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Antioxidant from maize and maize fermented by *Marasmiellus* sp. as stabiliser of lipid-rich foods

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Abstract

Mycelia of *Marasmiellus* sp. (KUM 50061) were grown on maize for antioxidant production. This formed the mycelial biomass which was then extracted with methanol. Antioxidant activity of methanolic extract was analysed by the TBARS assay, using egg yolk or palm cooking oil as a source of lipid, rather than the conventional rat liver microsomes or linoleic acid. Results showed that at low concentrations of extracts, inhibition of lipid peroxidation in buffered egg yolk was marginal, but significant inhibitory response was evident as the concentrations was increased. The concentration of extract of fermented maize that caused 50% inhibition of lipid peroxidation of buffered egg yolk was 6 mg/ml. Results also indicated a decrease in peroxidation in heated cooking oil supplemented with dried extract compared to unsupplemented cooking oil. The concentration range of dried extract supplementation was 0.2–5 mg/ml. Increasing the extract concentration did not significantly alter the inhibition of peroxidation. The inhibition effect was still evident even at the lowest concentration tested, and was found to be better than catechin and BHA. This pattern of observation was consistent over the 12-day period of observation. Therefore, the possibility of substituting synthetic antioxidants such as BHA and BHT, which are known to be carcinogenic, with antioxidants of natural origin is suggested.
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Keywords: Antioxidant; Lipid peroxidation; *Marasmiellus*; Thiobarbituric acid reactive substance (TBARS)

1. Introduction

Damage caused by free radicals and reactive oxygen species has been linked to some neurodegenerative disorders and cancers. Oxidation of low-density lipoprotein is a major factor in the promotion of coronary heart disease and atherosclerosis (Mathew & Abraham, 2006). In our society, whether it is cooking at home or eating out in a fast-food restaurant, preparation of most foods involves the use of cooking oil, for shallow or deep frying. Cooking oil, rich in polyunsaturated lipids, is easily oxidised when subjected to such high heat. The presence of lipid free radicals may oxidise flavours, pigments and vitamins. Hydroperoxides, the primary products of autoxidation, may form dark-coloured, possibly toxic products or decompose to yield rancid off-flavour compounds. Halliwell and Gutteridge (1999) stated that the food deterioration was caused mainly by lipid peroxidation. Autoxidation occurred between molecular oxygen and unsaturated lipid, leading to lipid deterioration. This process was initiated by exposure to the enzyme lipoxygenase, heat, ionising radiation, light, metal ions and metallo-protein catalysts. The food industries are well aware of these reactions and are concerned with rancidity and the oxidative spoilage of foodstuff (Shahidi & Wanasundara, 1992).

In the food industry, the rate of autoxidation is reduced by freezing, refrigeration, packaging under inert gas in the absence of oxygen, and vacuum packaging. In cases where these methods are neither economical nor practical, the use of antioxidants is employed to extend shelf life of food, reduce wastage and nutritional losses (Coppen, 1983;

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Schuler, 1990; Shahidi & Wanasundara, 1992). The oxidative deterioration of fats and oils in foods is prevented by synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylated hydroxyquinone (TBHQ). THBQ was reported by Shahidi and Wanasundara (1992) to be the best antioxidant for protecting frying oils against oxidation. Although efficient in preventing autoxidation, only a few synthetic compounds are currently approved for use in the food industry because of their potential toxicity and carcinogenicity. BHA and BHT are both anticarcinogenic and carcinogenic (Botterweck, Verhagen, Goldbohm, Kleinjans, & Brandt, 2000; Löliger, 1991). Thus, to satisfy consumers' preference for natural food additives over synthetic ones, there is increasing importance in searching for natural antioxidants from herbs, fruits, vegetables and spices as a less harmful alternative to synthetic antioxidants.

From our previous investigation, *Marasmiellus* sp. (KUM 50061) grown on rice contained high antioxidant levels, based on ferric reducing antioxidant potential assay (Noorlidah et al., 2005; Pavalamalar, Noorlidah, Vikineswary, & Kuppusamy, 2003). The mycelia growth rate is quite high and a previous study revealed that extract of maize fermented by *Marasmiellus* sp. had high ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, compared to extracts from fermented chickpea, lentil, maize, rice and soy bean (Daker, 2006). The fruiting bodies of *Marasmiellus* sp. studied are white but brown centrally and thin. The diameter of cap ranged from 6 to 7 cm with gills uneven and free. The stipe is dark brown, solid, tough and centrally attached.

The objective of this study was to evaluate the antioxidant property of a methanolic extract obtained from the mycelial biomass of *Marasmiellus* sp. (KUM 50061), based on its ability to inhibit lipid peroxidation and scavenge 1,1diphenyl-2-picrylhydrazyl (DPPH) radicals. The ability of natural antioxidants to inhibit lipid peroxidation in cooking oil was assessed.

2. Materials and methods

2.1. Chemicals

Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from AppliChem (Darmstadt, Germany). Hydrogen peroxide (H_2O_2) and dimethyl sulfoxide (DMSO) were obtained from Fisher Scientific, (Loughborough, UK) while ferrous sulphate ($FeSO_4$) and DL-catechin were from Sigma (St. Louis, MO) and Sarsynthèse (Genay, France) respectively. Palm cooking oil (Labour brand from Lam Soon Edible Oils, Hong Kong) was purchased from a local supermarket.

2.2. Preparation of antioxidant extract

Maize soaked in water for 24 h was drained, and 4% (w/w) malt extract, 4% (w/w) yeast extract and 4% (w/w) rice bran were added as nitrogen sources for solid substrate fermentation. Fifty-gram aliquots were distributed into 250-ml Erlenmeyer flasks, covered with non-absorbent cotton plugs and aluminium foil, and autoclaved for 20 min at 121 °C. Five 7-mm diameter plugs of five-day old *Marasmiellus* sp. mycelia were inoculated into the cooled maize and then incubated for 14 days. After 14 days, the mycelia grew to a high density, covering the entire surface of the substrate. The nutritional content of the mycelia alone was not analysed but a proximate analysis on the mycelia plus substrate was carried out. The mixture contained 81% carbohydrate, 11.4% protein and 3.5% fat. Maize without the mycelial plugs served as the control. Unfermented maize and fermented maize were broken up, and approximately 250 ml methanol was added into each Erlenmeyer flask. The mixture was shaken for 48 h, rotating at 150 rpm at room temperature. Methanolic extracts were collected by filtration, followed by rotary evaporation under vacuum to obtain the extract, and finally freeze dried.

2.3. Inhibition of lipid peroxidation of buffered egg yolk by extracts

The method of Kuppusamy, Indran, and Balraj (2002) was modified, to determine the conditions which would induce a high amount of thiobarbituric acid reactive substance (TBARS), a secondary product of lipid peroxidation. Fowl egg yolk – comprising mainly of phospholipids, triacylglycerols and proteins – was used as an alternative to rat liver microsomes and linoleic acid, which were more costly and required tedious preparation (Kuppusamy et al., 2002). Lecithin – a major component of cellular systems – is suitable for lipid peroxidation assay because unsaturated lipids are more prone to oxidation. The use of buffered fowl egg yolk was cost effective.

The reaction mixture for inducing lipid peroxidation contained 1 ml fowl egg yolk emulsified with 0.1 M phosphate buffer (pH 7.4), to obtain a final concentration of 25 g/l and 100 μl of 1000 μM Fe^{2+}, both concentrations of which were higher than that of Kuppusamy et al. (2002). Extracts of unfermented maize and fermented maize were assayed for their effect on lipid peroxidation. A stock solution of 360 mg/ml was prepared by dissolving 3.6 g extracts in 10 ml distilled water. This stock solution was then diluted to final extract concentrations of 0.1–30 mg/ml. Each assay was carried out in triplicate. The mixture was incubated at 37 °C for 1 h, after which it was treated with 0.5 ml of freshly prepared 15% TCA and 1.0 ml of 1% TBA. The reaction tubes were kept in a boiling water bath for 10 min. Upon cooling, the tubes were centrifuged at 3500*g* for 10 min, to remove precipitated protein. The formation of TBARS was measured by removing 100 μl of supernatant and measuring the absorbance at 532 nm. The control was buffered egg with Fe^{2+} only. DL-Catechin ranging from 5 to 500 μg/ml was used as the standard. The percentage inhibition ratio was calculated from the following equation:

$$\% \text{ inhibition} = \left(\frac{A_0 - A_s}{A_0}\right) \times 100$$

where A_0 refers to the absorbance of the control and A_s is the absorbance of the sample.

To determine the concentration required to achieve 50% inhibition of phospholipid oxidation in egg yolk, the percentage of lipid peroxidation inhibition was plotted against extract concentration.

2.4. Inhibition of lipid peroxidation of cooking oil by extracts

Methanolic extract of *Marasmiellus* sp. mycelial biomass was redissolved in analytical grade DMSO to obtain a stock solution of 25 mg/ml. DMSO was used due to its insolubility in water. This was diluted accordingly with DMSO then mixed with 12 ml palm cooking oil to yield final extract concentrations of 0.2, 1.0 and 5.0 mg/ml. In addition, Erlenmeyer flasks containing DMSO-added oil, oil supplemented with 0.225 mg/ml catechin and oil supplemented with 0.2 mg/ml BHA were prepared. Erlenmeyer flasks were heated over a Bunsen burner and the contents brought to boil for 10 min to simulate cooking/frying conditions.

Lipid peroxidation assay was conducted in reaction tubes, which contained 250 μl 15% TCA and 500 μl 1% TBA and 500 μl of cooking oil heated with catechin, BHA or different concentrations of extracts. Unheated oil only and unheated DMSO-added oil were also assayed for lipid peroxidation. Each assay was carried out in triplicate. The reaction tubes were incubated in a boiling water bath for 10 min. Upon cooling, the tubes were centrifuged at 3500*g* for 10 min, to properly separate the aqueous phase from the oil phase. The formation of TBARS was measured by removing 100 μl of supernatant and reading its absorbance at 532 nm. The absorbance was plotted against sample concentration in order to determine the effect on TBARS formation. The oil mixtures were kept in Erlenmeyer flasks, sealed with parafilm and stored in the dark. The assay was carried out, to determine the effect of storage of used oil for 12 days.

2.5. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

The extracts obtained from fermented maize as well as the extracts of the unfermented maize, which served as the control, were analysed for 1,1-diphenyl-2-picrylhydrazyl radical scavenging ability (Brand-Williams, Cuvelier, & Berset, 1995). A 0.06 mM DPPH$^{\cdot}$ solution in methanol was prepared. A 1 mM solution of L-ascorbic acid was prepared. Stock solutions of each extract were prepared by dissolving 0.5 g of extract in 10 ml methanol. This produced stock solutions with a concentration of 50 mg/ml. An aliquot of 3.9 ml methanolic solution containing 0.06 mM DPPH$^{\cdot}$ was added to 0.1 ml of methanol-dissolved extracts. The solution was mixed vigorously and absorbance was then measured at 515 nm with methanol as the blank. The decrease in absorbance was recorded at 0, 1, 2 min and every 15 min until the reaction reached a plateau. The time taken to reach steady state was determined by one-way analysis of variance (ANOVA), to compare the decrease in absorbance values. Antioxidant activity was compared with L-ascorbic acid (0.1–1.0 mM) as a positive standard. All determinations were performed in triplicate. The scavenging activity on DPPH$^{\cdot}$ was expressed as percentage radical-scavenging, calculated as follows:

$$\% \text{ radical scavenging effect} = \left(\frac{A_0 - A_s}{A_0}\right) \times 100$$

where A_0 refers to the absorbance of 0.06 mM DPPH$^{\cdot}$ methanolic solution only, whereas A_s is the absorbance of the reaction mixture.

2.6. Statistical analysis

Data were recorded as means ± standard deviations and analysed by SPSS (version 12 for Windows 2000: SPSS Inc., Chicargo, IL). One-way analysis of variance and Tukey multiple comparisons were carried out to test for any significant differences between the means; the mean values of antioxidant activities between two extracts or two treatments were analysed by independent-samples *t*-test. *P*-values less than 0.05 were considered statistically significant. All analyses were performed in triplicate except for the assay on inhibition of lipid peroxidation in buffered egg yolk by the fermented maize extract, which was done in duplicate.

3. Results and discussion

3.1. Inhibition of lipid peroxidation of buffered egg yolk by extracts

The assay was carried out to determine the ability of the extracts to inhibit peroxidation of phospholipids present in egg yolk and thus to assess the potential of the extracts as a source of natural antioxidants. At lower concentrations of extracts, the lipid peroxidation inhibition was marginal but significant inhibitory response was evident as the concentrations were increased. The percentage lipid peroxidation inhibition values of extracts of fermented maize were always higher then those of extracts of unfermented maize. However, at 20 and 30 mg/ml, the values of peroxidation inhibition were comparable (Fig. 1). According to Shahidi and Wanasundara (1992), the effect of antioxidant concentration on autoxidation rates depended on the antioxidant structure, nature of sample being oxidised and the oxidation conditions. Phenolic antioxidants often lose their activity at high concentrations. Table 1 shows the EC_{50} value (mg/ml) of extract of fermented maize and extract of unfermented maize, as determined by the lipid peroxidation assay in buffered egg yolk. Although the extract of fer-

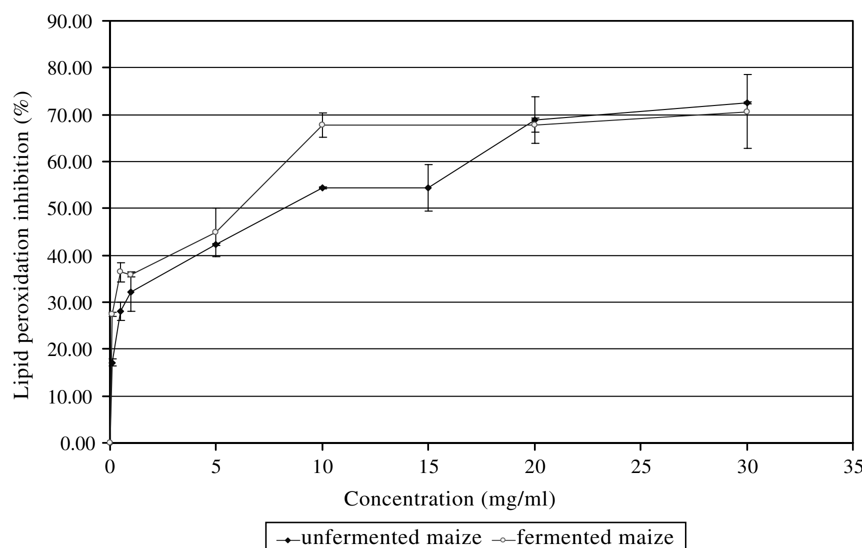

Fig. 1. Lipid peroxidation inhibition abilities of extract of unfermented maize and maize fermented by *Marasmiellus* sp. (KUM 50061) mycelia via solid substrate fermentation.

mented maize exhibited an EC_{50} value of 6.00 mg/ml, better than that of the extract of unfermented maize at 8.25 mg/ml, this difference was not significant. Further evaluation of oxidative stability of natural antioxidants was difficult because the crude extracts were used directly (Frankel, 1993), rather than isolating the antioxidative compounds from other components.

Lipid peroxide levels were progressively suppressed by the addition of increasing amounts of catechin. With an EC_{50} of 225 µg/ml or 0.225 mg/ml (Fig. 2), catechin was more effective than either extract at all tested concentrations. This indicated a lower inhibitory activity against lipid peroxidation in both extracts, relative to catechin.

Kuppusamy et al. (2002) analysed the antioxidant activity of extracts of various fruits, vegetables and whole plants at a final concentration of 0.42–4.17 mg/ml, using the lipid peroxidation of buffered egg yolk, as in this study. The EC_{50} value was 0.3–3.97 mg/ml and the lipid peroxidation inhibitory potency decreased in the order: curry leaf > ginger > okra > Chinese parsley > Chinese kale > spearmint > cabbage > Chinese mustard > spinach > Chinese radish.

In 2005, Cheung and Cheung fractionated methanol extracts of *Lentinus edodes* and *Volvariella volvacea* into dichloromethane, ethyl acetate and *n*-butanol sub-fractions; and aqueous extracts into low molecular weight and high molecular weight sub-fractions. In general, *V. volvacea* showed a higher inhibition of lipid peroxidation, compared to *L. edodes* fractions. In lipid peroxidation assay using rat brain tissue, the dichloromethane sub-fraction of *V. volvacea* had the lowest IC_{50} value (0.109 mg/ml). Lo and Cheung (2005) reported the lipid peroxidation of rat brain homogenate induced by Fe^{2+}/ascorbate, was strongly inhibited by the ethyl acetate fraction of the methanolic extract of *Agrocybe aegerita* var. *alba* with an IC_{50} value of 0.05 mg/ml. Song et al. (2003) evaluated *Phellinus linteus* for the inhibition of lipid peroxidation in rat brain homogenate, initiated by $FeCl_2$. The IC_{50} value was reported to be 0.485 mg/ml. *P. linteus* has attracted great attention due to its anti-tumour effect and other medicinal properties. In a study on *Pleurotus pulmonarius* (=*P. sajor-caju*) carried out by Jose, Ajith, and Jananrdhanan (2002), significant lipid peroxidation inhibition activity was demonstrated by the methanolic extract; with 50% inhibition at 0.960 mg/ml.

Table 1
Concentration of extracts of unfermented maize and maize fermented by *Marasmiellus* sp. (KUM 50061) mycelia via solid substrate fermentation that caused 50% inhibition of lipid peroxidation of buffered egg yolk

Extract	Unfermented maize	Fermented maize
EC_{50} (mg/ml) Means ± SD	8.25 ± 0.35	6.00 ± 0.71

Average values are not significant at $P = 0.05$.

3.2. Inhibition of lipid peroxidation of cooking oil by extracts

Frankel (1993) stated that lipid oxidation which led to rancidity played an important factor in determining the useful storage life of food products. Tests involving evaluation of oxidative stability in food lipids often employ accelerated oxidation conditions. However, for practical purposes, predictions of oxidative stability in foods and oils should be measured under conditions maintained as close as possible to those under which protection against oxidation was required. Screening of plant or fungal antioxidants and comparing their antioxidant potential with that of commercial food preservatives and synthetic products may help find new sources of natural antioxidants (Caillet et al., 2007).

This study was designed to investigate if the extract of fermented maize was able to protect palm cooking oil from

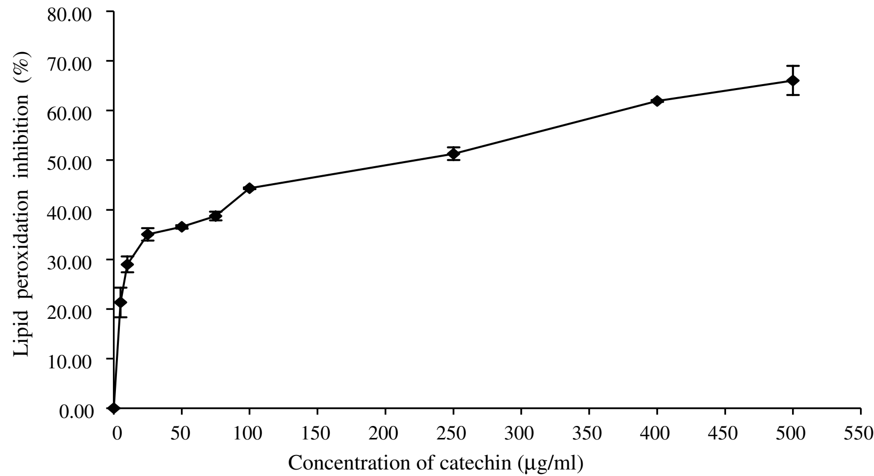

Fig. 2. Lipid peroxidation inhibition ability of catechin.

peroxidation that occurred during preparation of food, and also to assess if this extract was a better antioxidant than the extract of unfermented maize. Unheated oil by itself experienced some amount of auto-oxidation. Heated oil, heated DMSO-added oil and heated oil supplemented with 0.2 mg/ml BHA gave the highest absorbance values (Fig. 3). Consequently, these mixtures experienced the highest level of peroxidation upon exposure to heat. Oils supplemented with extract of unfermented maize and extract of fermented maize showed no significant differences in their absorbance values. This means that the presence of the mycelia neither enhances nor decreases the ability to inhibit lipid peroxidation of palm cooking oil. This is in accordance with the non-significant EC50 values (Table 1). Comparison was made against catechin, a natural antioxidant present in wine and tea, used to retard lipid peroxidation in oils, fats and animal tissues (Yilmaz, 2006) and a synthetic antioxidant, BHA, used in controlling the oxidation of short-chain fatty acids such as coconut and palm kernel oils (Shahidi & Wanasundara, 1992). On Day 0 and Day 4, the six different combinations of oil with extract, and also oil with 0.2 mg/ml catechin recorded absorbance values which were all significantly lower than that of oil with 0.2 mg/ml BHA. However, by Day 8, oil with 0.225 mg/ml catechin exhibited increased oxidation and short-term protective effects. There were significantly lower absorbance values for the six combinations of oil and extracts compared to that of oil with 0.225 mg/ml catechin and oil with 0.2 mg/ml BHA, indicating a higher inhibition of peroxidation. However, on Day 12, pink TBARS were observed in oil with 0.2 mg/ml extract of fermented maize, indicating the occurrence of lipid peroxida-

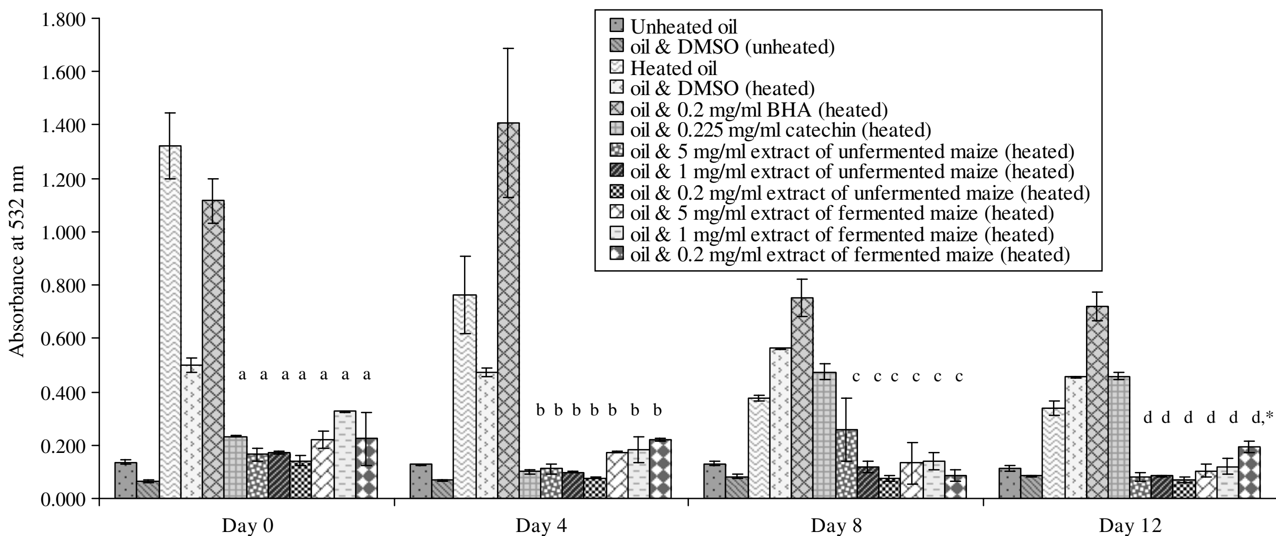

Fig. 3. Absorbance values of different reaction mixtures to demonstrate inhibition of lipid peroxidation in palm cooking oil by butylated hydroxyanisole (BHA), catechin, extract of unfermented maize and extract of maize fermented by *Marasmiellus* sp. (KUM 50061) mycelia. (a) mean absorbance significantly lower in comparison to oil with 0.2 mg/ml BHA; (b) mean absorbance significantly lower in comparison to oil with 0.2 mg/ml BHA; (c) mean absorbance significantly lower in comparison to oil with 0.2 mg/ml BHA and oil with 0.225 mg/ml catechin. (d) mean absorbance significantly lower in comparison to oil with 0.2 mg/ml BHA and oil with 0.225 mg/ml catechin. *Pink TBARS formed; mean absorbance significantly higher than other oil with extract combination except oil with 1 mg/ml extract of fermented maize.

tion. Also, this combination recorded an absorbance value which was significantly higher than the other combinations of oil and extract except for oil with 1 mg/ml extract of fermented maize.

DMSO used to dissolve the dried extract does not contribute to oil peroxidation. The absorbance values of unheated DMSO-added oil were comparable to those of oil heated in the presence of extracts and those of unheated oil undergoing auto-oxidation.

When heating of oil was done in the presence of various concentrations of extracts, the extent of oxidation was clearly and significantly lowered. Statistical analysis showed that inhibition of oil peroxidation by addition of extract was not dosage-dependent. Whether the extract concentration was 5 mg/ml, 1 mg/ml or 0.2 mg/ml, the absorbance values were not significantly different and the inhibition of peroxidation was still evident even at the lowest concentration tested. This suggests that a potent antioxidant property exists even in very small amounts of the extracts. This is important because if the extracts were to be used in the industry as an antioxidant, then only a small amount of the extracts is necessary for addition into a large volume of palm cooking oil, thus contributing to cost-effectiveness.

This indicated that the extracts of unfermented or fermented maize are good potential sources of natural antioxidants, to replace chemicals such as BHA and catechin. The concentration of 0.225 mg/ml for catechin was chosen because this was its EC_{50} value determined by lipid peroxidation assay using buffered egg yolk. As for BHA, the concentration of 0.2 mg/ml was chosen because the FDA considers it a generally recognised as safe (GRAS) compound if the content does not exceed 0.02% or 200 ppm of the fat or oil content of the food product. This study indicates that catechin and BHA may require higher concentrations, to exert any inhibitory effect on oil peroxidation. Moreover, heating oil supplemented with extracts released a pleasant fragrance, which suggested a possibility of employing the extracts to enhance prophylactic features to make food more appealing and acceptable to consumers, as opposed to BHA, which emits irritating fumes when heated to decomposition.

In this study, the storage effect of heated oil over 12 days was examined to determine the term of protective effect. Even after 12 days, the oil with extracts gave absorbance values which were still always lower than those of oil without any extract (Fig. 3). Throughout the 12-day duration, oil with 0.2 mg/ml BHA always recorded absorbance values significantly higher than that of oil with 0.225 mg/ml catechin. The former produced far more intense pink TBARS, indicating greater lipid peroxidation. This shows that the efficacy of BHA as an antioxidant was far less than that of catechin. This could be due to the nature of BHA being a monohydric phenolic compound whereas catechin is a flavonoid with three reactive phenolic groups. It is known that the inhibition of lipid oxidation is generally higher in polyphenolic compounds.

In a work involving medicinal mushrooms, i.e., *Ganoderma lucidum, G. tsugae* and *Coriolus versicolor*, Mau, Lin, and Chen (2002) reported that by using the 1,3-diethyl-2-thiobarbituric acid (DETBA) method and at 0.6 mg/ml, *G. lucidum*, *G. lucidum* antler and *G. tsugae* exhibited excellent antioxidant activity. The percentage of lipid peroxidation was expressed compared to the control containing no mushroom extract (100%) and a lower percentage indicated a higher antioxidant activity. The antioxidant activity of the three *Ganoderma* mushrooms were between 2.30% and 6.41% while *Coriolus versicolor* showed only 58.6% antioxidant activity. BHA showed only 66.1% of the lipid peroxidation of the control at 10 mg/ml. Using the same method, Mau, Lin, and Song (2002) worked on the antioxidant properties of specialty mushrooms such as *Dictyophora indusiata* (basket stinkhorn), *Grifola frondosa* (maitake), *Hericium erinaceus* (lion's mane) and *Tricholoma giganteum* (white matsutake). They reported that the methanolic extract of *D. indusiata* had an excellent antioxidant activity, with 2.26% lipid peroxidation at 1.2 mg/ml. At the same concentration, *G. frondosa*, *H. erinaceus* and *T. giganteum* showed 29.8%, 48.5% and 67.0% lipid peroxidation, respectively. At 10 mg/ml, BHA only inhibited lipid peroxidation by 33.9%. Yang, Lin, and Mau (2002) studied the antioxidant properties of freshly obtained commercial edible mushrooms consisting of *Lentinus edodes* (shiitake), *Flammulina velutipes* (enokitake), *Pleurotus cystidiosus* (abalone mushroom) and *P. ostreatus* (tree oyster mushroom). Using also the DETBA method, the authors demonstrated that the lipid peroxidation of methanolic extracts from these commercial mushrooms fell within 24.7–62.3% at 1.2 mg/ml, and thus are classified as moderate to high peroxidation inhibitors. BHA showed only 66.1% of lipid peroxidation at 10 mg/ml.

3.3. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

Antioxidant activity was defined as the amount of antioxidant necessary to decrease the initial $DPPH^{\cdot}$ concentration by 50% (EC_{50}; unit = mg extract/ml methanol). The lower the EC_{50} is, the higher the antioxidant power. Unfermented and fermented maize exhibited EC_{50} values of 3.87 mg/ml and 1.88 mg/ml, respectively, as shown in Fig. 4. Thus, although the abilities of both extracts to inhibit lipid peroxidation were similar, their difference in ability to scavenge free radicals is of significance, the extract of fermented maize being the better scavenger.

4. Conclusion

The assays on inhibition of lipid peroxidation of buffered egg yolk or palm cooking oil were convenient modifications to the more tedious methods involving rat brain tissue, rat liver microsomes or linoleic acid. Both provided practical techniques to assess inhibition or delay of autoxidation and permitted reproducible results. In the assay to

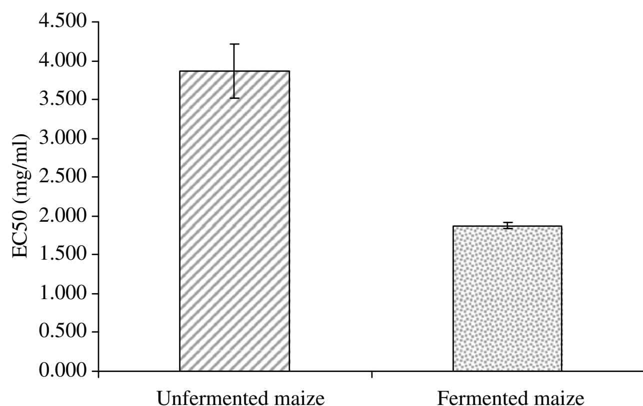

Fig. 4. DPPH˙ scavenging activity of extracts of unfermented and fermented maize. Average values are significant at $P = 0.05$.

investigate the effect on inhibition of palm cooking oil peroxidation by extracts of fermented or unfermented maize, 0.2 mg/ml was sufficient to protect the cooking oil from peroxidation by heat. The potency of the extracts, which was even greater than that of catechin, a natural antioxidant, coupled with the release of a pleasant aroma upon heating, suggests a possibility of employing the extracts to make food more appealing and acceptable to consumers to replace synthetic chemicals, in addition to increasing shelf life, reducing wastage and nutritional losses, by inhibiting and delaying oxidation.

Extract of fermented maize was able to produce bioactive compounds with enhanced radical-scavenging ability, having an EC_{50} value of 1.88 mg/ml, determined by the DPPH˙ method and lipid peroxidation inhibitory effect, with an EC_{50} value of 6.00 mg/ml, determined by the lipid peroxidation assay. Taking into consideration the antioxidant activity demonstrated in the DPPH˙ method and lipid peroxidation method, it may be possible to suggest that the predominant antioxidant mechanism of the extracts tested was free radical scavenging, rather than inhibition of lipid oxidation. If any one of the extracts was used as a stabilizer for lipid-rich food, then it should be that of fermented maize because of its enhanced ability to scavenge free radicals.

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