J Food Sci Technol (February 2015) 52(2):773–782
DOI 10.1007/s13197-013-1060-5

ORIGINAL ARTICLE

Polyphenolic rich traditional plants and teas improve lipid stability in food test systems

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Revised: 31 January 2013 / Accepted: 4 June 2013 / Published online: 27 June 2013

Abstract The deleterious effects of lipid autoxidation are of major concern to the food industry and can be prevented by food antioxidants. In this vein, the phenolic contents and antioxidant potential of traditional plants of Mauritius such as *P. betle* L. (Piperaceae), *M. koenigii* L. Sprengel. (Rutaceae), *O. gratissimum* L. (Lamiaceae), *O. tenuiflorum* L. (Lamiaceae), and commercially available Mauritian green and black teas were evaluated. Their ferric reducing antioxidant power (FRAP) were compared to that of butylated hydroxytoluene (BHT) with the following order of potency: BHT > "Natural" commercial green tea > "Black Label" commercial black tea > *O. gratissimum* > *P. betle* > *O. tenuiflorum* > *M. koenigii*. The trolox equivalent antioxidant capacity (TEAC) assay reflected a similar antioxidative order for BHT and "Natural" commercial green tea, with however *P. betle*, *O. tenuiflorum* and *O. gratissimum* exhibiting higher activities than "Black Label" commercial black tea and *M. koenigii*. Based on their potent antioxidant capacity, *P. betle* (0.2 % m/m) and *O. tenuiflorum* (0.2 % m/m) extracts, and green tea (0.1 % m/m) infusate were compared with BHT (0.02 % m/m) on their ability to retard lipid oxidation in unstripped sunflower oil and mayonnaise during storage at 40 °C. *P. betle* and green tea were more effective than BHT in both food systems. Moreover, odour evaluation by a sensory panel showed that the plant extracts and green tea infusate effectively delayed the development of rancid odours in unstripped sunflower oil and mayonnaise ($p<0.05$).

Keywords Lipid autoxidation · Antioxidants · Polyphenolics · Traditional plants · Tea · Sensory analysis

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Introduction

Lipids occur in practically all raw food materials, or may be added as fats to a wide range of foods as part of food formulations (Gordon 2001). However, they are prone to both hydrolytic and oxidative deterioration. Lipid oxidation, mediated by the free radical chain reaction, generates free radicals and compounds that affect the nutritional value and sensory attributes of foods namely colour, odour, flavour and texture (Hamlinton 2003). Quality deterioration due to oxidative rancidity has been reported to affect food companies' reputation thereby resulting in loss of market share and competitiveness (Lemov and Hewitt 1999). Consequently, controlling lipid autoxidation contributes to ensure food quality and safety towards meeting consumer expectations in the light of the rising demand for convenience and ready-to eat products, thereby protecting consumer health and securing brand confidence for business growth.

In food systems, antioxidants can delay the onset of oxidation through several mechanisms including scavenging of free radicals, removal and/or inactivation of pro-oxidants or quenching of secondary oxidation products (Belitz et al. 2004). Synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG)

and tert-butylhydroquinone (TBHQ) have found extensive applications in the food industry and have been effective in maintaining food quality (Shahidi 2008). In recent years, the gradual shift from synthetic to natural antioxidants has been elicited by toxicological reports highlighting the potential carcinogenic effects of BHA and BHT (Porkorny and Korczak 2001). Moreover, the relative high volatility and thermal instability of BHA and BHT at high temperatures, as well as increasing consumer demand for natural products, have prompted research and application of natural antioxidants as alternative food additives in new food product development.

The search for natural antioxidant sources and the evaluation of their activity in different multiphase systems is thus of major interest (Gordon 2001; Jacobsen et al. 2008). The antioxidant importance of aqueous extracts of traditional plants like *Piper betle, Murraya koenigii*, *Ocimum tenuiflorum* and *Ocimum gratissimum* has been expounded in recent investigations (Arambewala et al. 2006; Gupta et al. 2009; Charles 2012). In Mauritius, they have been used as phytotherapeutics (Gurib-Fakim 2002). There is still however, insufficient data on their phytophenolic profile and antioxidant potential as opposed to the Mauritian teas which have been profoundly studied (Luximon-Ramma et al. 2005, 2006; Bahorun et al. 2010, 2012).

These plants therefore represent novel potential antioxidant sources that need to be evaluated in real food systems. Moreover, despite the comprehensive research on the phenolic, antioxidant profiles and clinical effects of Mauritian tea, the latter's contribution as effective antioxidants in food systems is still unclear and therefore warrants in depth attention. As such, it is suggested that a multi-dimensional approach is best suited to efficiently evaluate the antioxidant propensity and protective influence of natural antioxidants (Frankel and Meyer 2000). Hence, this study aims at evaluating the antioxidant activity of aqueous extracts of Mauritian *P. betle, M. koenigii*, *O. tenuiflorum*, *O. gratissimum* and tea infusates, using the FRAP and TEAC assay. Based on their potent antioxidant capacity, *P. betle, O. tenuiflorum* and "Natural" commercial green tea extracts were selected and further examined for their ability to retard lipid oxidation in unstripped sunflower oil and mayonnaise during storage at 40 °C.

Materials and methods

Samples and reagents

P. betle, O. tenuiflorum, *O. gratissimum* were collected from Vacoas (Central Mauritius) and *M. koenigii* from Grand Baie (North coast of Mauritius). Plant materials were identified and authenticated by the Herbarium of the Mauritius Sugar Research Institute, Mauritius. "Natural" commercial green and "Black Label" black teas were from Bois Chéri Tea Factory of Mauritius. Briefly, "Natural" commercial green tea was produced as follows: freshly harvested green tea leaves underwent panning in rolling drum to stop leaf oxidation. Rolling and drying were then carried out to prepare the green tea powder which was made into tea bags. Black tea, on the other hand, was obtained after complete fermentation at high humidity (95–98 %) and a temperature of 25–30 °C prior to rolling and drying. Ingredients for mayonnaise preparation, namely sugar, table salt, eggs, sunflower oil, vinegar, and processed lemon juice concentrate were purchased from the local hyper-market. Gallic acid, quercetin and trolox were of analytical grade. Cyanidin chloride was of HPLC grade, BHT and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) salt were obtained from Sigma Chemicals (UK). Folin-ciocalteau reagent and TPTZ were purchased from SD Fine-Chem limited (India) and aluminium chloride from Techno Pharmchem (India). All the other reagents used for analysis were of analytical grade.

Preparation of aqueous plant extracts and tea infusates

Plant extracts

Mature leaves of the plants were air-dried in the shade for 2 days. The extraction procedure was adapted from Arambewala et al. (2006). Briefly, 500 g of leaves were cut in small pieces and added to 2.5 L of boiled distilled water. This mixture was allowed to simmer on low heat for 1 h. The extraction temperature was kept between 85 °C to 95 °C and the extraction mixture was filtered after 1 h. The yields obtained for the extracts were: *P. betle*-76.8 %; *O. tenuiflorum*-76.9 %; *O. gratissimum*-76.4 % and *M. koenigii*-64.0 % and were calculated as follows: (Initial concentration(g/mL)/Final concentration(g/mL))∗100.

Green and black tea infusates

Tea infusates were freshly prepared on the same day of purchase. 200 mL of boiling distilled water was added to each green or black tea bag in a conical flask and covered with a petri-plate. The infusate was filtered after 5 min.

Methanolic plant/tea/BHT extracts

One fourth of the total volume of aqueous plant extracts/tea infusate was freeze-dried at −20 °C. The freeze-dried powder was dissolved in methanol to give a ratio of 1 g fresh weight per 5 mL methanol. The methanolic plant/tea extracts were used for total flavonoids and total proanthocyanidins assays. BHT was dissolved in methanol for the analyses. The concentration of BHT in the food systems was 0.02 % which is the maximum permissible level used either individually or in combination (Shahidi and Zhong 2005).

Total phenolics

Total phenolic content was determined by the method adapted from Singleton and Rossi (1965). To each tube, 0.25 ml of the extract was added followed by 3.5 ml of distilled water and 0.25 ml of Folin-Ciocalteu reagent. After 3 min, 1 ml of 20 % sodium carbonate was added. The tubes were mixed thoroughly and heated at 40 °C for 40 min. The blue coloration was read at 685 nm against a blank standard. Results were expressed in mg of gallic acid equivalent (GAE)/g dry weight (DW) for plant extracts and tea infusates.

Total flavonoids

The $AlCl_3$ method (Lamaison and Carnet 1990) was used for total flavonoids determination of methanolic plant and tea extracts. 0.5 mL of a solution of 2 % methanolic $AlCl_3.6H_2O$ was added to equal volumes of methanolic extract. The tubes were vortexed and the absorbance was read at 440 nm after 10 min incubation at room temperature. Flavonoids were expressed in mg of quercetin equivalent (QE)/g DW for plant extracts and tea infusates.

Total proanthocyanidins

The HCl/Butan-1-ol assay, adapted from Porter et al. (1986) was used for the quantification of total proanthocyanidins. 3 mL of n-BuOH/HCl solution (95:5, v/v) was added to 0.125 μL of methanolic extract in capped test tubes, followed by 100 μL of a 2 % solution of $NH_4Fe(SO_4)_2.12H_2O$ in 2 M HCl. The tubes were incubated at 95 °C for 40 min and the absorbance of the red coloration was read at 550 nm against a blank standard after cooling. The proanthocyanidin content is reflected by the difference in the absorbance obtained prior to and after hydrolysis, and was expressed in mg of cyanidin chloride equivalent (CCE)/g DW for plant extracts and tea infusates.

Measurement of antioxidant activity

FRAP assay

The ferric reducing antioxidant power (FRAP) assay was carried out as recommended by Benzie and Strain (1996). The FRAP reagent was prepared by mixing 10 mM of 2,4,6-tripyrdyl-5-triazine (TPTZ) in 40 mM HCL and 20 mM $FeCl_3$ in 0.25 M acetate buffer of pH 3.6. The reagent was incubated at 37 °C for 4 min. The reaction mixture was prepared by adding 100 μl of diluted extract to 3 ml of distilled water, followed by 3 ml of FRAP reagent. The absorbance was read at 593 nm after incubation at room temperature for 4 min. Results were expressed as μmol Fe^{2+}/g DW for plant extracts and tea infusates.

TEAC assay

The trolox equivalent antioxidant capacity (TEAC) of the plant and tea extracts was measured in terms of their radical-scavenging ability according to the method of Campos and Lissi (1997). ABTS radical cation was prepared by reacting 0.5 mM 2,2-azino-bis (3-ethyl-benzythiazoline-6-sulphonic acid) diammonium salt (ABTS) with activated 1 mM MnO_2 in 0.1 M phosphate buffer of pH 7. After vigourous shaking for 15 min, the solution was filtered and the absorbance was adjusted to 0.500±0.010 at 734 nm. 0.5 ml of diluted extract was added to 3 ml $ABTS^{\cdot+}$ solution. The decay in absorbance was monitored for 15 min at 734 nm. Results were expressed as μmol Trolox/g DW for plant extracts and tea infusates.

Assessment of antioxidant activity in unstripped sunflower oil and mayonnaise

Preparation of methanolic solution of plant extracts and green tea infusate

Plant extracts/tea infusate were dissolved in methanol as the latter, is miscible in oil and ensures homogeneity in both unstripped sunflower oil and mayonnaise systems. The following methanolic solutions were prepared by dissolving the freeze-dried powder or the synthetic antioxidant in 100 % methanol: *Piper betle* extract (10 % mass/volume (m/v)); *Ocimum tenuiflorum* extract (10 % m/v); green tea infusate (5 % m/v); butylated hydroxytoluene, BHT (1 % m/v). Freeze-dried extract powder used in low amounts were readily soluble in methanol. Centrifugation at 3,000 rpm for 5 min was necessary only for methanolic extracts with slight turbidity.

Preparation of unstripped sunflower oil samples

48.01 g of unstripped sunflower oil was placed in clean and dry glass pot of 100 mL. 960 μL of methanolic solution of BHT or aqueous plant extract or green tea infusate was added to sunflower oil and stirred thoroughly to obtain 0.02 % mass/mass (m/m) BHT, 0.2 % (m/m) plant extracts and 0.1 % (m/m) green tea infusate concentration in the sunflower oil samples. The sunflower oil samples were prepared in triplicates. 2.5 g of each oil sample was transferred to a 15 mL graduated centrifuge tube and labelled as day 0 samples and then frozen at −45 °C. The remaining oil was covered and kept in the dark at 40 °C in an incubator.

Preparation of mayonnaise samples

The method used to prepare one batch of mayonnaise was adapted from Jacobsen and Meyer (1999). 2.88 mL of

methanolic solution of BHT or aqueous plant extracts or green tea infusate was added to sunflower oil and stirred thoroughly to obtain 0.02 % (m/m) BHT, 0.2 % (m/m) plant extracts and 0.1 % (m/m) green tea infusate concentration in the mayonnaise samples. 74.7 % (m/m) sunflower oil was incorporated into the 21.7 % (m/m) egg yolk, 30 drops at a time using a pasteur pipette, while mixing homogenously with an electric hand beater (model: Philips HR 1456, China), at high speed for 8 min. 7.0 % (m/m) distilled water, 0.1 % (m/m) potassium sorbate, 1.0 % (m/m) unsweetened lemon juice, 3.4 % (m/m) white vinegar (3^0 Spirit), 0.8 % (m/m) refined sugar and 0.3 % (m/m) refined salt were mixed and then added to the oil and egg yolk mixture, while mixing for another 1 min. The mayonnaise samples were prepared in triplicates by distributing each batch of mayonnaise equally among three pots. The samples were covered and kept in the dark at 40 °C in an incubator.

Chemical Analysis of unstripped sunflower oil and mayonnaise samples during storage at 40 °C

During storage at 40 °C, sunflower oil and mayonnaise samples were stirred at regular intervals. 2.5 g of each of the oil samples and 5 g of each of the mayonnaise samples were transferred to 15 mL graduated centrifuge tubes. These centrifuged tubes were stored at −45 °C until analysed. Prior to chemical analysis, the oil and mayonnaise samples were thawed at room temperature. Mayonnaise samples were centrifuged at 3,600 rpm for 3 min at 10 °C, and the supernatant oil fraction was separated. The oil samples or isolated oil fractions from mayonnaise samples were used for peroxide value (PV) (AOCS 1997a) and conjugated diene value (CD) (AOCS 1997b).

Odour assessment of unstripped sunflower oil and mayonnaise samples

Odour assessment was carried out on one replicate of sunflower oil or mayonnaise samples. 2.5 g of sunflower oil sample or 4 g of mayonnaise sample was transferred to a 15 mL plastic container with cover, and stored at 5 °C prior to odour assessment.

Recruitment, screening and training of assessors

The recruitment, screening and training of assessors was implemented according to the guidelines of the standard ISO 8586–1 (ISO 1993).

Recruitment of potential assessors Twenty-one undergraduate Food Science students completed a questionnaire designed to gather the following information: health status; degree of liking and frequency of consumption of mayonnaise; past experience in sensory analysis; understanding of relevant sensory descriptors; knowledge of quality characteristics of mayonnaise; motivation and availability to participate in the research project.

Screening of potential assessors Based on the information provided in the recruitment questionnaire, 14 students were shortlisted for the odour recognition test to evaluate their ability to detect and recognise specific odours. The students were asked to describe the odour of 10 samples coded with 3-digit random numbers. The samples were: fresh mayonnaise; rancid mayonnaise; fresh ground coconut kernel; rancid ground coconut kernel; fresh coconut oil; rancid coconut oil; fresh sunflower oil; vinegar; freshly squeezed lemon juice and raw egg. All the ingredients used to prepare these samples were standardised products from the market.

Development of vocabulary and training of assessors Seven assessors were selected according to their odour recognition test performance. They were trained to further develop their ability to perceive the rancid odour consistently. The training involved detection practice and rancid odour measurement using a five-point category scale with score range: 1-Not rancid; 2-Slightly rancid; 3-Moderately rancid; 4-Very rancid; 5-Extremely rancid. Reference samples for mayonnaise and sunflower oil, with different levels of rancidity were provided to develop the assessors' ability to discriminate small differences in rancid odour intensity. The reference samples for mayonnaise were: freshly prepared (Not rancid); 5 days in incubator at 40 °C (Slightly rancid); 7 days in incubator at 40 °C (Moderately rancid) and 14 days in incubator at 40 °C (Extremely rancid). The reference samples for sunflower oil were: unheated (Not rancid); 7 days in incubator at 40 °C (Moderately rancid) and 14 days in incubator at 40 °C (Extremely rancid). For mayonnaise samples, the assessors were carefully trained to assess rancidity, independently of egg and vinegar odours. There was adequate interaction and discussion with the assessors during training to ensure objective and consistent responses.

Odour evaluation of unstripped sunflower oil and mayonnaise samples during storage at 40 °C

On each sampling day, 2.5 g of sunflower oil or 4 g of mayonnaise were respectively transferred to plastic containers with covers coded with 3-digit random numbers. The order of presentation for sunflower oil or mayonnaise samples to assessors was randomised. The assessors were separated in booths and were asked to rate the rancidity level in each sample, using a five-point category scale. The assessors were instructed to take short sniffs while assessing rancid odour intensity in the samples provided. Reference samples were provided to assessors during the evaluation of test samples, to

remind them of the rancid odour intensity associated with specific points on the category scale.

Data analysis

The mean and standard deviation for phenolics, FRAP, TEAC, PV and CD values were computed using Microsoft Excel (2007). Statistical analysis was carried out using one-way ANOVA, followed by LSD test at 5 % to assess significant differences among plant extracts and tea infusates. Time-taken to reach a peroxide value of 10 meq/kg was calculated from exponential curves generated using Microsoft Excel. Mean number of days for triplicates was analysed by one-way ANOVA and LSD at 5 %. Scatter diagrams with linear trendlines were generated and the mean gradient for triplicates was analysed by one-way ANOVA and LSD at 5 %.

Results and discussion

In this study, hot water plant extracts were prepared according to traditional usage while teas were infused as per common practice. The total phenolic content varied widely for plant extracts and tea infusates (Table 1) and significant differences in phenolic content were observed between them ($p<0.05$). The tea infusates showed the most important levels, with green tea having a higher total phenolic content than black tea. This was found to be consistent with observations from previous studies assessing phenolic subclasses and antioxidant propensities of commercially available Mauritian teas (Luximon-Ramma et al. 2005, 2006).

In the present investigation, the relatively higher phenolic content observed in the tea infusates compared to the plant extracts is attributed to the concentrated nature of the teas, prepared from dried tea leaves, unlike the plant extracts which were from fresh leaves. Amongst the plant extracts, highest total phenolics were measured in *P. betle* and lowest in *O. tenuiflorum*. The total phenolic content of *O. gratissimum* reported by Hakkim et al. (2008) (168.2±3.2 mg GAE/g dry weight) was much higher than the value reported in this study. Variations in climatic conditions and soil composition generally affect the biosynthesis of phenolics and their composition in plant organs (Heldt 2005). Furthermore, the choice of solvents and extraction conditions also influence measured phenolic levels (Bahorun et al. 2013).

Flavonoids were present in all the extracts (Table 1). High amounts occurred in *O. tenuiflorum* and black tea while moderate concentrations were measured in green tea, *O. gratissimum* and *M. koenigii*. Flavonoids were found to be the predominant class of polyphenols in both *Ocimum* species. The levels in *O. tenuiflorum* leaves were higher than values reported by Aqil et al. (2006) in the same organs. Compared to the other extracts, *P. betle* had the lowest flavonoid content ($p<0.05$). The plant extracts were generally poorer in proanthocyanidins compared to the tea infusates which had higher amounts in green tea (Table 1). However, the observed concentration in black tea was much lower than the corresponding data published by Luximon-Ramma et al. (2005), who also reported green tea to be richer than black tea.

Plant extracts, tea infusates and BHT showed significant antioxidant activity ($p<0.05$) as evidenced by their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion in the FRAP assay system (Table 2). The highest antioxidant activity was observed for BHT, followed by green and black tea. *O. gratissimum* was more potent than *P. betle, O. tenuiflorum* and *M. koenigii* in the FRAP assay ($p<0.05$) (Table 2). The highest TEAC activity was observed in green tea followed by *P. betle* and *O. tenuiflorum* extracts while the lowest activity was noted in *M. koenigii*. The TEAC values recorded for green and black tea extracts were comparable to those published by Luximon-Ramma et al. (2005, 2006) on Mauritian green tea leaves and black teas. The strong antioxidant activities of green tea have been largely attributed to the high

Table 1 Total phenol, total flavonoid and total proanthocyanidin content of plant extracts and tea infusates

Plant extracts/Tea infusates	[I,*]Total Phenol (mg GAE/g DW)	[II,*]Total Flavonoid (mg QE/g DW)	[III,*]Total Proanthocyanidin (mg CCE/g DW)
Piper betle	50.0±0.09[d]	9.4±0.04[f]	0.05±0.001[d]
Murraya koenigii	24.5±0.04[f]	59.1±0.17[e]	0.14±0.002[c]
Ocimum gratissimum	32.7±0.10[e]	69.1±0.22[d]	0.02±0.004[f]
Ocimum tenuiflorum	59.4±0.07[c]	189.3±0.22[a]	0.04±0.002[e]
"Natural" commercial green tea	300.0±0.23[a]	80.2±0.24[c]	2.1±0.02[a]
"Black Label" commercial black tea	72.5±0.10[b]	148.8±0.21[b]	1.9±0.02[b]

DW dry weight, *GAE* Gallic acid equivalent, *QC* Quercetin equivalent, *CCE* Cyanidin chloride equivalent;

[I] LSD = 0.212, [II] LSD = 0.348, [III] LSD = 0.020, at 5 % significance;

[a,b,c,d,e,f] Different superscripts within columns represent significant differences among the extracts ($p<0.05$)

*Data are expressed as mean ± standard deviation, ($n=3$)

Table 2 FRAP and TEAC profile of plant extracts, tea infusates and BHT

Plant extracts	[I,*]FRAP (μmol Fe^{2+}/g DW)	[II,*]TEAC (μmol Trolox/g DW)
Piper betle	496.0±0.63[e]	815.2±4.18[c]
Murraya koenigii	263.4±0.39[g]	173.3±0.67[g]
Ocimum gratissimum	670.9±3.69[d]	512.8±5.20[e]
Ocimum tenuiflorum	294.2±0.73[f]	597.7±12.59[d]
"Natural" commercial green tea	2084.2±2.40[b]	1024.7±8.78[b]
"Black Label" commercial black tea	910.7±1.30[c]	444.4±2.06[f]
BHT	4107.5±7.73[a]	122,222.2±0.00[a]

DW dry weight, *FRAP* Ferric Reducing Antioxidant Power, *TEAC* Trolox Equivalent Antioxidant Capacity;

[a,b,c,d,e,f,g] Different superscripts within columns represent significant differences among the extracts ($p<0.05$)

[I] LSD = 5.988, [II] LSD = 11.174, at 5 % significance;

*Data are expressed as mean ± standard deviation, ($n=3$)

contents of flavan-3-ol derivatives and gallic acid (Kang et al. 2010). Green tea contains more catechins than black tea since catechins are usually altered during fermentation through oxidation to form theaflavins and thearubigins. Furthermore, the fermentation process also increases the liberation of gallic acid from catechin gallates as indicated by the high levels of this acid in the Mauritian black teas (Luximon-Ramma et al. 2005).

BHT, *P. betle* and *O. tenuiforum* extracts showed high trolox equivalent antioxidant activity (TEAC) but lower ferric reducing potential (FRAP). The reverse trend was noted for green and black teas, *O. gratissimum* and *M. koenigii*. Literature reports ascribe these differences to the varied types and nature of phytochemicals present in complex extracts, which influence antioxidant efficacies in different assay systems based on the mechanisms underlying the test systems (Yanishlieva-Maslarova 2001). Furthermore, previous authors have discussed that polyphenolic rich plant extracts seem to express their TEAC potency in terms of hydrogen-donation, a property inherent of phenolic compounds (Bors et al. 1990).

Whilst the antioxidant efficacy of the extracts in non-food assay systems was clearly demonstrated, the assessment of the antioxidant activity of *P. betle* and *O. tenuiflorum* extracts and green tea infusate in unstripped sunflower oil and mayonnaise revealed their potency to inhibit hydroperoxide formation during storage at 40 °C in oil and emulsion systems. The early stage of lipid oxidation is characterised by the rate of hydroperoxide formation exceeding that of hydroperoxide decomposition (Gordon 2001). In fact, the peroxide value peaks during the propagation phase of lipid autoxidation and then decreases during the termination phase when the rate of hydroperoxide decomposition is higher than that of formation (Laguerre et al. 2007).

The maximum permissible peroxide value (PV) for refined oils is 10 meq/kg (Codex Alimentarius Commission 2011). All the unstripped sunflower oil samples were characterised by a rise in PV, as from day 2 onwards, with increasing storage time (Fig. 1). Control oil sample had reached a PV greater than 20 meq/Kg by day 6 while the oil sample containing *P. betle* extract had a PV less than 10 meq/Kg. The oil samples with BHT, plant extracts or tea infusate had lower PV than control oil sample, throughout storage. Figure 1 expounds on the differences in both the rate and extent of increase in PV, for the different treatments. The slowest increase in PV was noted for *P. betle*, followed by green tea. As from day 4, a greater rise in PV was observed for both BHT and *O. tenuiflorum* extract, compared to the *P. betle* extract and green tea infusate.

The highest mean number of days to attain a PV of 10-meq/Kg was obtained for oil sample with 0.2 % *P. betle* extract (Table 3). Both the oil samples with *P. betle* (0.2 %) extract and green tea (0.1 %) infusate took a longer time than BHT (0.02 %) to reach a PV of 10 meq/Kg. Similarly, the PV of all mayonnaise samples increased with increasing storage time (Fig. 1). By day 6, the control-mayonnaise sample, had reached a PV greater than 20 meq/Kg. Furthermore, the PV values of mayonnaise samples containing *P. betle* or green tea were still below 20 meq/Kg, by the end of storage period. The control-mayonnaise sample showed a drastic increase in PV from day 9 to day 13 (Fig. 1). Mayonnaise with *P. betle* took the highest mean number of days to achieve a PV of 10 meq/Kg, which was about thrice the time taken by the control mayonnaise sample ($p<0.05$) (Table 3).

It is reported that over 90 % of hydroperoxides formed as a result of lipid oxidation, undergo a stabilisation of the radical state by double bond rearrangement to give a conjugated diene system (Laguerre et al. 2007). As such, a sharp rise in conjugated diene value (CD) was observed in the control sample for both sunflower oil and mayonnaise after 2 days storage at 40 °C (Fig. 1). The lowest rise in CD was exhibited by *P. betle* oil extract, as evidenced by the lowest mean slope value by the end of storage time. The mean CD for both *P. betle* and green tea were more or less stable over the 15 day period. For the mayonnaise samples, a greater rise was observed for sample with BHT, compared to those containing green tea and *P. betle* (Fig. 1).

P. betle extract offered very good protection against lipid autoxidation and was more effective than green tea infusate and BHT (0.02 %) in both food systems. *O. tenuiflorum* (0.2 %) extract, though not as potent as *P. betle* and green tea infusate, also conferred protection by delaying lipid autoxidation. The exact protective antioxidant mechanism warrants an in depth study. The polar paradox theory has highlighted that hydrophilic antioxidants are more effective

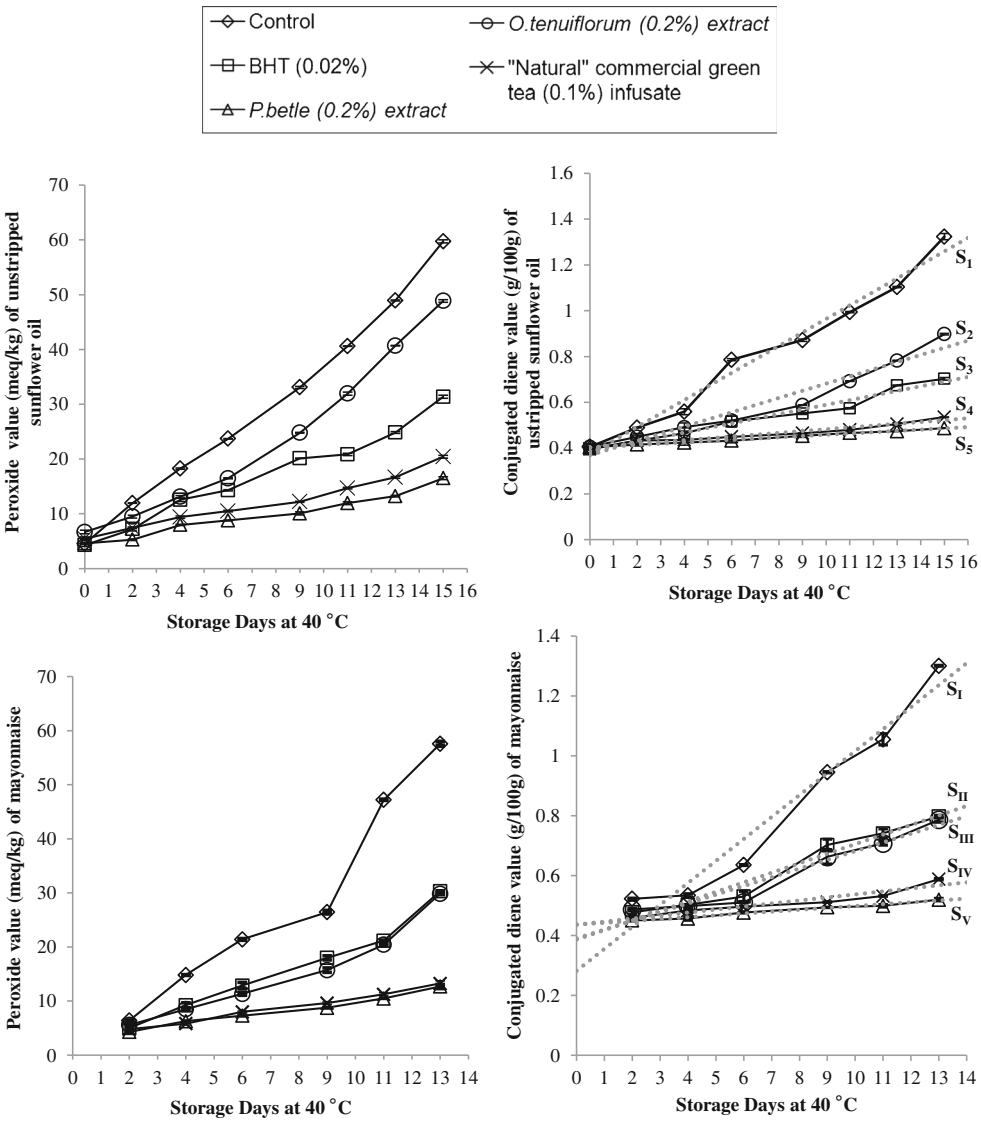

Fig. 1 The effect of *P. betle*, *O. tenuiflorum* extracts, "Natural" commercial green tea infusate and BHT on oxidation of unstripped sunflower oil and mayonnaise samples, as assessed by peroxide value and conjugated diene value (n=3)

S_1 (Control-Slope$_1$)=0.059^a ± 0.0006;
S_2 (*O. tenuiflorum*-Slope$_2$)=0.031^b ± 0.0018;
S_3 (BHT-Slope$_3$)=0.020^c ± 0.0003;
S_4 ("Natural" commercial green tea -Slope$_4$)=0.008^d ±0.0002;
S_5 (*P. betle*-Slope$_5$)=0.006^e ± 0.0001; LSD= 8.45 x 10^{-4} at 5% level significance; Different superscripts on the mean slope values represent significant differences in the rates of increase in conjugated diene value for the treatments.

S_I (Control-Slope$_1$)= 0.073^a ± 0.0006;
S_{II} (BHT-Slope$_2$)= 0.032^b ± 0.0009;
S_{III} (*O. tenuiflorum*-Slope$_3$)= 0.029^c ± 0.0011;
S_{IV} ("Natural" commercial green tea -Slope$_4$)=0.010^d ±0.0003;
S_V (*P. betle*-Slope$_5$)=0.006^e ± 0.0007; LSD= 1.40 x 10^{-3} at 5% level significance; Different superscripts on the mean slope values represent significant differences in the rates of increase in conjugated diene value for the treatments.

in bulk oils than in emulsion systems, and confer effective protection against lipid oxidation by orienting themselves at the oil–air interfaces (Porter 1980; Porter 1993). In this study, water was the solvent used for the preparation of *P. betle* extract which was expected to contain a majority of polar compounds potentially responsible for the effective retardation of lipid oxidation in the unstripped sunflower oil.

Roedig-Penman and Gordon (1997) assessed the antioxidant properties of catechins and green tea extracts in emulsions prepared from unstripped sunflower oil, during prolonged storage (40 days) at 30 °C where the tea extract (0.03 %) was found to have similar antioxidant properties as BHT (0.02 %). In the present study, green tea infusate (0.1 %) was found to be more effective than BHT in retarding autoxidation in both food systems during storage at 40 °C. The antioxidant activity of green tea in emulsions has been ascribed to several components, namely less polar catechins like EGC and EC, flavonols like myricetin (Roedig-Penman and Gordon 1997). Moreover, oxidation products of EGCG, flavonol glycosides and proanthocyanidin dimers, have also

Table 3 Time (Mean number of days) taken for unstripped sunflower oil and mayonnaise samples to reach a peroxide value (PV) of 10 meq/Kg during storage at 40 °C

	[I,*]Unstripped sunflower oil	[II,*]Mayonnaise
Control	1.8±0.08[e]	3.0±0.01[e]
BHT-0.02 %	4.5±0.16[c]	5.3±0.19[d]
P. betle extract-0.2 %	8.8±0.19[a]	10.2±0.10[a]
O. tenuiflorum extract-0.2 %	2.4±0.20[d]	5.6±0.13[c]
"Natural" commercial green tea infusate-0.1 %	6.1±0.21[b]	9.6±0.15[b]

[a,b,c,d,e] Different superscripts within the columns represent significant differences between the samples

[I] LSD = 0.316, [II] LSD = 0.241 at 5 % level significance;

*Data in table represents mean ± standard deviation (n=3)

been identified as contributing factors (Luximon-Ramma et al. 2005). The antioxidant activity of the green tea extract in unstripped sunflower oil, is more likely to be due to the synergistic effects of more polar catechins namely ECG, EGCG, together with EGC and EC which have been shown to effectively inhibit oxidation in bulk oils (Wanasundara and Shahidi 1996).

Conversely to the polar paradox theory, *P. betle* (0.2 %) was also found to be equally effective in inhibiting oxidation in mayonnaise. A number of factors including the nature and characteristics of the emulsifying agent, chemical and physico–chemical composition of both aqueous and lipid phases as well as extrinsic factors like temperature and pressure have been reported to affect antioxidant activity in emulsions (Di Mattia et al. 2009; Mei et al. 1999; Coupland and McClements 1996; Mancuso et al. 1999). In the current investigation, the chemical composition of mayonnaise might have influenced the observed overall antioxidant activity as constituents like lemon juice contribute to the presence of citric acid and ascorbic acid, which are chain-breaking antioxidants, and subsequently leading to possible synergistic reactions (Hudson 1990).

Despite a significantly higher phenol content, green tea infusate showed a lower antioxidant activity than *P. betle* extract in the food systems studied. This advocates that antioxidant efficacy not only depends on the phenolic content but is also strongly mediated by the mechanism of action of the antioxidant within the food system (Laguerre et al. 2007). Moreover, in multiphase food systems, polarity is not the sole parameter in determining efficiency of natural antioxidants in emulsions. Other complex factors are believed to be involved in antioxidant behaviour of compounds with different polarity and the polar paradox phenomenon may be applicable only over certain concentration ranges (Shahidi and Zhong 2011). In addition to the concentration of the phenolic compounds in the food system, their structure and their rapidity in donating a hydrogen atom can significantly affect their protective role as antioxidants against lipid autoxidation (Di Mattia et al. 2009; Zhong and Shahidi 2012).

Table 4 Sensory rancid odour scores of unstripped sunflower oil and mayonnaise samples during storage at 40 °C

	[*] Storage period (Days)							
Unstripped sunflower oil	0	2	4	[I]6	[II]9	[III]11	[IV]13	[V]15
Control	1.0±0.00	1.0±0.00	1.6±0.53	2.7±0.49[a]	3.1±0.38[a]	3.7±0.49[a]	4.7±0.49[a]	4.9±0.38[a]
BHT (0.02 %)	1.0±0.00	1.0±0.00	1.1±0.38	1.3±0.49[b]	1.4±0.53[b]	1.9±0.38[c]	2.4±0.53[c]	2.9±0.38[c]
P. betle extract (0.2 %)	1.0±0.00	1.0±0.00	1.1±0.38	1.1±0.38[b]	1.1±0.38[b]	1.1±0.38[d]	1.1±0.38[e]	1.3±0.49[e]
O. tenuiflorum extract (0.2 %)	1.0±0.00	1.0±0.00	1.1±0.38	2.3±0.49[a]	1.7±0.49[b]	2.7±0.49[b]	3.1±0.38[b]	3.7±0.49[b]
"Natural" commercial green tea infusate (0.1 %)	1.0±0.00	1.0±0.00	1.1±0.38	1.3±0.49[b]	1.7±0.76[b]	1.3±0.49[d]	1.7±0.49[d]	2.1±0.38[d]
Mayonnaise	0	2	4	[VI]6	[VII]9	[VIII]11	[IX]13	15
Control	ND	1.0±0.00	1.3±0.49	2.4±0.53[a1]	3.4±0.53[a1]	3.7±0.49[a1]	4.9±0.38[a1]	ND
BHT (0.02 %)		1.0±0.00	1.1±0.38	1.1±0.38[b1]	1.6±0.53[b1]	2.3±0.49[b1]	2.7±0.49[b1]	
P. betle extract (0.2 %)		1.0±0.00	1.3±0.76	1.3±0.76[b1]	1.1±0.38[b1]	1.1±0.38[c1]	1.1±0.38[c1]	
O. tenuiflorum extract (0.2 %)		1.0±0.00	1.1±0.38	1.1±0.38[b1]	1.4±0.53[b1]	2.3±0.49[b1]	2.4±0.53[b1]	
"Natural" commercial green tea infusate (0.1 %)		1.0±0.00	1.1±0.38	1.1±0.38[b1]	1.1±0.38[b1]	1.1±0.38[c1]	1.3±0.49[c1]	

Score Range: 1-Not rancid, 2-Slightly rancid, 3-Moderately rancid, 4-Very rancid, 5-Extremely rancid

ND not determined

[a,b,c,d,e,a1,b1,c1] Common superscripts within columns represent no significant differences between samples

LSD at 5 % was carried out where ANOVA, showed significant differences: Unstripped sunflower oil (storage period: 0–15): [I] LSD = 0.646, [II] LSD = 0.740, [III] LSD = 0.540, [IV] LSD = 0.540, [V] LSD = 0.510;

Mayonnaise (storage period: 2–13): [VI] LSD = 0.627, [VII] LSD = 0.640, [VIII] LSD = 0.537, [IX] LSD = 0.555;

*Data in table represents mean ± standard deviation (n=7 assessors)

The odour evaluation was a means of monitoring secondary products of lipid oxidation, which resulted from decomposition of hydroperoxides and was detected by the presence of off-odours. Addition of the plant extracts and green tea infusate to sunflower oil and mayonnaise, significantly contributed in delaying development of rancid odours ($p<0.05$). Rancid odour was not detected in the sunflower oil samples until day 6, when the control-oil sample was rated close to "moderately rancid" (Table 4). The observed trend in mean rancid odour scores of oil samples after 15 days storage at 40 °C, decreased as follows: Control > *O. tenuiflorum* extract > BHT > green tea > *P. betle* extract ($p<0.05$). Table 4 illustrates that on day 6, the mayonnaise control sample was rated as "slightly to moderately rancid", while the other samples were given a mean score close to "not rancid". After 13 days storage at 40 °C, the observed mean rancid odour scores of mayonnaise samples decreased in the following order: Control > BHT and *O. tenuiflorum* extract > *P. betle* extract and green tea infusate ($p<0.05$).

According to Belitz et al. (2004), the nature of off-odours or off-flavours formed from autoxidation, depends on the fatty acid composition of the oil. Hexanal which is formed in large amounts from the autoxidation of linoleic acid, along with other volatile products contribute to the off-odours and flavours perceived during sensory assessment of oxidised oils (Gordon 2001). Since sunflower oil, contains a high proportion of linoleic acid (48.3–74.0 %) (Codex Alimentarius Commission 2009), the volatiles formed from its oxidation, could be potentially responsible for the strong rancid odour perceived by assessors in the control sample of both sunflower oil and mayonnaise.

Conclusion

This study lays particular emphasis on the potential applications of antioxidant plant extracts to modulate lipid autoxidation in food systems during storage. Our data clearly suggest that *Piper betle*, *Ocimum tenuiflorum* and commercial green tea are good sources of natural antioxidants liable to prevent and/or minimise food deterioration and could contribute to enhance safety, quality and nutritional value of lipid foods.

Acknowledgments The authors wish to thank University of Mauritius for providing the laboratory facilities and logistics required to conduct the experimental work.

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