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Extracts of cocoa (*Theobroma cacao* L.) leaves and their antioxidation potential

H. Osman *, R. Nasarudin, S.L. Lee

School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Malaysia

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Abstract

Cocoa shoot (CS), young leaves (CL) and tea leaves (GT) were processed according to green tea processing procedures. Polyphenol components was extracted and analysed using high pressure liquid chromatography. The total polyphenol of CS, CL and GT were 19.0, 28.4 and 17.3 mg/100 mg, respectively. The main catechin-polyphenols in extracts were epicatechin (EC), epigallocatechin gallate (EGCG), epigallocatechin (EGC), gallic acid (GA) and epicatechin gallate (ECG). The concentrations of caffeine for CS, CL and GT were 2.24, 1.33 and 3.34 mg/100 mg, respectively. The concentrations of EGCG, in both cocoa leaves, were lower than commercial green tea. However, the concentrations of EC in CS (5.93 mg/100 mg) and in CL (2.82 mg/100 mg) were significantly higher that those found in green tea (0.65 mg/100 mg). The antioxidation properties of the polyphenol extracts were tested, using ferric chloride reduction, and compared against a synthetic antioxidant (BHA). The polyphenol extracts (CS and CL) showed similar antioxidation powers to GT and BHA throughout the entire concentration range (100–2000 ppm). In the oil-based test medium; the antioxidative performance of polyphenol extracts were better than BHA at 50 ppm. At 200 ppm, the performance is quite similar to BHA. At higher concentration (400 ppm) the antioxidant activities are much better than BHA. In the presence of Cu^{2+} prooxidant (20 ppm), BHA (200 ppm) and all the extracts (200 pmm) showed similar performances. Since the oxidation test was conducted at 65 °C, the 8 days of stability provided by 200 ppm addition of CL and CS extracts, can be equated to 8 months of room temperature (25 °C) stability. Hence, the cocoa leaves extracts have the potential to complement or replace synthetic antioxidants in aqueous and oil-based food applications.

Keywords: Cocoa leaf; Antioxidant activity; Oil emulsion

1. Introduction

Numerous investigators have shown that foods containing phytochemicals with antioxidation potential have strong protective effects against major disease risks, including cancer and cardiovascular diseases (Ames, Shigenga, & Hagen, 1993; Block, Patterson, & Subar, 1992; Byers & Guerrero, 1995; Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Ho, Ferraro, Chen, Rosen, & Huang, 1994; Knekt et al., 1997; Steinberg, 1991). In most cases, the majority of the antioxidant activity may be from compounds such as flavonoids, isoflavone, flavones, anthocyanin and catechins rather than from Vitamin C, E and β-carotene (Kahkonen, Hopia, & Vuorela, 1999; Wang, Cao, & Prior, 1996). Epidemiological studies have shown that consumption of food and beverages rich in phenolic content can reduce the risk of heart disease, slowing the progression of atherosclerosis by acting as antioxidants towards low-density lipoprotein (LDL) (Frankel, Kanner, German, Parks, & Kinsella, 1993; Kinsella, Frankel, German, & Kanner, 1993). The antioxidant activity of phenolics is mainly because of their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evans, Miller, & Paganga, 1997). The emergence of natural extracts possessing antioxidation properties will help in reducing the current

* Corresponding author.
E-mail address: osman@ukm.my (H. Osman).

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dependency on synthetic antioxidants in food applications. In line with the efforts to balance the conservation of biodiversity and encouraging controlled exploitation of plant resources for economic gains, especially in biopharming, wastage of valuable resources should be minimized. Thus, the objective of this investigation is an attempt to assess the antioxidative potential of phenolic extracts from cocoa leaves which are normally wasted during frequent pruning.

2. Materials and methods

2.1. Chemicals

Epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), gallic acid (GA) and caffeine (Caff) were purchased from the Sigma Chemical Co. (St. Louis, MO). Methanol, and acetic acid were purchased from E. Merck Co. (Darmstadt, Germany).

2.2. Preparation of the leaves

Fresh tea leaves (GT) were obtained from Boh Plantation, Banting, Malaysia and they were sampled from leaves normally plucked for commercial tea production. Two types of cocoa leaves were collected, namely the cocoa shoot (CS) (the apex to 4th leaf) and young cocoa leaf (CL) (5th leaf to the 8th leaf). The leaves were generally processed in accordance with the principles of green tea processing (Stahl, 1962). The fresh cocoa shoot (CS) and young cocoa leaves (CL) and tea leaves were steam-blanched for $4\frac{1}{2}$ min, to inactivate peroxidase enzymes (Fields, 1977). The leaves were then dried in a convection oven (45 ± 1.0 °C, air velocity 6.6–16.5 $m^3\ min^{-1}$, 18 h) until the moisture content reaches 8% (w/w).

2.3. Extraction of polyphenol

The polyphenols were extracted according to Todd and Paul (1996). The dried leaves (100 g) were extracted with anhydrous methanol, enough water added to keep the mass liquid. After standing for 90 min, the methanol was evaporated using a rotary evaporator. Hexane (90 ml) was added, the mixture agitated. The water-insoluble hexane phase was separated from the water phase and the water phase was again extracted with 30 ml of hexane, the hexane phase separated, 10 g of sodium chloride added to the water layer and the pH adjusted to 3.5 with phosphoric acid. The aqueous phase then extracted twice with 150 ml of ethyl acetate. The ethyl acetate was evaporated until a dry solid catechin-rich fraction was obtained. The final ethyl acetate extract was kept in a capped bottle.

2.4. Determination of total phenolic content

Total phenolics were determined using the Folin-Ciocalteau reagent (Singleton & Rossi, 1965). Samples (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged in the cold at 10,000*g* for 15 min and the supernatant was saved. The residue was re-extracted, twice, with 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. The residue was dissolved in 5 ml of distilled water. 100 μl of this extract was diluted to 3 ml with water and 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% of sodium carbonate were added and the contents were mixed thoroughly. After 60 min of standing, the colour was measured at 650 nm (Pye-Unicam UV2 Spectrophotometer). The results were expressed as mg catechol/100 mg of fresh weight material.

2.5. HPLC analysis

The analysis was performed according to the method of Kim and Keeney (1983), using a Waters Symmetry C18 column (4 m particle, 3.8×250 mm), with the eluent comprising of 87% water: 8% methanol: 5% acetic acid, flowing at 0.8 ml/min^{-1} (Jasco 980 HPLC Pump) and detected using a Waters 484 Variable wavelength detector, set at 280 nm. All solvents (HPLC Grade) and samples were filtered through a nylon 0.45μ membrane.

2.6. Reducing power

The antioxidative potential of the infusion was determined using the Fe^{3+} reduction ("reducing power") (Langley-Evans, 2000; Oyaizu, 1986). The concentrations of polyphenol extracts used for this experiment were 0.00 (Control), 50, 100, 200 and 400 ppm. The absorbance was measured at 725 nm (Pye-Unicam UV2 spectrophotometer).

2.7. Peroxide value

In separate vials, sufficient amounts of extracted polyphenols were added to 0.5 ml of ethanol and the mixture was added to 100 g palm olein containing 1 g monoglyceride (EMULDAN HP, Grindsted PLC). The mixture was homogenised to emulsify the measured amounts of polyphenol into the palm olein. The final polyphenol concentrations were 0 (Control), 50, 100, 200 ppm. The emulsions-with-polyphenol were kept in an oven (65 °C) and peroxide values were determined at 0, 6, 8, 15, 22, 29 and 36 days using the AOAC Official Method 965.33 (AOAC, 1995). All experiments were carried out in triplicate.

2.8. Analysis of data

The statistical analysis was performed using SAS 6.12 package (SAS Institute).

3. Results and discussion

The cocoa leaves were given a similar treatment to that in green tea processing. To assess the antioxidation potential of dried cocoa leaves, they were compared to green tea and butylated hydroxyanisole (BHA), a common synthetic antioxidant widely used in edible oil industries.

The blanching parameters were sufficient to inactivate peroxidase in the leaves. The inactivation of peroxidases is an essential preliminary step in the process to turn the leaves into a green tea-like product. Unlike black tea, green tea is a product that does not allow extensive oxidation to occur (Stahl, 1962). The dehydration process was conducted in a controlled atmosphere chamber, where the temperature, wind speed and humidity can be controlled and monitored. Generally, the final product at 8% moisture was obtained after an 8 h drying treatment.

Table 1 shows the composition of the leaves. The total phenolic content is significantly higher in CL (28.4%), than in CS (19.0%) and GT (17.3%). The higher phenolic content may be due to accumulation of phenolic compounds in the "older" or more mature leaf physiology, as found in CL. The results of HPLC analyses show that all the marker catechins were present in all the extracts. As expected, the EGCG content was highest in GT (9.13%), followed by CS (1.93%) and CL (1.62%). The level of EGCG in GT is generally in agreement with other published data obtained from extract of commercial green tea (Lin, Lin, Liang, Lin-Shiau, & Juan, 1998). With the exception of epicatechin, the catechin content in GT is also quantitatively superior to CS on CL. The lower value of total catechin compared to total polyphenols could be due the presence of other phenols, not accounted for in the HPLC analyses, but detected by the Folin-Denis reagent. The higher concentration of total polyphenols in CL (28.4%) could be due to the presence of other phenolics since the fresh CL samples are reddish brown in colour, indicating the presence of cyanidins.

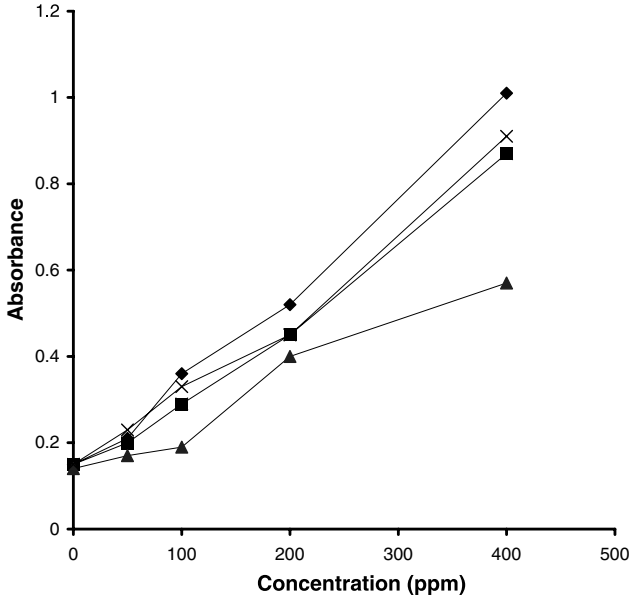

Fig. 1. Reducing power of the leaves extracts and synthetic antioxidant: (–◆–) green tea extract; (–■–) cocoa shoot extract; (–▲–) cocoa leaf extract; (– × –) BHA.

Fig. 1 shows the antioxidation potential of each extract compared to an established synthetic-antioxidant (BHA) for oil products. Palm oil was chosen because the final aim of this investigation was to reveal the application/efficacy of natural plant antioxidant in the prevention of oxidation in palmoil-based food products, especially blended/emulsified products. According to Duh, Yen, Du, and Yen (1997), reducing power has a direct, positive correlation with antioxidation properties. The storage temperature was set at 65 °C to accelerate the reaction and, at this condition, 1 day is equivalent to 1 month of storage at ambient temperature (Wanasundra & Shahidi, 1994). At similar concentration, the best antioxidation activity was achieved by the green tea (GT) extract, followed by the BHA. The difference is statistically significant ($p < 0.05$). The higher

Table 1
The total and individual polyphenol contents of green tea and cocoa leaves extracts

Sample	Total polyphenols (%)	Polyphenols (%)						Caffeine (%)
		EGCG	EGC	ECG	EC	GA	Total catechin	
CS	19.0[b]	1.93[a]	0.69[b]	0.62[b]	5.93[c]	0.58[b]	9.75[b]	2.24[b]
CL	28.4[c]	1.62[a]	0.26[a]	0.16[a]	2.82[b]	0.39[a]	5.25[a]	1.33[a]
GT	17.3[a]	9.13[b]	2.35[c]	2.74[c]	0.65[a]	0.33[a]	15.2[c]	3.34[c]

Different superscripts in the same column indicate significant difference ($p < 0.05$).

The % is based on mg/100 mg sample.

CS, cocoa shoot; CL, young cocoa leaves; GT, green tea; GA, gallic acid; EGCG, epigallocatechin gallate; EGC, epigallocatechin; EC, epicatechin.

performance by the GT extract can be attributed to the high concentration of EGCG, EGC and ECG (Penman & Gordon, 1997). However, the performance of CS was very similar to the BHA (statistically insignificant). The lowest antioxidation protection was afforded by the CL extract. The antioxidant activities from CS and CL can be attributed to the presence of epicatechin, and other un-identified phenolics. Epicatechin is known to possess strong antioxidant activity (Kris-Etherton & Keen, 2002)

Oxidation of oil can be monitored by the development of peroxide value. According to Economou, Oreopoulou, and Thomopoulos (1991), the oil will become noticeably rancid when the peroxide value reaches 20–40 meq/kg. Secondary oxidation products were not measured because the aim of this investigation was to gauge the potential of the natural extracts to prevent the onset or initial stage of oxidation. The antioxidation protection of the extracts was further evaluated in an oil system kept at 65 °C and the results are shown in Figs. 2–4. Clearly, the additives have marked positive effects on the oils compared to the control. At the lower concentration of 50 ppm (Fig. 2) all additives provided oxidative protection to oil until the 8th day of storage. After the 8th day, there was a dramatic increase in PV for oil treated with BHA. The oil with phenolic extracts (CL, CS and GT) still showed relative stability until the 15th day, after which oil treated with CL and CS extracts started to show significant increase in PV, although the magnitude was more pronounced in CL. The experiments were continued until the 36th day because the PV levels of the emulsions treated with GT extract reached

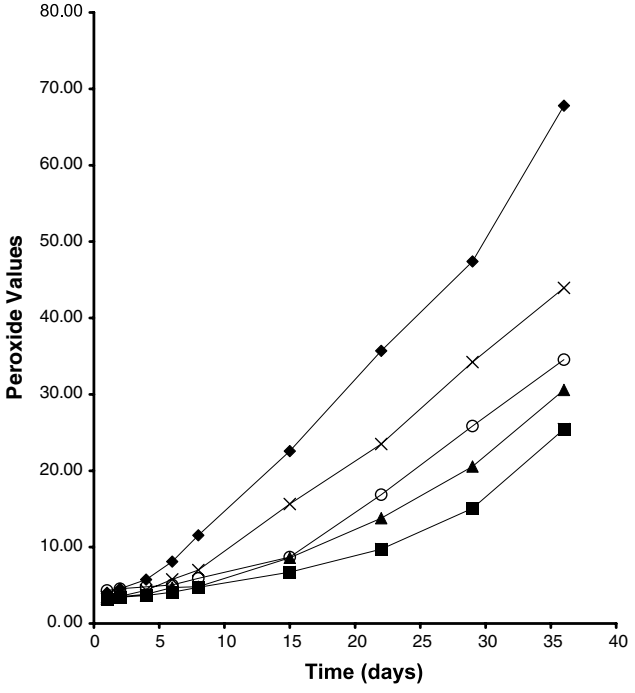

Fig. 2. The change in peroxide value in palm olein with addition of leaf extracts (50 ppm) and synthetic antioxidant, BHA (50 ppm). The oil is stored at 65 °C: (–◆–) control; (– × –) BHA; (–■–) green tea extract; (–○–) cocoa leaf extract; (–▲–) cocoa shoot extract.

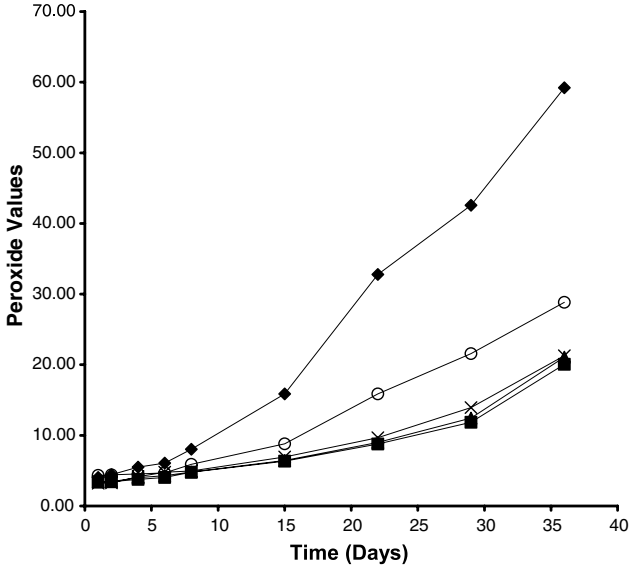

Fig. 3. The change in peroxide value in palm olein with addition of leaf extracts (200 ppm) and synthetic antioxidant, BHA (200 ppm). The oil is stored at 65 °C: (–◆–) control; (– × –) BHA; (–■–) green tea extract; (–○–) cocoa leaf extract; (–▲–) cocoa shoot extract.

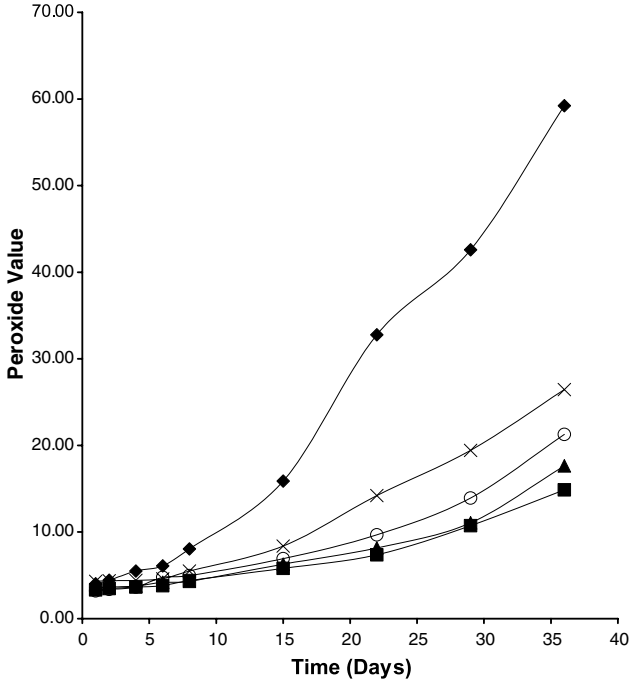

Fig. 4. The change in peroxide value in palm olein with addition of leaf extracts (400 ppm) and synthetic antioxidant, BHA (200 ppm). The oil is stored at 65 °C: (–◆–) control; (– × –) BHA; (–■–) green tea extract; (–○–) cocoa leaf extract; (–▲–) cocoa shoot extract.

20 meq/kg after the 30th day. At this concentration, the best performance was achieved by the GT extract, although the performance of CS extract was not too far off.

In conformation with the worldwide standard, the Malaysian Food Act (1983) has set the maximum safety limit of BHA in oil at 200 ppm. Thus, in this experiment, addition of 200 ppm antioxidant is considered as a benchmark for suitability of an antioxidant. Fig. 3 shows that the oil was least stabilized by the CL extract. However, relative to the control, the oil with CL extract only showed significant increase in PV after 15 days of storage. The PV profiles of the other antioxidants (CS, GT and BHA) were very similar, and the differences between then are statistically insignificant. The data suggest that botanical extracts from CS and GT are equally as effective as BHA in protecting the oil against oxidation. At 400 ppm, the CS, CL and GT extracts showed significant improvements ($p < 0.05$), relative to 200 ppm BHA, in slowing down the build-up of peroxide in the oil during storage. In the case of botanical extracts, such as CS, CL and GT, exceeding the 200 ppm limit cannot be considered advantageous, because these products can be considered as GRAS until proven otherwise. In actual application, the maximum usage level in food will be self-limiting in term of sensory acceptability, due to slight bitterness of the extract.

To further verify this observation, the oil was premixed with prooxidant Cu^{2+} (20 ppm) and the antioxidants were added. Thus, any contributory prooxidant effect of carry-over chlorophyll will be totally overwhelmed by the much stronger activity of Cu^{2+}. Fig. 5 shows the rapid oxidation of the control oil. As for the treated oils, the PV reached 20 meq/kg after the 4th day and on the 6th day the PV reached around 40 meq/kg; thus the oil can be considered as rancid. In terms of equivalence, the oils (stored at 65 °C) were stabilised for nearly 6 months, even in the presence of 20 ppm prooxidant.

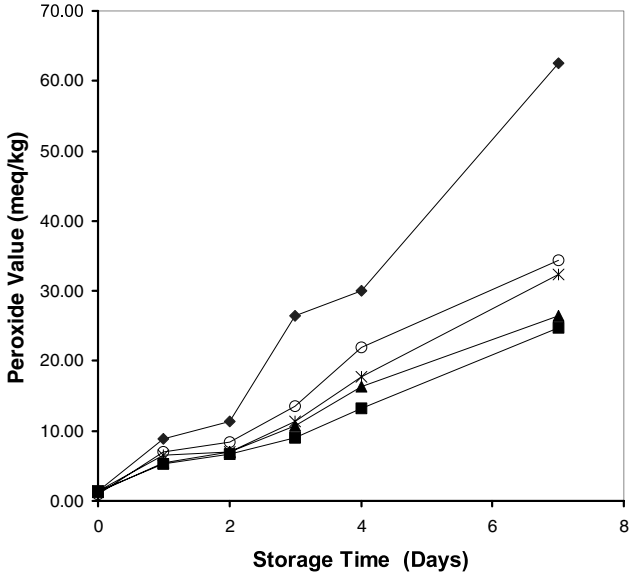

Fig. 5. The effect of Cu^{2+} prooxidant (20 ppm) on the oxidation of palm olein emulsion stored at 65 °C and stabilized with natural polyphenols (200 ppm) and synthetic antioxidant (200 ppm): (–◆–) control; (– × –) BHA; (–■–) green tea extract; (–○–) cocoa leaf extract; (–▲–) cocoa shoot extract.

4. Conclusion

The cocoa leaves extracts, especially the cocoa shoot, have similar antioxidant activities to green tea. With proper pruning management, the "waste" leaves from cocoa plantations can be utilised as a new source of natural bioactive extract.

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