



Antioxidant Potential of *Pleurotus porrigens* Extract and Application in Sunflower Oil during Accelerated Storage

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ABSTRACT

The oxidative stability of refined sunflower oil supplemented with edible wild mushroom, *Pleurotus porrigens* crude extract, its n-butanol fraction (n-BUT, from liquid-liquid partitioning), and semi-purified sub-fraction (SF) III and SFIV, were tested under accelerated storage conditions compared with BHA and α -tocopherol, by measuring their peroxide value, *p*-anisidine value, thiobarbituric acid-reactive substance, and iodine value. The total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing/antioxidant power (FRAP) were also evaluated. *P. porrigens*' SFIII exhibited highest DPPH scavenging, while n-BUT showed highest FRAP; TPC was found highest in crude extract. Generally, BHA and α -tocopherol are more protective in stabilizing the sunflower oil. *P. porrigens*' SFIII and SFIV had short-term protective effect in secondary oxidation for 1/2-year; whilst crude extract retarded secondary oxidation (TBARS value) and extend the shelf-life upon 1 1/2-year. *P. porrigens* extracts did not show similar retardation of lipid oxidation in sunflower oil as compared to α -tocopherol and BHA at 200 ppm. However, the higher concentration of *P. porrigens* extracts that provided the protective effect in stabilizing sunflower oil can be further studied.

Keywords: antioxidant activity, lipid oxidation, *Pleurotus porrigens*, sunflower oil

1. INTRODUCTION

Oxidation is essential to living organisms for the purpose of generating energy for fuel biological processes, but at the same time free radicals are produced in normal and pathological cell metabolism. Lipid oxidation occurs through complex mechanisms induced

by oxygen in the presence of initiators such as free radicals, light, high temperature, photo-sensitizing agents, and metal ions [1]. Lipid oxidation and its associated changes are responsible for the quality deterioration of lipids and lipid-containing foods that

result in losses of nutritional value as well as flavour, colour, texture, and other physiological properties of foods [2].

Auto-oxidation is the major damaging reaction that causes spoilage of fats or oils and unsuitable for consumption. Temperature is likely to be the most influential factor of lipid oxidation, decomposing hydroperoxides, and forming secondary oxidation products such as carbonyl compounds, unsaturated aldehydes, nonvolatile aldehydes, alcohols, and hydrocarbons [3]. Secondary oxidation products formed can alter the taste (rancid odors and flavors) and nutritional quality (loss of essential fatty acids and vitamins) of foods [4]. Thus, it is essential to evaluate the primary and secondary oxidation products in order to monitor food lipid oxidation, which is a major problem in the storage of fat-containing foods.

Some of the synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG) are commonly incorporated into food products to prevent the oxidative degradation of lipid in foods. However, possible carcinogenesis risk from the usage of synthetic antioxidants has been reported and one of it, namely TBHQ is not allowed for food application in certain countries [5]. The demand for antioxidants from natural resources has increased over the years for their presumed safety. Natural antioxidants extracted from plant origins or residual sources of agricultural and industrial waste have been well documented [6].

In Asian countries, edible mushrooms have been valued as functional foods and as medicine resource due to their antioxidative, preventive, and therapeutic properties [7]. A variety of secondary metabolites that include phenolics, polyketides, terpenes, and steroids, are accumulated in mushrooms

[8]. The phenolic compounds in Shiitake mushroom (*Lentinus edodes*) and straw mushroom (*Volvarella volvacea*) are believed to have contributed to their ability to scavenge free radicals, chelate metals, and inhibit lipoxygenase [9]. Mushrooms can be used as natural antioxidants in the forms of extracts, concentrates, powders, or dietary supplements [10].

Numerous studies of extracts from plants and herbs have demonstrated promising antioxidant activity, those extracts included green leafy vegetables (Amaranth and Coriander) [11], *Vites negundo* L. leaf [12], and bitter melon [13]. In addition, potato peels, corncob and garlic extracts in high concentration have been shown to delay lipid peroxidation in cooking oil compared to those synthetic antioxidants [14, 15, 16]. However, there is limited literature available on the antioxidative potential of edible wild mushroom, *Pleurotus porrigens* extracts from Sabah, Malaysia and its effect on stabilizing the cooking oil has not yet been reported. Thus, the aims of the present work were to evaluate the antioxidant activity of *P. porrigens* extracts, and to investigate the oxidative stability of refined sunflower oil supplemented with *P. porrigens* crude extracts, its n-butanol fraction (from liquid-liquid partitioning), sub-fraction (SF) III and SFIV (that possess high free radical scavenging activity from purification process by silica gel open column chromatography) under accelerated storage conditions, compared with synthetic antioxidant, BHA as well as α -tocopherol.

2. MATERIALS AND METHODS

2.1 Materials

Refined sunflower oil without additives or antioxidants was sponsored by Lam Soon Edible Oils Sdn. Bhd. (Selangor, Malaysia). The wild mushroom *P. porrigens*

was purchased from indigenous people who collect from forest and sell at local market in Kota Kinabalu, Sabah, Malaysia. The mushroom was identified by Dr Markus Atong of the School of Sustainable Agriculture, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia, where a voucher specimen was deposited. The mushroom was washed, air dried followed by drying in an oven at 45°C for 24 h, and then grounded to powder using a miller (MF 10 basic; IKA®-Werke, Staufen, Germany) with 0.5 mm mesh size and vacuum packaged into a nylon-linear low density polyethylene film by using vacuum packaging machine (DZQ 400/500, Zhejiang, China) prior to analysis. BHA and α-tocopherol were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-Thiobarbituric acid (TBA), trichloroacetic acid (TCA), and 2,4,6-tripyridyl-s-triazine (TPTZ) were from Merck (Darmstadt, Germany). All other reagents were of analytical grade. Water used throughout the study was of Millipore quality (Millipore, MA, USA).

2.2. Extraction Process

Dried *P. porrigens* powder (~ 10 g) was extracted with 100 ml of distilled water. The mixture was shaken at 37.4°C for 315.5 min at 150 rpm (Memmert, Schwabach, Germany). The extraction time and temperature was based on the optimized values determined previously by response surface methodology [17]. The mixture was centrifuged for 10 min at 4000 rpm (Universal 320R, Hettich Zentrifugen, MA, USA) and the supernatant was filtered through a Whatman No. 1 filter paper. Re-extraction was done on the residue; both extracts were combined and freeze-dried (ALPHA 1-4 LD Plus, Christ, Germany).

2.3. Determination of Total Phenolic Content

The total phenolic content (TPC) analysis was performed using the Folin-Ciocalteu method by Zhao and Hall [18] with slight modification. A 1 ml sample was mixed with 1 ml of Folin-Ciocalteu's solution. After 3 min, 1 ml of 7.5% sodium carbonate solution was added to the mixture and adjusted to 10 ml with deionized water. The mixture was allowed to stand at room temperature in a dark environment for 90 min. Absorbance was measured against the blank reagent at 725 nm using an XTD 5 spectrophotometer (Secomam, Alés Gard, France). Gallic acid was used for the calibration curve with a concentration range of 50-1000 µg/ml ($R^2 = 0.99$) and analyzed as above. Results were expressed as mg gallic acid equivalent (GAE)/g of extract. All experiments were performed in triplicate.

2.4. DPPH Radical Scavenging Activity

Radical scavenging activity by antioxidant in the mushroom extract was evaluated using DPPH radicals based on the method by Xu and Chang [19] with slight modification. The DPPH· solution was prepared by dissolving 5.9 mg of DPPH· in ethanol (100 ml). Accurately, 3.8 ml of ethanolic DPPH· solution was added to 0.2 ml of mushroom extract. The mixture was shaken vigorously for 1 min and left to stand at room temperature in the dark for 30 min. Absorbance was measured against the blank reagent at 517 nm (XTD 5, Secomam, Alés Gard, France). All determinations were carried out in triplicate. The radical scavenging activity was calculated according to the Eq. (1) below:

$$\text{Radical Scavenging activity (\%)} = [1 - (\text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}})] \times 100 \quad (1)$$

2.5. Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing activity of mushroom extract was estimated based on the methods by Benzie and Strain [20] and Xu and Chang [19] with slight modification. The FRAP reagent was prepared by adding 2.5 ml of 10 mM TPTZ into 40 mM HCl. After dissolving TPTZ in HCl, 2.5 ml of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (ferric trichloride hexahydrate) was added followed by 25 ml of 0.3 M acetate buffer at pH 3.6. The freshly prepared FRAP working reagent was warmed to 37°C. Then, approximately 3 ml of the FRAP reagent was added to 100 μl of mushroom extract and let to stand for 4 min. The absorbance was measured at 593 nm against the blank (XTD 5, Secomam, Alés Gard, France). FRAP value was calculated and expressed as Trolox equivalent antioxidant capacity (TEAC, mg/g of extract). Linearity range of the calibration curve was 20-1600 mM ($R^2 = 0.99$). All determinations were carried out in triplicate.

2.6 Qualitative Phytochemical Screening

The phytochemical tests were carried out on the crude extract of *Pleurotus porrigens*, its n-butanol fraction, and selected sub-fraction III and IV using standard procedures. Presence of alkaloids was tested by using Mayer's and Wagner's reagents; and flavonoids by using sodium hydroxide-hydrochloric acid solution as described by Onwukaeme *et al.* [21]. Terpenoids, saponins, and tannins were determined using Salkowski test, frothing test, and 5% ferric chloride solution, respectively according to Edeoga *et al.* [22].

2.7 Oxidative Stability Determination

2.7.1 Sample Preparation

The oxidative stability of sunflower oil was monitored under accelerated storage at 65°C over a 24-day period according to Iqbal

and Bhangar [14] with slight modification. The accelerated storage condition involves placing a known volume of fat or oil in a forced-draft oven at 65°C, where each day under such oven storage test at 65°C is equivalent to one month of the storage at ambient temperature [23]. *P. porrigens* crude extract, its liquid-liquid partitioning n-butanol (n-BUT) fraction, as well as its sub-fraction (SF) III and SFIV were added to sunflower oil at a concentration of 200 ppm. In brief, the respective extract, fraction, and sub-fractions were mixed with 150 μl absolute ethanol and added into 250 ml pre-heated sunflower oil (50°C for 3 h). The oil samples were mixed with a magnetic stirrer on a hot plate and stirrer (HMS100, Labentech, Korea) for 3 h to ensure homogenous dispersion. BHA at its legal limit of 200 ppm and α -tocopherol (200 ppm) were applied as reference. All the samples (250 ml) were placed in dark brown reagent bottles with narrow neck and stored in an oven at 65°C. Oxidative changes were monitored by peroxide value, *p*-anisidine value, 2-thiobarbituric acid reactive substances, and iodine value at 0-day and at regular intervals of 6-day for 24-day.

2.7.2 Analysis of Peroxide Value

The peroxide value (PV) was determined according to AOAC method 965.33 [24]. Sunflower oil samples ($5.00 \pm 0.05\text{g}$) were dissolved in 30 ml glacial acetic acid-chloroform (3:2, v/v). Then, 0.5 ml of saturated KI solution was added and the mixture was left to stand with occasional shaking for 1 min. Followed by adding 30 ml of distilled water and slowly titrate against 0.1 M sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) solution with vigorous shaking until the yellow color almost disappeared. Then, 0.5 ml 1% starch solution was added and the titration was continued and shaken

vigorously until the blue color of the mixture just disappeared, the endpoint was recorded. The blank was analyzed under similar conditions. Determination of PV of each sample was carried out in triplicates and PV (meq/kg) was calculated according to the Eq. (1) below:

$$PV = [S \times M \times 1000] / \text{sample weight (g)} \quad (1)$$

where, S is the volume of $\text{Na}_2\text{S}_2\text{O}_3$ solution used (blank corrected); M is the molarity of $\text{Na}_2\text{S}_2\text{O}_3$ solution.

2.7.3 Measurement of *p*-Anisidine Value

The *p*-anisidine value (AnV) was determined according to AOCS method Cd 18-90 [25]. Sunflower oil samples (2 g) were dissolved in 25 ml isooctane, then, 5 ml of this mixture was mixed with 1 ml 0.25% *p*-anisidine in glacial acetic acid (w/v). The mixture was shaken vigorously and kept in the dark for 10 min, and the absorbance was measured at 350 nm using a spectrophotometer (XTD 5, Secomam, Alés Gard, France). The blank consists of 5 ml isooctane was measured under similar condition. The AnV was calculated according to the Eq. (2) as follows:

$$\text{AnV} = [25 \times (1.2 A_s - A)] / \text{sample weight (g)} \quad (2)$$

where, A_s is the absorbance of test solution after reaction with the *p*-anisidine reagent; A is the absorbance of blank.

2.7.4 Thiobarbituric Acid Reactive Substances (TBARS) Assay

Lipid oxidation of all samples was determined by 2-thiobarbituric acid (TBA) method according to Ahn and Kim [26] with slight modification. In brief, 1 ml oil sample was mixed with 2 ml TBA-TCA

solution. The TBA-TCA stock solution was prepared by dissolving 15% TCA (w/v) and 20 mM TBA in distilled water. The mixture was vortexed and then incubated in water bath (90°C) for 15 min for pink color development. Then, the samples were cooled in ice bath for 10 min and centrifuged for 15 min at 4500 rpm. A blank solution was prepared under similar conditions with 1 ml distilled water. The absorbance of the supernatant was measured at 531 nm using a spectrophotometer against blank solution. A standard curve of malondialdehyde (MDA) was prepared using 1,1,3,3-tetraethoxypropane (TEP) and TBARS values were expressed as mg of MDA per kg sample.

2.7.5 Analysis of Iodine Value

The iodine value (IV) in oil samples was determined by Wijs method, as described in AOAC official method 993.20 [24]. In brief, 0.2 g of sample was dissolved in 15 ml of cyclohexane-acetic acid (1:1, v/v) solvent. Then, the mixture was added with 25 ml of Wijs solution (16.5 g ICl was dissolved in 1 L acetic acid) and kept in dark at room temperature for 1 h. Then, the mixture was added with 20 ml of 15% KI solution and 150 ml of distilled water. The mixture was gradually titrated against 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ solution with continuous vigorously shaking until the dark brown color disappeared. The blank was analyzed under similar conditions. All determinations were carried out in triplicates. The IV was expressed as the gram of iodine absorbed per 100 g sample ($\text{g I}_2/100 \text{ g}$) and was calculated using Eq. (3) below.

$$IV = [(B-S) \times M \times 12.69] / \text{sample weight (g)} \quad (3)$$

where, B is the titration of blank (ml); S is the titration of test solution (ml); M is the molarity of $\text{Na}_2\text{S}_2\text{O}_3$ solution (mol/L).

2.8 Statistical Analysis

Experiments were replicated twice and all analyses were carried out in triplicates for each replicate, which the results were expressed as mean \pm standard deviation. One-way analysis of variance (ANOVA) and Tukey's multiple comparisons were carried out to compare the mean values by MINITAB (Minitab Inc., PA, USA) version 14 for Windows. Significant levels were based on the confidence level of 95% ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content, DPPH Radical Scavenging Ability and Reducing Power

The total phenolic content (TPC) of *Pleurotus porrigens*' crude extract (CE), its n-butanol (n-BUT) fraction and the selected sub-fraction (SF) III and IV was determined using Folin-Ciocalteu method as shown in Table 1. Results showed CE (28.37 ± 1.20 mg GAE/g) had significantly higher ($p < 0.05$) TPC than that of n-BUT fraction, SFIII, and SFIV (with lowest TPC of 4.06 ± 0.42 mg GAE/g). This could possibly indicate that more extractable phenolic compounds were presence in crude extract of *P. porrigens*.

Table 1. Total phenolic content, DPPH radical scavenging ability and reducing power of *Pleurotus porrigens* extracts at concentration of 200 ppm.

Sample	TPC (GAE, mg/g)	DPPH· scavenging ability		FRAP (TEAC, mg/g)
		%	EC_{50}^{\dagger} (mg/ml)	
Crude extract	28.37 ± 1.20^a	39.26 ± 2.04^d	0.317	58.10 ± 3.80^d
n-Butanol fraction	22.08 ± 1.10^b	42.89 ± 0.88^d	0.230	80.77 ± 4.74^c
Sub-fraction III	9.86 ± 0.60^c	67.52 ± 0.23^b	0.170	23.79 ± 0.71^e
Sub-fraction IV	4.06 ± 0.42^d	40.03 ± 0.48^d	0.243	16.33 ± 0.38^e
α -Tocopherol	NA	98.15 ± 0.94^a	<0.001	584.66 ± 40.66^b
BHA	NA	64.70 ± 0.62^c	0.011	1478.55 ± 131.51^a

Experiments were replicated twice and all analyses were carried out in triplicates for each replicate, which the results were expressed as mean \pm standard deviation ($n = 6$). NA: not applicable

*Different superscripts within the column denote significantly different ($p < 0.05$).

†Defined as effective concentration that was able to scavenge 50% of the total DPPH radicals; EC_{50} was calculated by interpolation of linear regression analysis (based on concentration-dependent result - data not shown).

The DPPH radical scavenging ability assay and reducing power based on FRAP were used to evaluate the antioxidant activity of *P. porrigens*' CE, its n-BUT fraction and the selected SFIII and SFIV (Table 1). In the present study, a concentration of 200 ppm was used as benchmark; α -tocopherol and BHA were used as reference. Higher EC_{50} (defined as effective concentration that was

able to scavenge 50% of the total DPPH radicals) indicates lower antioxidant activity. Scavenging ability of α -tocopherol and BHA ($\text{EC}_{50} = < 0.001$ and 0.011 mg/ml, respectively) were higher than that of *P. porrigens*' crude extract, its n-BUT fraction, SFIII and SFIV. Amongst the *P. porrigens* extracts, SFIII showed greatest DPPH radical scavenging ability with EC_{50} value of

0.170 mg/ml, followed by n-BUT, SFIV and crude extract with EC₅₀ of 0.230, 0.243 and 0.317 mg/ml, respectively. The reducing powers of *P. porrigens* crude extract, its n-BUT fraction, SFIII and IV at 200 ppm were greatly lower than that of BHA (1478.55 ± 131.51 mg TEAC/g) and α-tocopherol (584.66 ± 40.66 mg TEAC/g) ($p < 0.05$). The reducing power of the test samples followed the order of BHA > α-tocopherol > n-BUT > CE > SFIII = SFIV.

3.2 Phytochemical Screening

Five types of phytochemical tests were carried out to identify the presence of various secondary metabolites in mushroom

crude extract, its n-butanol fraction, and sub-fraction III and IV (Table 2). The presence of phytochemicals are indicated by as (+) and absence was represented by (-) based on the color changes of the tests. Results indicate the presence of all tested phytochemicals except alkaloid in crude extract and n-butanol fraction, while only tannin and terpenoid were found in sub-fraction III; and only flavonoid was present in sub-fraction IV. Crude extract and n-butanol fraction consist most of the phytochemical constituents, which explained their high antioxidant activity as compared to others.

Table 2. Phytochemical tests of crude extract, n-butanol fraction, and selected sub-fractions of *Pleurotus porrigens*.

Sample	Phytochemical tests					
	Flavonoids (NaOH)	Saponin	Tannins (FeCl ₃)	Terpenoids (Salkowski test)	Alkaloids Wagner's	Mayer's
Crude extract	+	+	+	+	-	-
n-Butanol fraction	+	+	+	+	-	-
SF-III	-	-	+	+	-	-
SF-IV	+	-	-	-	-	-

+/-: Indicates presence or absence of phytochemical constituents. SF: Sub-fraction.

3.3 Peroxide Value

In the present study, the primary oxidation was assessed by measuring the PV of sunflower oil samples supplemented with 200 ppm of *P. porrigens* extract, BHA and α-tocopherol at 65°C for 24 days as shown in Figure 1. Generally, a gradual increase in PV as a function of storage time that showed the progression of oxidation was observed at all intervals. PV of sunflower oil samples with additives at day-24 were in the range of 67-94 meq/kg for *P. porrigens*, whereas, the PV for control (without antioxidant added) was about 130 meq/kg.

Figure 1 shows that the PV of control sunflower oil was significantly higher ($p < 0.05$)

compared to the *P. porrigens* extracts and antioxidants treated samples throughout the 24 days analysis. The PV of sunflower oil added with *P. porrigens*' CE, n-BUT fraction, SF-III and SF-IV, α-tocopherol and BHA at the end of 24 days were found to be 75.25 ± 1.77, 77.96 ± 4.31, 94.07 ± 2.92, 83.08 ± 1.39, 63.98 ± 1.45 and 64.98 ± 1.45 meq/kg, respectively. The antioxidant effect of BHA and α-tocopherol were better than that of *P. porrigens* with exception of CE of *P. porrigens* that showed the ability in delaying primary oxidation products up to day-12 (equivalent to 1-year storage at ambient temperature). The antioxidant effect is possibly attributed by the presence of

phenolic compounds in crude extract of *P. porrigens*, as the phenolic compounds present in wild edible mushroom are believed to be the main components responsible for their antioxidant activity [27]. Phenolic plant secondary metabolites have been shown to play an important role in numerous biochemical activities including the antioxidative properties that provide the oxidative protective effect in cooking oil system [28].

Throughout the storage period from day-12 onwards, results showed that *P. porrigens*' n-BUT fraction, SF-III and SF-IV were not effective in delaying primary oxidation of sunflower oil compared to α -tocopherol and BHA. This less protective effect may be due to their low concentration added in the sunflower oil, as other studies had shown that 1000 ppm of garlic extract [14] and 1000 ppm of corncob methanolic extract [15] possess better protective effect

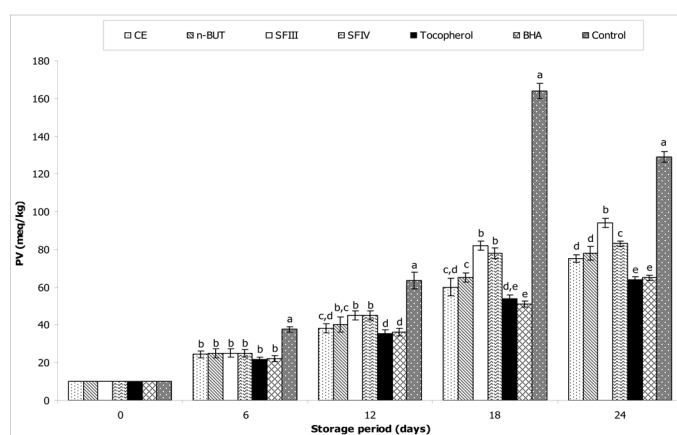


Figure 1. Peroxide value of sunflower oil added with *Pleurotus porrigens* extracts and antioxidants under accelerated storage at 65°C for 24 days.

compared to synthetic antioxidant (BHA/BHT) under accelerated storage. Rehman *et al.* [16] revealed a comparable antioxidative effect difference between synthetic antioxidant (BHA and BHT at 200 ppm) and potato peel extract (1600 ppm) in inhibition of soybean oil peroxidation.

A remarkable rise in PV after day-6 was observed in control sunflower oil and continued to rise to reach maximum at day-18 (163.94 ± 4.16 and 161.83 ± 9.89 meq/kg), followed by a decrease on day-24 (128.96 ± 2.89) for *P. porrigens* (Figure 1). This observation could be due to the occurrence of volatilization of some oxidation products, namely hydroperoxides and peroxides along the heating storage period [14]. Furthermore, the rate of decomposition

of primary products may supersede the formation rate of secondary products, thus, decreasing the PV in control samples [29].

3.4 *p*-Anisidine Value

A gradual increase of *p*-anisidine value (AnV) was observed in sunflower oil samples treated with *P. porrigens* (Figure 2) extracts, α -tocopherol, BHA and control. The AnV of control sample increased significantly throughout the storage time, accelerated after day-6 and reached the maximum value of 41.49 ± 0.80 and 45.34 ± 1.77 for *P. porrigens*. Throughout the observation, sunflower oil samples in the presence of *P. porrigens* (except for SF-IV) extracts and antioxidants exhibited comparable AnV within day-6. Sunflower oil samples added

with *P. porrigens*' extracts were found less effective (higher AnV) as compared to α -tocopherol and BHA from day-12 onwards and the AnV increased drastically thereafter (Figure 2). The results indicate that *P. porrigens*' CE, n-BUT and SFIII are able to retard the formation of secondary oxidation product in sunflower oils up to 1/2-year

(*P. porrigens*) storage at ambient temperature. This short protective effect could possibly due to the low concentration of additives (200 ppm) being supplemented in the sunflower oil, as the phenolic antioxidants present to inhibit the lipid peroxidation decompose and deteriorate themselves with the course of storage time [30].

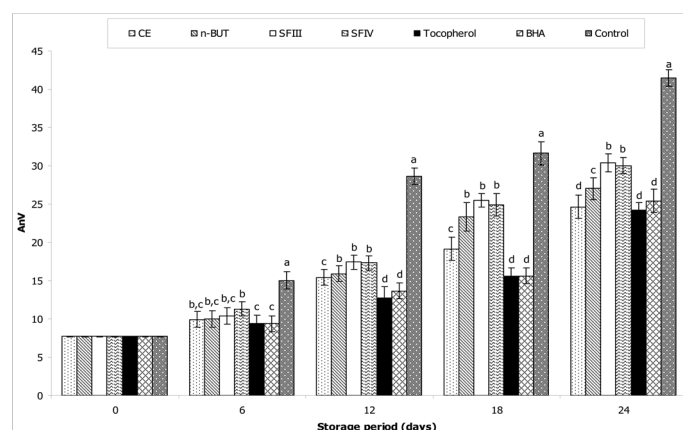


Figure 2. p-Anisidine value of sunflower oil added with *Pleurotus porrigens* extracts and antioxidants under accelerated storage at 65°C for 24 days.

The *Polygonum cuspidatum* crude extract has been shown to retard secondary oxidative deterioration (as measured by AnV) in peanut oils comparable to that of BHT [24]. However, higher concentration of *P. porrigens* extracts may provide better protective effect, as reported by Sultana *et al.* [15] that 1000 ppm corncob extract was more effective in retarding the formation of secondary oxidation products compared to BHT. *Pandanus amaryllifolius* leaf extract as low as 0.1%, on the other hand was found to be as effective of retarding secondary oxidation in palm olein as synthetic antioxidant [29].

3.5 Determination of Total Oxidation (TOTOX Value)

After determining the PV and AnV afore-mentioned, the values obtained are often used to calculate the TOTOX value, which indicates the total oxidation of a

particular oil sample. The lower TOTOX value indicates that the oil sample is more stable against oxidative rancidity [3]. TOTOX value was given as:

$$\text{TOTOX value} = 2\text{PV} + \text{AnV} \quad (4)$$

The TOTOX values of sunflower oil samples added with *P. porrigens* extracts are shown in Figure 3. Sunflower oil samples supplemented with α -tocopherol and BHA showed significant lower TOTOX values than *P. porrigens* extracts from day-12 till day-24, except for CE (92.01 ± 4.24) that showed no significant difference compared to BHA (85.67 ± 3.62) at day-12 (Figure 3). Sunflower oil contains high amount of polyunsaturated fatty acids (linoleic and linolenic acids) that are susceptible to oxidative rancidity. The results showed that the oxidative stability of sunflower oil samples supplemented with

P. porrigens' CE is similar to that of BHA up to 1-year at ambient temperature storage, respectively.

TOTOX value is a useful indicator of oil oxidation as it takes into consideration the secondary oxidation (*p*-anisidine value) and the present state (the primary oxidation, peroxide

value) [3]. The *P. porrigens* CE remained effective over a specific period of time and finally became ineffective could possibly be explained by its antioxidants inhibit lipid peroxidation at the cost of their own life, followed by decomposition and deterioration over the course of time [30].

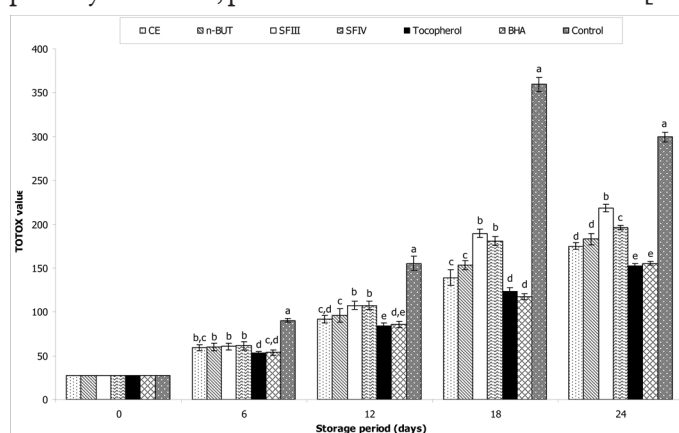


Figure 3. TOTOX value of sunflower oil added with *Pleurotus porrigens* extracts and antioxidants under accelerated storage at 65°C for 24 days.

3.6 Thiobarbituric Acid Reactive Substances (TBARS)

Malondialdehydes (MDA) is the major product of lipid peroxidation and has been used as an index of lipid peroxidation and as a marker of oxidative stress [31, 32]. The concentration of MDA can be assessed by reaction with thiobarbituric acid (TBA) forming red condensation product that can be measured spectrophotometrically (531 nm), and the value obtained is commonly described as TBARS [24]. The effects of supplementing *P. porrigens* extracts, α -tocopherol and BHA on TBARS values of sunflower oil at 65°C for 24-day were shown in Figure 4. TBARS values of sunflower oils supplemented with *P. porrigens* extracts, and antioxidants increase gradually over the 24-day storage and were in the range of 0.39-0.63, with 0.66 mg/kg oil for both control, respectively.

Figure 4 shows no significant differences were found on TBARS formation up to

day-6 storage among *P. porrigens*' CE (0.11 ± 0.01 mg/kg), n-BUT (0.12 ± 0.02 mg/kg), SF-III (0.13 ± 0.02 mg/kg) and SF-IV (0.13 ± 0.01 mg/kg) to that of α -tocopherol (0.11 ± 0.02 mg/kg) and BHA (0.11 ± 0.01 mg/kg). Whilst at day-12 storage, only *P. porrigens*' CE (0.20 ± 0.01 mg/kg) is able to retard the formation of TBARS comparable to that of BHA (0.17 ± 0.01 mg/kg). The results suggested that *P. porrigens*' CE were as effective as α -tocopherol and/or BHA (200 ppm) in stabilizing the formation of TBARS in sunflower oil for 1-year and 2-year at ambient temperature.

A similar stabilizing effect on TBARS formation was reported in four *Chrysanthemum morifolium* Ramat water extracts (200 ppm) that have better antioxidative properties than 0.02% of tocopherol and BHA in soybean oil emulsion [33]. Daker *et al.* [34] reported that as high as 5000 ppm and 1000 ppm of unfermented or fermented maize extracts showed better oxidative stability

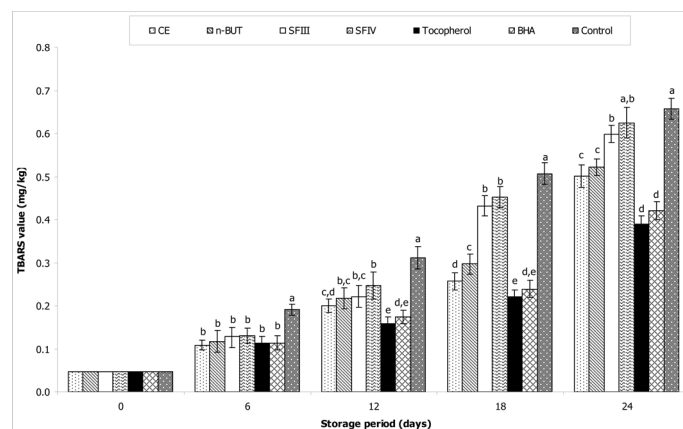


Figure 4. TBARS value of sunflower oil added with *Pleurotus porrigens* extracts and antioxidants under accelerated storage at 65°C for 24 days.

effects compared to BHA (200 ppm). Two Chinese herbs, *Cortex fraxini* and *Polygonum cuspidatum* extracts have been shown to exhibit strong antioxidant activity in peanut oil and had similar protective effect as BHT [31, 35]. Wanasundara and Shahidi [36] reported that the addition of ethanolic extract of canola at 500 ppm and 1000 ppm to refined and bleached canola oil exhibited a better lipid oxidative stability compared to BHA and BHT under Schaal oven test conditions at 65°C over a 17 days period.

In addition, the results showed that only crude extract of *P. porrigens* is able to provide the oxidative stability up to 1 year, indicating a possible combination of antioxidative components that provide synergistic protective effect on sunflower oil. The synergistic effect of combination of *Pistacia lentiscus* resin with citric acid that exhibited a synergistic effect to retard oxidation in sunflower and corn oils has been reported [37]. As shown in the phytochemical screening (Table 2), the crude extract of *P. porrigens* contains all of the phytochemicals tested except for alkaloid, which may provide such synergistic effect. In addition, a combined 200 ppm rosemary extract and 200 ppm ascorbyl palmitate; and 200 ppm rosemary extract,

200 ppm tocopherol and 1000 ppm lecithin provide comparable oxidative stability as TBHQ and can be used to substitute TBHQ to preserve the oxidative stability of margarine [38].

3.7 Iodine Value

The sunflower oil comprises high amount of polyunsaturated fatty acids (linoleic acid) that is susceptible to oxidation, therefore, a decreasing trend in iodine value (IV) can be observed under accelerated storage conditions. There was a slow decreasing trend observed in sunflower oil samples, IV of CE and n-BUT of *P. porrigens* (Figure 5), and CE, SF-II, at day-6 did not differ significantly when compared to α -tocopherol and BHA. However, at day-12 only CE (108.77 ± 1.84 g I₂/100g) and n-BUT (107.21 ± 1.22 g I₂/100g) of *P. porrigens* showed no significant difference IV than that of BHA (111.90 ± 0.98 g I₂/100g and 125.60 ± 1.78 g I₂/100g, respectively). Thereafter, at day-18 and day-24, greater inhibitory effect was observed in α -tocopherol and BHA, compared to *P. porrigens* extracts. The results indicated that *P. porrigens*' CE and n-BUT, with the presence of flavonoid, saponin, tannin, and terpenoid are shown to be effective in

stabilizing the rancidity similar to that of BHA up to 1-year of storage under normal conditions.

A decreased in IV indicates the development of oil rancidity due to destruction of double bonds by oxidation and the formation of secondary oxidation

products during storage [16]. The standard of IV in sunflower oil falls between 110-143 g I₂/100g [39]. Based on this, α -tocopherol and BHA were effectively retarded the decreasing of IV in sunflower oils throughout 1½-year storage under ambient temperature.

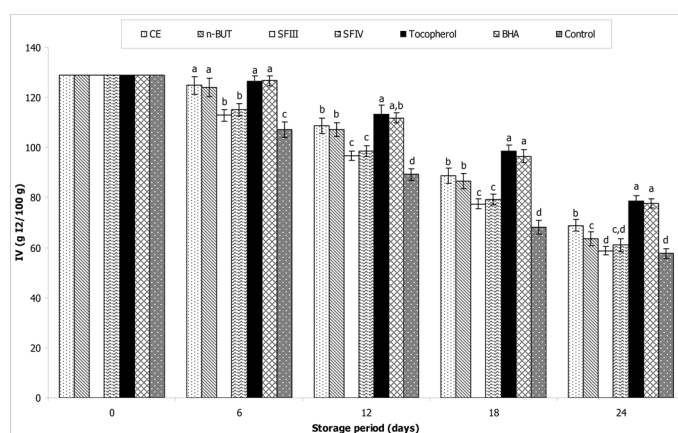


Figure 5. Iodine value of sunflower oil added with *Pleurotus porrigens* extracts and antioxidants under accelerated storage at 65°C for 24 days.

4. CONCLUSION

BHA and α -tocopherol are commonly used antioxidants and possess higher inhibitory ability on lipid oxidation compared to *P. porrigens* extracts. Sunflower oil samples supplemented with crude extracts of *P. porrigens* and its n-butanol fraction showed better oxidative stability than their purified sub-fractions in different extent under accelerated storage. This raises the perspective of whether those potent antioxidants present in the crude extracts and n-butanol fraction of *P. porrigens* would produce synergistic or compounded antioxidative effect on sunflower oil stability.

Overall, the present study showed that the addition of 200 ppm of *P. porrigens* extracts were found to be able to prolong the shelf-life of sunflower oils and inhibit oxidative deterioration by retarding the formations of primary and secondary oxidation products, and reducing the losses

of polyunsaturated fatty acids between ½-year and 1½-year. The total oxidation (as determined by TOTOX value) found that crude extract of *P. porrigens* provide oxidative stability of sunflower oil up to 1 year of storage under normal conditions, similar to that of BHA. However, higher concentration (> 200 ppm) of *P. porrigens* extracts may show comparable or better protective effect than α -tocopherol and BHA.

AUTHORS DISCLOSURE STATEMENT

No competing financial interests exist.

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