

Antioxidative properties of *Pandanus amaryllifolius* leaf extracts in accelerated oxidation and deep frying studies

Fatihanim Mohd Nor^a, Suhaila Mohamed^{a,*}, Nor Aini Idris^b, Razali Ismail^b

^a Functional Food Laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

^b Malaysian Palm Oil Board, No. 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor Darul Ehsan, Malaysia

Received 17 August 2007; received in revised form 21 November 2007; accepted 4 February 2008

Abstract

The potential uses of *Pandanus amaryllifolius* leaf extract as a natural antioxidant were evaluated in refined, bleached and deodorized (RBD) palm olein, using accelerated oxidation and deep frying studies at 180 °C from 0 to 40 h. The extracts (optimum concentration 0.2%) significantly retarded oil oxidation and deterioration ($P < 0.05$), comparably to 0.02% BHT in tests such as peroxide value, anisidine value, iodine value, free fatty acid, oxidative stability index (OSI), polar and polymer compound contents. In sensory evaluation studies, different batches of French fries were not significantly different ($P < 0.05$) from one another for oiliness, crispiness, taste and overall acceptability when the same oil was used for up to the 40th hour of frying. *P. amaryllifolius* leaf extract, which had a polyphenol content of 102 mg/g, exhibited an excellent heat-stable antioxidant property and may be a good natural alternative to existing synthetic antioxidants in the food industry.

© 2008 Elsevier Ltd. All rights reserved.

Keywords: Frying; *Pandanus amaryllifolius* leaf extract; Palmolein; Sensory evaluation antioxidant; Accelerated oxidation study

1. Introduction

Pandanus amaryllifolius leaves, commonly known as pandan, are often used to give a refreshing, fragrant flavour to both sweet and savoury South-East-Asian dishes (rice, chicken, jellies, drinks, puddings, custard or sweets). Pandan leaves are also used in cooking ordinary non-aromatic rice to imitate the more expensive aromatic Basmati and Jasmine rices. The fragrant leaves are often used as food wrappers, in potpourri and as air fresheners. The long, narrow, blade-like monocotyledon leaves, emit a pleasant aroma, mainly due to the presence of 2-acetyl-1-pyrroline (Laksanalamai & Ilangantileke, 1993). The leaves are sometimes put into frying oils to impart flavour to fried food. The plant grows prolifically in tropical areas, including the pacific islands, Africa, South Asia, South East Asia and Australia.

The leaves are used medicinally in South East Asia to refresh the body, reduce fever, and relieve indigestion and flatulence (Cheeptham & Towers, 2002). The oil of the leaf is described as stimulant and antispasmodic and is effective against headaches, rheumatism, and epilepsy and as a cure for sore throats (Quisumbing, 1951). The leaf contains essential oils, carotenoids, tocopherols and tocotrienols (Lee, Su, & Ong, 2004), quercetin (Miean & Mohamed, 2001), alkaloids (Busque, March, Figueredo, Font, & Sanfeliu, 2002), fatty acids and esters (Zainuddin, 2004) and non-specific lipid transfer proteins (Ooi, Wong, Sun, & Ooi, 2006).

Deep fat frying affects the flavour, texture, shelf life and nutrients of fried food. Palm olein is one of the most common frying media and is used extensively, both domestically and on a commercial scale. At high temperatures in the presence of air and food, oxidation, hydrolysis and polymerization of oil takes place, leading to the formation of desirable and undesirable secondary products, affecting both oil and finished product qualities.

* Corresponding author. Tel.: +60 3 89468391; fax: +60 3 89423552.

E-mail address: mohamed.suhaila@gmail.com (S. Mohamed).

Low cost synthetic antioxidants propyl gallate, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) are often used to retard fat oxidation. Dietary administration of BHT to rats caused fatal haemorrhages in pleural and peritoneal cavities and organs, such as epididymis, testes and pancreas (Deshpande, Deshpande, & Salunkhe, 1996). BHA too, exhibits toxic and carcinogenic effects. They are allowed for use within legal limits in the food industry. They are very effective during storage and transport of oils and fats, but are less effective at frying temperatures due to their volatility. Natural antioxidative substances from the polyphenols of edible herbs are believed to be safer and may provide additional health benefits, compared to synthetic antioxidants. It is an area worth investigating due to current consumer concerns about health.

The phenolic compounds in herbs act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators. Several herbs reportedly retard lipid oxidation during frying (Che Man & Jaswir, 2000; Naz, Siddiqi, Sheikh, & Sayeed, 2005), in heated oil (Khan & Shahidi, 2001; Nogala-Kalucka et al., 2005; Shyamala, Gupta, Lakshmi, & Prakash, 2005) and in food (Jaswir, CheMan, & Kitts, 2000). This study reports the beneficial effects of *P. amaryllifolius* leaf extract in palm olein during accelerated oxidation and frying in order to understand its potential use as an antioxidant in the food industries.

2. Materials and methods

2.1. Herbs and oil

P. amaryllifolius leaves were obtained from the local market. Refined, bleached and deodorized (RBD) palm olein was obtained from Golden Jomalina while prefried frozen French fries were supplied by Simplot. All chemicals and reagents were of A.R. grade and were obtained from Merck, System and Fischer.

2.2. Preparation of herbs extract

The cleaned leaves were dried in a hot air oven at 45 °C for 24 h, and ground to fine powder before extracting into ethanol for 8 h at 50 °C, at 1:10 ratio of powder to ethanol. The solvent was removed using a rotary evaporator.

2.3. Determination of polyphenols and antioxidant activities

The total phenolics content in the extract was determined according to Mattheus (2002) with modification. Herbs (10 mg) were dissolved in 10 ml of methanol. A 200 µl aliquot of the resulting solution was added to 1 ml of Folin–Ciocalteu reagent, 0.8 ml of 0.2% Na₂CO₃ was added and the volume made up to 10 ml using water–methanol (4:6). After 30 min, the absorbance was measured at 765 nm using a spectrophotometer. The concentration

was calculated using gallic acid as a standard, and the results were expressed as gallic acid equivalents per gramme of extract.

The free radical-scavenging activity was assayed, based on the reduction of DPPH radicals in methanol, which causes an absorbance drop at 517 nm. The extract's activity against the DPPH radical was evaluated using the method of Blois (1958). To 4 ml of DPPH[•] in methanol (0.1 mM) was added 1 ml of 50 or 100 ppm herbs extract solution. After 20 min, the absorbance was measured at 517 nm. Radical-scavenging activity was expressed as the percentage inhibition.

The antioxidative activities of the extracts in the linoleic acid model system were also determined (Lingnert, Vallentin, & Eriksson, 1979). In the linoleic acid model system, the autoxidation rate of linoleic acid was determined by measuring the increase of conjugated diene and decrease of linoleic acid content in the sample. The autoxidation of linoleic acid was accompanied in the early stages by formation of hydroperoxides that exhibited absorption at 234 nm. Linoleic acid ester (10 mM), emulsified with an equal amount of Tween 20 in sodium phosphate pH7 buffer, was homogenized for about 1 min. Extracts of 10 µl aliquots (1000, 2000, 3000 ppm) were mixed with 5 ml of the emulsion. Control was the emulsion without addition of the sample. The samples were incubated at 50 °C for 20 h; absorbance was measured at 234 nm before and after oxidation by taking 0.2 ml of the solution and dissolving in 5 ml of methanol. The activity of antioxidant (AOA) was defined as the difference in absorbance between sample and control, divided by the absorbance of the control.

2.4. Accelerated oxidation study

The palm olein was heated to 60 °C before addition of extract (at 0.1%, 0.2%, 0.3% and 0.4%) and stirred to ensure that it completely dissolved. BHT-containing and control samples (without any antioxidants) were used as the positive and negative controls. All samples were heated at frying temperature (180 °C) for 0, 8, 16, 24 and 32 h. Samples were collected, cooled to 60 °C and flushed with nitrogen, and then kept at –20 °C before analysis.

2.5. Frying experiment

Deep frying experiments were carried out simultaneously, using a stainless steel electrical open fryer (Fry-master brand, model H14-2SC) with split pot of 11.5 kg capacity (for each pot) and equipped with an autolift stainless steel basket and automatic portable filter system. The frying media used were, (i) palm olein containing the optimum concentration of 0.2% *P. amaryllifolius* leaf extract based on the accelerated oxidation study (estimated 6.6 mM, based on the most abundant flavonoid quercetin m.w. 302.236), (ii) palm olein containing 0.02% BHT (about 1 mM) and (iii) control palm olein without any additive.

Oil (10 kg) was introduced into a separate fryer, and heated to 60 °C before adding 0.2% of extract, and stirred to ensure that it completely dissolved. The frying medium was heated at 180 ± 2 °C and was allowed to equilibrate at this temperature for 30 min. About 14 batches at 200 g per batch of French fries were fried for 2.5 min per day at 30 min intervals for 8 h daily. The fryers were turned off at the end of the frying experiment each day and the oil was allowed to cool to 60 °C. The oil in each fryer was filtered to remove debris using separate filters. Accurately weighed 400 g oil samples were sampled from each frying medium to represent 0, 8, 24 and 40 h of frying, and were kept in amber bottles. All oil samples were flushed with slow bubbles of nitrogen from the bottom of the bottles and stored in a freezer at -20 °C for physical and chemical analysis.

After frying, the French fries were removed from the fryer. Sensory evaluation was conducted on the same day using the fifth and sixth batches of fried French fries. The fryers were topped up to 10 kg with oil containing antioxidants (0.02% BHT or 0.2% extract), depending on the oil loss. The whole procedure was repeated consecutively for 5 days.

2.6. Analysis of oil quality

Changes in the quality of the oil, such as by peroxide value, anisidine value, iodine value, free fatty acid, oxidative stability index (OSI), polar compounds, polymers and colour test, were analysed by the American oil chemists' society official methods (Firestones, 1993). Determination of French fries colour was done using a colorimeter.

Peroxide value, a measurement of the primary oxidation product of fats and oil, was determined, based by their ability to liberate iodine from potassium iodide. Anisidine value is a measure of secondary products of fat oxidation, formed by the breakdown products of hydroperoxides. They react with *p*-anisidine giving an absorption that can be measured at 350 nm. Iodine value represents the degree of unsaturation of fats and oils. Free fatty acid represents the percentage of fatty acid liberated from the triglyceride chain. Fat was dissolved in neutralized ethanol and the fatty acids were neutralized with standard alkali. The oxidative stability instrument traps volatiles from the oil sample and a probe continuously measures conductivity due to the increase in organic acids as autoxidation proceeds. Oxidative stability index (OSI) is measured as the time before there is a dramatic increase in the rate of lipid oxidation. Polar compounds or the non-volatile products formed during fat and oil oxidation were determined via separation of polar and nonpolar compounds on column chromatography, followed by elution of the nonpolar compounds, and then the differences between weight of sample added to the column and eluted nonpolar compounds were calculated. Polymers, products of polymerization of triglycerides, were determined by methanolysis with sulphuric acid in methanol.

Colour of the oil sample was determined using the Lovibond Tintometer, by matching of the colour of the light transmitted through a specified depth of oil to the colour of the light, originating from the same source, transmitted through standard colour slides. The colour of the French fries was measured using a Minolta Chroma Meter, by taking triplicate readings on each of three equidistant locations on the fries. The colorimeter converts colours into numbers, with readings using the CIE Lab L^* , a^* and b^* colour scale. The a^* represents redness, while b^* represents yellowness.

2.7. Sensory evaluation

Sensory attributes of fried French fries, including colour, flavour, oiliness, crispiness, taste and overall quality, were evaluated using a 9-point hedonic scale where 1 = very poor and 9 = very good. The sensory was done by 10 trained panellists selected from staffs of the Malaysian Palm Oil Board (MPOB) on days 1, 3 and 5, equivalent to 8, 24 and 40 h of frying.

2.8. Statistical analysis

Each analysis was done in triplicate. The MINITAB 14 software was used to analyse data for determining ANOVA, standard deviation and Duncan's multiple range test for significance at 5% level.

3. Results

3.1. Polyphenol content and antioxidative properties

The phenolic content of *P. amaryllifolius*, after refluxing with ethanol, was found to be 102 ± 0.4 mg gallic acid equivalents/g extract ($n = 6$). The antioxidative activity of *P. amaryllifolius* extracts is lower than BHT for both (i) free radical-scavenging (DPPH \cdot method) and (ii) in the linoleic acid model system, and was concentration-dependent (Fig. 1).

3.2. Accelerated oxidation studies

3.2.1. Peroxide value, anisidine value and free fatty acid

Peroxide value represents primary reaction products of lipid oxidation, which can be measured by their ability to liberate iodine from potassium iodide. Increasing herb extract concentration significantly ($P < 0.05$) reduced oil oxidation (Fig. 2). It was generally observed that the extract lowered the peroxide value significantly ($P < 0.05$) compared to the control. Organic peroxides then decompose to secondary products, including alcohols, carboxylic acids, aldehydes and ketones, measured as anisidine value. The anisidine value was independent of the extract concentration, but was significantly different from the control (Fig. 3).

The free fatty acid value, at 0.1% extract concentration, was significantly different from 0%, 0.2%, 0.3% and 0.4% (Fig. 4).

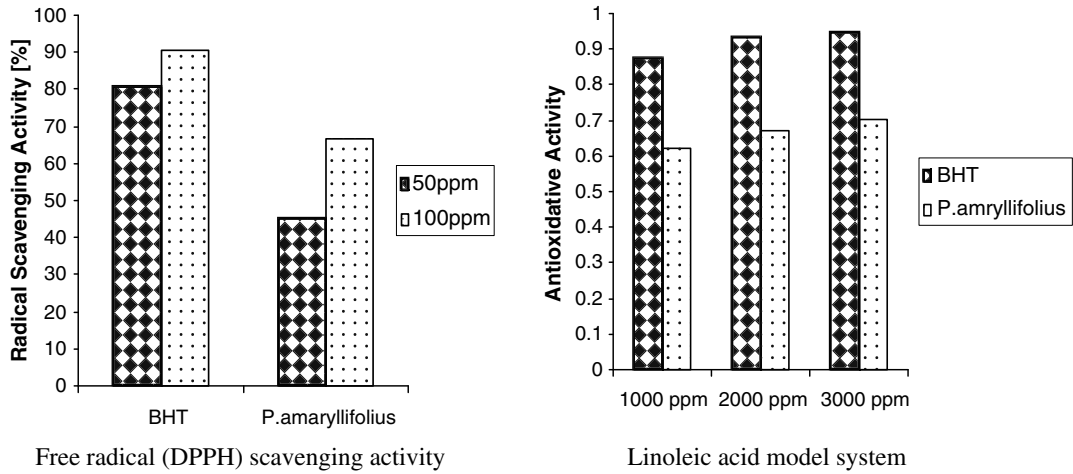


Fig. 1. Antioxidative activities of *P. amaryllifolius* extract compared to BHT.

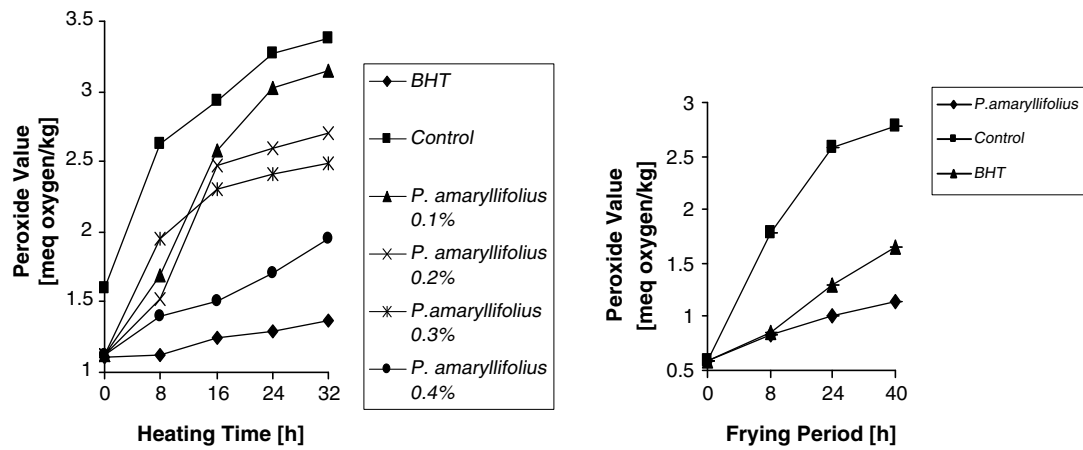


Fig. 2. Changes in peroxide value (PV) over time in RBD palm olein.

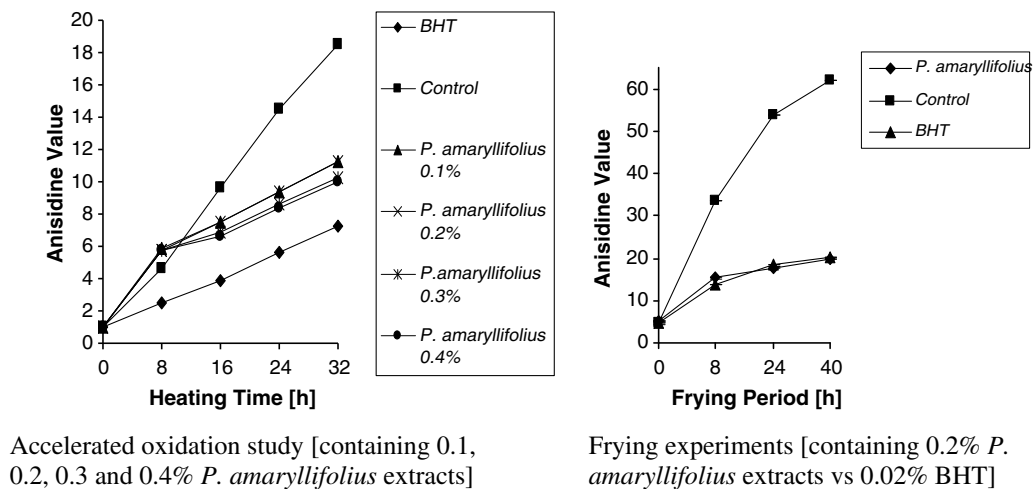


Fig. 3. Changes in anisidine value (AV) over time in RBD palm olein.

3.2.2. Oxidative stability index

The time before there is a dramatic increase in the rate of lipid oxidation is a measure of oxidative stability and is referred to as the induction period. Fig. 5 shows the OSI analysis of *P. amaryllifolius* compared to control and BHT. The herb extracts showed significantly higher OSI than the control and BHT. There was no significant difference in OSI between RBD palm olein containing 0.1 and 0.2% *P. amaryllifolius* extracts, or between 0.3% and 0.4% *P. amaryllifolius* extracts.

3.3. Frying experiments

3.3.1. Chemical analysis

Fig. 2 shows that *P. amaryllifolius* extract and BHT significantly ($P < 0.05$) lowered the peroxide formation compared to the control. The antioxidative activities of natural and synthetic antioxidants were not significantly different ($P < 0.05$) from one another from 0 to 8 h of

frying. However, after 24 h of frying, *P. amaryllifolius* significantly ($P < 0.05$) exhibited better activities than did BHT.

Fig. 3 shows that the anisidine value increased significantly ($P < 0.05$) with the time of frying. *P. amaryllifolius* extract significantly ($P < 0.05$) lowered the anisidine value during frying compared to the control. The ability to lower the anisidine value was as good as BHT.

Fig. 4 shows that BHT and *P. amaryllifolius* were significantly ($P < 0.05$) capable of lowering free fatty acid formation during the 24–40 h of frying. This indicated that the natural antioxidant was able to retard oxidation. Free fatty acid formation was low (less than 0.4% after 40 h of frying, or less than 0.25% after 32 h in the accelerated oxidation study) but increased significantly ($P < 0.05$) with frying time and storage.

Fig. 5 shows the oxidative stability index (OSI) for all samples during the 40 h of frying. Overall results suggested that both natural and synthetic antioxidants were capable

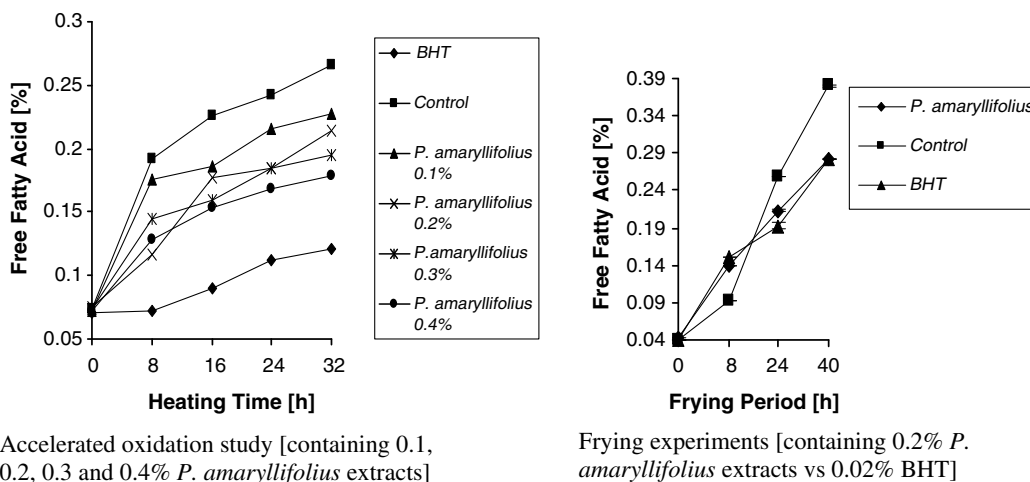


Fig. 4. Changes in free fatty acid (FFA) over time in RBD palm olein.

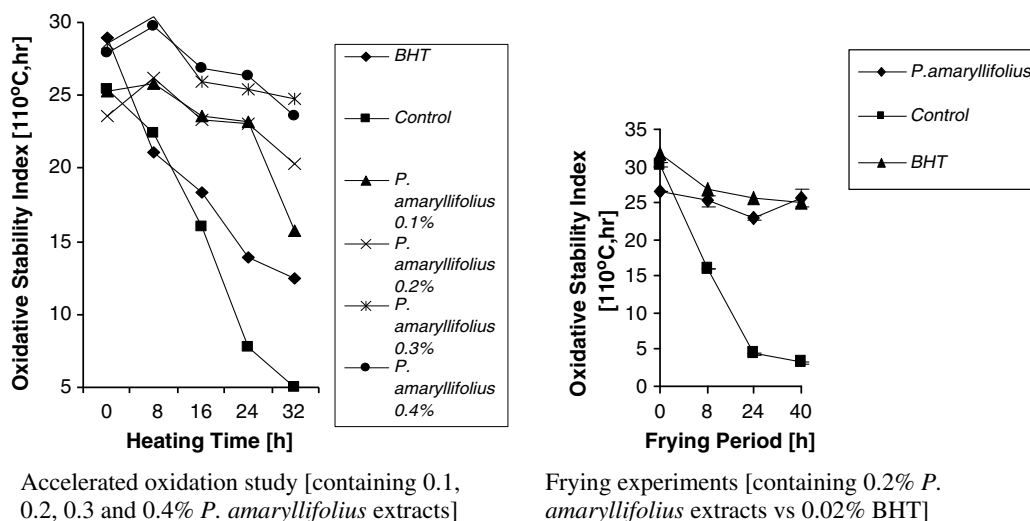


Fig. 5. Changes in oxidative stability index (OSI) over time in RBD palm olein.

of protecting the oil from further oxidation, compared to the control. *P. amaryllifolius* showed an OSI value that was comparable to BHT. It is interesting to note that, although the OSI for *P. amaryllifolius*, on day 0, was significantly lower than those for BHT and control, the OSI values for *P. amaryllifolius*-treated oil at 0, 8 and 40 h of frying were not significantly different ($P < 0.05$) from one another. This might be due to the topping up effect of oil and extract, indicating that this extract can be used in the industry as it has the capability of prolonging the shelf life of cooking oil.

Iodine value is a measure of the unsaturated linkages in fats and is expressed in terms of percentage of iodine absorbed. Fig. 6 shows that the iodine value decreased significantly ($P < 0.05$) with the time of frying for all samples. *P. amaryllifolius* and BHT could significantly ($P < 0.05$) protect the oil from further oxidation, from 24 to 40 h of frying with better protection by BHT.

Fig. 6 also shows colour changes in oil during the 40 h of frying. Darkening of oil samples occurred significantly ($P < 0.05$) for *P. amaryllifolius* throughout the study. This could be due to the presence and oxidation of phenolic

antioxidants themselves while heating. Colour of oil sample, for control and BHT, was not affected by the frying time. BHT showed significantly ($P < 0.05$) lower intensity of redness when compared to the control.

Polar compounds represent the non-volatile products that are formed during fat and oil oxidation. Fig. 7 shows that both *P. amaryllifolius* and BHT were capable of lowering percentage of polar compounds in oil during frying. The activities were not significantly different ($P < 0.05$) from 0 to 24 h of frying. The low polar compounds indicated that the oil was still of good quality.

Triglycerides in dimeric, trimeric and polymeric form are polymer compounds, and Fig. 7 shows that both natural and synthetic antioxidants were capable of lowering the percentage of polymer compounds in oil during frying. The activities were not significantly different ($P < 0.05$) from 0 to 24 h of frying, with better protection provided by BHT at 40 h of frying.

Table 1 shows the colour changes in French fries during 40 h of frying. Significant increase in French fries colour was observed for the *P. amaryllifolius*-treated sample as frying time increased. Samples fried in BHT oil were found

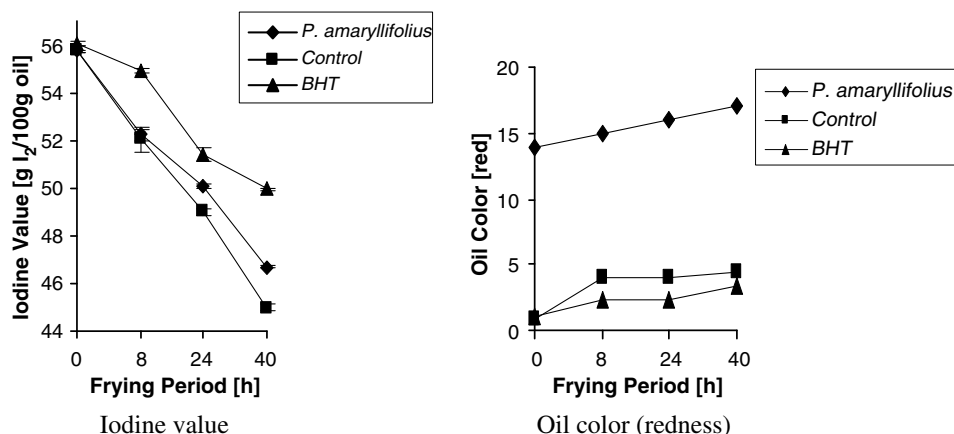


Fig. 6. Changes in iodine value and oil colour (redness) over time in frying experiments of RBD palm olein containing 0.2% *P. amaryllifolius* extracts, compared to 0.02% BHT.

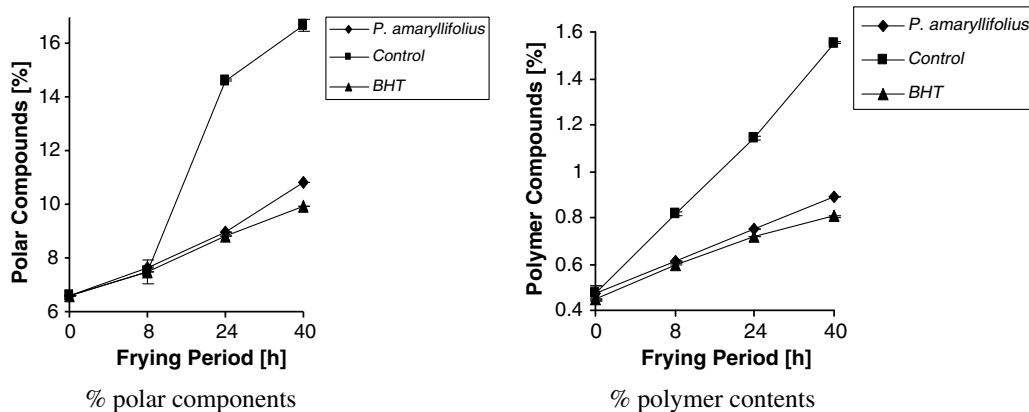


Fig. 7. Changes in % polar components and % polymer contents over time in frying experiments of RBD palm olein containing 0.2% *P. amaryllifolius* extracts, compared to 0.02% BHT.

Table 1
Effect of *P. amaryllifolius* and BHT on colour and sensory acceptability of French fries during deep-fat frying

Fries colour	<i>L</i>	<i>a</i> (+ <i>a</i> means red direction, – <i>a</i> means green direction)	<i>b</i> (+ <i>b</i> means yellow direction, – <i>b</i> means blue direction)
Pandan 8 h	66.13 ± 0.02	–2.53	23.53 ± 2.14
Pandan 24 h	69.37 ± 0.00	–0.85	25.73 ± 0.00
Pandan 40 h	70.10 ± 0.00	–2.03	25.84 ± 0.00
Control 8 h	61.56 ± 0.93	–0.45	16.16 ± 0.36
Control 24 h	60.67 ± 1.43	0.77 ± 0.20	12.92 ± 1.13
Control 40 h	58.09 ± 0.68	1.00 ± 0.09	13.80 ± 0.49
BHT 8 h	57.23 ± 1.30	–0.52	17.50 ± 1.68
BHT 24 h	62.22 ± 2.41	0.70 ± 0.16	18.58 ± 1.96
BHT 40 h	66.50 ± 0.34	0.75 ± 0.015	15.62 ± 0.32

Sensory Q ^{*,#}	Time (h)	Pandan	Control	BHT
Colour	8	5.33 ± 0.58Bb	8.00 ± 0.00Aa	8.00 ± 0.00Aa
	24	7.33 ± 0.58Aa	6.67 ± 1.16Aa	7.67 ± 0.58ABa
	40	6.00 ± 1.00ABa	6.33 ± 0.58Aa	6.00 ± 0.00Ba
Flavour	8	5.67 ± 1.15Ab	8.00 ± 0.00Aa	7.00 ± 0.00Aa
	24	6.00 ± 0.00Aa	6.67 ± 1.16Aa	7.00 ± 0.00Aa
	40	6.00 ± 0.00Aa	6.33 ± 0.58Aa	5.33 ± 0.58Aa
Oiliness	8	6.00 ± 1.00Aa	6.33 ± 0.58Aa	7.00 ± 1.00Aa
	24	6.00 ± 0.00Aa	7.00 ± 0.00Aa	6.00 ± 1.00Aa
	40	6.33 ± 1.16Aa	5.33 ± 0.58Aa	4.33 ± 0.58Aa
Crispiness	8	5.00 ± 1.00Aa	5.67 ± 1.16Aa	7.67 ± 1.16Aa
	24	5.67 ± 2.31Aa	6.33 ± 1.53Aa	6.33 ± 1.16Aa
	40	5.67 ± 1.16Aa	5.33 ± 0.58Aa	4.67 ± 0.58Aa
Taste	8	6.67 ± 0.58Aa	7.67 ± 0.58Aa	7.67 ± 0.58Aa
	24	6.67 ± 1.16Aa	7.00 ± 1.00Aa	7.00 ± 1.00Aa
	40	5.67 ± 1.16Aa	6.00 ± 0.00Aa	5.33 ± 0.58Aa
Overall quality	8	7.00 ± 0.00Ab	8.00 ± 0.00Aa	7.00 ± 1.00Aab
	24	6.33 ± 0.58Aa	7.00 ± 1.00ABa	7.00 ± 1.00Aa
	40	5.67 ± 0.58Aa	5.67 ± 0.58Ba	4.67 ± 0.58Ba

a–b, Means within a row with different small letters are significantly different ($P < 0.05$).

A–B, Means within a column with different Capital letters are significantly different ($P < 0.05$).

* Using a 9-point hedonic scale (1 = very poor and 9 = very good).

Mean ± Standard deviation from 10 trained panelists.

to be significantly different at the 40th hour. Samples fried in the control oil were not affected by the frying time, although there was a slight decrease in the colour intensity, indicating discolouration.

3.3.2. Sensory evaluation

Table 1 also shows the sensory evaluation scores at 8, 24 and 40 h for samples fried in oil containing synthetic and natural antioxidants. There were no significant differences ($P < 0.05$) in scores for oiliness, crispiness and taste observed between samples throughout the frying experiment. Evaluation of colour of French fries shows that *P. amaryllifolius* was able to improve the colour of French fries significantly ($P < 0.05$) at 24 h of frying and maintained it until the 40th hour of frying. The control fries was not significantly different ($P < 0.05$) from one another throughout the 40 h of frying. Samples fried in BHT oil showed a slight decrease in score as the hours of frying increased. Flavour scores for samples fried in *P. amaryllifolius* treated oil at 8 h were significantly lower ($P < 0.05$) than those of the control and BHT. The scores for all fries

were not affected by the increase of frying time. For overall quality, the control scored highest, followed by both synthetic and natural antioxidants. *P. amaryllifolius* extract was capable of maintaining its score as there was no significant difference ($P < 0.05$) observed with increasing frying time, unlike the control and BHT oil, where the score decreased significantly ($P < 0.05$) with the frying time. All fries were acceptable up to the 40th hour of frying.

Although *P. amaryllifolius* did not show any specific improvement of sensory score when compared to the control and BHT, it is interesting to note that the scores for colour, flavour, oiliness, crispiness, taste and overall quality were not significantly different ($P < 0.05$) throughout the 40 h of frying. This suggested that the *P. amaryllifolius* extract is capable of maintaining the quality, not only of the oil, but also that of the fried products.

4. Discussion

The development of oxidative rancidity was accelerated, so that the useful life of an oil-containing material may be

determined. From this, the effects of various levels and types of anti- or pro-oxidants could be studied. Phenolic compounds from plants are known to be good natural antioxidants. However, the activity of synthetic antioxidant was often observed to be higher than that of natural antioxidants (Ningappa, Dinesha, & Srinivas, 2007). Phenolic compounds, at certain concentrations, markedly slowed down the rate of conjugated diene formation (Chimi & Cillard, 1991). In their absence, linoleic acid concentration decreased dramatically, indicating oxidation. The antioxidant effectiveness of these compounds seemed to be related to their ability to quench peroxy radicals.

The peroxide values obtained in this study were similar to the trends of the antioxidative effect of *Phlomis* and *Stachys* species in sunflower oil, which were also concentration-dependent (Morteza-Semnani, Saeedi, & Shanani, 2006). The peroxide value decreased after some hours of heating, indicating formation of secondary oxidation products, such as ketones, aldehydes, hydrocarbons and epoxides, which could be measured using the anisidine test. Hindered phenols (caffeic acid, vanillic acid and ferrulic acid) and crude tea extract reportedly lower the peroxide value and anisidine value at 0.02% concentration in oil (Naz et al., 2005).

Iodine value decreases are indicative of decreased unsaturation during frying and are attributed to reactions involving double bonds, whether through direct interaction across the bond to form 1,2-diols or through chain reactions adjacent to the double bond to form volatile degradation products. When food is fried, water, steam and oxygen initiate the oxidation and hydrolysis degradation reactions in the frying oil. Water, a weak nucleophile, attacks the ester linkage of triacylglycerols and produces di- and monoacylglycerols, glycerol, and free fatty acids. Other plant extracts, such as rosemary and sage oleoresin, reportedly retard the release of free fatty acid in RBD palm olein at 0.4% concentration (Che Man & Jaswir, 2000).

Oxidation was slow until a point at which oxidation accelerated and became very rapid. The length of time before rapid acceleration of oxidation is the measure of the resistance to oxidation and is commonly referred to as the 'induction period, or oxidative stability index. It is interesting to note that the OSI for pandan is significantly higher than those of both control and BHT, even at 0.1% (Fig. 5). This is probably due to the greater volatility of BHT at high temperatures (Frankel, 1993). Rosemary extract at 500 ppm, similarly showed better protection of rapeseed oil in Rancimat and Oxidograph tests than did BHT at 100 ppm (Nogala-Kalucka et al., 2005).

Additives and phenolic compounds, such as catechin and composites of catechin, also caused significant improvement in the stability of peanut oil, and in palm superolein frying performance (Razali, Johari, & NorAini, 2003). Composites of several antioxidants showed synergistic effects in increasing oil stability (Kikugawa, Kinugi, & Kurochi, 1990). Interaction between the compounds and existing tocopherols and tocotrienols in RBD palm olein

may have resulted in some synergistic effect that retarded oil degradation.

Analysis of percentage polar compounds is considered to be one of the reliable indicators of the state of oil deterioration (Guttierrez, Gonzalez, & Dobarganes, 1988). On the other hand, formation of dimers and polymers depends on the oil type, frying temperature, and number of fryings (Takeoka, Full, & Lao, 1997).

The compounds that exist in leaves of, *P. amaryllifolius* showed antioxidative activities as good as BHT in certain tests, probably because of the daily topping up of oil and antioxidants. Frequent replenishment of fresh oil decreases the formation of polar compounds, diacylglycerols, and free fatty acids and increases the frying life and quality of oils (Romero, Cuesta, & Sanchez-Muniz, 1998). The increase in colour content was mainly attributed to the alpha, beta-saturated carbonyl compounds that have the ability to absorb the energy of visible light (Guttierrez et al., 1988).

This work shows another good property and potential use of *P. amaryllifolius* leaves, as an antioxidant for food and probably health.

5. Conclusion

Based on oxidative stability index, pandan extracts are capable of retarding oxidation, even at 0.1%. *P. amaryllifolius* extract was found to be capable of retarding oxidation in palm olein as effectively as BHT in tests such as PV, AV, FFA, and OSI. In sensory evaluation, the extract was able to maintain the sensory quality of French fries. Due to its easy growing requirements, it is suggested that this herb could be used and exploited, not only as flavouring, but also as a natural food antioxidant.

Acknowledgements

The authors would like to thank MPOB for financially supporting this work and the Director of MPOB for permission to publish this paper.

References

- Blois, M. S. (1958). Antioxidant determination by the use of stable free radical. *Nature*, *181*, 1199–1200.
- Busque, F., March, P. D., Figueredo, M., Font, J., & Sanfeliu, E. (2002). Total synthesis of four Pandanus alkaloids: pandamarilactonine-A and -B and their chemical precursors norpandamarilactonine-A and -B. *Tetrahedron Letters*, *43*, 5583–5585.
- Cheeptham, N., & Towers, G. H. N. (2002). Light-mediated activities of some Thai medicinal plant teas. *Fototerapia*, *73*, 651–662.
- Che Man, Y. B., & Jaswir, I. (2000). Effect of rosemary and sage extracts on frying performance of refined, bleached and deodorized (RBD) palm olein during deep-fat frying. *Food Chemistry*, *69*, 301–307.
- Chimi, H., & Cillard, J. (1991). Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidant. *Journal of American Oil Chemist Society*, *68*(5), 307–311.
- Deshpande, S. S., Deshpande, U. S., & Salunkhe, D. K. (1996). Nutritional and health aspects of food antioxidants. In *Food antioxidant, technological, toxicological, and health perspectives* (pp. 361–469). New York: Marcel Dekker Inc.

- Firestones, D. (Ed.). (1993). *Official methods and recommended practices of the American Oil Chemists' Society* (4th ed.). Champaign, IL: AOCS Press.
- Frankel, E. N. (1993). In search of better methods to evaluate natural antioxidant and oxidative stability in food lipids. *Trends in Food Science and Technology*, *11*, 20–25.
- Gutierrez, R., Gonzalez, O., & Dobarganes, M. C. (1988). Analytical procedures for the evaluation of used frying fats. In G. Varela, A. E. Bender, & I. D. Morton (Eds.), *Frying food: Principles, changes, new approaches* (pp. 141–154). London, England: VCH Publishers Ltd.
- Jaswir, I., CheMan, Y. B., & Kitts, D. D. (2000). Use of natural antioxidants in refined palm olein during repeated deep-fat frying. *Food Research International*, *33*, 501–508.
- Khan, M. A., & Shahidi, F. (2001). Effects of natural and synthetic antioxidants on the oxidative stability of borage and evening primrose triacylglycerols. *Food Chemistry*, *75*, 431–437.
- Kikugawa, K., Kinugi, A., & Kurochi, T. (1990). In B. J. F. Hudson (Ed.), *Food antioxidants* (pp. 85). Elsevier Science Publisher.
- Laksanalamai, V., & Ilangantileke, S. (1993). Comparison of aroma compound (2-acetyl-1-pyrroline) in leaves from pandan (*Pandanus amaryllifolius*) and Thai fragrant rice (Khao Dawk Mali-105). *Cereal Chemistry*, *70*, 381–384.
- Lee, B. L., Su, J., & Ong, C. N. (2004). Monomeric C18 chromatographic method for the liquid chromatographic determination of lipophilic antioxidants in plants. *Journal of Chromatography*, *1048*, 263–267.
- Lingert, H., Vallentin, K., & Eriksson, C. E. (1979). Measurement of antioxidative in model system. *Journal of Food Processing and Preservation*, *3*, 87–103.
- Matthaus, B. (2002). Antioxidant activity of extracts obtained from residues of different oil seeds. *Journal of Agricultural and Food Chemistry*, *50*, 3444–3452.
- Miean, K. H., & Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry*, *49*, 3106–3112.
- Morteza-Semnani, K., Saeedi, M., & Shanani, S. (2006). Antioxidant activity of the methanolic extracts of some species of *Phlomis* and *Stachys* on sunflower oil. *African Journal of Biotechnology*, *5*(24), 2428–2432.
- Naz, S., Siddiqi, R., Sheikh, H., & Sayeed, S. A. (2005). Deterioration of olive, corn and soybean oils due to air, light, heat and deep-frying. *Food Research International*, *38*, 127–134.
- Ningappa, M. B., Dinesha, R., Srinivas, L. (2007). Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf extract (*Murraya koenigii* L.). *Food Chemistry*, doi:10.1016/j.foodchem.2007.06.057.
- Nogala-Kalucka, M., Korczak, J., Dratwia, M., Lampart-Szczapa, E., Siger, A., & Buchowski, M. (2005). Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. *Food Chemistry*, *93*, 227–235.
- Ooi, L. S. M., Wong, E. Y. L., Sun, S. S. M., & Ooi, V. E. C. (2006). Purification and characterization of non-specific lipid transfer protein from the leaves of *Pandanus amaryllifolius* (Pandanaceae). *Peptides*, *27*, 626–632.
- Quisumbing, E. (1951). *Medicinal plants of the Philippines*. Manila: Bureau of Printing.
- Razali, I., Johari, M., & NorAini, S. (2003). Effects of additives on quality and frying performance of palm superolein during frying. *Palm Oil Developments*, *38*, 1–4.
- Romero, A., Cuesta, C., & Sanchez-Muniz, F. J. (1998). Effect of oil replenishment during deep fat frying of frozen foods in sunflower oil and high-oleic acid sunflower oil. *Journal of the American Oil Chemists Society*, *75*, 161–167.
- Shyamala, B. N., Gupta, S., Lakshmi, A. J., & Prakash, J. (2005). Leafy vegetable extracts – Antioxidant activity and effect on storage stability of heated oils. *Innovative Food Science and Emerging Technologies*, *6*, 239–245.
- Takeoka, G. R., Full, G. H., & Lao, L. T. (1997). Effect of heating on the characteristics and chemical composition of selected frying oil and fat. *Journal of Agricultural and Food Chemistry*, *45*, 3244–3249.
- Zainuddin, H. (2004). Flavonoids and volatile compounds in 29 types of tropical plants from different anatomical parts using gas chromatography–mass spectrometry (pp. 32, 33 and 57). Unpublished work. Faculty of Food Science and Technology, Universiti Putra Malaysia.