inactivation of our myrosinases (Fig. 2).

The consecutive step model is based on the succession of two irreversible first order reactions, as shown in Fig. 2. In the first step, the native enzyme converts to an intermediate enzyme form with a lower activity than the native one, and subsequently, the intermediate form converts to the inactive enzyme form, with each step having a distinct inactivation rate constant (Robert et al. 1995; Shalini et al. 2008; Ghawi et al. 2012). Meanwhile, the distinct two enzymes model assumes the existence of two isozymes with different thermal stabilities: one enzyme is heat labile, while the other is heat stable. Both enzymes follow the first order inactivation model (Weemaes et al. 1998).

Enzyme activity loss on heating is described by the following kinetic equations (Ghawi et al. 2012):

$$A_t/A_0 = [A_1 - A_2(k_1/k_1 - k_2)]\exp(-k_1 t) + A_2(k_1/k_1 - k_2)\exp(-k_2 t)$$

for the consecutive step model, and

$$A_t/A_0 = A_L\exp(-k_L t) + A_S\exp(-k_S t)$$

for the distinct two enzymes model,
where A_t=residual enzyme activity at time t, A_0=enzyme activity at time 0, A_1=activity of the native enzyme form at time 0, A_2=activity of the intermediate enzyme form at time t, A_L=activity of the labile enzyme at time 0, A_S=activity of the stable enzyme at time 0, k_1=inactivation rate constant of the native enzyme, k_2=inactivation rate constant of the intermediate enzyme, k_L=inactivation rate constant of the labile enzyme, and k_S=inactivation rate constant of the stable enzyme.

All experimental data are expressed using the mean value of three replications.

Results and Discussion

1. Isolation of myrosinase

Myrosinases were extracted from pulverized white mustard

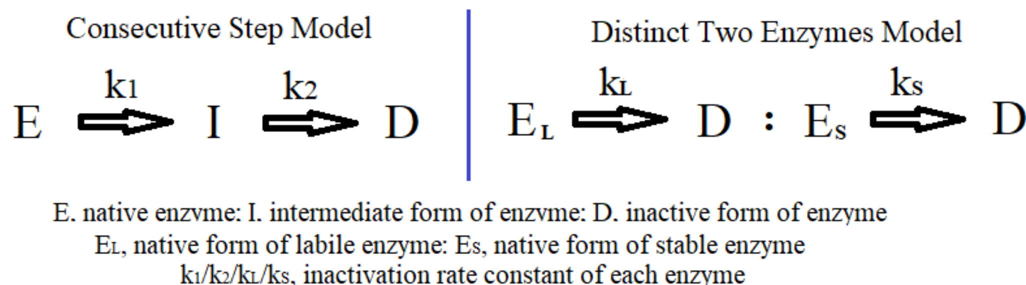

Fig. 2. Kinetic models to explain the inactivation of the enzyme myrosinase on heating.

seeds with 50 mM phosphate buffer (pH 6.5). From the extract, protein precipitates between 50% and 85% ammonium sulfate saturation were obtained, dialyzed and concentrated. In order to purify the myrosinases further, the ammonium sulfate precipitates were loaded onto a carbohydrate-binding Concanavalin A-Sepharose column and eluted with methyl-α-D-glucopyranoside as shown in Fig. 3. Three peaks with myrosinase activity were observed in the chromatogram, which suggests the existence of at least three isozymes. The major activity peak (myrosinase Ⅰ) appeared between elution volumes 60 and 85 mL, and two other activity peaks followed between elution volumes 90~100 mL for myrosinase Ⅱ, and 105~115 mL for myrosinase Ⅲ, respectively. In an earlier report by Ohtsuru & Hata (1972), four glycoproteins with myrosinase activity were found in a mustard seed. They differed in carbohydrate contents: 8.6, 15.8, 17.8 or 22.5%. Our three isozymes also seemed to differ in carbohydrate moieties since they were separated through a carbohydrate-binding affinity column. Moreover, Shin et al. (1996) reported three myrosinase isozymes in brown mustard (*Brassica juncea*) seeds, which is analogous to our findings.

The fractions corresponding to the myrosinase Ⅰ (major myrosinase) in Fig. 3 were combined and subjected to further purification by gel permeation chromatography. The major myrosinase preparation was loaded onto a Sephadex G-100 column, and its elution pattern is shown in Fig. 4. Enzyme activity and protein concentration peaks coincided between elution volumes 39 and 57 mL.

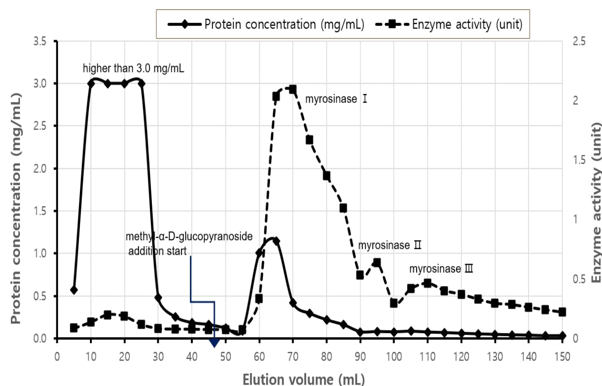

Fig. 3. Affinity chromatography of ammonium sulfate precipitates from white mustard seeds on Concanavalin A-Sepharose column. Elution was carried out with 500 mM methyl-α-D-glucopyranoside in equilibration buffer (20 mM Tris-HCl, pH 7.4). Column size, 1.5×16.5 cm; Flow rate, 0.17 mL/min; Elution volume, 5 mL/fraction.

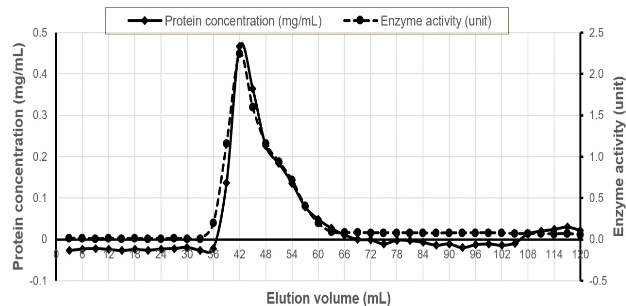

Fig. 4. Gel permeation chromatography of myrosinase Ⅰ on Sephadex G-100 column. Elution was carried out with 50 mM phosphate buffer (pH 6.5). Column size, 1.5×62 cm; Flow rate, 0.075 mL/min; Elution volume, 3 mL/fraction.

The major myrosinase obtained from the Sephadex G-100 column was examined by native polyacrylamide gel electrophoresis to check its purity (Fig. 5). It contained proteins mainly with molecular weights of 171 kDa and some higher molecular weight proteins, whereas 50~85% ammonium sulfate precipitate had various proteins (Fig. 5A). It was quite unexpected that the 171 kDa proteins were not separated from the higher molecular weight proteins by either Concanavalin A-Sepharose or Sephadex G-100 column, but resolved on polyacrylamide gel. When the

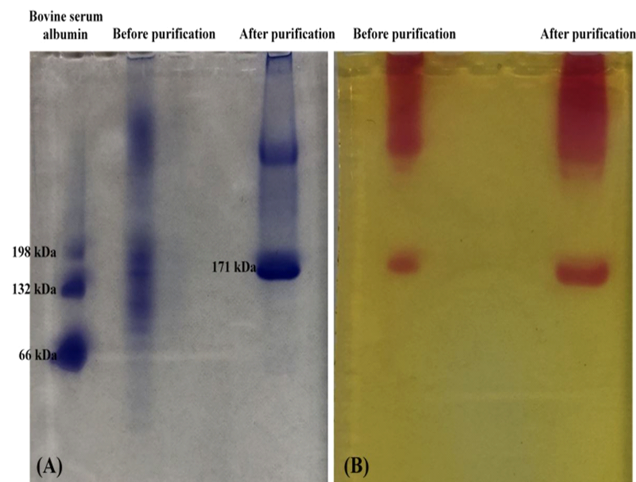

Fig. 5. Native polyacrylamide gel electrophoresis of the myrosinase from white mustard seeds and protein staining with Coomassie Brilliant Blue R-250 (A) and myrosinase activity staining with methyl red (B). Bovine serum albumin monomer (66 kDa), dimer (132 kDa) and trimer (198 kDa); Before purification, 50~85% ammonium sulfate precipitate; After purification, major myrosinases from Sephadex G-100 chromatography.

same gel was stained for myrosinase activity with sinigrin and methyl red, active red bands were observed at the locations of 171 kDa and higher molecular weight proteins. In addition, both protein samples before and after purification showed the same activity staining patterns (Fig. 5B).

Several reports about major plant myrosinases have appeared: The myrosinase isolated from white mustard seed (*Sinapis alba*, L.) was found to be a glycoprotein with a molecular weight of 151 kDa, consisting of two identical polypeptide subunits with a molecular weight of 62 kDa each and a carbohydrate part (Björkman & Janson 1972). Later, Pessina et al. (1990) reported the presence of at least three myrosinase isozymes in *Sinapis alba*. The main isozyme has a molecular weight of 135.1 kDa and consists of two identical subunits with a molecular weight of 71.7 kDa. Myrosinase exists in the leaves of *Lepidium latifolium*, as a dimer in native form (160 kDa) with a subunit size of 70 kDa (Bhat et al. 2015). Mahn et al. (2014) identified myrosinase in broccoli whose molecular weight was 157 kDa, and which was composed of three subunits, each with a molecular weight of 50~55 kDa.

The 171 kDa protein of the major myrosinase is thus suggested to be a major native dimeric enzyme. However, the higher molecular weight proteins are inferred to be multimeric forms of the myrosinase. In addition, the broad band of red near the top of the gel could be explained as the result of aggregation of myrosinase glycopolypeptides. In support of this interpretation, the myrosinases from plant sources including *Brassica napus*, *Sinapis alba* and Wasabi *japonica* are known to be glycopolypeptides with multiple forms with different molecular weights (135~480 kDa) and numbers (2~12) of subunits (Bones & Rossiter 1996). Bellostas et al. (2008) also reported that myrosinases in the *Brassicas* formed complexes with different molecular weights (140~200 kDa, 270~350 kDa and 500 - 600 kDa). They only isolated a myrosinase with a molecular weight of 140~200 kDa from seeds of *Sinapis alba* and purified it 570-fold through Concanavalin A-Sepharose affinity chromatography along with Sephadex G-200 gel filtration.

The data suggest that 171 kDa dimeric enzymes and higher molecular weight multimeric enzymes are contained in the major myrosinase preparation.

2. Thermokinetics

An increase in reaction temperature speeds up not only the catalytic reaction rate, but also the enzyme inactivation (denaturation) rate. After heating the major myrosinase preparation over a temperature range of 50~70℃, the residual activity was measured to study thermal inactivation kinetics (Fig. 6). The thermal inactivation curves showed no first-order reaction kinetics, which should appear as a straight line when residual activity is plotted versus heating time. The first order kinetic model assumes the existence of a single unique enzyme, and the disruption of its single bond or structure is sufficient to inactivate the enzyme (Shalini et al. 2008). Instead of the first order kinetic model, the consecutive step model and the distinct two enzymes model appeared to be suitable since a higher loss of activity occurred in the early stage of heating and the inactivation rate decreased in the late stage. Thus kinetic parameters were estimated by fitting the two nonlinear models to the experimental data (Table 1).

Inactivation energy (E_a) was calculated from the Arrhenius plot (Fig. 7) based on the kinetic parameters. When the consecutive step model was used (Fig. 7A), the inactivation energy of the myrosinases was 44.388 kJ/mol for the early stage of destruction and 32.019 kJ/mol for the late stage of destruction. On the other hand, when the distinct two enzymes model was used (Fig. 7B), the inactivation energy was 77.772 kJ/mol for the labile enzyme and 95.145 kJ/mol for the stable enzyme. Even though the coefficient of determination (R^2) was close to 1.0 and residual sum of squares (RSS) was very low when the consecutive step model was used, the inactivation energy (E_a) for the early destruction step was higher than that for the late destruction step (Table 1). The estimated E_a values were inconsistent

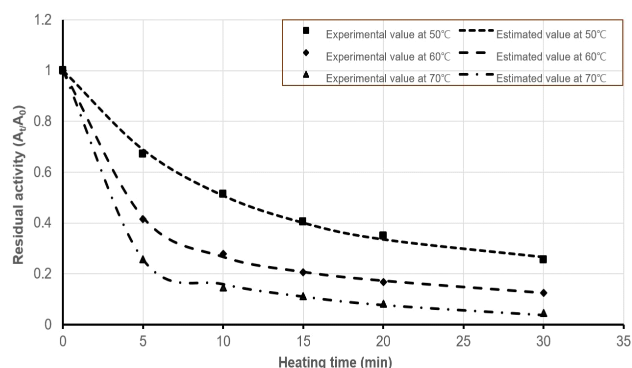

Fig. 6. Residual activity versus heating time at fixed temperature for the myrosinase from white mustard seeds. Estimated values were from the distinct two enzymes model. A_t, enzyme activity at time t; A_0, enzyme activity at time 0.

Table 1. Inactivation model and kinetic parameters of the myrosinase from white mustard seeds

Consecutive step model				
		50℃	60℃	70℃
Parameter	k_1 (min^{-1})	0.183285204	0.331583197	0.479161967
	k_2 (min^{-1})	0.027595583	0.035844534	0.055379833
	A_1	0.99972652	0.999791807	1.000014545
	A_2	0.495438608	0.315954471	0.219906447
Residual sum of squares (RSS)		8.79035E-05	0.000150888	1.8458E-05
Coefficient of determination (R^2)		0.999758718	0.999717735	0.999972023
Activation energy (E_a)(kJ/mol)	E_a for k_1	44.388 (inactivation of native enzyme)		
	E_a for k_2	32.019 (inactivation of intermediate enzyme)		
Distinct two enzymes model				
		50℃	60℃	70℃
Parameter	k_L (min^{-1})	0.115480715	0.307689236	0.622670633
	k_S (min^{-1})	0.009109742	0.03153754	0.071501659
	A_L	0.677005	0.677005	0.677005
	A_S	0.323256	0.323256	0.323256
Residual sum of squares (RSS)		0.000601909	0.000199527	0.000249088
Coefficient of determination (R^2)		0.998422539	0.999627274	0.999630634
Activation energy (E_a)(kJ/mol)	E_a for k_L	77.772 (inactivation of labile enzyme)		
	E_a for k_S	95.145 (inactivation of stable enzyme)		

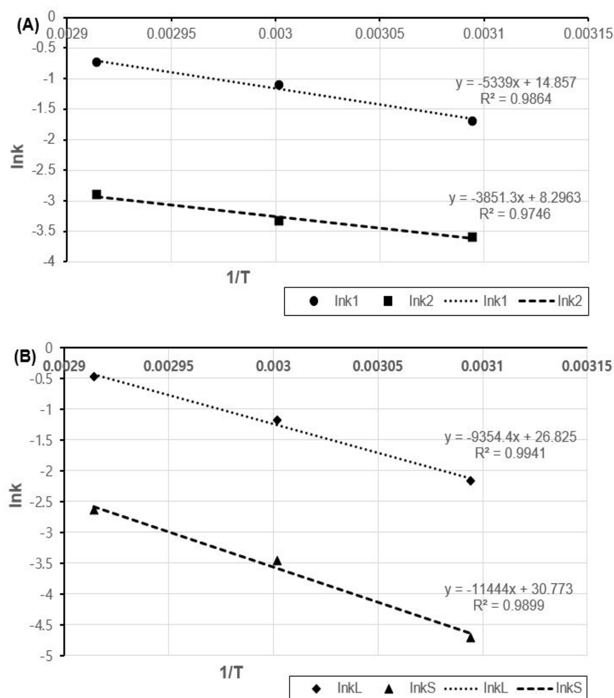

Fig. 7. Arrhenius plot for temperature dependence of inactivation rate of the myrosinase from white mustard seeds. (A) consecutive step model; (B) distinct two enzymes model.

with the assumption of the model in which the early destruction rate is faster than the late destruction rate (Robert et al. 1995; Shalini et al. 2008; Ghawi et al. 2012). Of the two nonlinear models, thus, the two distinct enzymes model was preferred to the consecutive step model. The coexistence of 171 kDa dimeric enzymes and higher molecular weight multimeric enzymes in the major myrosinase preparation could result in thermokinetics of the distinct two enzymes model. By fitting the distinct two enzymes model to our experimental data, residual activity upon heating was estimated and compared with the measured value as shown in Fig. 6. The experimental value and estimated value matched well.

Ghawi et al. (2012) reported that thermal inactivation of green cabbage myrosinase followed the consecutive step model, and that the activation energy for the destruction of myrosinase from green cabbage is 58.3 and 99.5 kJ/mol, respectively, for the first and the second inactivation steps. They used ammonium sulfate precipitates as a myrosinase preparation which was likely to be mixed with isozymes. The consecutive step model was shown to be efficient in modeling thermal inactivation of myrosinase from broccoli (Ludikhuyze et al. 1999). More heat-resistant myrosinases than ours were isolated from red cabbage (155

kJ/mol) using the first order kinetic model (Verkerk & Dekker 2004). Thermal inactivation of myrosinase from mustard seed produced different levels of inactivation energy depending on kinetic models applied. It was 327 kJ/mol, when the first order reaction model was used (Van Eylen et al. 2008); 425.5 kJ/mol for the labile fraction and 493.8 kJ/mol for the stable fraction when the distinct two enzymes model was used, and 446.4 kJ/mol for the first inactivation step and 496.0 kJ/mol for the second inactivation step when the consecutive step model was used (Van Eylen et al. 2006).

The thermal inactivation energies (77.772~95.145 kJ/mol) of the major myrosinase lie within energy range causing nutrient degradation on heating (Ling et al. 2015). The myrosinase has lower thermostability than other food-related enzymes such as polyphenol oxidase, lipoxygenase, pectinmethylesterase, and peroxidase (Ludikhuyze et al. 1999). The low level of inactivation energy suggests that the myrosinase of white mustard seeds has a high possibility of activity loss during ordinary thermal processing. Other way than ordinary thermal processing needs to be established to preserve myrosinase activity. Hydrolysis of glucosinolates in edible plants by myrosinase produces aglycones which have been expected to give beneficial effects in humans (Halkier & Gershenzon 2006; Albena et al. 2012). As a way to alleviate its thermal inactivation, simultaneous pressurization with heating brought less loss of catalytic activity than heating only in mustard myrosinase (Van Eylen et al. 2006; Okunade et al. 2015). High hydrostatic pressure treatment is an alternative way of food processing to reduce destruction of heat-labile nutrients and improve food quality (Kim et al. 2018).

Conclusion

Myrosinases were isolated from white mustard (*Sinapis alba*) seeds and their thermal stabilities were investigated. At least three isozymes of myrosinase are suggested to exist in white mustard seeds. When the consecutive step model and the distinct two enzymes model were tested for their applicability to the thermal inactivation of the myrosinases, the kinetic parameters of the inactivation differed between the models. The distinct two enzymes model fit well the thermal inactivation of the major myrosinase preparation which contained 171 kDa dimeric enzymes and higher molecular weight multimeric enzymes. The thermal inactivation energies lie within energy range causing nutrient degradation on heating. It is desirable to establish other way than ordinary thermal processing for hydrolysis and biological functions of glucosinolates.

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