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ORIGINAL PAPER

Antioxidative Properties of *Curcuma longa* Leaf Extract in Accelerated Oxidation and Deep Frying Studies

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Abstract The antioxidative properties of *Curcuma longa* (turmeric) leaf extract were evaluated in refined, bleached and deodorized (RBD) palm olein using accelerated oxidation and deep frying studies at 180 °C for up to 40 h. The extract was capable of retarding oil oxidation and deterioration significantly ($P < 0.05$) at 0.2% concentration, better than 0.02% BHT for the Oxidative Stability Index (OSI) in an accelerated oxidation study and also the peroxide value in deep frying studies. In sensory evaluation, the French fries were acceptable and were not significantly different ($P < 0.05$) from one another for color, oiliness and crispiness throughout the 40-h frying study. *Curcuma longa* leaf extract, which had a polyphenol content of 116.3 ± 0.2 mg/g, possessed heat-stable antioxidant properties and may be a good natural alternative to existing synthetic antioxidants in the food industry.

Keywords Frying · *Curcuma longa* leaves · Palm olein · Sensory evaluation · Antioxidant · Accelerated oxidation study

Introduction

Curcuma longa Linn. (*C. longa*) is a tropical herb, with a long and extensive use in Asia, for food and medicine, for coloring, flavoring and healing properties. Although the rhizomes have been used to treat hepatitis, sepsis, dyspepsia and various other disorders, not much literature is available on the use of the leaves. In South East Asia, turmeric leaves are added to various meats, and other savoury dishes for flavor, to be served with rice, and are also believed to be beneficial for health. Although the rhizomes contain diarylheptane derivatives (curcumin, demethoxycurcumin, bisdemethoxycurcumin and dihydrocurcumin) and volatile oil components (sesquiterpenes such as turmerone, curlone, α-turmerone, β-turmerone, bisacumol, zingiberene, curcumenone, curcumenol, procurcumenol, dehydrocurdione and germacrone-13-al [1], the leaves contain lambda-8(17), 12-diene-15,16 dial with antifungal activity [2] and protocatechuic acid, syringic acid and vanillic acid [3]. Turmeric rhizomes are known to have anti-bacterial, antioxidant, anti-inflammatory and anti-tumor properties [4].

Deep fat frying is a complex oil application, which influences the finished product flavor, texture, shelf-life and nutritional attributes. The high temperature used in deep frying accelerates oxidation and oil quality loss, thus, a protective antioxidant is necessary to prolong its shelf life and maintain product quality. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylated hydroxyquinone (TBHQ) are commonly added to retard oxidation, but they are volatile at frying temperatures [5]. Antioxidants from basil, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary and sage have been extracted and tested in various mediums [6–8], and some have activities that are comparable to existing synthetic antioxidants. Not much scientific study has been conducted on *C. longa* leaves. The objective of this study was to evaluate on the antioxidative properties of *C. longa* leaf

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extract in palm olein during accelerated oxidation and frying.

Methodology

Herbs and Oil

Curcuma longa leaves were obtained from the local market. Refined, bleached and deodorized (RBD) palm olein was obtained from Golden Jomalina, GoldenHope Sendirian Berhad, Kuala Lumpur, Malaysia, while prefried frozen French fries were supplied by Simplot Bhd. Kuala Lumpur, Malaysia. All chemicals and reagents were of A.R. grade and were obtained from Merck, Systerm and Fischer Bhd. Kuala Lumpur, Malaysia.

Preparation of Herbs Extract

The cleaned leaves were dried in a hot air oven at 45 °C for 24 h, ground to a fine powder before extracting with 10 times its weight in ethanol for 8 h at 50 °C. The solvent was evaporated using a rotary evaporator.

Determination of Polyphenols and Antioxidant Activities

The total phenolics content in the extract was determined [9] with minor modifications. A 200 µl aliquot of the extract solution (1:100 w/v in methanol) was added to 1 mL of Folin-Ciocalteau solution and 0.8 ml of 0.2% Na_2CO_3, and made up to 10 mL using water–methanol (4:6). After 30 min, the absorbance was read at 765 nm using a spectrophotometer. The concentration was quantified using gallic acid as a standard, and the results were expressed as gallic acid equivalents (GAE) per gram of extract.

The free radical scavenging activity was expressed as the percentage inhibition of the reduction of α-α-diphenyl-β-hydrazyl radical (DPPH) radicals in methanol [10]. To 4 mL of DPPH in methanol (0.1 mmol/L) was added 1 mL of 50 or 100 µg/g herbs extract solution. After 20 min being kept in the dark, the absorbance was measured at 517 nm.

The antioxidative activities of the extracts were determined [11] in the linoleic acid model system. The autoxidation rate of linoleic acid was measured by the increase in conjugated diene and decrease in linoleic acid in the sample. Linoleic acid ester (10 mM), emulsified with an equal amount of Tween 20 in sodium phosphate buffer pH 7, was homogenized for about 1 min. Aliquots of 10 µL (0, 1,000, 2,000, 3,000 µg/g extracts) were mixed with 5 mL of the emulsion. The samples were incubated at 50 °C for 20 h. Absorbance of the hydroperoxides formed was measured at 234 nm before and after oxidation by taking 0.2 mL of the solution and dissolving in 5 mL methanol. The activity of antioxidant (AOA) was defined as the difference in absorbance between sample and blank control, divided by the absorbance of the blank control.

Accelerated Oxidation Study

The palm olein was heated to 60 °C before addition of the extract (at 0%, 0.1%, 0.2%, 0.3% and 0.4%) and stirred until completely dissolved. BHT (0.02%) was used as a positive control. Samples were heated at 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.

Frying Experiment

Deep frying were carried out in a stainless steel electrical open fryer (Frymaster brand, model H14-2SC) with split pots of 11.5 kg capacity (for each pot) and equipped with an autolift stainless steel basket and an automatic portable filter system. The treatments conducted simultaneously were, (1) palm olein containing 0.2% *C. longa* leaf extract (10 kg oil was introduced into separate fryers, and heated to 60 °C before adding 0.2% extract, and stirred until completely dissolved) (2) palm olein containing 0.02% BHT, and (3) palm olein without any additive (i.e. control).

Approximately 400 g oil samples were collected from each fryer to represent the sample for day 0 before frying. The remaining oil was heated at 180 ± 2 °C and was allowed to equilibrate at this temperature for 30 min. About 14 batches of 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 cooled to 60 °C before filtering using separate filters to remove debris. Frying oil (400 g) from each fryer was sampled into amber bottles at the end of each day. All oil samples were flushed with slow bubbles of nitrogen from the bottom of the bottles and stored at −20 °C prior to physical and chemical analysis. The fryers were topped off 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.

Sensory evaluation was conducted on the same day using the fifth and sixth batches of fried French fries. They were evaluated using a 9-point hedonic scale (1 = very poor; 9 = very good) by 10 trained sensory panelists from the Malaysian Palm Oil Board (MPOB) on day 1, 3 and 5; for color, flavor, oiliness, crispiness, taste and overall quality.

Analysis of Oil Quality

Changes in oil quality were monitored using the peroxide value (Cd 8b-90), the anisidine value (Cd 18-90), the iodine value (Cd 1-15), free fatty acids (Ca 5a-40), the Oxidative Stability Index (OSI) (Cd 1 2b-92), polar (Cd 20-91), polymer (Cd 22-91) and color tests (Cc 13E-92), all tests based on The American Oil Chemists' Society Official Methods [12].

The color of the oil sample was measured using the Lovibond Tintometer by matching the color of the light transmitted through a specified depth of oil to the color of the light, originating from the same source, transmitted through standard color slides. The color 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 convert colors into numbers, using the CIE Lab L^*, a^* and b^* color scale. L^* represents lightness, a^* represents redness, while b^* represents yellowness.

Statistical Analysis

Each experiment and analysis, including leaves sampling and extraction, were conducted in triplicate. The MINITAB 14 software was used to analyze data for determining ANOVA, standard deviation and Duncan's multiple range test for significance at a 5% level [13].

Results and Discussion

Polyphenol Content and Antioxidative Properties

The phenolic content of *C. longa* leaves after refluxing with ethanol was found to be 116.3 ± 0.2 mg GAE/g extract ($n = 6$). The activity of *C. longa* extract was lower than BHT in both the DPPH radical scavenging assay and linoleic acid model system, and was concentration dependent for free radical scavenging assay (Fig. 1).

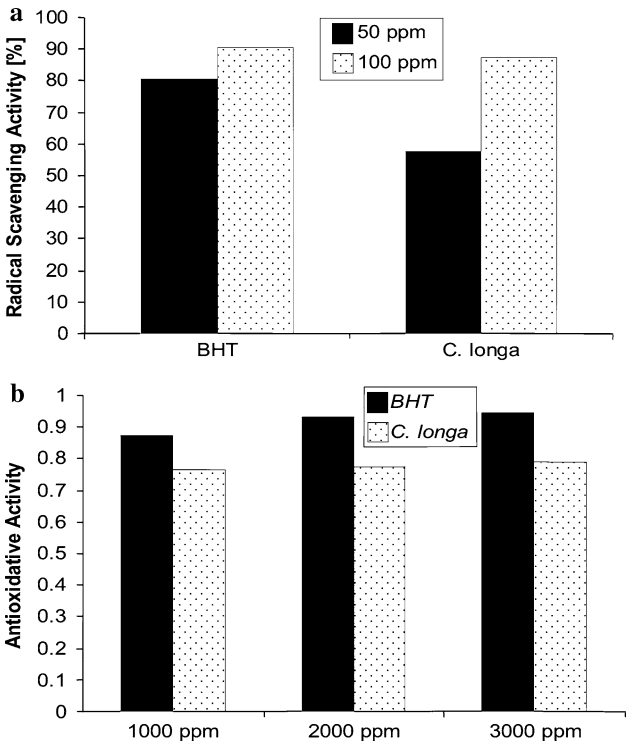

Fig. 1 Antioxidative activities of *C. longa* extract compared to BHT in free radical (DPPH) scavenging activity (**a**) and the linoleic acid model system (**b**)

Accelerated Oxidation Studies

Peroxide Value, Anisidine Value and Free Fatty Acid

The peroxide values were significantly reduced by the extract in a dose dependent manner with the optimum concentration at 0.4% (Fig. 2a). At 0.1–0.3% extract concentration, the peroxide values decreased after prolonged heating, indicating the conversion to secondary oxidation products such as ketones, aldehydes, hydrocarbons and epoxides. Anisidine value and free fatty acid value were significantly different from the control (Figs. 3a, 4a) but were independent of the extract concentration. Interestingly, the OSI was not significantly affected by extract concentration within the extract range studied (Fig. 5). However, OSI values for extract treatments were significantly ($P < 0.05$) better than 0.02% BHT.

Frying Experiments

Both natural and synthetic antioxidants significantly retarded peroxide formation with better effects observed in 0.2% *C. longa* leaf extract compared to 0.02% BHT after 8 h of frying (Fig. 2b). Both antioxidants also reduced the rate at which AV increased significantly ($P < 0.05$) (Fig. 3b), with better inhibition by BHT throughout the frying experiment. *Curcuma longa* leaf extract was better than BHT in lowering the free fatty acid value after 24 h of frying (Fig. 4b). Although the occurrence of hydrolysis were very low, it was significant, being about 0.25% after 32 h of storage at 180 °C and 0.39% after frying at 180 °C for 40 h.

The OSI protection was better during frying with BHT while the extract protected oil from degradation (i.e. protect iodine values) after 24 h of frying. Nevertheless, the OSI and iodine value for all samples were significantly higher for oil treated with both antioxidants during the 40 h of frying (Figs. 5b, 6a). The oil color was not greatly

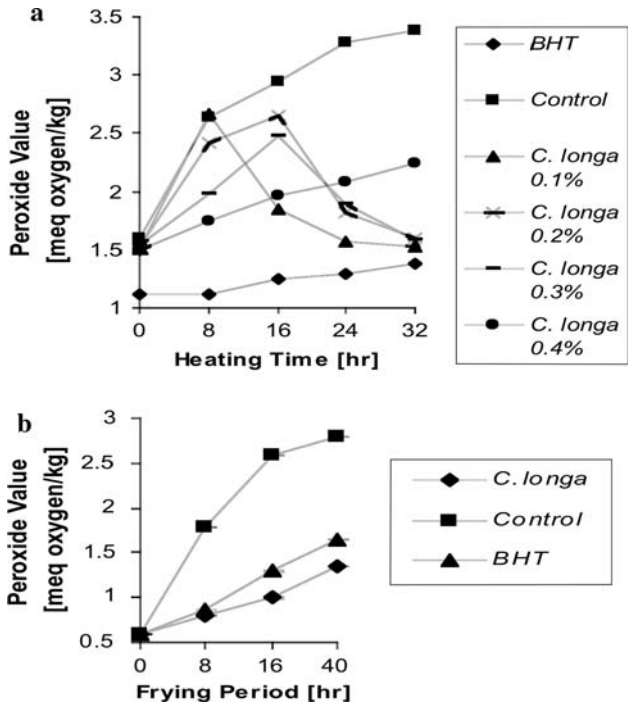

Fig. 2 Changes in peroxide value (PV) of palm oil treated with various level s of *C. longa* leaves extract in accelerated oxidation studies (**a**) and frying experiments (**b**)

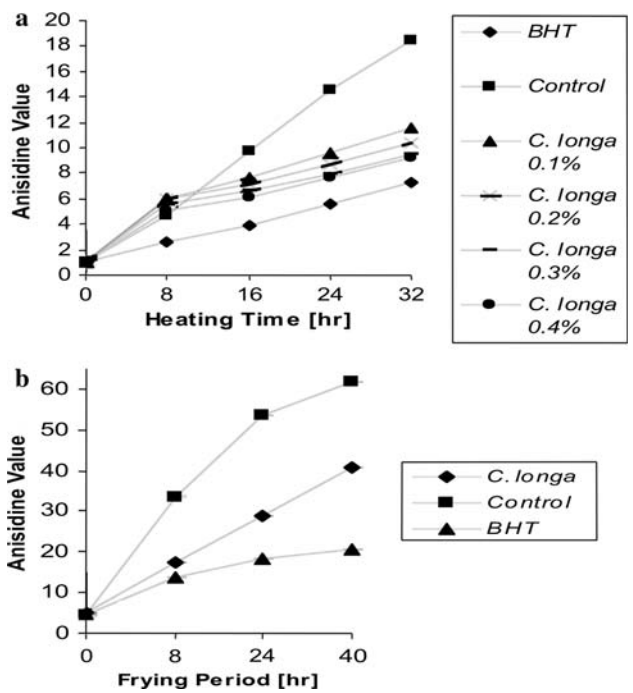

Fig. 3 Changes in anisidine value (AV) of palm oil treated with various levels of *C. longa* leaves extract in accelerated oxidation studies (**a**) and frying experiment (**b**)

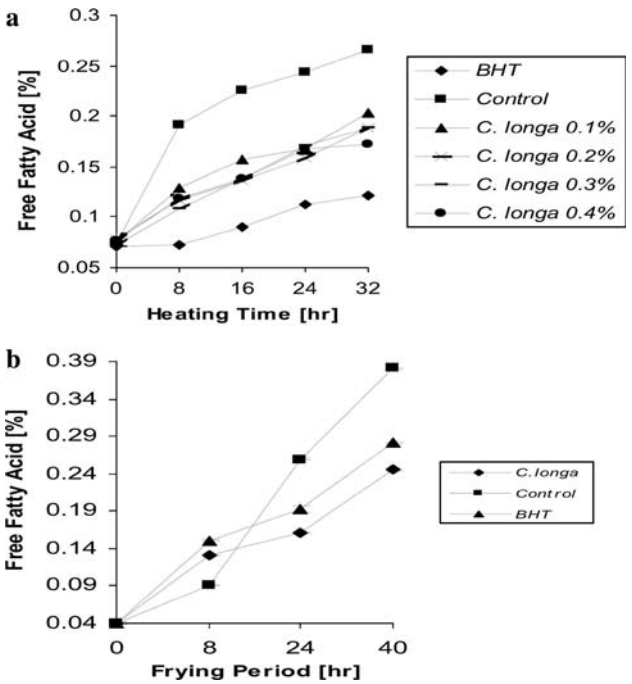

Fig. 4 Changes in free fatty acid (FFA) of palm oil treated with various levels of *C. longa* leaves extract in accelerated oxidation studies (**a**) and frying experiments (**b**)

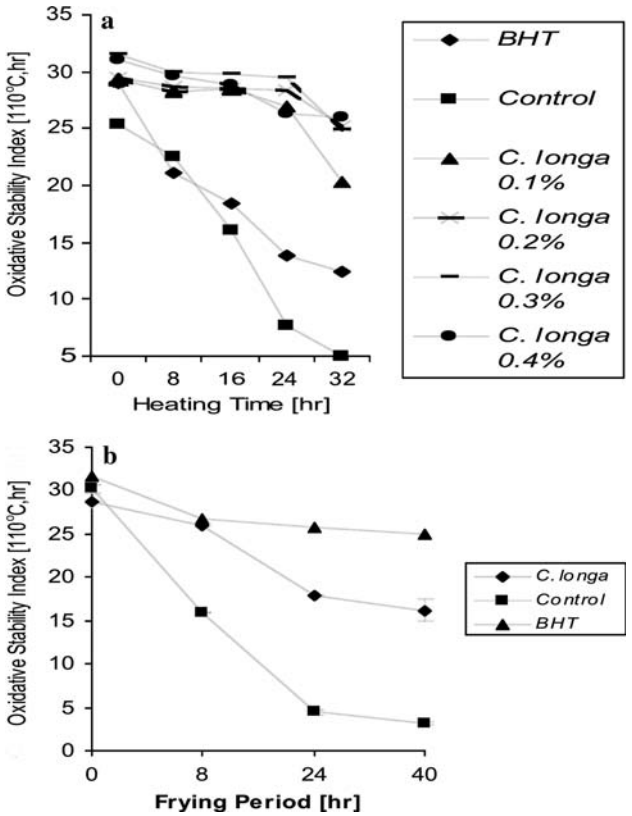

Fig. 5 Changes in Oxidative Stability Index (OSI) of palm oil treated with various levels of *C. longa* leaves extract in accelerated oxidation studies (**a**) and frying experiments (**b**)

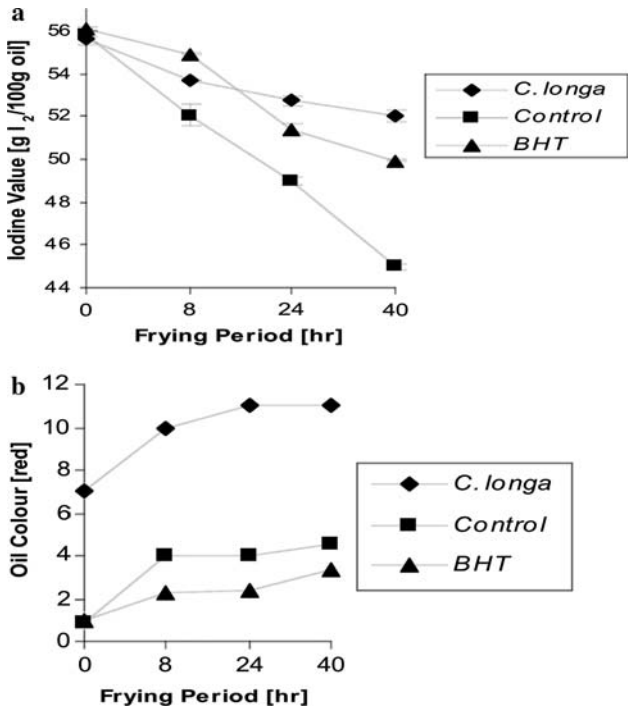

Fig. 6 Changes in Iodine value (**a**) and oil color (**b**) of palm oil treated with 0.2% *C. longa* leaves extract compared to 0.02% BHT during frying experiments

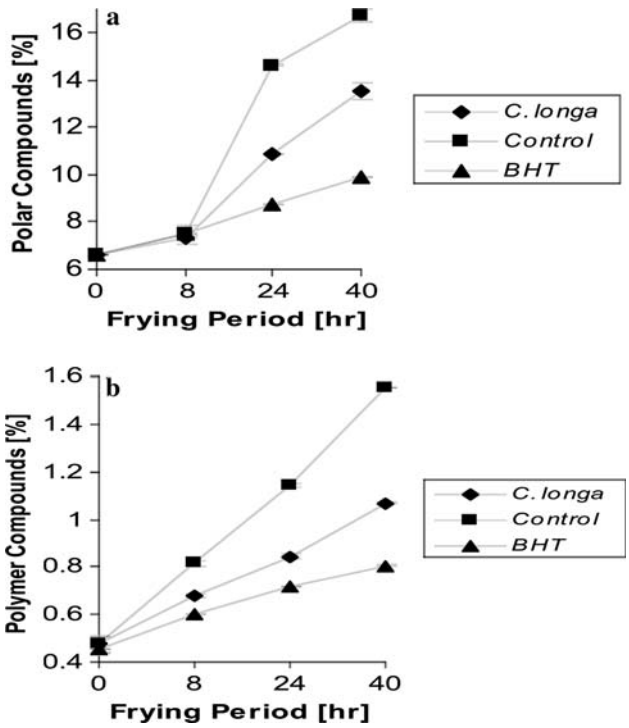

Fig. 7 Changes in polar components (**a**) and polymer compounds (**b**) of palm oil treated with 0.2% *C. longa* leaves extract compared to 0.02% BHT during frying experiments

affected by frying time for the control and BHT samples, with BHT showing significantly ($P < 0.05$) lower redness intensity than the control (Fig. 6b). Oil darkening were significantly ($P < 0.05$) higher in the presence of the extract throughout the frying, due to presence of pigments and phenolic compounds and their breakdown products during heating and frying.

Both antioxidants were capable of lowering the percentage of polar and polymer compounds in the frying oil, with BHT performing better than the extract, after 8 h frying (Fig. 7). All samples contained low polar compounds indicating that the oil is still in good quality, far below the level for rejection and replacement of cooking oil (24% and 26% limit) in most European countries [5]. The high molecular weight polymer molecules were created by the repetitive oxidative reaction of hundreds of triacylglycerides at the high frying temperatures. Both natural and synthetic antioxidants were capable of retarding polymer compounds formation significantly ($P < 0.05$) with better activity by BHT.

The color changes in the French fries, fried in the presence of *C. longa* leaf extract were not significantly different throughout the frying experiment (Table 1). Products fried in the control oil showed a slight increase in the color intensity. Samples fried in the oil containing BHT showed significantly different ($P < 0.05$) color after the 40th hour of frying, indicating discoloration of the product.

Sensory Evaluation

Sensory evaluation scores on day 1, 3 and 5 for samples fried in oil containing BHT and extracts (Table 2) were not significantly different for color, oiliness and crispiness between samples throughout the frying experiment. The flavor scores for all products were not affected by frying time, but were slightly different from one another by the fifth day of frying. There was no significant difference ($P < 0.05$) in taste scores between potatoes fried in the control and BHT-treated oils throughout the experiment. Products fried in the presence of *C. longa* leaves extract scored significantly lower ($P < 0.05$) than the control and BHT on the third day of frying but scored slightly higher on the fifth day of frying. Samples fried in *C. longa* leaf extract-treated oil were acceptable up to the fifth day of frying.

The natural phenolic compounds in the herbs may have retarded the rate of conjugated diene formation and protected against linoleic acid degradation [14]. Phenolics transfer hydrogen atoms to lipid peroxyl radicals and thus form the aryloxyl, which is incapable of acting as a chain carrier, and subsequently couples with another radical to quench the radical process [15]. The order of natural antioxidant efficacy studied in palm olein was reportedly rosemary oleoresin > BHA > sage extract > BHT [16]. Rosemary extract (0.1%) in refined rapeseed oil markedly

Table 1 Effect of *C. longa* and BHT on French fries color

Oil treatment	*L*	a (+a means red direction, −a means green direction)	b (+b means yellow direction, −b means blue direction)
C. longa 8 h	63.65 ± 3.26Aa	−0.85 ± 0.16Ba	19.25 ± 1.65Aa
C. longa 24 h	64.97 ± 1.89Aa	−0.69 ± 0.09Bb	15.50 ± 1.43ABab
C. longa 40 h	61.31 ± 5.14Ab	0.76 ± 0.61Aa	16.41 ± 3.35Aa
Control 8 h	61.56 ± 0.93Aab	−0.45 ± 0.13Ba	16.16 ± 0.36Aa
Control 24 h	60.67 ± 1.43Aa	0.77 ± 0.20Aa	12.92 ± 1.13Ab
Control 40 h	58.09 ± 0.68Ab	1.00 ± 0.09Aa	13.80 ± 0.49Aa
BHT 8 h	57.23 ± 1.30Bb	−0.52 ± 0.27Ba	17.50 ± 1.68Aa
BHT 24 h	62.22 ± 2.41ABa	0.70 ± 0.16Aa	18.58 ± 1.96Aa
BHT 40 h	66.50 ± 0.34Aa	0.75 ± 0.02Aa	15.62 ± 0.32Aa

a and b, Means with different lowercase letters are significantly different ($P < 0.05$) between treatments

A and B, Means with different capital letters are significantly different ($P < 0.05$) between days

Table 2 Effect of *C. longa* leaves extract and BHT on sensory characteristics of French fries during deep-fat frying[a, b]

Sensory quality	Time (day)	*C. longa*	Control	BHT
Color	1	8.00 ± 1.00Aa	8.00 ± 0.000Aa	8.00 ± 0.00Aa
	3	8.00 ± 0.00Aa	6.67 ± 1.16Aa	7.67 ± 0.58ABa
	5	8.00 ± 1.00Aa	6.33 ± 0.58Aab	6.00 ± 0.00Bb
flavor	1	5.33 ± 1.53Aa	8.00 ± 0.00Aa	7.00 ± 0.00Aab
	3	5.00 ± 0.00Aa	6.67 ± 1.16Aa	7.00 ± 0.00Aa
	5	6.67 ± 1.53Aa	6.33 ± 0.58Aa	5.33 ± 0.58Ab
Oiliness	1	5.67 ± 1.16Aa	6.33 ± 0.58Aa	7.00 ± 1.00Aa
	3	6.67 ± 1.16Aa	7.00 ± 0.00Aa	6.00 ± 1.00Aa
	5	6.67 ± 1.15Aa	5.33 ± 0.58Aa	4.33 ± 0.58Aa
Crispiness	1	5.33 ± 1.16Aa	5.67 ± 1.16Aa	7.67 ± 1.16Aa
	3	7.33 ± 0.58Aa	6.33 ± 1.53Aa	6.33 ± 1.16Aa
	5	7.00 ± 1.00Aa	5.33 ± 0.58Aa	4.67 ± 0.58Aa
Taste	1	5.33 ± 1.16ABa	7.67 ± 0.58Aa	7.67 ± 0.58Aa
	3	4.00 ± 2.00Bb	7.00 ± 1.00Aa	7.00 ± 1.00Aa
	5	6.67 ± 1.12Aa	6.00 ± 0.00Aa	5.33 ± 0.58Aa
Overall quality	1	5.33 ± 1.16Aa	8.00 ± 0.00Aa	7.00 ± 1.00Aa
	3	6.00 ± 1.00Aa	7.00 ± 1.00ABa	7.00 ± 1.00Aa
	5	7.00 ± 1.73Aa	5.67 ± 0.58Bab	4.67 ± 0.58Bb

a and b, Means with different lowercase letters are significantly different ($P < 0.05$) between days for a specific sensory quality

A and B, Means with different capital letters are significantly different ($P < 0.05$) between treatments for a specific sensory quality

[a] Using a 9-point hedonic scale (1 = very poor and 9 = very good)

[b] Mean of 10 trained panelists

reduced the rate of tocopherol degradation during deep fat frying of potato chips [17].

The free fatty acid level was found to be significantly lower ($P < 0.05$) for samples treated with 0.2% *C. longa* leaf extract, as similarly, reported for 0.4% rosemary and sage oleoresin extract [18]. The iodine value changes during frying (Fig. 6a), which is indicative of reactions involving double bonds, through direct interaction to form 1,2-diol or through chain reactions adjacent to the double bond to form volatile degradation products. *C. longa* leaf contains protocatechuic acid, syringic acid and vanillic acid [3]. vanillic acid, caffeic acid and ferulic acid (hindered phenols) and 0.02% crude tea extract reportedly lowered the peroxide value and anisidine value of oils [19].

Curcuma longa leaf extract (0.1%) extended the OSI even at 0.1% better than 0.02% BHT in accelerated oxidation studies, probably because BHT is more volatile [20] than phenolic compounds at high temperatures [21]. Rosemary extract (500 ppm) reportedly also showed better protection in rapeseed oil triacylglycerols in Rancimat and Oxidograph tests compared to BHT (100 ppm) [22]. The rate of dimer and polymer formation depends on antioxidants content, the oil type, frying temperature, and number of frying runs and is another indicator of fats and oil

oxidation. The phenolic compounds are antioxidative due to their redox properties; acting as reducing agents, hydrogen donors, free radical quenchers and metal chelators. The use of natural antioxidants is somewhat limited in industry due to lack of availability, cost, knowledge about their molecular composition, amount of active ingredients in the source material and the availability of relevant toxicity data.

The sensory evaluation showed that the *C. longa* leaf extract in the frying oil was capable of maintaining fried products quality, and was not significantly affected by the colored pigments. The darker oil color was most likely due to the alpha, beta-saturated carbonyl compounds that have the ability to absorb energy of visible light [23].

Curcuma longa leaf extract exhibited good antioxidant activity. The topping up of the oil and natural antioxidant, may explain why *C. longa* extract showed better antioxidant protection in the frying experiments compared to the accelerated oxidation study, at similar concentrations of 0.2% (Fig. 2). Interaction between the polyphenol compounds and existing tocopherols and tocotrienols in RBD palm olein may have resulted in synergistic antioxidant effects. This study demonstrated the antioxidant properties of *C. longa* leaves and it may be another potential source of natural antioxidants to be exploited in the food and nutraceutical industries.

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