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Antioxidant capacity of pitcher (*Nepenthes*) extract on oxidative rancidity of sun-dried squid

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Abstract

Consumers demand new biologically natural-active ingredients with a great capacity to promote a healthier advantage and free from toxicity. Pitcher (*Nepenthes)* is one of the naturally carnivorous climber herbs. Extract from *Nepenthes* plant contains numerous beneficial bioactivity characteristics. This research compared the impact of 0.05% pitcher extract demonstrated with 10 ppm butylated hydroxyanisole on the accumulation of acid value, peroxide value, carbonyl value and thiobarbituric acid during 12 months of storage. Results showed that there was no significant difference between 0.05% pitcher extract and 10 ppm butylated hydroxyanisole in respect of acid value, peroxide value, carbonyl value and thiobarbituric acid during preservation of the dried squid. Pitcher extract would be a promising alternative to replace synthetic antioxidants to overcome oxidative rancidity in the dried seafood.

1. Introduction

Pitcher (*Nepenthes*) is an important tropical carnivorous pitcher plant widely distributed in Vietnam and Southeast Asian countries. Pitcher (*Nepenthes*) has a unique pitcher organ growing from the tip of the leaf (Wang *et al*., 2009). It is able to trap and digest insects to obtain nutrients (Wan-Nor-Adibah-Wan *et al*., 2016). *Nepenthes* are commonly utilized as a folk remedy to cure different ailments. It has been reported to be effective to cure gastrointestinal discomfort, regulate the menstrual cycle, ease child-birth, relieve asthma, treat eye inflammation, gastric ulcer, jaundice, high blood pressure and astringent (Aung *et al*., 2002; Schwallier *et al*., 2015; Van Thanh *et al*., 2015; Thao *et al*., 2016; Sanusi *et al*., 2017). Its extract contains numerous phytochemical constituents like steroids, flavonoids, alkaloids, terpenoids, tannins (Shuaibu *et al*., 2017). Extract from this plant is responsible for pharmacological properties like cytotoxicity, anti-inflammatory, antibacterial, antidiabetic, antifungal, antioxidant, antimalarial, antiosteoporotic and hypolipidemic potentials (Likhitwitayawuid *et al*., 1998; Shil *et al*., 2010; Ismail *et al*., 2015; Van *et al*., 2015).

Lipid oxidation induces decomposition and degradation in three steps: initiation, propagation, and termination (Frankel, 1984; Fraser and Sumar, 1998). Rancidity in seafood could be initiated and propagated by peroxidase, lipoxygenase, auto-oxidation, photosensitized oxidation (Hsieh and Kinsella, 1989). Squid is one of the most important seafood species. There is increased consumer demand for dried squid in the world. It is rich in unsaturated fatty acids therefore it is susceptible to oxidative rancidity (Losada *et al*., 2007). Lipid oxidation results in protein denaturation leading to lower quality of the dried product (Lugasi *et al*., 2007; Beck, 2014). Artificial antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, tertiary butylated hydroxyl quinone and propyl gallate are commonly used in the seafood industry to control lipid oxidation. However, there are different health concerns when using these substances (Patel *et al*., 2010; Carocho and Ferreira, 2013; Reddy and Grace, 2016). Natural extracts with bioactive functions are highly preferred to replace synthetic antioxidants (Barros *et al*., 2007; Thao *et al*., 2016). Phenol-rich plant extracts such as the summer savoury flavoured powder, beetroot leaf extract and olive polyphenol successfully inhibited lipid and protein oxidation (Stina *et al*., 2020). Natural antioxidants from fruits, vegetables, herbs, and spices included beneficial essential oils suitable for seafood preservation by preventing oxidative chain reactions and prolonging the stability of these products (Lorenzo *et al*., 2018; Smaou *et al*., 2019; Sood *et al*., 2020; Alirezalu *et al*., 2020; Munekata *et al*., 2020; Dominguez *et al*., 2020; Mirian *et al*., 2021). Plant extract had antifungal,

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antioxidant, antimutagenic activities, and retard lipid oxidation in seafood (Cando *et al*., 2014; Ghayempour *et al*., 2015; Ashrafi *et al*., 2018; Kharchoufi *et al*., 2018). There was not any research that mentioned the utilization of pitcher extract to control lipid oxidation in the dried squid during storage. Therefore, the purpose of our study focused on the influence of pitcher extract demonstrated with butylated hydroxyanisole on the accumulation of acid value, peroxide value, carbonyl value and thiobarbituric acid during 12 months of storage.

2. Materials and methods

2.1 Materials

Raw squids were obtained from Tran De district, Soc Trang province, Vietnam. *Nepenthes* was collected from Da Lat district, Lam Dong province, Vietnam. A 100 g of *Nepenthes* was extracted under an alcoholic-aqueous solution (70% v/v) at 45°C for 3 hrs. The *Nepenthes* extract was obtained via filtration (Whatman paper No. 1). Chemical reagents were all analytical grade purchased from Fluka (Switzerland) and Sigma Aldrich (USA).

2.2 Methods

Squids were preliminarily gutted and washed under tap water to remove the ink. They were soaked in *Nepenthes* extract, butylated hydroxyanisole (BHA) solution (10 ppm) and control (without any additive) for 90 mins. They were dried under sunlight for 3 consecutive days to obtain a final moisture content of around 18%. The dried squids were kept in plastic bags for 12 months. In 3 month-interval, they were sampled to analyse the acid value, peroxide value, carbonyl value, thiobarbituric acid (TBA) value.

Free radical scavenging activity (DPPH, mg Trolox/100 g) of *Nepenthes* extract was evaluated by the method described by Bakar *et al*. (2017). Acid value (mg KOH/g) was determined by titration by 0.1 N KOH with phenolphthalein indicator to pink appearance. Peroxide value (meq/kg) was estimated by weighing 5 g of sample, adding 15 mL acetic acid and 10 mL chloroform, supplementing 1 mL KI solution. This mixture was kept in a dark place for 15 mins and a starch indicator was added. The final titration was defined by adding 0.01 N $Na_2S_2O_3$ until colourless. Carbonyl value (meq/kg) was determined by high-performance liquid chromatography with ultraviolet (UV) detection (Vanessa and Zenilda, 2013). Thiobarbituric acid (TBA) value (mg malonaldehyde/kg) was measured by the 2-thiobarbituric acid spectrophotometric method (Anna *et al*., 2017).

2.3 Statistical analysis

The experiments were run in triplicates with different groups of samples. The data were presented as mean±standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI.

3. Results and discussion

Free radical scavenging activity or DPPH (mg Trolox/100 g) of *Nepenthes* extract was shown at 75.83±0.03 (mg Trolox/100 g) compared to butylated hydroxyanisole (BHA) solution 77.09±0.01 (mg Trolox/100 g). *Nepenthes* extract has excellent antioxidant capacity ideal for lipid oxidative prevention. Excellent antioxidant activity of pitcher extract might have originated from a synergistic impact of terpenes, terpenoids, essential oils and other available metabolites such as flavonoids (Adhikari *et al*., 2003; Grassmann, 2005; Ozturk, 2012). Tiewlasubon *et al*. (2014) reported that *Nepenthes* extract showed powerful antioxidant capacity with 23.33±0.441 (mg Trolox/100 g). The leaf *Nepenthes* extract was demonstrated to be more effective than standard butylated hydroxytoluene (Ismail *et al*., 2015). In another research, the radical scavenging activity of the pitcher extract was noted at 0.148±0.04 mg/mL (Muhammad *et al*., 2018).

The acid value is commonly applied as an important indicator of fat rancidity. Under the incorporation of *Nepenthes* extract in the soaking solution, the dried squid had a low acid value similar to the sample treated by BHA during 12 months of storage. Meanwhile, the control sample had accumulated acid value every month (Table 1). At the end of 12 months of storage, the dried squid treated with *Nepenthes* extract still had a low acid value (2.70±0.03 mg KOH/g). It is suggested that *Nepenthes* extract significantly retarded the rancidity in dried squid. These results were in accordance with other findings such as the herbal extract that was demonstrated to be effective in the control of lipid oxidation in dried eel (Song, 2019). Furthermore, green tea extract was also suitable to retard fat rancidity in mackerel (Nam *et al*., 2011). While the combination of spices (turmeric, chilli) greatly improved the quality of dried Taki (Rahman *et al*., 2017). *Eryngium caucasicum* extracts were also as effective to slow down the acid value of silver carp fillets (Raeisi *et al*., 2017).

Primary lipid oxidation was followed by the peroxide value (Servet and Hudayi, 2011). Peroxide values (meq/kg) of the dried squid treated by *Nepenthes* extract, butylated hydroxyanisole and control were all presented in Table 2. The supplementation of *Nepenthes* extract was able to maintain the peroxide value of the dried squid at a low amount (2.91±0.01 meq/kg) after 12

Table 1. Acid value (mg KOH/g) of the dried squid

Storage (month)	0	3	6	9	12
Nepenthes extract 0.05%	1.06±0.02[ab]	1.45±0.00[ab]	1.94±0.01[b]	2.32±0.00[b]	2.70±0.03[b]
Butylated hydroxyanisole 10 ppm	0.98±0.01[b]	1.29±0.03[b]	1.85±0.02[b]	2.21±0.01[b]	2.59±0.02[b]
Control	1.17±0.00[a]	1.97±0.02[a]	4.83±0.03[a]	7.69±0.02[a]	10.85±0.01[a]

Values are presented as mean±SD, n = 3. Values with the same superscript within the same row are not significantly different (α = 5%).

months of storage. Meanwhile, the peroxide value of the control sample increased dramatically from the 3rd to the 12th month. There was no significant difference in the peroxide value between those treated with *Nepenthes* extract and butylated hydroxyanisole. It is suggested that *Nepenthes* extract could safely be used and effectively minimize lipid oxidation. The effectiveness of rancidity retardation by *Nepenthes* extract was similar to other plant extracts such as the green tea extract that strongly retards the oxidative rancidity in eel oil (Song and Kim, 2018). The peroxide value of anchovy oil was kept at low content by green tea extract (Kang *et al*., 2007). Basil leaf essential oil was also effective to limit peroxide value in Sea bass slices (Arfat *et al*., 2015). Pomegranate peel extract was also appropriated for Nile tilapia fillets to slow down peroxide value (Alsaggaf *et al*., 2017). *Eryngium caucasicum* extract was found to be a beneficial substance to decrease peroxide value in Silver carp fillets (Raeisi *et al*., 2017).

Carbonyl is commonly originated from proteolysis and amino acid oxidation to release malondialdehyde. Carbonyl value (meq/kg) of the dried squid soaked in *Nepenthes* extract, butylated hydroxyanisole and control were all presented in Table 3. It was thoroughly realized that with the addition of *Nepenthes* extract, low traces of carbonyl values of the dried squid were obtained (2.66±0.00 meq/kg) after 12 months of storage. Meanwhile, the carbonyl value of the control sample increased steeply from the 3rd to the 12th month. There was no significant difference in carbonyl value between the squids that were treated by *Nepenthes* extract and butylated hydroxyanisole. It was recommended that *Nepenthes* extract could be utilized to replace butylated hydroxyanisole to control the carbonyl substance. This result was relevant to another study when eels treated with green tea extract had a low carbonyl value (Choi, 2006).

In the evaluation of oxidative rancidity, the thiobarbituric acid (TBA) value should be mentioned. It is an important quality indicator to measure fat oxidation for dried and semi-dried seafood (Bahar *et al*., 2006). The basic mechanism of this procedure is the interaction of 1 molecule of malonaldehyde and 2 molecules of TBA to release a purple appearance of the malonaldehyde-TBA complex. Table 4 shows the influence of *Nepenthes* extract demonstrated with butylated hydroxyanisole to control the TBA value. It was found that the *Nepenthes* extract effectively retarded TBA formation in the dried squid during 12 months of storage. These results were relevant to fennel extracts

Table 2. Peroxide value (meq/kg) of the dried squid

Storage (month)	0	3	6	9	12
Nepenthes extract 0.05%	1.34±0.03[a]	1.75±0.02[b]	2.03±0.00[b]	2.48±0.02[b]	2.91±0.01[b]
Butylated hydroxyanisole 10 ppm	1.28±0.02[a]	1.69±0.01[b]	1.98±0.03[b]	2.37±0.00[b]	2.80±0.00[b]
Control	1.42±0.03[a]	4.27±0.03[a]	7.93±0.01[a]	11.86±0.03[a]	18.70±0.02[a]

Values are presented as mean±SD, n = 3. Values with the same superscript within the same row are not significantly different (α = 5%).

Table 3. Carbonyl value (meq/kg) of the dried squid

Storage (month)	0	3	6	9	12
Nepenthes extract 0.05%	0.83±0.01[a]	1.15±0.03[b]	1.59±0.01[b]	1.94±0.03[b]	2.66±0.00[b]
Butylated hydroxyanisole 10 ppm	0.79±0.00[a]	1.04±0.02[b]	1.48±0.02[b]	1.87±0.03[b]	2.59±0.01[b]
Control	0.84±0.00[a]	3.68±0.03[a]	8.53±0.00[a]	14.73±0.02[a]	19.93±0.00[a]

Values are presented as mean±SD, n = 3. Values with the same superscript within the same row are not significantly different (α = 5%).

Table 4. Thiobarbituric acid value (mg malonaldehyde/kg) of the dried squid

Storage (month)	0	3	6	9	12
Nepenthes extract 0.05%	1.07±0.03[a]	1.70±0.00[b]	2.02±0.02[b]	2.68±0.01[b]	2.95±0.03[b]
Butylated hydroxyanisole 10 ppm	1.02±0.01[a]	1.63±0.00[b]	1.97±0.01[b]	2.59±0.00[b]	2.93±0.02[b]
Control	1.13±0.02[a]	5.54±0.01[a]	9.13±0.03[a]	16.07±0.00[a]	23.46±0.01[a]

Values are presented as mean±SD, n = 3. Values with the same superscript within the same row are not significantly different (α = 5%).

that were ideal to increase the stability of silver carp fillets by slowing down TBA value (Mazandrani *et al*., 2016). *Eryngium caucasicum* extract was also effective to limit the TBA value of silver carp fillets (Raeisi *et al*., 2017).

4. Conclusion

Pitcher (*Nepenthes)* extract was rich in bioactive components, mainly polyphenols, which are valuable alternatives to replace synthetic antioxidants in seafood processing and preservation. Findings of this research concluded that Pitcher (*Nepenthes*) extract revealed excellent antioxidant potential to retard an accumulation of lipid oxidation in the dried squid. Its incorporation during soaking fresh squid would maintain the acid value, peroxide value, carbonyl value and TBA value at the low amount in the dried squid. It would be considered as an upcoming alternative to reduce the consumption of synthetic additives due to concerns of the possible toxic effects.

Conflict of interest

The author strongly confirms that this research was conducted with no conflict of interest.

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