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EFFECT OF HEATED ONION EXTRACT ON WHITE BUTTON MUSHROOM (*AGARICUS BISPORUS*) POLYPHENOL OXIDASE

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ABSTRACT

Heated onion extract exhibited a more potent inhibitory effect towards the browning of button mushroom (*Agaricus bisporus*) compared with the fresh onion. The inhibitions were 65.10% and 25.33% for fresh onion extracts for pyrocatechol and 4-methylcatechol, respectively. The percentage of inhibition increased to 68.51% for pyrocatechol and 42.33% for 4-methylcatechol when added with the heated onion extracts. Onion extracts inhibited the white button mushroom PPO non-competitively. The inhibitory efficiency of the onion extracts increased with increasing heating temperature and time. The percentage of inhibition for the non-heated onion extracts declined drastically from 89% to 50% for pyrocatechol and 77% to 31% for 4-methylcatechol after 9 days of storage at 4°C. Meanwhile, percentage of inhibition declined from 93% to 64% and 83% to 36% for pyrocatechol and 4-methylcatechol for heated onion extracts. Onion extract could be considered as a potential natural inhibitor for preventing browning of fruits and vegetables.

1. Introduction

Browning usually occurs in vegetables and fruits during handling, processing and storage. Food browning is normally undesirable due to a reduction in nutritional value, change in sensory perceptions, and decreased consumer acceptance of food (Lim *et al.* 2020). The enzyme that is responsible for the enzymatic browning of fruits and vegetables is known as polyphenol oxidase (PPO). PPO (EC 1.14.18.1) catalyzes hydroxylation of monophenols to *o*-diphenols, followed by oxidation of *o*-diphenols to *o*-quinones in the presence of oxygen, which lead to the formation of brown pigments (Ercili-Cura *et al.* 2015).

White button mushrooms (*Agaricus bisporus*), also known as champignon mushrooms and common mushrooms, which belongs to family Agaricaceae (Rachappa *et al.* 2020). It is one of the most popular edible fungi in the world. White button mushrooms are a good source of protein, minerals and vitamins such as vitamin C, B complexes and D. Besides the nutritional value, these edible mushrooms are also famous for their medicinal properties (Rachappa *et al.* 2020; Sinha *et al.* 2021). However, white button mushrooms have very short shelf life of between 3 to 5 days which is mainly due to the post-harvest enzymatic browning (Gholami *et al*. 2017). Therefore, it is important to study and control enzymatic browning of mushrooms in order to extend their commercial shelf life while preserving their nutritional value.

Chemicals anti-browning agents such as L-cysteine, sodium metabisulfite, have been the most studied for the used in processed foods. However, consumers are concern about sulphite

containing browning agents could cause problem to human health. Thus, this present research was to look for alternative ways to prevent and delay enzymatic browning of white button mushrooms. Onion was used as a natural inhibitor to investigate its inhibition towards white button mushroom PPO.

2. Materials and methods

2.1. Plant materials and chemicals

Fresh white button mushrooms (*Agaricus bisporus*) were bought from a local market in Kuala Lumpur. They were supplied by Champ Fungi Sdn. Bhd. in Telok Gong, Port Klang, Selangor, Malaysia. Red onions were bought from local market (Giant Hypermarket, Taman Connaught, Cheras, Kuala Lumpur). All chemicals used were analytical grade and were used as obtained.

2.2. Enzyme extraction

The extraction method was adopted from Wong and Lee (2014) with slight modification. White button mushrooms (60g) were washed and sliced. The samples were then homogenized with 600 mL of 0.05M sodium phosphate buffer (4°C, pH6.8) and 1% (w/v) of polyvinylpyrrolidone using an LB-8011ES industrial blender (Waring Laboratory, Torrington, CA, USA) at maximum speed (22,000 rpm) for 3 minutes. The homogenates were then subjected to centrifugation at 8000 rpm for 15 min at 4°C using a Universal 320R centrifuge (Hettich, Tuttlingen, Germany). The supernatant containing PPO was filtered under vacuum by Buchner filter (WP6211560 Vacuum pressure pump, Millipore Sigma, Burlington, MA, USA). The filtrate obtained was pipetted drop by drop into 600 mL of cold acetone (-20°C). The precipitates obtained were centrifuged at 8000 rpm for 15 min at 4°C. The white resultant powder was dried overnight at room temperature and stored at 4°C. In order to obtain the enzyme extracts, 1 g of acetone powder was suspended in 10 mL of pre-chilled 0.05M sodium phosphate buffer (pH 6.8) and stirred until all the powders were dissolved. The suspension was then centrifuged at 8000 rpm for 15 min at 4°C. The supernatant was used as the crude PPO extract.

2.3. Onion extract preparation

Red onions were used as natural inhibitors and the preparation of red onions was slightly modified from Lim *et al.* (2019). Red onions (100 g) were rinsed and sliced into small pieces. All the pieces of red onions were then homogenized with 100 mL of 0.05M sodium phosphate buffer (pH 6.8) at 22, 000 rpm for 3 min. Homogenized onion was centrifuged at 8000 rpm for 15 min. After centrifugation, the supernatant was then filtered and the filtrate was used as the fresh onion extract. Heated onion extract was prepared by incubating the fresh onion extract at 100°C for 10 minutes.

2.4. Influence of heat treatment temperature for onion extract on the inhibitory effect of white button mushroom PPO

Onion extracts were immersed in a water bath (Memmert Lab Companion, Jeio Tech, Selangor, Malaysia) in a temperature range of 30-100°C for 10 min prior to the PPO assay.

2.5. Influence of heat treatment time for onion extract on the inhibitory effect of white button mushroom PPO

This assay was done by preparing the heated onion extracts by immersing the onion extract into a water bath (Memmert Lab Companion, Jeio Tech, Selangor, Malaysia) at 100°C. An aliquot of onion extract was removed from the water bath at every 2 min interval until 14th min. The onion extract was then immediately cooled down to room temperature prior to the addition into the reaction mixture for PPO assay.

2.6. Influence of storage time of fresh and heated onion extracts on the inhibitory effect of white button mushroom PPO

Fresh onion and heated onion extracts prepared were kept in the refrigerator for 9 days. Aliquots of the onion extracts were taken on day 1, 3, 5, 7, 9 to be added into the reaction mixture. The efficacy of the inhibition was determined by measuring the absorbance by using a

spectrophotometer (Secoman, Champigny-sur-Marne, France).

2.7. Assay of PPO activity

PPO activity was determined via measurement of an increase in absorbance at 400 nm for pyrocatechol and 410 nm for 4-methylcatechol, respectively by using a PRIM Light spectrophotometer (Secoman, Champigny-sur-Marne, France) at 15 seconds interval. The reaction mixture contained 0.1 mL of enzyme solution, 0.9 mL of 0.05M sodium phosphate buffer (pH 6.8), 1 mL of onion extract (1 g/mL) as inhibitor and 1 mL of substrate. A control contained of 0.1 mL of enzyme solution, 1.9 mL of 0.05M sodium phosphate buffer (pH 6.8) and 1 mL of substrate. The initial velocity was calculated from the slope of the absorbance *vs.* time curve, where a single unit of PPO activity was defined as the amount of enzyme that caused a 0.001 absorbance change per min (Lim *et al.* 2019).

2.8. Statistical analysis

All the experimental data was performed by using Microsoft Office Excel. The data were presented as mean ± standard deviation (SD) (n=3) and also as percent relative activity.

3. Results and discussions

3.1. Inhibitory effect of onion extract on white button mushroom PPO

Table 1 shows the inhibitory effect of onion extract on white button mushroom PPO activity. Regardless of the substrate used, the heated onion extract at 100°C for 10 min exhibited a stronger inhibitory effect on white button mushroom PPO than did the fresh onion extract. These results were in agreement with those reported by Lim *et al.* (2019), Lim and Wong (2018), Wong and Lee (2014) and Kim et al. (2005) when sweet potato PPO, ginger PPO, cassava leaves PPO and pear PPO, were treated with heated onion extracts respectively.

However, a higher inhibition percentage of white button mushroom PPO by heated onion extract was found from this study (68.51%) than that of sweet potato (41.47%) (Lim *et al.* 2019) as well as ginger (33.11%) as reported by Lim and Wong (2018). Maillard reaction products produced during heating of onion extract probably would increase the inhibitory effect on browning of white button mushroom (Kim et al. 2005). It was also reported that the thiol compounds contained in onion might be the active components responsible for the inhibition of browning (Phisut and Jiraporn 2013; Akhtar 2015).

Table 1. The effect of natural inhibitors on white button mushroom PPO

Substrate	Inhibitor	Inhibition (%)	K_m (mM)	V_{max} (EU/min/ml)	Type of inhibition
Pyrocatechol	Fresh onion extract	64.74 ± 3.76	21.22	1414.43	Non-competitive
	Heated onion extract	68.51 ± 2.19	21.93	1096.49	Non-competitive
	Control	-	21.05	3508.77	-
4-methylcatechol	Fresh onion extract	25.33 ± 0.65	24.92	778.82	Non-competitive
	Heated onion extract	42.33 ± 1.77	25.58	639.39	Non-competitive
	Control	-	25.25	1010.10	-

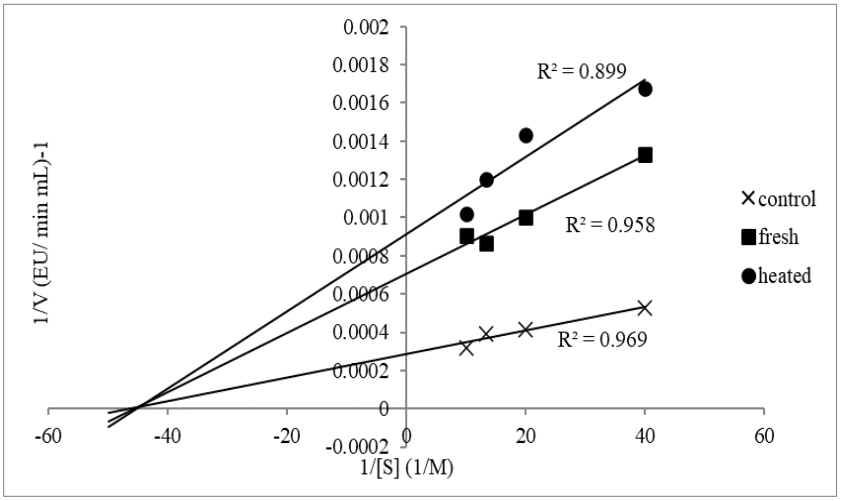

Figure 1. Lineweaver-Burk plot of fresh onion extract and heated onion extract on white button mushroom PPO using pyrocatechol.

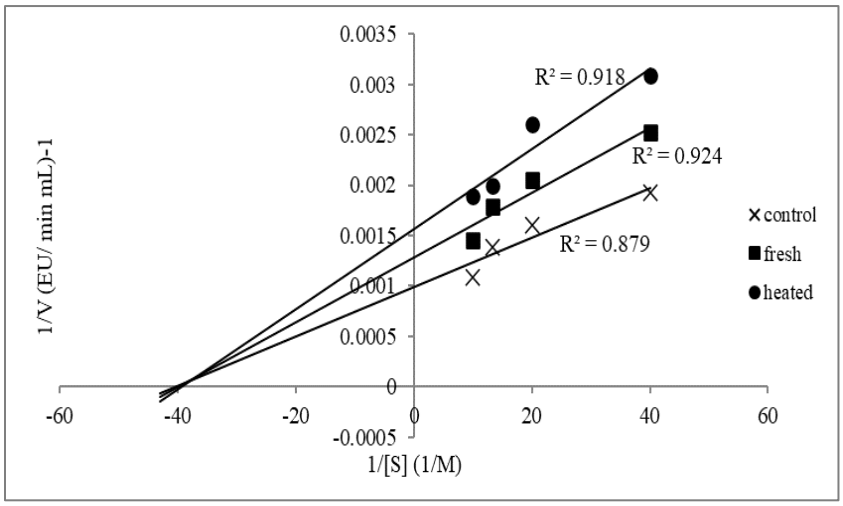

Figure 2. Lineweaver-Burk plot of fresh onion extract and heated onion extract on white button mushroom PPO using 4-methylcatechol.

Figures 1 and 2 show the Lineweaver-Burk plots of white button mushroom PPO in the presence of fresh onion extract and heated onion extract using pyrocatechol (Figure 1) and 4-methylcatechol (Figure 2), respectively. It can be seen that both the fresh and heated onion extracts inhibited the white button mushroom PPO non-competitively as the K_m of the enzyme was similar; while the V_{max} values decreases with the addition of both fresh and heated onion extracts. Similar type of inhibition has been found by Wong and Lee (2014) for cassava leaves PPO, which was non-competitive. According to Mohan *et al.* (2013), a non-competitive inhibitor binds to the enzyme other than active site and does not affect the affinity of the enzyme for the substrate. However, different type of inhibitions were found when onions were used to inhibit yam and ginger PPO (Yapi *et al.* 2015; Lim and Wong 2018).

The V_{max} values dropped drastically from an initial of 3508.77 EU/min ml to 1414.43 EU/min mL and 1096.49 EU/min mL, respectively when fresh and heated onion extracts were added with pyrocatechol as the substrate. The V_{max} decreased to 778.82 EU/min ml and 639.39 EU/min ml respectively from 1010.10 EU/min ml for 4-methylcatechol, when fresh and heated onion extracts were added (Table 1). 35% and 31% of the PPO activities retained when the

fresh and heated onion extracts for pyrocatechol and 75% and 58% of the PPO activities retained when the fresh and heated onion extracts for 4-methylcatechol (Table 1). These results show that onion extracts posed a higher inhibition power when pyrocatechol was used as substrate as higher percentages of PPO activities were inhibited.

3.2. Influence of heat treatment temperature for onion extract on the inhibitory effect of white button mushroom PPO

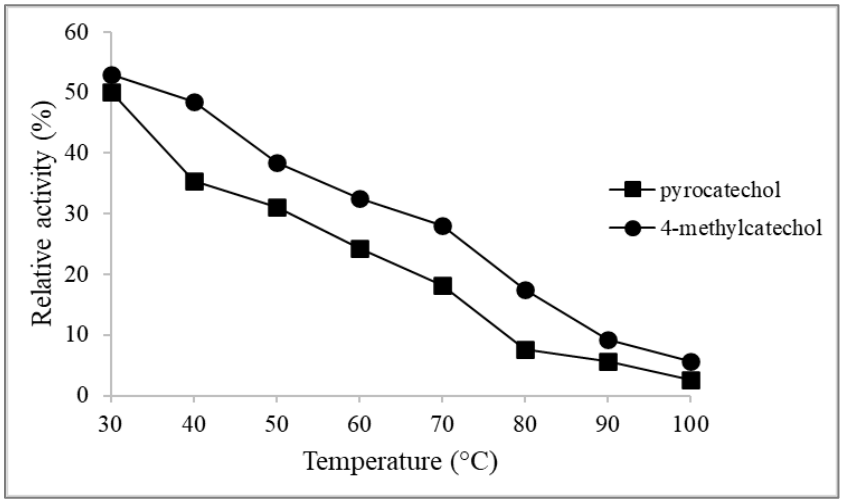

Figure 3. Effect of heating temperature of onion extract on the inhibitory effect of white button mushroom PPO

Figure 3 exhibits the inhibitory effect of onion extract after heating at various temperatures (30-100°C) for 10 min using pyrocatechol and 4-methylcatechol, respectively. As shown in Figure 3, onion extracts treated at a higher temperature exhibited a stronger inhibition towards white button mushroom PPO. This phenomenon was probably due to the inhibitory effect of Maillard reaction products increased with increasing treatment temperature (Lim *et al.* 2019).

The percentage of inhibition for the heated onion extracts increased from 47% to 94% and 50% to 97% with the increased of incubation temperatures from 30 to 100°C using 4-methylcatechol and pyrocatechol, respectively in this study. Similar results were obtained by Lee (2007) whereby the banana PPO activity markedly inhibited (22% to 65%) when the onion extracts were added after heating from 50 to 100°C.

3.3. Influence of heat treatment time for onion extract on the inhibitory effect of white button mushroom PPO

As shown in Figure 4, the longer the heating time on the onion extracts, the higher the decrease in residual PPO activity. Only 3% and 12% of relative PPO activities were found for 4-methylcatechol and pyrocatechol, respectively after 14 minutes heating of the onion extracts. These could be caused by the prolonged heating times may produce an increasing amount of inhibitory compounds, already formed at shorter heating times and or generate additional inhibitory compounds. The findings from this study was in agreement with those reported by Lee et al. (2007), whereby inhibition of taro PPO was increased with increasing of heating time for onion extracts.

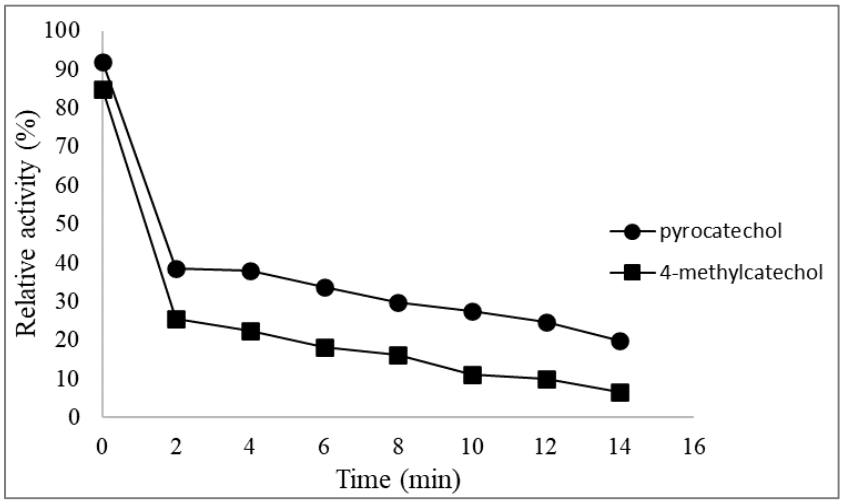

Figure 4. Effect of heating time of onion extract on the inhibitory effect of white button mushroom PPO

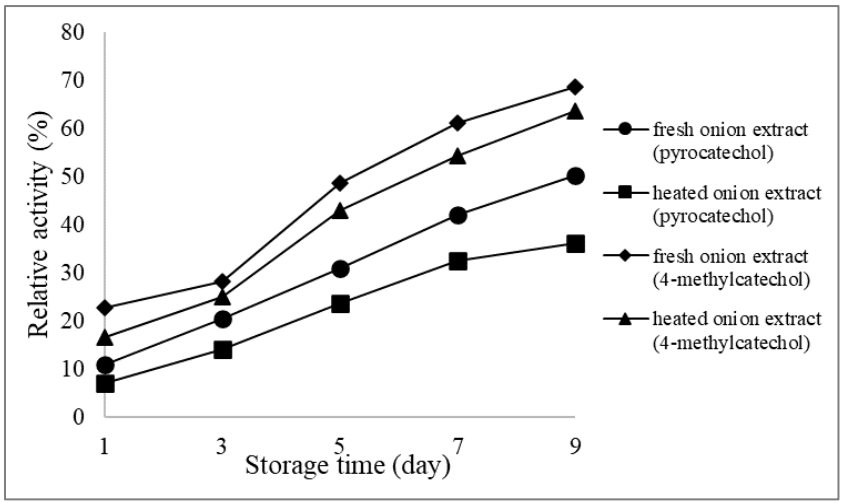

Figure 5. Effect of storage time of fresh and heated onion extracts on the inhibitory effect of white button mushroom PPO

3.4. Influence of storage time of fresh and heated onion extracts on the inhibitory effect of white button mushroom PPO

According to Figure 5, the relative PPO activities increased with the increasing of storage time, which indicated that the inhibitory effect of the onion extracts decreased with increased of storage time. It was noted that the relative PPO activity of white button mushroom increased from 11% to 50% in the presence of non-heated onion extracts, whereas 36% of the PPO activity was retained at day 9 of storage for the heated onion extracts when pyrocatechol was used. The fresh onion extracts showed nearly 77% inhibition when 4-methylcatechol was employed in the reaction mixture. However, the inhibitory effect drastically dropped to 31% after 9 days of storage. Similar pattern were observed when heated onion extracts were used, whereby a lower relative activity was obtained with 4-methylcatechol. The explanation for this could be due to the decreased in antioxidant activity of onion extracts with the increased of storage time (Lanzotti 2006).

4. Conclusions

This concluded that onion extract can be used as a natural inhibitor to prevent browning of white button mushroom. It can be potentially used to replace sulphite-containing anti-

browning agents and other chemical inhibitors which would possibly cause undesirable side effects. Inhibitory effect markedly increased with increase in temperature for a definite time and with heating time at a fixed temperature, while inhibitory effect decreased with storage time.

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