Atmosphere-ocean coupled processes in the Madden-Julian oscillation

Thermal and pressure stability of myrosinase enzymes from black mustard (*Brassica nigra* L. W.D.J Koch. var. *nigra*), brown mustard (*Brassica juncea* L. Czern. var. *juncea*) and yellow mustard (*Sinapsis alba* L. Subsp *Maire*) seeds

Olukayode Adediran Okunade, Sameer Khalil Ghawi, Lisa Methven, Keshavan Niranjan

Department of Food and Nutritional Sciences, University of Reading, Whiteknights,

P.O Box 226, Reading, RG6 6AP, UK

Abbr. Running Title:

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Corresponding Author;

Olukayode Adediran Okunade

Department of Food and Nutritional Sciences, University of Reading, Whiteknights,

P.O Box 226, Reading, RG6 6AP, UK E-mail: fc030053@reading.ac.uk

Abstract

This study investigates the effects of temperature and pressure on inactivation of myrosinase extracted from black, brown and yellow mustard seeds.

Brown mustard had higher myrosinase activity (2.75 un/mL) than black (1.50 un/mL) and yellow mustard (0.63 un/mL).

The extent of enzyme inactivation increased with pressure (600-800 MPa) and temperature (30-70 °C) for all the mustard seeds. However, at combinations of lower pressures (200-400 MPa) and high temperatures (60-80 °C), there was less inactivation. For example, application of 300 MPa and 70 °C for 10 minutes retained 20%, 80% and 65% activity in yellow, black and brown mustard, respectively, whereas the corresponding activity retentions when applying only heat (70 °C, 10min) were 0%, 59% and 35%. Thus, application of moderate pressures (200-400 MPa) can potentially be used to retain myrosinase activity needed for subsequent glucosinolate hydrolysis.

Keywords: Processing, Myrosinase activity, Inactivation, Mustard seed.

1 Introduction

Mustard plant belongs to the *Brassicaceae* family, the dry seeds being the main part used in food processing (canned mustard leaves are available). Common types of mustard are yellow mustard (*Sinapsis alba*), brown (*Brassica juncea*) and black mustard (*Brassica nigra*). Mustard has a rich chemical composition and its seed flour is widely used in food processing (Abul-Fadl, El-Badry, & Ammar, 2011; Wanasundara, 2008). Mustard is also used for its spicy flavour, produced from the hydrolysis of glucosinolates by myrosinase enzymes (Wanasundara, 2008). Mustard seed is widely used as a condiment, however, its advantageous chemical composition and relatively low price offer wide possibilities for utilization as additives in human food and in animal feeds (Abul-Fadl, El-Badry, & Ammar, 2011; Wanasundara, 2008).

The glucosinolates (thioglucosides) in mustard seeds are pseudo-thioglucosides containing nitrogen and sulphur. Myrosinase enzymes (thioglucoside) are glucohydrolases (EC 3.2.3.1) (Bones & Rossiter, 1996; Fahey, Zalcmann, & Talalay, 2001; Thangstad & Bones, 1991). According to Thangstad *et al.* (1991) and Bones *et al.* (1996), glucosinolates and myrosinase enzymes coexist in segregated compartments of the plant. After plant tissue damage, glucosinolates are hydrolysed to produce a variety of compounds; some of which are bioactive (isothiocyanates, indoles) (Bongoni, Verkerk, Steenbekkers, Dekker, & Stieger, 2014), thiocyanates, oxazolidine-2-thiones and others of which are potentially toxic (nitriles, epithionitriles) by myrosinase enzymes. The nature of the hydrolysis products depends on the structure of glucosinolate and the reaction conditions (Fahey, Zalcmann, & Talalay, 2001; Lambrix, Reichelt, Mitchell-Olds, Kliebenstein, & Gershenzon, 2001). These compounds are of immense interest in food processing (taste, aroma, and flavour attributes) and human health (anticarcinogenic and antimicrobial properties) (Drewnowski

& Gomez-Carneros, 2000; Fahey, Zhang, & Talalay, 1997; Johnson, Koh, Wang, Yu, & Yuan, 2010; Tang & Zhang, 2004; Wanasundara, 2008).

Myrosinase enzymes are significantly inactivated at normal cooking temperatures regardless of the method used (Ludikhuyze, Ooms, Weemaes, & Hendrickx, 1999; Van Eylen, Oey, Hendrickx, & Van Loey, 2007; Yen & Wei, 1993), although, the same processing conditions rarely affect glucosinolates if leaching out can be avoided (Oerlemans, Barrett, Suades, Verkerk, & Dekker, 2006). Microflora in the human gut can hydrolyse glucosinolates into bioactive compounds, but the yield is much lower compared to that resulting from plant myrosinases (Conaway, Getahun, Liebes, Pusateri, Topham, Botero-Omary, *et al*., 2001). Hence, the control of myrosinase activity is important to determine the bioavailability of hydrolysis products.

Myrosinase enzymes in *Brassica* vegetables are known to exhibit varying degree of thermal stability (Ghawi, Methven, Rastall, & Niranjan, 2012; Ludikhuyze, Ooms, Weemaes, & Hendrickx, 1999; Matusheski, Juvik, & Jeffery, 2003; Van Eylen, Indrawati, Hendrickx, & Van Loey, 2006; Yen & Wei, 1993). Yellow mustard and rapeseed myrosinases are known to have the highest thermal stability of *Brassica* plant species (Kozlowska, Nowak, & Nowak, 1983; Ludikhuyze, Ooms, Weemaes, & Hendrickx, 1999; Pérez, Barrientos, Román, & Mahn, 2014; Van Eylen, Indrawati, Hendrickx, & Van Loey, 2006; Van Eylen, Oey, Hendrickx, & Van Loey, 2007; Verkerk & Dekker, 2004; Yen & Wei, 1993). The use of high pressure processing (HPP) as an alternative to thermal processing has been suggested and it has been found to reduce thermal inactivation of certain enzymes, including myrosinase (Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998; Van Eylen, Oey, Hendrickx, & Van Loey, 2008). Although a narrow range of thermal and pressure stability in *Brassica* vegetables has been reported, no

data is yet available for the thermal and pressure inactivation of brown and black mustard seed myrosinase enzymes; and yet, they may be more stable than myrosinase in other *Brasiccas* as they are related to yellow mustard which has already been shown to have higher heat stability (Van Eylen *et al.*, 2006; 2008).

In recent studies, it was found that addition of an exogenous source of myrosinase (Daikon radish root and mustard seeds) to processed *Brassica* can reinitiate the hydrolysis of glucosinolates (Dosz & Jeffery, 2013; Ghawi, Methven, & Niranjan, 2013). Hence, evaluating other sources of myrosinase that are more stable under processing conditions is of importance.

The hypothesis of this study were that (1) myrosinase from different mustard species differ in thermal and pressure stability and (2) lower pressure processing can be used to decrease thermal inactivation rate of mustard myrosinase. The study aimed to investigate thermal, pressure and combined thermal and pressure inactivation of myrosinase enzymes from black, brown and yellow mustard seeds in order to ascertain the possible extent of degradation that would occur under food processing conditions.

2 Materials and methods

2.1 Sample preparation and myrosinase extraction

Yellow mustard (*Sinapsis alba* L. *subsp maire*), black mustard (*Brassica nigra* L. W.D.J Koch. *var*. *nigra*) and brown mustard (*Brassica juncea* L. Czern. *var. juncea*) were obtained from the I.P.K Gene bank (Gatersleben, Germany). All samples were obtained as dried seeds.

Myrosinase enzyme extraction was done as described by Ghawi *et al.* (2012). The process involved grinding dry mustard seeds in a coffee grinder. 10mL of buffer (Tris HCl 0.2M, pH 7.5 containing EDTA 0.5mM, dithiothreitol 1.5mM and 0.4g Polyvinylpolypyrrolidone) was then added to 0.5g powdered mustard and blended on ice (15 minutes) and then centrifuged (11,738 ×g) for 15 minutes at 4 °C. The supernatant was filtered (0.45µm). The filtrate was made up to 10mL using the buffer solution and 90% precipitation of protein was achieved using 6.2g ammonium sulphate with slow blending on ice for 30 minutes. The mixture was then centrifuged (13,694 ×g) for 15 minutes at 4 °C. The pellet obtained was suspended in 2mL of 10mM Tris HCl buffer, pH 7.5.

The mix was extensively dialysed at low temperature (4 °C) using cellulose membrane (Medicell International Ltd, Molecular weight cut-off 12,000-14,000 Da) and 10mM Tris HCl buffer for 24 hours to remove excess ammonium and sulphate ions and centrifuged (11,738 ×g) at 4 °C for 15 minutes to remove insoluble materials. Finally, the supernatant was frozen (-80 °C) and then lyophilised, the resulting powder was stored at -20 °C until further analysis.

2.2 Myrosinase enzyme activity assay

Myrosinase activity was measured according to the coupled enzymatic procedure with some modifications (Gatfield & Sand, 1983; Ghawi, Methven, Rastall, & Niranjan, 2012;

Wilkinson, Rhodes, & Fenwick, 1984). A D-glucose determination kit was used (R-Biopharm Rhone, Heidelberg, Germany). The reaction mixture (1.51mL) included 0.5mL NADP/ATP, 10 µL hexokinase/glucose-6-phosphate dehydrogenase, 0.9mL of water containing ascorbic acid 7mM (cofactor) and 50 µL sample. The mix was allowed to equilibrate for 5 minutes and 50 µL sinigrin solution (0.6M) was added. The change in absorbance due to the formation of NADP was measured at 340nm. Myrosinase activity was determined from the initial linear rate of increase in the curve of absorbance against reaction time. A standard myrosinase enzyme (Sigma Aldrich, UK) was used to establish the calibration curve of absorbance against concentration. One unit (un) of myrosinase was defined as the amount of enzyme that produces 1 µmol of glucose per minute at 25 °C and pH 7.5.

2.3 Heat treatment

Thermal inactivation was done under isothermal conditions at different temperatures, between 10-80 °C. 150 µL sample (25mg lyophilised powder/mL de-ionised water) was pipetted into clean durham tube (6.5mm internal diameter, 1mm thickness and 30mm in length, Fischer Scientific, Loughborough, U.K) and sealed. These were carefully placed in heated water bath fitted with a thermometer for pre-set time of 5 and 10 minutes at different temperature (10-80 °C). After each determination, the samples were quickly immersed in an ice bath and the enzyme activity was measured not later than an hour after each heat treatment. Treatments were done in triplicate.

2.4 Pressure treatment

Pressure treatments were performed between 100-900 MPa using a high pressure unit (37mm diameter and 246mm length Food Lab 300 Stansted Fluid Power, Stansted, UK). 1, 2-Propanediol (30%) (sigma-Aldrich, Poole, U.K) was used as the pressure transmitting

fluid. The processing temperature was controlled by liquid circulation in the outer jacket of the high pressure vessel. 200 µL samples (25mg/mL) were placed in flexible polyethylene bags (LDPE) and air was carefully removed from the bags before sealing. Pressure treatment at different levels for pre-set time of 5 and 10 minutes was applied with temperature controlled at 15°C. Samples were removed from the vessel and rapidly cooled in an ice bath and the enzyme activity was measured not later than an hour after pressure treatment.

Combined pressure and temperature treatments were performed using a combination of high pressure (600-800MPa) with moderate temperature (30-70 °C) for 10 minutes and low pressure (200- 400MPa) with slightly higher temperature (60-80 °C) for different pre-set times of 5, 10 and 15 minutes. Pressure build up is usually accompanied with increase in temperature due to adiabatic heating. About 3-5 minutes was needed to reach equilibrium (desired temperature and pressure) and this was added to the holding time. All treatments were done in triplicate.

2.5 Protein assay

Total protein content (unprocessed samples) was determined using the Bradford procedure (Bradford, 1976). This is based on formation of a complex with Brilliant Blue G. The samples (25mg/mL) were mixed with the reagent and the absorbance was measured at 595nm after 20 minutes of incubation at 23 °C. Bovine serum albumin BSA (0 - 1.4mg/mL) (Sigma Aldrich, UK) was used in constructing a standard curve.

2.6 Statistical analysis

The statistical differences between the values obtained under different experimental conditions were established by undertaking ANOVA followed by Tukey's HSD multiple

pairwise comparison test using SPSS software (PASW Statistics 17.0, IBM, UK). Differences were considered significant at $P<0.05$.

3 Results and discussion

3.1 Enzyme activity, protein content and specific enzyme activity of myrosinase enzymes from black, brown and yellow mustard seeds

Table 1 shows the enzyme activity, protein content and specific enzyme activity of myrosinase enzyme from black, brown and yellow mustard. Brown mustard had higher myrosinase activity (2.75 un/mL) than black mustard (1.50 un/mL) and yellow mustard had the least myrosinase activity (0.63 un/mL). The protein content of all studied samples was similar. Myrosinase from brown mustard had the highest proportion of specific activity (2.04un/mg) and yellow mustard the lowest (0.48un/mg). The differences between mustard species in overall enzyme activity were not related to differences in protein content and hence, differences between species in specific activity prevailed. Variations in myrosinase enzyme activity within and between *Brassica* species have been reported previously and have been attributed to genetic and/or environmental factors (agronomic and climatic conditions) (Pocock, Heaney, Wilkinson, Beaumont, Vaughan, & Fenwick, 1987; Wilkinson, Rhodes, & Fenwick, 1984). In addition, Rask *et al*. (2000) had also reported that the difference in thermal stability of myrosinase in *Brassica* plants was probably due to the existence of different isoforms of myrosinase, where some of them interact with myrosinase-binding proteins (a group of proteins found in *Brassica* plants) to form complexes that may improve stability. It is therefore possible that the different species of mustard may have genetic differences, or have adapted to different environmental conditions that have resulted in different isoforms of myrosinase. It has also been suggested that myrosinase activity for similar *Brassica* samples may vary between studies merely due to the different protocols employed (Piekarska, Kusznierewicz, Meller, Dziedziul, Namiesnik, & Bartoszek, 2013). In this study, the observed differences in enzyme activity between the

three species cannot be specifically attributed to one or more of the above factors because, even though the seeds were obtained from the same gene bank, they may not have been produced under strictly controlled conditions for drawing such inferences. However, the main purpose of this study is to evaluate the effects of processing on enzyme activity retention, which can robustly be undertaken for a given seed variety by normalising the enzyme activity after processing with the corresponding initial activity.

3.2 Effect of temperature on black, brown and yellow mustard seed myrosinase

Figure 1 shows the effect of temperature and exposure time on myrosinase activity at atmospheric pressure. Exposure time of 10 minutes at temperature ranging between 10-80 °C were employed. Recent studies have shown that myrosinase enzymes extracted from broccoli are stable up to 70 °C by blanching intact broccoli florets (Pérez, Barrientos, Román, & Mahn, 2014) whereas the enzyme extracted from yellow mustard is stable up to 60 °C (Ludikhuyze, Ooms, Weemaes, & Hendrickx, 1999; Van Eylen, Indrawati, Hendrickx, & Van Loey, 2006). Results of heating for 5 minutes (data not included) showed no significant inactivation up to 60 °C for black mustard myrosinase whereas the myrosinase from brown and yellow mustard showed activity loss of approximately 28% and 17% respectively. Myrosinase activity decreased significantly at above 60 °C in all the mustards studied.

When the exposure time was 10 minutes, the same trend, as observed for 5 minutes processing time, was observed in the case for both black and brown mustard. However, yellow mustard myrosinase was only substantially stable up to 50 °C (27% loss in activity) and lost about 79% of its activity at 60 °C. Heating up to 70 °C led to about 41% loss in activity for black mustard, 65% for brown mustard while there was no myrosinase activity

for yellow mustard. At 80 °C, there was no significant myrosinase activity in the case of all three seeds.

These results are similar to those in previous studies; Van Eylen *et al.* (2006 & 2008) concluded that the inactivation of myrosinase extracted from yellow mustard occurs at temperatures above 60 °C at an exposure time of 10 minutes. Stoin *et al.* (2009) had earlier reported that myrosinase from black mustard exhibited maximum activity at temperature ranging from 45-50 °C and even at a range of 70-85 °C, a small amount of enzyme activity could be observed. However, the authors suggested that activity at temperature above 85 °C may be from other sources for example thermostable desulphatase enzyme using sinigrin as a substrate. The current study has concluded that myrosinases from black and brown mustard are fully inactivated at temperatures above 80 °C and that myrosinase from these mustard sources is more stable than that from yellow mustard.

Comparing to heat stability from other *Brassica* sources, studies have shown that in both white and red cabbage, 90% loss in myrosinase activity was observed after heating at 70 °C for 30 minutes (Yen and Wei, 1993), while Matusheski *et al.* (2004) discovered high sulforaphane content in broccoli after treating at 60 °C for 10 minutes, implying that the myrosinase in broccoli was intact at 60 °C. However, in both studies, intact cabbage and broccoli were used, therefore, actual temperatures the myrosinase was exposed to may have been lower. In other studies, only rapeseed has been shown to have a higher inactivation temperature compared to yellow mustard, where inactivation typically occurs above 60 °C at a holding time of 10 minutes (Kozlowska, Nowak, & Nowak, 1983; Stoin, Pirsan, Radu, Poiana, Alexa, & Dogaru, 2009; Van Eylen, Indrawati, Hendrickx, & Van Loey, 2006; Van Eylen, Oey, Hendrickx, & Van Loey, 2008). Overall, myrosinase enzyme is

temperature sensitive and thermal treatments like blanching or heat processing are known to cause a decrease in myrosinase activity (Ghawi, Methven, Rastall, & Niranjan, 2012; Ludikhuyze, Ooms, Weemaes, & Hendrickx, 1999; Van Eylen, Indrawati, Hendrickx, & Van Loey, 2006; Van Eylen, Oey, Hendrickx, & Van Loey, 2008). Inactivation of myrosinase leads to decrease in formation of beneficial hydrolysis products from glucosinolate-myrosinase hydrolysis, although myrosinase inactivation in *Brassicas* can be used to control sensory characteristics in *Brassica* vegetables.

3.3 Effect of pressure treatment on black, brown and yellow mustard seed myrosinase

Figure 2 depicts pressure inactivation of myrosinase from yellow, black and brown mustard seeds. Pressure stability was determined at 10 minutes exposure time at pressures ranging from 100-900 MPa at 15 °C. Myrosinase was substantially stable at 600 MPa with about 19% loss in activity for brown mustard after 5 minutes exposure time (data not included) while loss in activity for black mustard was 31%. Yellow mustard myrosinase was observed to be notably stable up to 500 MPa (14% loss in activity) and there was 79% loss in myrosinase activity at 600 MPa. At 700 MPa, there was 50 and 60% loss in myrosinase activity for black and brown mustard respectively. However, at 700 MPa, myrosinase from yellow mustard was completely inactivated, whilst there was no myrosinase activity at 900 MPa for black and brown mustard.

Pressure treatment for 10 minutes showed similar trend to those observed for 5 minutes. Myrosinase activity decreased significantly above 600 MPa for both black and brown mustard. At 800 MPa, the loss in enzyme activity was over 70% for both black and brown mustard and there was no enzyme activity at 900 MPa. Yellow mustard myrosinase was

however only considerably stable up to 500 MPa (21% loss in activity) and at 600 MPa, about 79% enzyme activity was lost. Van eylen *et al.* (2006) reported that myrosinase from yellow mustard was inactivated at pressures above 600 MPa, however, this is not in agreement with the current study where 79% loss in activity was observed at 600 MPa processing. Compared to some other *Brassicas*, myrosinase from mustard have much higher pressure stability. Pressure stabilities of myrosinase from other *Brassica* types have only been reported for broccoli (300-500 MPa), green cabbage (250-300 MPa) and yellow mustard (above 600 MPa) (Ghawi, Methven, Rastall, & Niranjan, 2012; Ludikhuyze, Ooms, Weemaes, & Hendrickx, 1999; Van Eylen, Oey, Hendrickx, & Van Loey, 2008).

3.4 Effect of combined temperature and pressure treatment on black, brown and yellow mustard seed myrosinase

Combined high pressure and temperature stability of myrosinase from brown, black and yellow mustard seed (Table 2) was studied at a temperature of 30-70 °C and pressure of 600 -800 MPa for an exposure time of 10 minutes. At low temperatures (30-40 °C) and 600 MPa, it was observed that loss in activity was about 30% for black mustard, 20% for brown and 50% for yellow mustard, respectively. Yellow mustard myrosinase showed no activity at 700 and 800 MPa. An increase in temperature up to 70 °C led to approximately 60% loss in myrosinase activity at 600 MPa for black mustard and 70% for brown mustard. Overall, there was a gradual loss in myrosinase activity as the temperature and pressure gradually increased**.** This trend is in agreement with previous studies (Van Eylen, Oey, Hendrickx, & Van Loey, 2008), where applying high pressure (over 600 MPa) increased thermal inactivation rate.

315 At a combined pressure of 800 MPa and 70 °C, there was no myrosinase activity in any of
316 the mustard samples studied. This indicates a synergistic effect of high pressure (600-800
317 Mpa) on thermal inactivation of myrosinase in mustard seeds. However, Ghawi *et al.* (2012)
318 reported a synergistic effect at lower pressure level in the case of green cabbage
319 myrosinase. Pressure stability of myrosinase from *Brassica*s is not widely reported. It is clear
320 that myrosinase is inactivated at combined high pressure and temperature, so applying
321 lower pressure and temperature could be more beneficial in retaining myrosinase activity
322 and enabling formation of hydrolysis products.

323 At low pressure (200-300 MPa), it was observed that myrosinase enzyme activity was
324 notably stable at 60 °C for black mustard while significant decrease in activity was observed
325 for brown and yellow mustard (30% and 50%). In earlier studies (Van Eylen, Indrawati,
326 Hendrickx, & Van Loey, 2006) an antagonistic effect of low pressure (200-300 MPa) on
327 thermal inactivation of myrosinase in broccoli juice was reported, while Ghawi *et al.* (2012)
328 reported a synergistic effect of pressure on thermal inactivation of myrosinase in green
329 cabbage. In this study, an antagonistic effect of low pressure on thermal inactivation of
330 mustard seed myrosinase was observed. The loss in myrosinase activity was lower using
331 combined low pressure and temperature than the application of only thermal treatment.
332 For black and brown mustard myrosinase, activity retention at 75 °C and 200-300 MPa for 10
333 minutes processing time was above 70% and 55% respectively . Whereas without pressure,
334 activity retention at 70 °C was approximately 59% for black and 35% for brown mustard
335 myrosinase. Thermal processing of black and brown mustard myrosinase at 80 °C led to full
336 inactivation, however, application of low pressure (200-300 MPa) at 80 °C retained
337 considerable levels of the activity, about 50% for black and 40% for brown mustard. At 400

MPa, 80 °C and 10 minutes processing time, myrosinase activity was observed for black (30%) and brown mustard (20%). Similarly, combining low pressure (200-300 MPa) with thermal treatment at 70 °C retained 20% activity of yellow mustard myrosinase, whereas, there was no myrosinase activity under thermal processing for yellow mustard myrosinase at the same temperature. However, at higher temperature levels, there was no protective effect of pressure on thermal inactivation for yellow mustard myrosinase.

The differences in initial activity between the mustard species, where brown had the highest activity and yellow the least, led to similar trends in enzyme stability with temperature and pressure, where myrosinase from yellow mustard was the least stable. As discussed earlier, the differences in stability between the mustard species might have resulted from genetic differences or responses to different environmental challenges.

4 Conclusion

Myrosinase from different mustard species varied in terms of specific enzyme activity as well as temperature and pressure stability. Brown mustard myrosinase had the highest overall myrosinase activity and specific activity. Brown and black mustard myrosinase were more resistant to pressure and thermal treatment than myrosinase from yellow mustard. Combined high pressure-thermal treatment (up to 70 °C and 800 MPa) completely inactivated myrosinase from the mustards studied. However, at low pressure (200-400 MPa), inactivation temperature increased in the mustard samples studied with lower rate of loss in myrosinase activity compared to any of thermal, pressure and combined high pressure-thermal treatment. This difference in myrosinase stability could be utilized to control the hydrolysis level of glucosinolates when mustard seeds are used as a condiment along with cooked *Brassica* vegetables. This could have important health implications through increasing the delivery of bioactive isothiocyanates from the *Brassica*. In addition, controlling enzyme activity can also be used to regulate sensory attributes of *Brassica* vegetables.

Acknowledgments

The authors thank The Federal Polytechnic, Ado Ekiti, Ekiti State, Nigeria and The Tertiary Education Trust Fund (**TETFUND**), Nigeria, for supporting the first named author, Dr Carol Wagstaff, Department of Food and Nutritional Sciences, University of Reading, Whiteknights, Reading, UK for her technical advice and IPK Gene Bank, Gatersleben, Germany for providing the mustard seeds used in this study.

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Figure and table captions

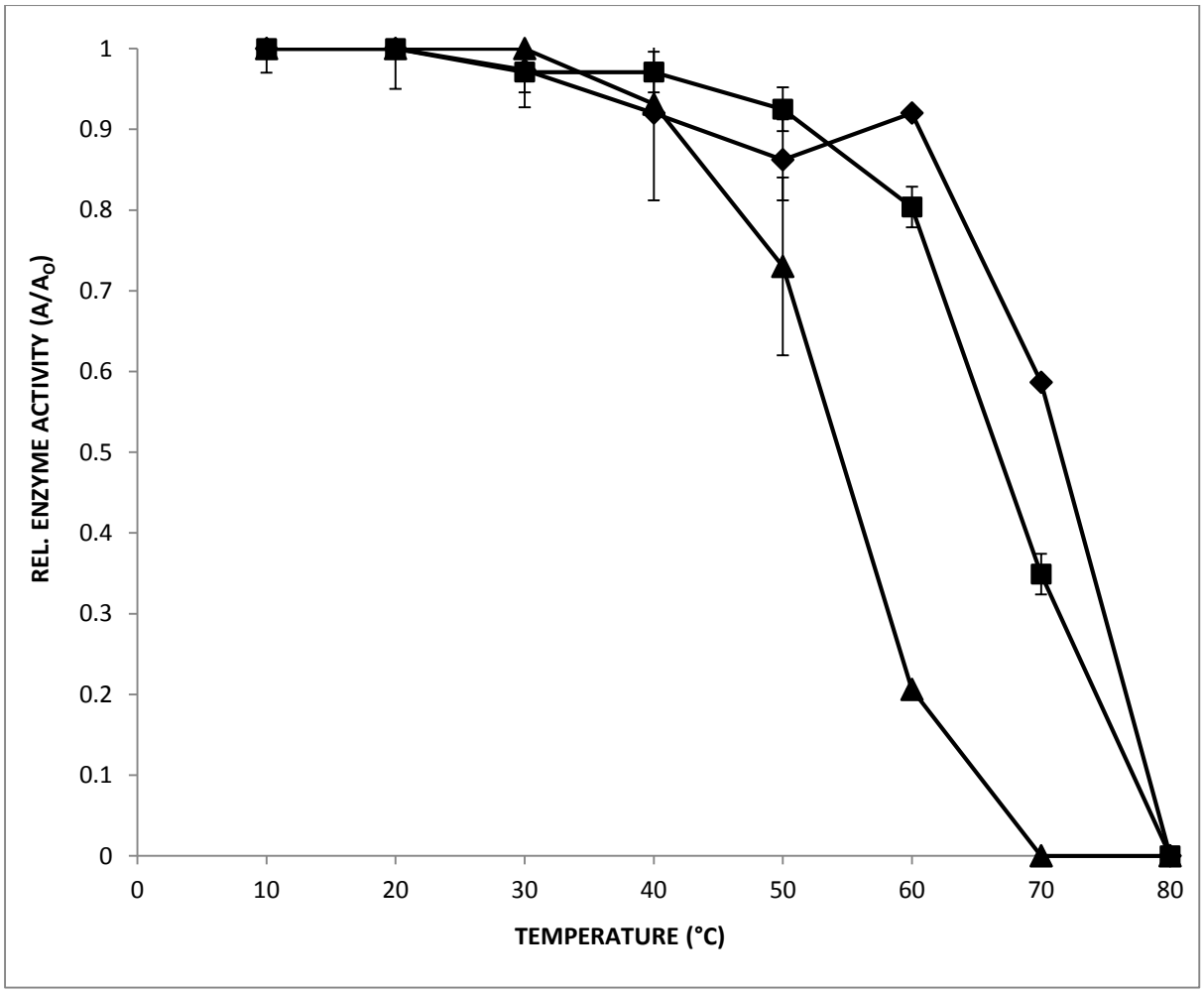
Figure 1: Effect of thermal processing on relative myrosinase activity in black, brown and yellow mustard seeds; where temperature exposure time was 10 minutes. (■) brown mustard; (♦) black mustard; (▲) yellow mustard. (A – enzyme activity after thermal treatment, Ao – Initial enzyme activity). Error bars represent standard errors of the means.

Figure 2: Effect of pressure on relative myrosinase activity in black, brown and yellow mustard seeds. Pressure holding time was 10 minutes and processing temperature was controlled at 15 °C. (■) brown mustard; (♦) black mustard; (▲) yellow mustard. (A – enzyme activity after pressure treatment, Ao – Initial enzyme activity). Error bars represent standard errors of the means.

Table 1: Myrosinase activity, protein content and specific activity of yellow, brown and black mustard seeds. (*un is activity units defined in section 2.2, lines 150-152).

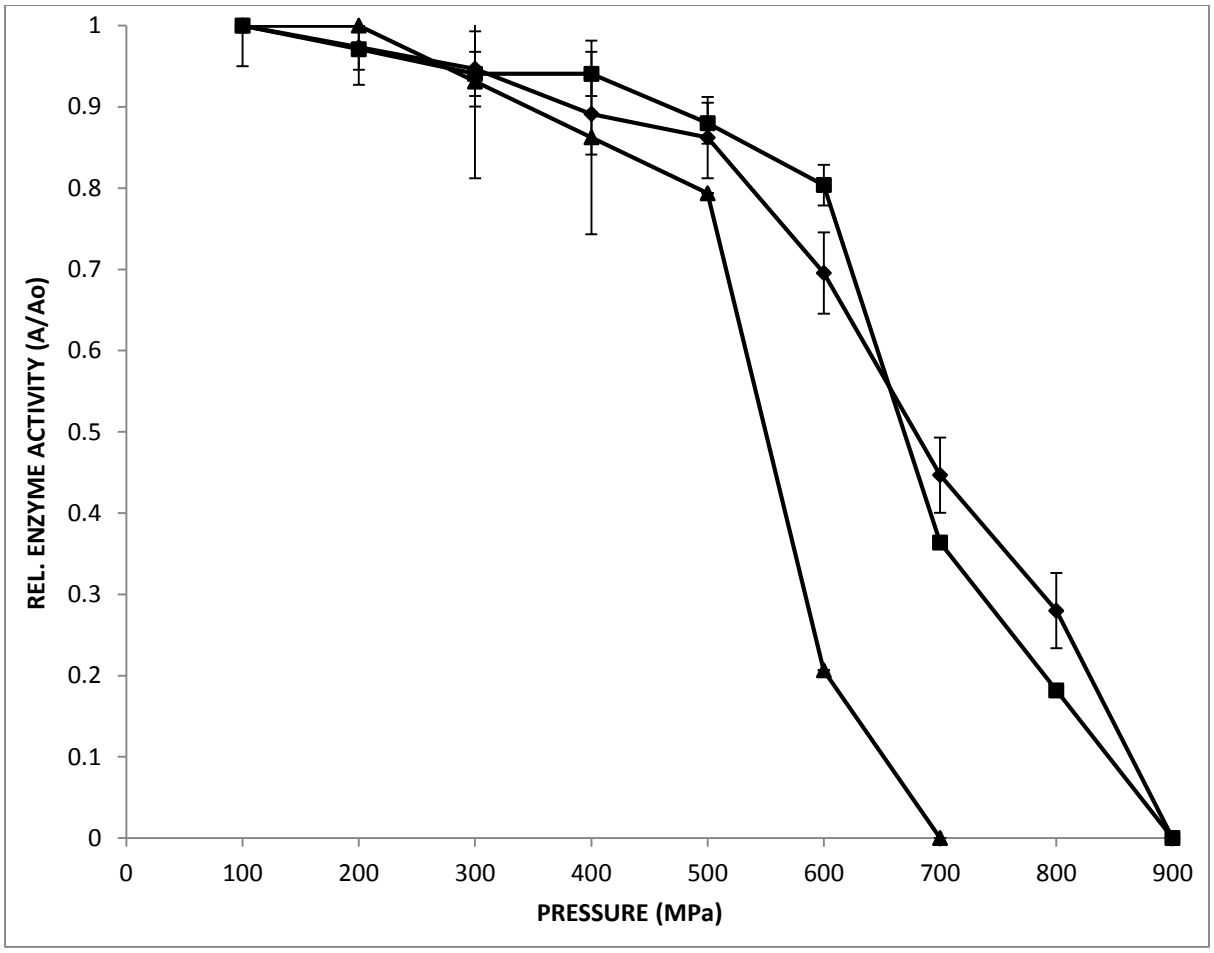
Table 2: Combined temperature and high pressure inactivation of myrosinase from black, brown and yellow mustard seeds at 10 minutes holding time.

Table 3: Effects of combined low pressure and temperature processing on myrosinase activity in black, brown and yellow mustard seeds at 5, 10 and 15 minutes holding time.



Error bars represent standard errors of the means

Figure 1



Error bars represent standard errors of the means

Figure 2

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Table 1.

Mustard Seed	Myrosinase Activity (un/mL)	Protein Content (mg/mL)	Specific Activity (un/mg)
Black	1.5±0.00	1.21±0.01	1.24
Brown	2.75±0.22	1.34±0.05	2.04
Yellow	0.63±0.00	1.32±0.01	0.48

Table 2

Pressure (MPa)	Temperature (°C)	Relative Enzyme Activity (A/A_o)		
		Black	Brown	Yellow
600	30	0.7 ±0.00[a]	0.8 ±0.00[a]	0.5 ±0.00[a]
	40	0.7 ±0.00[a]	0.8 ±0.00[a]	0.5 ±0.00[a]
	50	0.7 ±0.04[a]	0.5 ±0.00[b]	0.4 ±0.00[b]
	60	0.7 ±0.00[a]	0.4 ±0.00[c]	0.2 ±0.00[c]
	70	0.4 ±0.00[b]	0.3 ±0.02[d]	-
700	30	0.4 ±0.00[a]	0.4 ±0.00[a]	-
	40	0.4 ±0.00[a]	0.4 ±0.00[a]	-
	50	0.3 ±0.04[b]	0.3 ±0.02[b]	-
	60	0.2 ±0.04[c]	0.1 ±0.00[c]	-
	70	0.2 ±0.00[c]	0.1 ±0.02[c]	-
800	30	0.3 ±0.00[a]	0.2 ±0.00[a]	-
	40	0.3 ±0.00[a]	0.2 ±0.00[a]	-
	50	0.2 ±0.00[b]	0.1 ±0.00[b]	-
	60	0.1 ±0.00[c]	0.1 ±0.00[b]	-
	70	-	-	-

Values not sharing a common letter are significantly different at $P<0.05$.

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554 Table 3.

P (MPa)	T(°C)	Relative Enzyme Activity (A/A_o)								
		Black			Brown			Yellow		
		Processing time (Minutes)			Processing time (Minutes)			Processing time (Minutes)		
		5	10	15	5	10	15	5	10	15
200	60	0.9±0.07^{a}	0.9±0.00^{a}	0.9±0.07^{a}	0.7±0.07^{a}	0.7±0.07^{a}	0.7±0.00^{a}	0.6±0.00^{a}	0.5±0.11^{a}	0.5±0.11^{a}
	65	0.9±0.05^{a}	0.9±0.05^{a}	0.9±0.05^{a}	0.7±0.03^{a}	0.7±0.03^{a}	0.7±0.00^{a}	0.4±0.00^{a}	0.4±0.00^{a}	0.4±0.00^{a}
	70	0.8±0.00^{b}	0.8±0.00^{b}	0.8±0.00^{b}	0.7±0.00^{a,}	0.7±0.00^{a}	0.6±0.05^{,b}	0.2±0.00^{b}	0.2±0.00^{b}	0.2±0.00^{b}
	75	0.8±0.00^{b}	0.8±0.00^{b}	0.7±0.05^{c}	0.6±0.00^{b}	0.6±0.03^{b}	0.6±0.03^{b}	-	-	-
	80	0.6±0.00^{c}	0.6±0.05^{c}	0.5±0.08^{d}	0.4±0.03^{c}	0.4±0.00^{c}	0.4±0.03^{c}	-	-	-
300	60	0.9±0.07^{a}	0.9±0.07^{a}	0.9±0.07^{a}	0.7±0.07^{a}	0.7±0.00^{a}	0.7±0.07^{a}	0.5±0.11^{a}	0.5±0.11^{a}	0.5±0.11^{a}
	65	0.9±0.05^{a}	0.9±0.05^{a}	0.9±0.05^{a}	0.7±0.03^{a}	0.7±0.03^{a}	0.7±0.00^{a}	0.3±0.11^{a}	0.3±0.11^{a}	0.3±0.11^{a}
	70	0.8±0.05^{b}	0.8±0.05^{,b}	0.8±0.05^{,b}	0.7±0.00^{a}	0.6±0.00^{b}	0.6±0.03^{b}	0.2±0.00^{b}	0.2±0.00^{b}	0.2±0.00^{b}
	75	0.7±0.05^{c}	0.7±0.05^{c}	0.7±0.05^{c}	0.6±0.03^{b}	0.6±0.00^{b}	0.6±0.03^{b}	-	-	-
	80	0.5±0.00^{d}	0.5±0.00^{d}	0.5±0.00^{d}	0.4±0.00^{c}	0.4±0.00^{c}	0.4±0.07^{c}	-	-	-
400	60	0.8±0.00^{a}	0.7±0.00^{a}	0.7±0.07^{a}	0.6±0.07^{a}	0.6±0.00^{a}	0.6±0.07^{a}	0.4±0.00^{a}	0.4±0.00^{a}	0.3±0.11^{a}
	65	0.7±0.00^{b}	0.7±0.00^{a}	0.7±0.00^{a}	0.6±0.00^{a}	0.6±0.00^{a}	0.6±0.03^{a}	0.3±0.11^{a}	0.3±0.11^{a}	0.2±0.00^{a}
	70	0.7±0.00^{b}	0.7±0.00^{a}	0.7±0.00^{a}	0.6±0.03^{a}	0.6±0.03^{a}	0.6±0.07^{a}	0.1±0.11^{b}	-	
	75	0.5±0.05^{c}	0.5±0.05^{b}	0.5±0.05^{b}	0.4±0.03^{b}	0.4±0.03^{b}	0.4±0.05^{b}	-	-	
	80	0.3±0.05^{d}	0.3±0.00^{c}	0.3±0.00^{c}	0.2±0.00^{c}	0.2±0.03^{c}	0.2±0.05^{c}	-	-	

555 Values not sharing a common letter are significantly different at $P<0.05$.

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