

DRUMSTICK (*MORINGA OLEIFERA* L.) LEAVES: A POTENTIAL SOURCE OF NATURAL LIPID ANTIOXIDANTS

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Accepted for Publication August 17, 2009

ABSTRACT

Antioxidant activity of methanolic extract of drumstick leaves (200, 500 and 1,000 ppm) in soybean oil was assessed by an accelerated oxidation test (70C, 10 days) and a heating test (180C, 1 h). The extract significantly ($P \leq 0.05$) suppressed the formation of peroxides and thiobarbituric acid-reactive substances (TBARS) during accelerated oxidation, even at a level of 200 ppm. On heating test, conjugated dienes, conjugated trienes and TBARS values of the oil treated with the extract were significantly ($P \leq 0.05$) lower than those of the control sample (oil without any antioxidant/extract). The antioxidant activity of the extract at 500 and 1,000 ppm was greater ($P \leq 0.05$) than that of synthetic antioxidant, butylated hydroxyanisole on both accelerated oxidation and heating tests. Results of this study revealed high thermal stability of antioxidant components of drumstick extract and the antioxidant potential of the extract in inhibiting lipid peroxidation of soybean oil. Thus, the extract of drumstick leaves is recommended as an alternative source of natural antioxidants.

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PRACTICAL APPLICATIONS

Toxicological effects of synthetic antioxidants and consumer preference for natural products have resulted in increased interest in the application of natural antioxidants with special focus on those available in edible plants. In this study, methanolic extract of drumstick leaves showed an excellent antioxidant activity in soybean oil on accelerated oxidation and heating tests. Its antioxidant activity was comparable or even greater with that of a synthetic antioxidant, butylated hydroxyanisole. Then, drumstick leaves extract can be regarded as a “natural” source of antioxidants, having potential application in edible oils and lipid-containing food products to retard or inhibit their lipid oxidation. In addition, consumption of such products can impart health benefits to the consumer.

INTRODUCTION

Lipid oxidation is of great concern to the food manufacturers and consumers as it leads to the development of undesirable off-flavors, loss in nutritional value of food and promotion of health disorders such as cancer, heart disease and aging (Namiki 1990). Hence, the presence of antioxidants in foods is essential for their quality retention and safety. Toxicological effects of synthetic antioxidants and consumer preference for natural products have resulted in increased interest in the application of natural antioxidants (Kaur and Kapoor 2001). Recently, natural plants have received much attention as sources of biologically active substances including antioxidants, antimutagens and anticarcinogens (Dillard and German 2000). Numerous studies have been carried out on some plants such as rosemary, sage and oregano, which resulted in the development of natural antioxidant formulations for food, cosmetic and other applications. Consumption of such products may enrich the innate antioxidative status of living cells and thus protect them against damage caused by free radicals (Shukla *et al.* 1997).

Drumstick (*Moringa oleifera* L.) is the most widely cultivated species of the Moringaceae family that is native to the Indo-Bangla subcontinent and cultivated throughout the tropical belt (Sastri 1962; Fahey 2005). This rapidly growing perennial, soft wood tree, also known as horseradish tree, and Ben-oil tree, has been utilized since ancient times in traditional medicine and industry. All parts of the tree are edible and have long been consumed by humans (Fahey 2005). The leaves are tri-pinnate, feathery, with many small leaflets and are rich in β -carotene, vitamin C, vitamin B, calcium, iron and essential amino acids. Thus, they have been advocated as an outstanding indigenous source of nutrients suitable for utilization in many of the so-called “developing” regions

of the world to combat malnutrition (Nambiar and Seshadri 2001; Fahey 2005). In the Indian indigenous system of medicine, drumstick leaves are used for the treatment of a variety of common ailments, e.g., anemia, anxiety, dry cough, bronchitis, joints pain, scurvy and psoriasis (Sastri 1962; Mathur 2005). Leaves juice is given as emetic, and leaves paste is used as an external application for wounds (Sastri 1962). In the scientific literature, a wide range of pharmacological properties such as anti-inflammatory, antitumor, antimicrobial, hypotensive, hypocholesterolemic and hypoglycemic have been reported for drumstick leaves (Fahey 2005). The major bioactive compounds of phenolic groups present in drumstick leaves have been reported to be flavonoids, such as quercetin and kaempferol (Siddhuraju and Becker 2003; Nambiar *et al.* 2005). Reports indicate that in South India, drumstick leaves are used in the preparation of clarified butter in order to increase the shelf life of product (Siddhuraju and Becker 2003). Such enhancement of the shelf life of clarified butter might be related to the presence of antioxidant compounds which are present in drumstick leaves. The potent antioxidant activities of various solvents (methanol, acetone and water) extracts of drumstick leaves have been indicated in different *in vitro* test systems and found that methanol was the most efficient solvent for the extraction of antioxidant components from the leaves (Arabshahi-Delouee 2006). Also, the efficiency of aqueous ethanol and aqueous methanol for the extraction of antioxidants from drumstick leaves has been reported by Siddhuraju and Becker (2003). Moreover, it is believed that the effectiveness of an antioxidant such as plant extracts in foods depends on various factors like thermal stability, pH and storage time. Methanolic extract of drumstick leaves has been found to have a high stability to heat, pH and storage time (Arabshahi-Delouee 2006). However, scientific literature is silent regarding the application of drumstick extract as a natural antioxidant in the oil systems. Thus, the present study was undertaken to evaluate the feasibility of using methanolic extract of drumstick leaves in retarding lipid oxidation of an edible oil containing a high proportion of polyunsaturated fatty acid (PUFA), i.e., soybean oil, which is also the most important vegetable oil produced in the world.

MATERIALS AND METHODS

Chemicals

Butylated hydroxyanisole (BHA) was obtained from Sigma Chemicals Co. (St. Louis, MO), Malonaldehyde-bis-dimethyl acetal from Merck-Schuchardt (Hohenbrunn, Germany), and thiobarbituric acid (TBA) from HiMedia Lab. Pvt. Ltd. (Bombay, India).

Materials

Drumstick leaves were obtained in bulk from a local market in Mysore, India. The leaves were washed and then dried in a hot air oven ($45 \pm 2\text{C}$) for 8–10 h. The moisture content of dried leaves was $8.20 \pm 0.56\%$ (on dry weight basis). The dried samples were ground to a fine powder, sieved through a 60-mesh and kept in airtight containers at 4C. Refined, bleached and deodorized (RBD) soybean oil was provided from Habib Agroindustries, Boothana Hosur, Mandya, Karnataka, India.

Extraction of Antioxidants from Drumstick Leaves

The dried drumstick leaves (15 g) were extracted overnight with 100 mL of methanol in a mechanical shaker at room temperature. The extract was filtered with Whatman No. 1 filter paper and the filtrate was evaporated to dryness at 40C in a rotary evaporator (Buchi Laboratoriums–Technik, Flawil/Schweiz, Switzerland). The residue obtained was weighed and then stored in an airtight container at 4C until further use.

Antioxidant Dispersion. Methanolic extract of drumstick leaves (MEDL) at different levels (200, 500 and 1,000 ppm), and BHA at 200 ppm were added to RBD soybean oil and kept stirred for 30 min on a magnetic stirrer to homogenize. The oil sample without addition of any antioxidant/extract was considered as control. The oil samples were subjected to accelerated oxidation and heating tests. These tests, which are used by most researchers to estimate the stability of oils to oxidation, are representative of two commonly used methods for food processing, i.e., cooking (50–100C) and frying (~180C).

Accelerated Oxidation Test. Oil samples (50 g) of each variation were stored in an oven at $70 \pm 2\text{C}$ for 10 days to induce accelerated oxidation. Aliquots of each sample were taken every day for the analysis of peroxide value (PV), and every 2 days for the analysis of thiobarbituric acid-reactive substances (TBARS) value.

Heating Test. Oil samples (50 g) of each variation were heated continuously in 100 mL Pyrex beakers (Wertheim, Germany) on a hot plate at $180 \pm 5\text{C}$ for 1 h (Allam and Mohamed 2002). At the end of thermal treatment, samples were set aside to cool and then analyzed for conjugated dienes (CD), conjugated trienes (CT) and TBARS values.

Analysis of PV. PV was determined according to standard method of AOCS (1997, Cd 8–53). Induction period (IP) values were expressed as the time when PV reaches 20 meq/kg (Povilaityte and Venskutonis 2000).

Analysis of CD and CT Values. Oil sample (50 mg) was dissolved in cyclohexane (5 mL). CD and CT values of oil samples were determined by measuring absorbance of solution at 234 and 268 nm, respectively (Abdalla and Roozen 1999). Inhibition of formation of CD and CT was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Value of control} - \text{Value of sample}}{\text{Value of control}} \times 100$$

Analysis of TBARS Value. The test was performed according to the method of Ranganna (1999) with small modifications. To 10 g of oil sample, 0.67% aqueous TBA solution (20 mL) and benzene (25 mL) were added. The mixture was shaken continuously for 2 h on a mechanical shaker. The aqueous layer was withdrawn and placed in a boiling water bath for 1 h. After cooling, absorbance of solution was measured at 532 nm. The TBARS values were expressed as malonaldehyde (MA) equivalents (mg/kg oil). Inhibition of formation of TBARS was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{TBARS value of control} - \text{TBARS value of sample}}{\text{TBARS value of control}} \times 100$$

Statistical Analysis

Data were recorded as means \pm standard deviation of triplicate measurements. Analyses of variance (ANOVA) were performed by ANOVA test and significance differences between the means were determined by Duncan's multiple-range test ($P \leq 0.05$) (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Accelerated Oxidation Test

PV. The PVs of various oil samples during accelerated oxidation are shown in Fig. 1. The control sample (oil without any antioxidant/extract) was oxidized at the highest intensity indicated by a higher PV (120 meq/kg) at the end of test period. Addition of MEDL in the oil contributed to the oxidative stability of the oil significantly, which is indicated by lower ($P \leq 0.05$) PVs than the control sample. However, this effect was dose-dependent. The PV of the control sample increased rapidly, by 46 times from zero to 10th day, whereas those of the oil samples containing 200, 500 and 1,000 ppm of

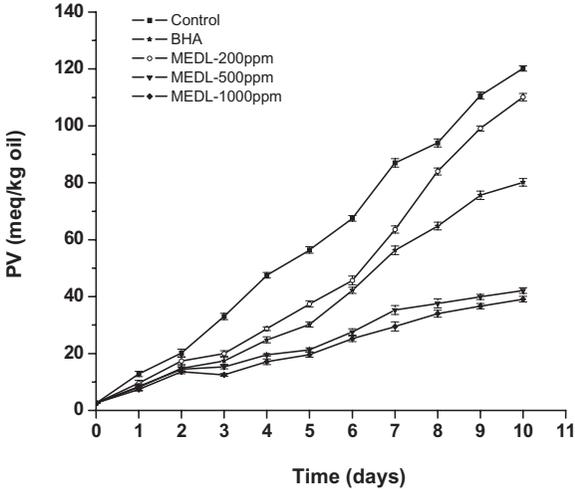


FIG. 1. EFFECT OF METHANOLIC EXTRACT OF DRUMSTICK LEAVES (MEDL), AND BUTYLATED HYDROXYANISOLE (BHA) ON THE FORMATION OF PEROXIDES IN SOYBEAN OIL DURING ACCELERATED STORAGE (70C, 10 DAYS) Control: oil samples free from any antioxidant/extract. PV, peroxide value.

MEDL, and 200 ppm of BHA increased 42, 16, 15 and 24 times, respectively, indicating the antioxidant power of these additives. It is evident that increasing the concentration of MEDL from 200 to 500 ppm had a considerable positive effect on the stability of the oil. Further increase in the concentration of the extract from 500 to 1,000 ppm did not make a considerable decrease in the PV of the oil, though the differences between PVs were statistically ($P \leq 0.05$) different from each other. Looking at the changes of PV in the oil samples during test, it is obvious that the protective effect of MEDL (500 and 1,000 ppm) was more pronounced in the later stages (after 3rd day) of oxidation compared with the initiation stage. It has been reported that with longer storage, hydroperoxides generated in the initiation stages can themselves catalyze formation of more hydroperoxides (Yang *et al.* 2005). The higher protective effect of MEDL in the later stages of oxidation might be attributed to the ability of the extract in stabilizing hydroperoxides, thus inhibiting the propagation stage of lipid oxidation.

The IP calculated as “the time required to reach a PV of 20 meq/kg at 70C” for different oil samples are shown in Table 1. Addition of BHA and MEDL prolonged the IP of soybean oil significantly ($P \leq 0.05$), indicating their ability to delay the onset of rancidity of oil. The order of antioxidant activity expressed as protection factor was: MEDL (1,000 ppm) > MEDL (500 ppm) > BHA (200 ppm) > MEDL (200 ppm).

TABLE 1.
EFFECT OF MEDL AND BHA ON THE STABILITY OF
SOYBEAN OIL EXPRESSED AS INDUCTION PERIOD (IP)
AND PROTECTION FACOR (PF) DETERMINED BY
PEROXIDE VALUE

Additive	IP (days)†	PF‡
None	1.75 ± 0.03 ^e	1.00
BHA	2.70 ± 0.01 ^c	1.60
MEDL (200 ppm)	2.51 ± 0.02 ^d	1.43
MEDL (500 ppm)	4.00 ± 0.01 ^b	2.28
MEDL (1,000 ppm)	4.47 ± 0.02 ^a	2.55

Values carrying different superscripts a,b . . . in each column differ significantly ($P \leq 0.05$) from each other.

† Time to reach a peroxide value of 20 meq kg⁻¹ at 70C.

‡ PF = IP (oil + additive)/IP (oil).

BHA, butylated hydroxyanisole; MEDL, methanolic extract of drumstick leaves.

TBARS Value. During oxidation of oil, peroxides are generally decomposed to lower molecular weight compounds such as alcohols, acids, ketones, aldehydes, etc. Measurement of secondary metabolites of lipid oxidation such as TBARS, e.g., MA is useful as an index of lipid peroxidation (Shahidi and Wanasundara 1997). In this study, the TBARS value of all the oil samples increased significantly ($P \leq 0.05$) as the time progressed (Fig. 2). However, the oil samples were treated with varying levels of MEDL, and BHA had always lower ($P \leq 0.05$) values than the control sample during test. It is interesting to note that the TBARS values of the oil samples treated with MEDL (500 and 1,000 ppm) were significantly ($P \leq 0.05$) less than that of BHA-treated sample during 10 days of storage. On day 10, MEDL at 200, 500, 1,000 ppm, and BHA reduced the formation of TBARS by 10.1, 34.8, 42.3 and 11.6%, respectively. Results indicated the ability of MEDL in retarding lipid oxidation even at the level of 200 ppm and suggested a relatively high thermal stability of antioxidant components present in the extract. The order of antioxidant activity in the TBARS assay was consistent with that obtained in the PV analysis. The inhibitory effect of the extract and BHA on the formation of TBARS appeared to be less than their effect on peroxide formation.

Several methods are available to assess the oxidative status of vegetable oils. Because the process of lipid oxidation is known to be very complex, use of different methods for its assessment can give comprehensive information, especially when the effectiveness of multicomponent natural extracts is to be investigated (Povilaityte and Venskutonis 2000). In this study, the antioxidant efficacy of MEDL in soybean oil was detected by measuring primary and secondary changes during accelerated oxidation of soybean oil. The extract

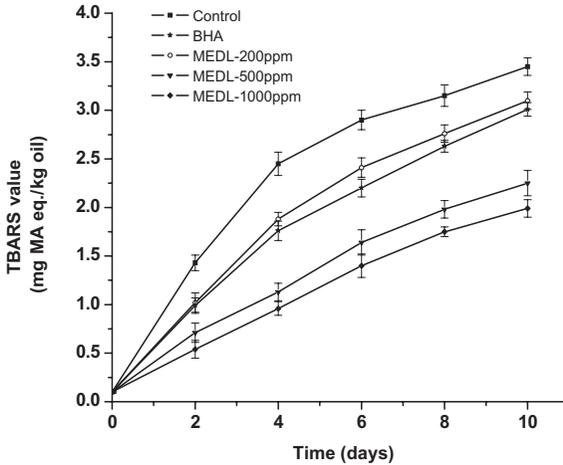


FIG. 2. EFFECT OF METHANOLIC EXTRACT OF DRUMSTICK LEAVES (MEDL), AND BUTYLATED HYDROXYANISOLE (BHA) ON THE FORMATION OF THIOBARBITURIC ACID-REACTIVE SUBSTANCES (TBARS) IN SOYBEAN OIL DURING ACCELERATED STORAGE (70C, 10 DAYS)

Control: oil samples free from any antioxidant/extract.

was effective in retarding formation of peroxides and TBARS in the oil. Moreover, the antioxidant efficacy of extract (500 and 1,000 ppm) was superior to that of a synthetic antioxidant, BHA. These results are consistent with the earlier reports stating that the extracts of some spices and herbs are more effective antioxidants than some major synthetic antioxidants (Larson 1988; Marinova and Yanishlieva 1997). The acetone extract of ajwain has been a more effective antioxidant than BHA and butylated hydroxytoluene in retarding the formation of primary and secondary oxidation products of linseed oil at 80C (Singh *et al.* 2004). Rosemary extract has been found to retard deterioration of rapeseed oil during storage at 80C (Gordon and Kourimska 1995). Addition of freeze-dried extract of durum wheat bran in soybean oil has decreased the PV of oil, significantly, under active oxygen method conditions for 9 h (Onyeneho and Hettiarachchy 1992). Green tea polyphenols have been shown to exhibit antioxidant activity in lard and soybean oil, greater than that of α -tocopherol (Koketsu and Satoh 1997). Mehta *et al.* (1994) found that formation of primary and secondary oxidation products of oxidized soybean oil (60C) was significantly suppressed in the presence of ajowan extract.

Heating Test

CD and CT Values. Oxidation of PUFAs is accompanied by a shift in their double bond position because of isomerization and conjugate formation.

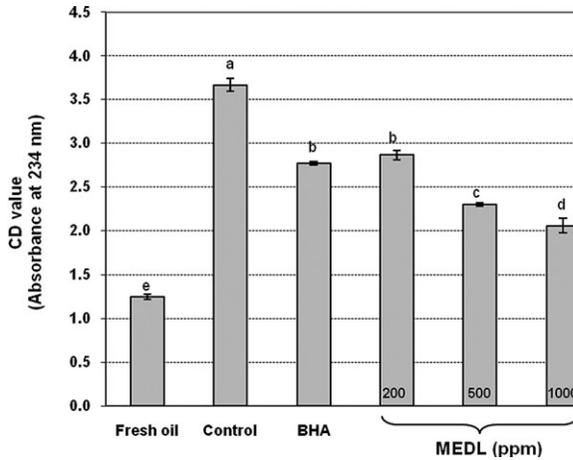


FIG. 3. EFFECT OF METHANOLIC EXTRACT OF DRUMSTICK LEAVES (MEDL), AND BUTYLATED HYDROXYANISOLE (BHA) ON THE FORMATION OF CONJUGATED DIENES (CD) IN SOYBEAN OIL ON HEATING AT 180C (1 H)

Bars carrying different superscripts a, b . . . differ significantly ($P \leq 0.05$) from each other.

The resulting CD and CT exhibit intense absorption at 234 and 268 nm, respectively (Shahidi and Wanasundara 1997). CD and CT values of various oil samples after heating (180C, 1 h) are shown in Figs. 3 and 4, respectively. All the samples absorbed more intensively at 234 and 268 nm, indicating the increased formation of dimers and trimers as a result of heating. CD and CT values of the control sample increased by 2.9 and 4.4-fold, respectively. Increase in CD and CT values of the oil samples treated with BHA, and MEDL (200, 500, 1,000 ppm) were significantly ($P \leq 0.05$) lower than that of the control sample, indicating antioxidant efficacy of these compounds in the oil during heating. The effect of MEDL was dose-dependent. Addition of MEDL in soybean oil decreased the formation of CD and CT, by 21.8–43.9%, and 28.4–56.3%, respectively. Interestingly, no significant ($P \leq 0.05$) differences were found between CD and CT values of the oil samples containing 200 ppm of BHA and MEDL. This indicates that MEDL exhibits antioxidant activity, which is comparable with that of a synthetic antioxidant, BHA, even at the lowest level. The higher levels of MEDL showed significantly ($P \leq 0.05$) higher antioxidant activity than BHA. Results indicated that the added antioxidants were more effective in inhibiting the formation of trimers compared with their relatively lower inhibition of dimers formation.

TBARS Value. The TBARS values (MA equivalents) of the oil samples heated at 180C for 1 h are presented in Fig. 5. Heating at 180C caused a

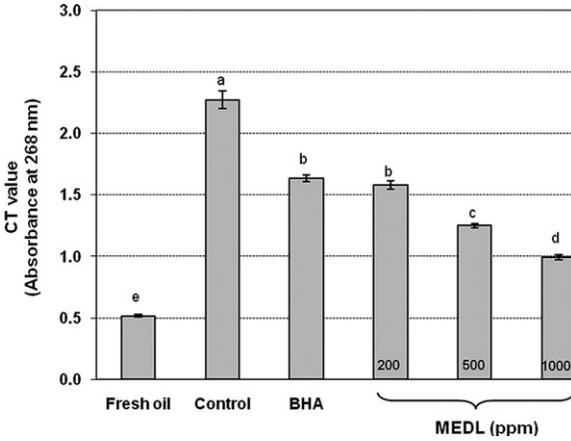


FIG. 4. EFFECT OF METHANOLIC EXTRACT OF DRUMSTICK LEAVES (MEDL), AND BUTYLATED HYDROXYANISOLE (BHA) ON THE FORMATION OF CONJUGATED TRIENES (CT) IN SOYBEAN OIL ON HEATING AT 180C (1 H) Bars carrying different superscripts a, b . . . differ significantly ($P \leq 0.05$) from each other.

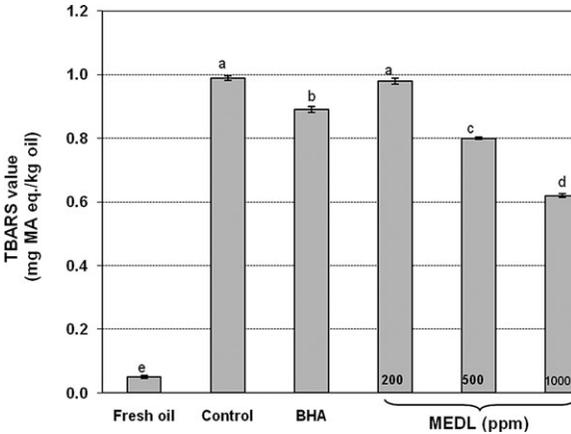


FIG. 5. EFFECT OF METHANOLIC EXTRACT OF DRUMSTICK LEAVES (MEDL), AND BUTYLATED HYDROXYANISOLE (BHA) ON THE FORMATION OF THIOBARBITURIC ACID-REACTIVE SUBSTANCES (TBARS) IN SOYBEAN OIL ON HEATING AT 180C (1 H) Bars carrying different superscripts a, b . . . differ significantly ($P \leq 0.05$) from each other.

remarkable increase ($P \leq 0.05$) in the TBARS value of the control sample, indicating the increased formation of secondary oxidation products, i.e., TBARS. MEDL at the level of 200 ppm did not exhibit inhibitory activity against formation of TBARS. Addition of 500 and 1,000 ppm of MEDL in

soybean oil significantly ($P \leq 0.05$) decreased the TBARS value of the oil, indicating the potency of extract in inhibiting the formation of TBARS even after heating at 180C. The TBARS values of MEDL-treated (500 and 1,000 ppm) oil samples were significantly ($P \leq 0.05$) lower than that of BHA-treated sample. This indicates that MEDL improved the oxidative stability of the oil to a greater extent compared with BHA. The inhibitory activity of MEDL and BHA against TBARS formation was less than their effect on the formation of CD and CT.

The results of CD, CT and TBARS analyses showed that MEDL was an effective antioxidant in suppressing the formation of primary and secondary oxidation products in soybean oil during heating at 180C. It has been reported that ginger extract (2,400 ppm) was able to retain chemical characteristics such as PV, free fatty acid and iodine value of sunflower oil when heated at 185C (Zia-ur-Rehman *et al.* 2003). Rosemary extract has been reported to reduce the formation of dimers in the rapeseed oil during frying (Gordon and Kourimska 1995). Mehta *et al.* (1994) found that treatment of soy oil with methanolic extract of ajowan was effective in reducing the amount of CD formed in the oil at frying temperature.

CONCLUSIONS

Food processing operations require antioxidants that survive high temperatures experienced during baking, cooking or frying, and provide protection to finished products. Methanolic extract of drumstick leaves showed an excellent antioxidant activity in soybean oil on accelerated oxidation and heating tests, in a dose-dependent manner. MEDL is a mixture of methanol-soluble materials that may include several classes of compounds other than antioxidative components, thus the net concentration of effective substances in MEDL is expected to be substantially lower than that of BHA. The strong antioxidant activity of MEDL in soybean oil emphasizes on the presence of potent antioxidative compounds with high thermal stability. Thus, the extract of drumstick leaves has the potential to be used as alternative source of natural antioxidants for increasing the shelf life of oils and oil-containing foods and are more efficacious than synthetic antioxidant, BHA.

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