IJBPAS, July, 2015, 4(7): 4891-4897

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

AN INVESTIGATION ON THE EFFECTS OF CLOVE ESSENCE ON DEACTIVATION OF HORSERADISH PEROXIDASE

MARYAM MOHSENI[1], ALI MOHAMADI SANI[*1], AMIR DARAEI GARMAKHANY[2]

1: Department of Food Science & Technology, Quchan Branch, Islamic Azad University, Quchan, Iran.

2: Department of Food Science & Technology, Buali Sina University, Hamadan, Iran

***Corresponding Author: Ali Mohamadi Sani' E Mail: mohamadisani@yahoo.com**

ABSTRACT

Production of organic crops follows an increasing trend due to the role they play in human health. Such an increase necessitates a reduction in using chemical substances in the course of post-harvesting stages and have led a progressive increase in the application of natural inhibitors. Considering their anti-microbial and anti-oxidant characteristics, natural essences are thought to be good alternatives in this scope. This research is aimed to evaluate the capabilities of clove essence in reducing the activity of peroxidase enzyme (as one of the important chemical degrading agents) within horseradish. For this purpose, an enzymatic essence containing peroxidase was mixed with a substrate (composed of guaiacol, hydrogen peroxide, sodium phosphate buffer and various concentrations of essence (50, 75, 100 and 200 µg/100ml) before being transferred into a kavat where kinetic absorption of the samples were observed at a wavelength of 470 nm for 400 seconds. Considering variation of this enzyme within different vegetables, the results showed that enzymatic activities of peroxidase were 4625 (gram of vegetable × second / unit activity) with clove essence causing a reduction of such a value by 1135, 1665, 1365 and 1675 percent of activity/gram/second within the horseradish.

Keywords: Horseradish, Essence, Peroxidase Enzyme

INTRODUCTION

Enzymes are protein-bearing compounds served as vital catalyzers which may cause desirable or undesirable physical and chemical modifications of food products. They also play an important role in food metabolism within fauna organs. Generally speaking, impact mechanism of enzymes works via reducing required energy which is necessary for many chemical reactions and is called "activation energy". The material influenced by the enzyme is called substrate with the enzyme being separated from the substrate after performing required modifications within it, before it can be reused again [1-2]. On the other hand, essences are a category of volatile and aromatic compounds within the plants which have a wide and varied range of applications within food, pharmaceutical and perfumery industries. Herbal essences include phenolic and terpenic compounds, aliphatic alcohols, aldehydes, ketones, acids and iso-flavonoids among which phenols are the main causes of anti-microbial characteristics [3-4]. Prasad and Joshi (1992) proposed a preservation methodology for the fruits through submerging them into a mix of clove powder and salt [9]. Hemeda and klein (1990) reported variations in the activity of peroxidase enzyme within essences of raw vegetables [5]. The activity of the peroxidase in carrot essence was higher than those in tomato and eggplant essences. Application of clove and rosemary essences on the essence of chard was associated with a reduction of 59% and 36% in the activity of peroxidase enzyme compared to the control sample, respectively, which is in fact an indication of anti-oxidant characteristics of such essences [10]. Ponec *et al.* (2004) investigated the effects of essences of eucalyptus, tea, lemon balm, rosemary, clove and sour lemon on the activity of peroxidase enzyme within the essences of vegetables like spinach, lettuce, and cabbage and reported a reduction in enzymatic activity within all essences except for that of eucalyptus, with the highest activity reduction being associated with the application of clove essence. Belonging to Myrtaceae family, clove is among the oldest and most valuable spices used in the East [8]. Its essence is one of the most important herbal essences due to its anti-microbial and anti-oxidant characteristics with a wide range of applications within the food industries [7]. Higher levels of eugenol essence of clove exhibited strong biological, anti-microbial and anti-oxidant contributions. Such phenolic compounds can denature proteins and react with cell membrane phospholipids to modify their permeability [9]. The

purpose of this research is to evaluate capability of native Gorgan clove essence in terms of deactivation of peroxidase within horseradish so that it can be longer preserved by using a natural resource. Considering anti-oxidant characteristic of herbal essences, this research can be seen as an attempt to verify the effects of such substances on reducing activity of effective enzymes in enzymatic (peroxidase) browning and hence, color and taste preservation of edible fruits and vegetables through deactivating this enzyme as a prerequisite for the activity of oxidase polyphenol enzymes.

MATERIAL AND METHODS

For the sake of this research, horseradish was purchased from the vegetable market of Gorgan (Iran). As a source of natural anti-oxidants, clove essence was obtained from Barij Essenes Company. Tween 80 was used to produce samples of various concentrations: 50, 75, 100 and 200 µg/100 ml.

2.1. Preparation of enzymatic extract of horseradish

For this purpose, a 10 g sample of the desired vegetable was weighted before being copped while introducing 30 ml of distilled water (at 4ºC). The resultant mixture was centrifuged (at 10,000 rpm) at 4ºC for 15 minutes. At the end, the upper liquid containing peroxidase enzyme was used as the enzymatic extract [10].

2.2. Reaction mixture

Substrate mixture included 10 ml of guaiacol at 1% concentration, 10 ml of hydrogen peroxide at 3% concentration, and 100 ml of sodium phosphate buffer. The pH value of the reaction mixture was set at 6.5 [6].

2.3. Activity measurement of peroxide enzyme

Enzymatic activity measurements were conducted in accordance with the methodology proposed by Hemeda and klein (1990) [5]. In order to investigate effects of the used essence at the applied concentrations, activity of peroxidase enzyme was measured using an spectrophotometer at a wavelength of 470 nm and a temperature of 25ºC while guaiacol (Merck) and hydrogen peroxide (Merck) were used as substrate and hydrogen donor, respectively. Accordingly, 2.87 ml of substrate mixture together with 0.1 ml of raw extract as well as 0.03 ml of distilled water (a sum of 3 ml) were transferred into the kavat where kinetic absorption was observed at a wavelength of 470 nm for 400 seconds. In order to evaluate the impact of herbal essences on enzymatic activity of peroxidase, 0.03 ml of the essence under study was added, at various concentrations, to the mixture of

substrate and enzymatic extract, before kinetic absorption of samples were observed at a wavelength of 470 nm [6]. In order to measure the activity of peroxidase enzyme in the next step, a 10 g sample of the desired vegetable was weighted and then submerged into the prepared solutions of herbal essence at 50, 75, 100 and 200 µg/100 ml concentrations for a period of 1 minutes after which they were taken out. One hour later, kinetic absorption of samples was observed at a wavelength of 470 nm for 400 seconds.

2.4. Statistical decomposition analysis

This experiment was performed under full stochastic scheme using SAS (2001) statistical software. Average values were compared via Duncan's multiple range test at 5% significance level, while Excel (2003) was used to draw the corresponding plots.

RESULTS AND DISCUSSION

Level of activity of peroxidase enzyme within raw extract of horseradish was measured on a spectrophotometer and found to be 4625 (gram of vegetable×second/unit activity) indicating a high level of activity by peroxidase within the horseradish.

The results regarding the effect of various concentrations of the essence on enzymatic activity of peroxidase within raw extract of horseradish under study are presented in Tables 1 which shows that all concentrations of clove had positively contributed to the reduction of enzymatic activity of peroxidase.

Activity measurement of peroxidase by direct introduction of essence into extract

As shown in Figure 1, all studied concentrations of clove essence including those of 50, 75, 100 and 200 µg/100 ml were associated with a significant reduction in enzymatic activity of peroxidase within horseradish, so as it was observed that by increasing clove concentration, a decreasing trend was evident in the plot of peroxidase activity with its slope decreased, so as it can be expressed that clove essence has an appropriate inhibitory contribution to the reduction of enzymatic activity of peroxidase.

3.2. Activity measurement of peroxidase enzyme by submerging the vegetables into the essence

As shown on Figure 2, all studied concentrations of clove essence including those of 50, 75, 100 and 200 µg/100 ml were associated with a significant reduction in enzymatic activity of peroxidase within horseradish with the highest reductions observed for three concentrations of 75, 100 and 200 µg/100 ml whose corresponding results are coincided on each other. Therefore, it can be expressed that clove essence has an appropriate inhibitory contribution to the reduction of enzymatic activity of peroxidase.

Table 1: Effects of Clove essence concentration on enzymatic activity (gram vegetable × second/unit activity) of peroxidase within raw extract of horseradish

Treatment	sample (without essence)	50 µg/100 ml	75 µg/100 ml	100 µg/100 ml	200 µg/100 ml
Activity of peroxidase	4625[d]	1135[a]	1665[c]	1365[b]	1675[c]

*** In each row, the difference among the numbers accompanied by the same alphabet is not significant (p > 0.05)**

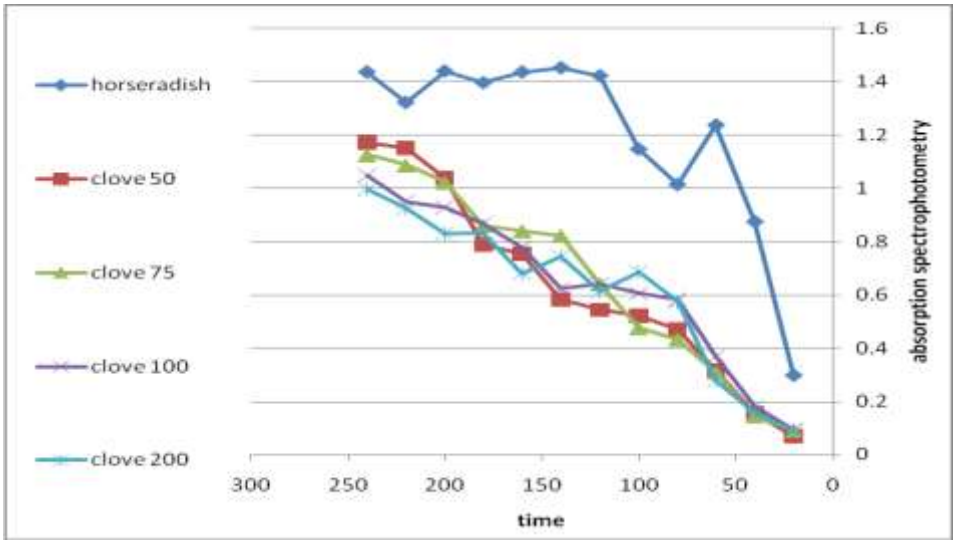

Figure 1. Effects of various concentration of clove essence on enzymatic activity of peroxidase within horseradish.

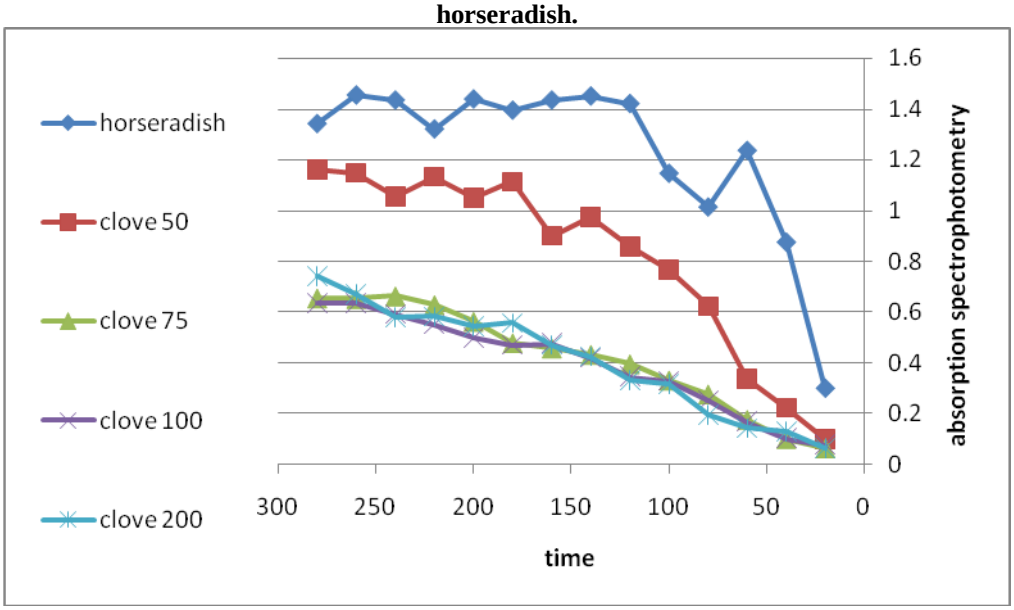

Figure 2. Effects of various concentration of clove essence on enzymatic activity of peroxidase within horseradish.

As is evident on Figures 1 and 2, at a given concentration of clove essence, enzymatic activity of peroxidase will increase by increasing the time. The results on the relationship between enzymatic activity and the time, indicate an increase in the availability of substrate for the enzyme which is confirmed by Garme Khani *et al.*

(2010), Shahabi Ghahfarokhi *et al.* (2013) [3, 10]. As the time span of enzymatic activity increases, its intensity and rate (absorption) are reduced. Such a reduction may be caused by lower availability of substrate for the enzyme or lower oxidative activity of enzyme. On the other hand, formation of enzymatic activity inhibitory compounds also have some contributions in this regard [5]. By reacting with oxygen in the environment, essences may prevent enzymatic browning reaction from being realized, hence controlling the activity of peroxidase enzyme. Peroxidase is composed of more than 2000 iso-enzymes distinguished by non-protein groups or metals attached to them, resulting in some differences in their resistance against thermal and/or non-thermal deactivation [1].

CONCLUSION

Different methods are employed to preserve food products. Due to their anti-microbial and anti-oxidant characteristics, natural essences can be an alternative for conventional methodologies. In fact, deactivating peroxidase enzyme by heat may be associated with loss of color or nutritional characteristics of food products, while the application of essences may not only be free of such problems, but also contributes to a reduction in enzymatic activity. Application of clove to horseradish was associated with a reduction in the performance of peroxidase ($P < 0.05$). The results of this research showed that natural essences can properly reduce enzymatic activity of peroxidase. Considering the fact that peroxidase enzymes are different in different vegetables, this research proved that clove essence may have a positive contribution toward reducing the performance of peroxidase within horseradish.

REFERENCES

1. Alikhani, M. and Daraei Garmakhany, A. 2012. Effect of microencapsulated essential oils on storage life and quality of strawberry (Fragaria ananassa cv. Camarosa). *Quality Assurance and Safety of Crops & Foods*, 4: 106–112.
2. Burt, S. 2004. Essential oils: their antibacterial propertied and potential application in food- a review. *Int J Food Microbiol*, 94: 223-253.
3. Daraei Garmakhany, A. Mirzai H. O. Aghajani, N. kashiri. M. 2010. Investigation of natural essential oil antioxidant activity on peroxidase enzyme in selected vegetable. *Journal of Agricultural Science and Technology*, ISSN1939-125, USA, volume 4 (3): 78-84.

4. Fatemi, H. 2006. Principles of Food preservation technology, Enteshar publication Co. Tehran (In Persian)

5. Hemeda, H. M. and Klein, B. P. 1990. Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. *Journal of Food Science*, 55: 184-186.

6. Kalemba, D. and Kunicka, A. 2003. Antibacterial and antifungal Properties of Essential oils. *Cur Medical Chem*, 10: 813-829.

7. Parthasarathy, V. A. Chempakam, B. and Zachariah, T. J. 2008. *Chemistry of Spices*. CAB International. UK, pp. 1-17.

8. Ponec, A. G. Del Valle, C. E. Roura, S. I. 2004. Natural essensial oil as reducing agents of perroxidase activity in leafy vegetable. *LWT*, 37: 199-204.

9. Prasad, H. and Joshi, N. 1992. The Perservative value of spices used in pickling raw fruits in India. *Agricultural Journal of India*, 24: 98-106.

10. Shahabi Ghahfarrokhi. I, Daraei Garmakhany. A, Kashaninejad. M, Dehghani. A. A. 2013. Estimation of Peroxidase Activity in red cabbage by Artificial Neural Network (ANN). *Quality Assurance and Safety of Crops & Foods*, 5(2): 163-167.