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RESEARCH ARTICLE

A COMPARATIVE STUDY OF MYROSINASE ACTIVITY IN SELECTED CRUCIFEROUS VEGETABLES AFTER CONVENTIONAL HEAT AND MICROWAVE TREATMENTS

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ABSTRACT

Diet rich in cruciferous vegetables contain glucosinolates associated with decreased incidence of many types of cancers. These health promoting effects are due to the breakdown products of glucosinolates formed by the action of myrosinase found in them. Myrosinase released from the damaged plant cells after cutting or steaming hydrolyze glucosinolates, breaks the β thioglucoside bond of glucosinolates, producing glucose, sulfate, nitrile, thiocyanates and isothiocyanates. Myrosinase in broccoli remained stable till 100°C for 5 min and till 75°C in red cabbage whereas upon microwave treatment the activity of myrosinase remained intact at 450W for 5min exposure in both of the samples.

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INTRODUCTION

Dietary isothiocyanates are phytochemicals primarily derived from cruciferous vegetables. It comprises many commonly consumed vegetables, condiments, forages and oil containing plants, such as cabbage, broccoli, horseradish, mustard, cauliflower, brussels sprouts and rape. Isothiocyanates are stored as glucosinolates in cruciferous vegetables. In plants, glucosinolates coexist with but are physically segregated from an endogenous enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.1.1). Myrosinase activity results in the release of the glucose moiety to leave an unstable intermediate, which spontaneously rearranges to produce several products. The products of glucosinolate hydrolysis include isothiocyanates, nitriles, thiocyanates, indoles and oxazolidinethiones; from which isothiocyanates and indoles in particular have been implicated to have anticarcinogenic properties (Mithen *et al*., 2000). Many steps in the food production chain, such as cultivation, storage, processing and preparation of vegetables, may have an impact on myrosinase stability (Dekker *et al*., 2000; Howard *et al*., l997; Vos *et al*., 1988). Boiling, one of the food processing step can be carried out by conventional heat treatment. Microwave cooking is another interesting alternative way of cooking with little or no water needed for preparation of the vegetables. In most studies effect of factors such as climate, soil, genotype, seasonal variation, processing, extraction quantification that can affect the enzyme activity and stability was studied.

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But comparative studies of myrosinase activity upon different heat treatments in broccoli and red cabbage was not investigated. Thus aim of this study was to compare the stability of myrosinase when subjected to conventional and microwave heat treatments.

MATERIALS AND METHODS

One batch of fresh broccoli (*Brassica oleracea var. italica*) and red cabbage (*Brassica oleracea* of Capitata*)* was purchased from the local vegetable grocery.

Sample preparation: Stems were removed from broccoli and the bigger florets were cut into smaller pieces (size approximately 5 X 5 X 5 cm), Red cabbage was cut into smaller pieces and frozen at 0°C overnight.

Processing of the vegetables to various heat treatments
Conventional heat treatment

The samples were transferred into metal tubes with caps that could be hermetically closed and kept on ice until heating. They were heated in a heating block with a thermostat at different temperatures 50°C, 75°C and 100°C for a time period of 5, 15 and 30min.

Microwave treatment: The samples were also subjected to microwave treatment without adding water at 180,450 and 800W for a time period of 5, 10 and 15min. All the heating experiments were carried out in duplicate.

Table 1(a). Effect of conventional heat treatment on Broccoli

Sample	Temperature exposed	Duration of exposure	Absorbance at 340nm	Glucose concentration in mg/dl
Broccoli	50^0C	5min	0.1433	13.90
		15min	0.1603	15.55
		30min	0.3870	37.54
Broccoli	75^0C	5min	0.2283	22.15
		15min	0.5503	53.38
		30min	0.3088	29.96
Broccoli	100^0C	5min	0.8910	86.44
		15min	0.4663	45.24
		30min	0.3045	29.54
Broccoli	0^0C(without heat treatment)		0.0861	8.35

Table 1(b). Effect of conventional heat treatment on Red cabbage

Sample	Temperature exposed	Duration of exposure	Absorbance at 340nm	Glucose concentration in mg/dl
Red cabbage	50^0C	5min	0.3971	38.52
		15min	0.5135	49.25
		30min	0.5472	53.08
Red cabbage	75^0C	5min	0.3771	36.58
		15min	1.3303	129.08
		30min	1.1230	108.94
Red cabbage	100^0C	5min	0.9275	89.98
		15min	0.6090	59.08
		30min	0.3039	29.48
Red cabbage	0^0C		0.0674	6.50

Table 1(c). Effect of microwave heat treatment on *Brassica oleracea*

Sample	Temperature exposed	Duration of exposure	Absorbance at 340nm	Glucose concentration in mg/dl
Broccoli	180W	5min	0.3730	37.56
		10min	0.3948	39.75
		15min	0.2940	29.60
Broccoli	450W	5min	0.4099	41.27
		10min	0.0980	9.87
		15min	0.0210	2.11
Broccoli	800W	5min	0.0101	1.02
		10min	0.0206	2.07
		15min	0.0027	0.27

Table 1(d). Effect of microwave heat treatment on Red cabbage

Sample	Temperature exposed	Duration of exposure	Absorbance at 340nm	Glucose concentration in mg/dl
Red cabbage	180W	5min	0.3828	38.55
		10min	0.4023	40.51
		15min	0.3912	39.39
Red cabbage	450W	5min	1.0463	105.36
		10min	0.4729	47.62
		15min	0.0821	8.27
Red cabbage	800W	5min	0.0519	5.23
		10min	0.04565	4.60
		15min	0.0176	1.77

Myrosinase Activity determination by d -glucose method

The myrosinase activity was determined according to a coupled enzymatic procedure (Van Eylen *et al*., 2006) with some modification. In this assay, the D -glucose formed by the reaction between myrosinase and glucosinolates as a substrate could be used to transform NADP+ to NADPH (D-Glucose kit, Enzyplus, Biocontrol). A reaction mixture containing 3.0 ml of distilled water, 100 µl of buffer solution (R1 – Imidazole buffer, magnesium chloride and sodium azide as a preservative, 100 µl of ATP/NADP+ (R2) and 100 µl of sample solution was prepared. The mixture was transferred to a glass cuvette of 1.00 cm light path and the absorbance (A1) was read after 3 minutes. After reading, 10 µl of hexokinase/glucose-6-P-dehydrogenase (R3) were added to the mixture and the absorbance (A2) read after 10 minutes. To calculate the glucose content, the difference in absorbance for both blank and sample solution was determined.

The absorbance difference of the blank was subtracted from the absorbance difference of the sample, thereby the Δ AD-glucose was obtained. A blank was also prepared, but instead of 100 µl of sample solution, 100 µl of distilled water were added.

The concentration of D-glucose can be calculated as: Δ A of test sample × 100
Δ A of Standard sample

RESULTS AND DISCUSSION

Glucosinolates are one of the most important bioactive compounds in cruciferous vegetables. Enzyme myrosinase (β-thioglucoside glucohydrolase), liberated when plant cells are damaged, promotes hydrolysis of glucosinolates to pungent and highly reactive isothiocyanates, hydrogen sulfate, and glucose. Glucose is released into an aqueous medium and is

equivalent to the total glucosinolates (Fahey *et al*., 1997). Like other vegetables, most Brassica vegetables are heat processed before consumption. Among food processing, high temperature treatments lead to myrosinase inactivation (Verkerk *et al*., 2009).

Effect of conventional heat treatments on Broccoli and Red cabbage

When broccoli and red cabbage were subjected to conventional heat treatment by exposing it to various temperatures 50°C,75°C and 100°C for a time period of 5min,10min and 15min, the concentration of released glucose in broccoli reached maximum at 100^{o}C for an exposure of 5min. There after the activity of the enzyme decreased along with time at the same temperature. Whereas 15min exposure of 75^{o}C resulted in maximum concentration of glucose in red cabbage thereafter with increased temperature and time resulted in diminishing of enzyme activity. The results are tabulated in the 3.1(a) and (b). Controls (vegetables without any of the heat treatment resulted minimum glucose concentration indicating that increase in temperature promotes the catalytic activity of the myrosinase at the same time excess temperature denatures the enzyme.

Effect of microwave heat treatments on Broccoli and Red cabbage

When the samples were subjected to microwave treatments at 180,450 and 800W for a time period of 5, 10 and 15min, the activity of myrosinase was reached maximum for broccoli and also for red cabbage at 450W for 5min microwave exposure indicating maximum stability at 450W. Results were tabulated in 3.1(C and D). Our findings are in line with previous findings. Myrosinase activity in cabbage was effectively lost after 2 min of microwave cooking at 750W or after 7min of steaming (Rungapamestry *et al*., 2007) or after microwave cooking for 4.8 min at 900 W (Verkerk *et al*., 2000).

Conclusion

Glucosinolates are a group of plant secondary metabolites found in highest concentrations Brassicaceae family. Broccoli and Red cabbage belonging to this family were subjected to heat treatments and stability of myrosinase enzyme present in them was investigated. Compared to microwave treatment, conventional heat treatment produced less degradation of myrosinase thus preserving isothiocyanates concentration. The parameters obtained during different treatments will enable the optimisation of domestic food processing with respect to an enhanced health promoting potential of processed products.

Conflicts of interest: The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript

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