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Exploitation of Catechin Extract from Pruned Tea Leaves as a Promising Food Preservative Against Lipid Oxidation

Lai Thi Ngoc Ha*, Tran Thi Hoai, Hoang Lan Phuong & Nguyen Van Lam

Faculty of Food Science and Technology, Vietnam National University of Agriculture, Hanoi 131000, Vietnam

Abstract

In Vietnam, a tea-producing country, the tea buds and top three leaves are normally used for tea production while older leaves are pruned and discarded as agricultural waste in the winter. The present study aimed to exploit catechins from pruned tea leaves and use them as natural antioxidants for applications in the food industry. Catechins were analyzed using the guideline of ISO 14502-2-2005 by HPLC-MWD. The contents of catechins in pruned tea leaves of ten popular tea varieties were relatively high, ranging from 65.57 to 136.88 mg/g dry weight. The optimized conditions for catechin extraction from Phuc Van Tien pruned tea leaves (one of the varieties with a high catechin content) were found using response surface methodology as follows: a liquid-to-solid ratio of 21.6/1 at 70^{o}C for 31 minutes. The catechin-rich extract powder was added to sesame oil to inhibit lipid oxidation. During oil accelerated oxidation at 60^{o}C, the catechin-rich extract powder inhibited the increase of the peroxide value compared with the negative and positive controls (no preservative and added tert-butylhydroquinone, respectively). Significant positive correlations between the decrease of catechin content and the inhibition of peroxide formation ($r = 0.91$, 0.94, 0.95, 0.97, and 0.96 for catechin, epigallocatechin gallate, epigallocatechin, epicatechin gallate, and epicatechin, respectively, $P < 0.05$) proved that the inhibition of peroxide formation in the sesame oil was essentially due to the antioxidant capacity of the catechins in the pruned tea leaf extract. Catechin extracts from pruned tea leaves are potential sources of natural antioxidants for oil preservation.

Keywords

Phenolic compounds, catechin extraction, response surface methodology, lipid oxidation

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Correspondence to
ltnha.cntp@vnua.edu.vn

ORCID
Lai Thi Ngoc Ha
https://orcid.org/0000-0003-1020-3752

Introduction

Tea (*Camellia sinensis*) is one of the most popularly consumed beverages in the world (Prasanth *et al.*, 2019). Tea leaves contain a

high quantity of catechins, which are considered strong natural antioxidants (Gulua *et al.*, 2019). These compounds have been reported to prevent lipid oxidation, a major problem in the food industry (Toschi *et al.*, 2000; Hara, 2001). Tea leaf polyphenols have been shown to be effective in preserving meat (Yang *et al.*, 2017; Bellés *et al.*, 2018), oil (Hara, 2001; Nain *et al.*, 2021), seafood (Fıçıcılar *et al.*, 2018), and even biodiesel (Bharti & Singh, 2019). Tea polyphenols have also been shown to have significant effects on delaying the increase of total volatile base nitrogen (TVB-N, the product produced by protein decomposition), inhibiting bacterial growth (Yang *et al*., 2017), reducing the formation of peroxide and secondary oxidation products in oil (Nain *et al*., 2021), and extending the shelf-life of foods (Yang *et al*., 2017; Nain *et al*., 2021).

The content of catechins is higher in young leaves that are used for drinking tea than in older ones. The young tea leaves are harvested in the spring, summer, and autumn. In winter, the tea plants are pruned to shape, which stimulates new growth buds in the spring. This source of pruned tea leaves (PTL) is discarded and used as mulch for the soil (**Figure 1**). Nonetheless, this leaf source has been analyzed and determined to have potential natural anti-oxidative compounds (Zandi & Gordon, 1999; Vuong *et al.*, 2012). Those were (+)-catechin (C), (-)-epicatechin (EC), epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCG). Interestingly, their antioxidant activity could be higher than that of butylated hydroxyanisole (BHA), an artificial antioxidant, when added to lard, fish oil, and soybean oil (Shahidi, 2015).

The objective of this study was to exploit the catechins in PTL and apply them in inhibiting lipid oxidation. To achieve this goal, firstly, the catechin content in the PTL of ten popular tea varieties in Vietnam was evaluated. Then, the variety containing the highest catechin quantity was used as the material for optimizing the catechin extraction process. Finally, the ability to inhibit lipid oxidation of catechin-rich extract powder from PTL was evaluated.

Materials and Methods

Chemicals and reagents

Standards, namely (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)- epicatechin gallate, (-)-epigallocatechin gallate, and caffeine at HPLC grade, were obtained from Sigma-Aldrich (Germany). Absolute ethanol, acetonitrile, and acetic acid were purchased from Merck (Germany). Vitamin C was supplied by Kanto Chemical (Japan). Ethylenediaminetetraacetic acid (EDTA) was bought from Norgen (Canada). Sodium thiosulfate was obtained from Samchun (Korea). Tert-butylhydroquinone (TBHQ) was obtained from Eastman Chemical (Kingsport, Tennessee). Virgin black sesame oil was obtained from the Bao Tam Company (Vietnam).

Leaf collection and preparation

PTL were collected in 2016 and 2017 for analyzing catechin content and for optimizing the catechin extraction conditions, respectively. To determine the catechin content, tea leaves from the pruned part (20cm from the top of the tea tree) of ten tea varieties were collected at the Northern Mountainous Agriculture and Forestry

Figure 1. Old tea leaves before pruning (A) and the PTL (B, C)

Science Institute (NOMAFSI), Phu Tho province, Vietnam, in November 2016. The ages of the tea trees ranged from 7-10 years and the heights were 0.8-1m. Leaves were then placed into paper bags and transported to the laboratory of NOMAFSI on the same day. Five biological replications (each of 200g) were taken.

At the laboratory of NOMAFSI, the polyphenol oxidases of the tea leaves were immediately deactivated by steaming for 4-5 minutes. After steaming, the tea leaves were dried at 73-80^{o}C in an oven until the moisture content was reduced to about 5-8%.

For the catechin extraction optimization, PTL of the Phuc Van Tien variety was collected in November 2017 and treated as described above.

Determination of catechins in pruned tea leaves

Catechins extraction

Catechin determination was conducted using the guidelines of ISO 14502-2-2005. Briefly, approximately 0.2g of ground dry tea leaves was mixed with 5mL of methanol 70% in a 15-mL centrifugation tube and shaken for 10 minutes at 70^{o}C. After centrifugation at 3,830*g* for 10 minutes at 4^{o}C, the supernatant was collected, and the residue was extracted one more time with the same volume of the same solvent. The supernatants from the two successive extractions were pooled in a volumetric flask of 10mL and methanol 70% was added to the 10mL mark. Extractions were done in triplicate. Each extract was then filtered through a 0.45µm pore-size syringe (Phenex™-NY, Utrecht, The Netherlands), and diluted with a stabilizing solution containing acetonitrile 10% (*v/v*), ascorbic acid 500 µg mL^{-1}, and EDTA 500 µg mL^{-1} before HPLC analysis.

HPLC analysis of catechins

Quantification of the catechins was performed by HPLC using an Aligent system 1260 (Santa Clara, CA) equipped with G1311B-Quat pumps, G1329B autosampler, G1330B thermostat, and G1365 MWD VL lamp. A 20-µL aliquot of an extract was injected into a Kinetex EVO C18 column (150x4.6mm i.d: 5µm particle size) equipped with a guard column of the same type (Phenomenex, Netherlands). The mobile phases were A (20 µg mL^{-1} EDTA, 2% acetic acid, 9% acetonitrile) and B (20 µg mL^{-1} EDTA, 2% acetic acid, 80% acetonitrile). The flow rate was 1 mL min^{-1} and the column temperature was 30^{o}C. The gradient elution was as follows: 0-7.5min, 0% B; 7.5-15min, 0-30% B; 15-20min, 30% B; 20-25min, 30-100% B; 25-29min, 100% B; 29-34min, 100-0% B; and 34-36min, 0% B. Monitoring was set at 278nm. Catechins were identified by their retention times as compared to the authentic standards (**Figure 2**) and were quantified using five-point calibration curves.

Optimization of catechin extraction from the PTL of the Phuc Van Tien variety by food grade solvent

The response surface methodology was used for optimizing the extraction conditions of catechins from PTL. A three-factor and rotatable central composite design (CCD) consisting of 21 experimental runs with eight factorial points, six axial points, and three replicates at the center point and maximal and minimal factorial points (**Table 1**) were employed. The design variables were the liquid-to-solid ratio (15/1-25/1, X_1), extraction temperature (45-85^{o}C; X_2), and the extraction time (30-90 min; X_3). The variables of ethanol concentration (50%) and particle size (< 0.3mm) were kept at constant values. Responses were the total catechins and EGCG content of the PTL.

For all runs, extractions were done in 15-mL falcon tube. Extractions were terminated by centrifugation at 3,830*g* for 10 minutes at 4^{o}C. The obtained extracts were collected, filtered, and analyzed by HPLC-MWD. The experimental data were fitted to the following second-order polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ij} x_i^2 + \sum_{i}^{k-1} \sum_{j}^{k} \beta_{ij} x_i x_j$$

where Y is the response, β_o, β_i, β_{ii}, and β_{ij} are the regression coefficients for the intercept, linear, quadratic and interactions terms, respectively, and x_i and x_j are the coded values of the independent variables. The formula used to convert the coded values to real values and vice versa was as follows $x_i = (X_i - X_0)/\Delta X_i$, *where* x_i

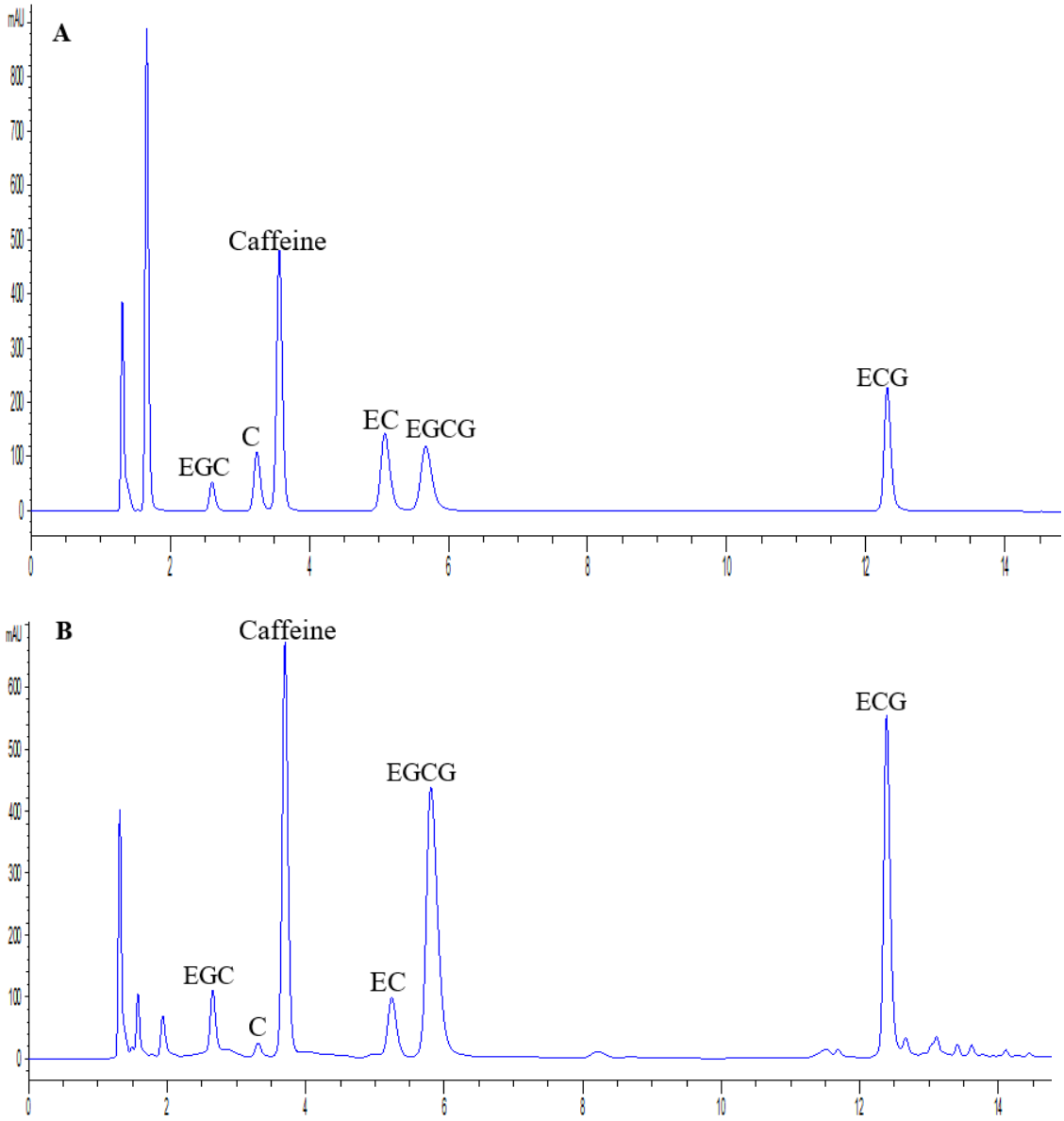

Figure 2. HPLC chromatograms of the catechin standards (A) and the PTL of the Phuc Van Tien variety (B)

and X_i are the dimensionless and the real values of the independent variable i (i = 1, 2 and 3), respectively, X_0 is the real value of the independent variable i at the central point, and ΔX_i is the step change of X_i corresponding to a unit variation of the dimensionless value.

The optimum conditions of catechin extraction were determined by using JMP 10 software. The software was set to search for the optimum desirability of the response variables (maximum EGCG content and total catechins). The validation of the model was carried out by determining the content of the interest compounds extracted at the optimal conditions with four replicates. The experimental results were compared with the predicted values.

Oxidative stability of sesame oil enriched with catechins from pruned tea leaf extract

Preparation of tea leaf extract powder

PTL from the variety with high total catechins were collected in large amounts to produce the extract powder. The extraction was performed in a dark glass bottle that contained 2 liters of ethanol 50% at the optimized conditions. After shaking, the mixture was centrifuged at

Table 1. Rotatable central composite design setting in the coded form (x_1, x_2, x_3) and real values of the independent variables (X_1, X_2 and X_3) with the experimental results for the response variables (EGCG content and total catechins of Phuc Van Tien PTL)

Run	Standard variables			Real variables			EGCG (mg/g DW)	Total catechins (mg/g DW)
	x_1	x_2	x_3	X_1 - Liquid/solid ratio	X_2 - Temperature (°C)	X_3 - Time (min)		
1A	1	1	1	25	85	90	43.15	103.92
1B	1	1	1	25	85	90	41.67	100.48
1C	1	1	1	25	85	90	45.84	109.93
2	-1	1	1	15	85	90	41.03	99.54
3	1	-1	1	25	45	90	51.25	124.47
4	-1	-1	1	15	45	90	47.41	116.92
5	1	1	-1	25	85	30	52.17	126.30
6	-1	1	-1	15	85	30	49.00	119.58
7	1	-1	-1	25	45	30	49.34	120.34
8A	-1	-1	-1	15	45	30	47.56	117.53
8B	-1	-1	-1	15	45	30	47.47	116.90
8C	-1	-1	-1	15	45	30	48.77	120.12
9	1.68	0	0	28.4	65	60	50.38	121.77
10	-1.68	0	0	11.6	65	60	47.29	115.86
11	0	1.68	0	20	98.6	60	44.21	106.18
12	0	-1.68	0	20	31.4	60	48.14	118.60
13	0	0	1.68	20	65	110.4	49.96	121.59
14	0	0	-1.68	20	65	9.6	48.92	120.54
15A	0	0	0	20	65	60	49.47	120.96
15B	0	0	0	20	65	60	51.24	125.01
15C	0	0	0	20	65	60	53.61	131.08

3,830*g* for 10min at 4°C, and the supernatant was then collected. The solvent in the extract was evaporated in a rotavapor at 40°C. Finally, the concentrated extract was freeze-dried to obtain the PTL extract powder.

Preparation of the catechins enriched sesame oil

Sesame oil was enriched with PTL extract powder at two levels: 100mg and 200mg of catechins per kilogram of oil. The tea extract powder was dissolved in absolute ethanol and added to the oil to reach the required catechin concentration. The ethanol was evaporated from the oil by degassing at 30°C for 15min. After that, the oil was transferred to glass bottles without lids (20 mL/bottle) and stored at 60°C in the dark for 18 days. Samples with 100ppm of TBHQ (positive control) and without preservatives (negative control) were prepared in the same way. Oil samples were taken and the peroxide value was analyzed every 3 days. Simultaneously, catechins in the oil were extracted and analyzed by HPLC.

Extraction of catechins from sesame oil and determination of peroxide value

The extraction of catechins from the sesame oil was conducted using the methods described by Salta *et al.* (2007) with minor modifications. Catechin enriched oil (1.2g) was extracted three times with methanol, 0.2mL for each extraction. The mixture of oil and methanol was shaken vigorously and then centrifuged at 3,508*g* for 1

minute, allowing the methanol layer to separate. Three methanolic extracts were combined and the methanol was evaporated in a vacuum rotavapor. Methanol 70% was added into the residue to re-dissolve the catechins. The solution was filtered and then analyzed by using HPLC.

The peroxide value of the stored oil was determined using ISO 3960:2007.

Statistical analysis

Data were analyzed using the statistical software SAS 9.4 (SAS Institute, Cary, NC). The catechin contents of the tea varieties were expressed as mean ± standard deviation of five biological replications. One-way analysis of variance (ANOVA) and Duncan's multiple range test were used to determine the differences among the means. The response surface methodology experiment was performed using the software JMP 10 (SAS Institute, Cary, NC) to obtain the optimal extraction conditions. In the experiment of the accelerated oxidation of sesame oil, analysis of variance was carried out using a generalized linear model (GLM) procedure to determine the effect of the sample (without or added preservative), oxidation time, and their interaction on the peroxide value. The model configuration was $Y = a + b_1X_1 + b_2X_2 + b_{12}X_1X_2$ (Y: the peroxide value; X_1: sample, and X_2: oxidation time). Pearson correlation coefficients were determined in order to evaluate the relationships between the reduction of catechin content and the inhibition of peroxide formation in the oxidized sesame oil.

Results and Discussion

Composition of catechins in the PTL from ten Vietnamese tea varieties

The catechin contents in the PTL from ten varieties were high (65.57-136.88 mg/g DW). Among the ten investigated varieties, PH11 had the highest total catechin content with the value of 136.88 mg/g DW, followed by Keo, LDP1, and Phuc Van Tien with values of 127.48, 122.86, and 117.83 mg/g DW, respectively. Kim Tuyen had the lowest total catechins with a content of 65.56 mg/g DM (**Table 2**).

The total catechin content in the PTL from the ten investigated varieties was similar to the level in aged tea leaves of *Camellia asamica* Kucha in China (10.9%) (Lu *et al.*, 2009), but it was higher than the Yabukita cultivar in Australia (5.98%) (Vuong *et al.*, 2012). Khoa *et al.* (2017) analyzed old tea leaves from the Shan variety in Vietnam and found a similar catechin content (9.63%). The total catechin content in PTL (or old tea leaves) was about two times lower than that of young tea leaves (21.54% or 113.81-279.43 mg/g DM) as reported by Khoa *et al.* (2017) and Wu *et al.* (2012), respectively. The decrease in total catechins of tea leaves during the growth period was confirmed by Song *et al.* (2012). However, it is important to note that the PTL had a higher quantity of polyphenols than some known phenolic sources such as sim (*Rhodomyrtus tomentosa*) fruits (Lai *et al.*, 2015) and grape seeds (Bucić-Kojić *et al.*, 2007) of which the phenolic contents were 4.92% and 1.47-6.68% of dry matter, respectively.

Regarding individual catechins, EGC and EGCG were the major ones identified in the PTL depending on the variety, while catechin was the minor one in all the varieties. Indeed, while EGCG was the major catechin of young tea leaves (Wu *et al.*, 2012), in the PTL of the ten investigated varieties in the present study, EGCG was the major one in only five of the varieties, namely Keo, Kim Tuyen, LDP1, PH11, and Phuc Van Tien, with the contents ranging from 33.39 to 57.24 mg/g DW. Keo had the highest concentration of EGCG in PTL (57.24 ± 6.02 mg/g DW). EGC was the main catechin of the five remaining varieties, namely PH1, Shan, LDP2, PH8, and PH10, with the contents ranging from 37.48 to 59.26 mg/g DW. Interestingly, both EGCG and EGC are catechins that have demonstrated antioxidant activities better than EC and ECG (Chen & Chan, 1996; Hara, 2001). Therefore, the PTL were not only the source of total catechins but also the source of EGC and EGCG, which are potential antioxidants. mW m^{-2}.

Optimization of catechin extraction from PTL by using response surface methodology

Among the five tea varieties having high total catechins (PH11, Keo, LDP1, Phuc Van

Table 2. Catechin content in ten tea varieties grown in Vietnam (mg/g DW)

Varieties	EGC	C	EC	EGCG	ECG	Total catechins
Keo	47.65^{bD} ± 4.32	1.41^{eC} ± 0.04	5.89^{dE} ± 0.26	57.24^{aA} ± 6.41	15.29cD ± 0.52	127.48^{B}± 10.68
Kim Tuyen	23.58^{bH} ± 1.71	0.15^{eG} ± 0.03	2.45^{dH} ± 0.55	33.39^{aE} ± 3.42	5.99cI ± 0.82	65.57^{G}± 5.46
LDP1	33.51^{bG} ± 2.14	1.35^{eC} ± 0.25	9.30^{dC} ± 0.61	53.06^{aB} ± 1.13	25.63cC ± 1.62	122.86^{B} ± 3.20
LDP2	59.26^{aA} ± 3.05	0.51^{eF} ± 0.03	3.16^{dG} ± 0.33	35.85^{bD} ± 2.32	8.16cH ± 1.00	106.94^{DE} ± 4.13
PH1	56.05^{aBC} ± 6.15	0.78^{eDE} ± 0.05	4.82^{dF} ± 0.47	27.97^{bF} ± 2.46	11.38^{cF}± 0.80	101.00^{F}± 9.53
PH10	37.48^{aF} ± 3.57	0.84^{eD} ± 0.11	5.68^{dE} ± 0.57	33.71^{bDE} ± 2.64	12.65^{cE}± 1.00	90.35^{G}± 7.57
PH11	44.55^{bE} ± 5.92	1.62^{eB} ± 0.34	10.24^{dB} ± 1.39	53.28^{aB} ± 2.44	27.19^{cB}± 1.16	136.88^{A}± 7.59
PH8	57.68^{aAB} ± 3.06	1.60^{dB} ± 0.11	7.95^{cD} ± 0.26	33.68^{bDE} ± 1.75	9.16^{cG} ± 0.45	110.08^{D} ± 4.75
Phuc Van Tien	31.77^{bG} ± 0.54	2.24^{eA} ± 0.25	8.29^{dD} ± 0.28	46.76^{aC} ± 2.20	28.76^{cA} ± 1.18	117.83^{C}± 4.06
Shan	53.44^{aC} ± 1.40	0.71^{dE} ± 0.12	12.68^{cA} ± 0.84	23.73^{bG} ± 1.06	12.53^{cE} ± 0.70	103.08^{EF}± 3.63

Note: Mean ± SD (n = 5). Means followed by the same lowercase letter in a row and same uppercase letter in a column do not statistically differ.

Tien, and PH8) and the five varieties owning high EGCG contents (Keo, PH11, LDP1, Phuc Van Tien, and LDP2), only the PTL from the Phuc Van Tien variety were able to be collected because the tea plants were pruned earlier than usual. We were not able to collect the pruned leaves from the variety with the highest total catechins (PH11) or the one with the highest EGCG content (Keo). Therefore, in 2017, a large amount of Phuc Van Tien PTL was collected and used as the material for optimizing the catechin extraction process and then producing catechin-rich extract powder.

The total catechins and the EGCG (main catechin) content were optimized through the response surface methodology approach. Aqueous ethanol was chosen because ethanol is a food-grade solvent. A fixed ethanol concentration of 50% was chosen based on the primary study. Three factors, namely the liquid-to-solid ratio, temperature, and extraction time, were considered as variables in the model. The experimental design with five-levels and three-variable CCRDs, and the experimental results of the extractions are shown in **Table 1**.

By applying multiple regression analysis and converting the coded values into real values, the relation between the investigated variables and the responses was explained by **Equations 1** and **2** for the total catechin and EGCG contents, respectively**,** in which X_i were the real variables.

$$Y_{\text{total catechins}} = 8.05 + 4.188X_1 + 1.886X_2 + 0.683X_3 + 0.00234X_1X_2 + 0.00295X_1X_3 - 0.0093X_2X_3 - 0.103X_1^2 - 0.012X_2^2 - 0.00199X_3^2 \quad \textbf{(Eq. 1)}$$

$$Y_{EGCG} = 2.837 + 1.657X_1 + 0.772X_2 + 0.281X_3 + 0.00039X_1X_2 + 0.00138X_1X_3 - 0.00377X_2X_3 - 0.0391X_1^2 - 0.0048X_2^2 - 0.0008X_3^2 \quad \textbf{(Eq. 2)}$$

To fit the response functions and experimental data, the linear and quadratic effects of the independent variables, as well as their interactions, on the response variables were evaluated by analysis of variance (ANOVA) (**Table 3**). The regression coefficients were determined and shown in **Table 4**.

The ANOVA of the regression models showed that the models were highly significant due to low probability values (P = 0.0021 and P = 0.0052 for total catechins and EGCG content, respectively) (**Table 3**). The coefficients of determination (R^2) were 0.85 and 0.82 indicating that 85% of the variation for the total catechins and 82% of the variation for EGCG content were attributed to the three studied factors. Lack of fit was absent for the two models (P = 0.3720 and 0.3554 for total catechins and EGCG content, respectively) indicating that the total error of the models was due to the pure error.

The effects of the liquid-to-solid ratio, temperature, and time of extraction on the catechin content are presented in **Table 4**. As

Table 3. Analysis of variance of total catechins and EGCG content in PTL

Source	DF	Sum of Squares	Mean Square	F Ratio
Total catechins				
Model	9	1208.128	134.236	6.830
Error	11	216.195	19.654	*P* = 0.0021
Lack of fit	5	112.731	22.546	1.3075
Pure error	6	103.464	17.244	*P* = 0.3720
Total	20	1424.323		
EGCG				
Model	9	178.638	19.849	5.495
Error	11	39.732	3.612	*P* = 0.0052
Lack of fit	5	21.110	4.222	1.360
Pure error	6	18.622	3.104	*P* = 0.3554
Total	20	218.371		

Table 4. Parameter estimates of the predicted second-order model for the response variables

Term	Estimate	Std Error	t Ratio	Prob>|t|
Total catechins				
Intercept	125.729	2.554	49.22	<0.0001
Liquid/solid ratio	1.907	1.102	1.73	0.1114
Temperature	-4.112	1.102	-3.73	0.0033
Time	-3.111	1.102	-2.82	0.0166
Liquid/solid ratio*Temperature	0.234	1.421	0.17	0.8719
Liquid/solid ratio*Time	0.442	1.421	0.31	0.7616
Temperature*Time	-5.601	1.421	-3.94	0.0023
Liquid/solid ratio*Liquid/solid ratio	-2.585	1.312	-1.97	0.0745
Temperature*Temperature	-4.856	1.312	-3.70	0.0035
Time*Time	-1.789	1.312	-1.36	0.1999
EGCG				
Intercept	51.457	1.095	46.99	<0.0001
Liquid/solid ratio	1.001	0.472	2.12	0.0577
Temperature	-1.410	0.472	-2.98	0.0124
Time	-1.165	0.472	-2.47	0.0313
Liquid/solid ratio*Temperature	0.039	0.609	0.06	0.9504
Liquid/solid ratio*Time	0.206	0.609	0.34	0.7412
Temperature*Time	-2.264	0.609	-3.72	0.0034
Liquid/solid ratio*Liquid/solid ratio	-0.978	0.562	-1.74	0.1100
Temperature*Temperature	-1.918	0.562	-3.41	0.0058
Time*Time	-0.764	0.562	-1.36	0.2016

illustrated in this table, the temperature and time of extraction showed significant linear effects for the catechin content (P = 0.0033 and 0.0166, respectively) and for the EGCG content (P = 0.0124 and 0.0313, respectively). Among them, the temperature appeared to be the most affecting

factor in the total catechins extraction process from old tea leaves since its coefficients had the highest absolute values (4.11 and 1.41 for total catechins and EGCG content, respectively).

The negative quadratic effects of X_1, X_2, and X_3 were found in both the total catechins and EGCG extraction models indicating that there were maximum catechin and EGCG contents at a certain liquid-to-solid ratio, temperature, and extraction time. To find the optimum conditions, the JMP 10 software was set to search for the optimum desirability of the two responses at the same time. The optimized conditions were found as follows: a liquid-to-solid ratio of 21.6, a temperature of 70°C, and a time of extraction of 31 minutes, as shown in **Figure 3**. For validating the models, four extractions were performed at the optimum conditions. The experimental values (49.94-50.18 mg/g DW of EGCG and 122.73-123.50 mg catechins/g DW) lay within a 95% mean confidence interval of the predicted values (49.94-54.29 mg/g DW for EGCG and 122.28-132.42 mg/g DW for total catechins) indicating the predictability of the two models.

Oxidative stability of sesame oil enriched with catechins from PTL extract powder

Table 5 shows the change in the peroxide values of sesame oil during accelerated oxidation at 60°C. Both factors, namely the sample (control and preservative added samples) and accelerated oxidation time, significantly affected the peroxide values of the sesame oil (P <0.0001 for both factors). In addition, a significant treatment*oxidation time interaction was observed (P <0.0001).

The peroxide values in all the samples at the beginning of oxidation were similar and equivalent to 5.33 meqO_2 /kg. Significant increases in the peroxide values were observed for all the samples over oxidation time (P <0.0001). However, the increases were different among the samples. The negative control sample (sesame oil without catechins and artificial preservatives) had a dramatic increase in its peroxide value followed by the TBHQ added sample (positive control). After 18 days of preservation, their peroxide values rose up to 45.84 and 28.53 meqO_2/kg, respectively.

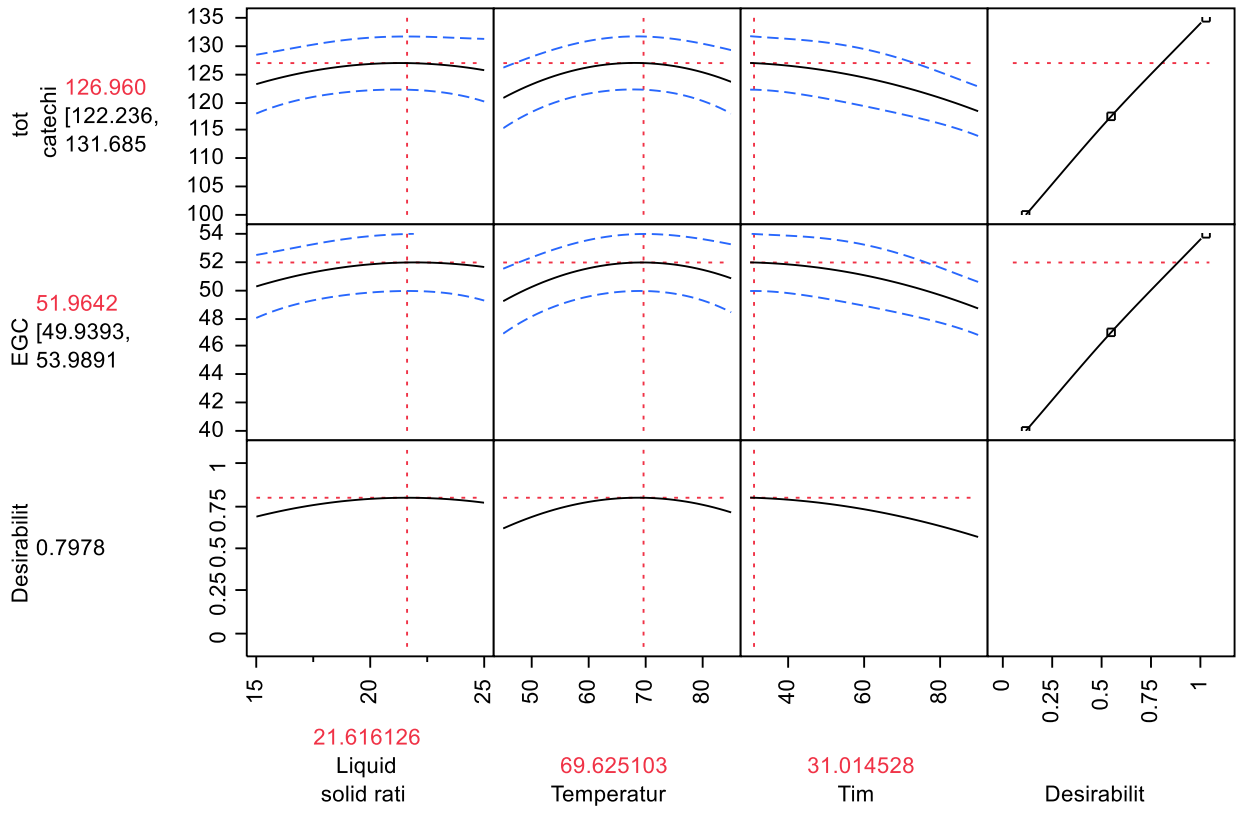

Figure 3. Optimum conditions for the extraction of EGCG and total catechins from PTL

Table 5. Change of peroxide values of sesame oil during accelerated oxidation at 60°C ($meqO_2$/kg)

Accelerated oxidation time (days)	Control sample	TBHQ (200 ppm)	PTL extract (100ppm of catechins)	PTL extract (200ppm of catechins)
0	$5.29^{aG} \pm 0.19$	$5.42^{aG} \pm 0.13$	$5.27^{aG} \pm 0.15$	$5.34^{aG} \pm 0.08$
3	$9.69^{aF} \pm 0.27$	$7.75^{bF} \pm 0.19$	$6.32^{cF} \pm 0.07$	$6.22^{cF} \pm 0.19$
6	$14.67^{aE} \pm 0.21$	$8.96^{bE} \pm 0.09$	$8.25^{cE} \pm 0.16$	$7.92^{dE} \pm 0.17$
9	$20.58^{aD} \pm 0.53$	$10.05^{bD} \pm 0.06$	$9.32^{cD} \pm 0.21$	$8.86^{cD} \pm 0.23$
12	$27.05^{aC} \pm 0.43$	$13.30^{bC} \pm 0.29$	$9.72^{cC} \pm 0.14$	$9.24^{cC} \pm 0.11$
15	$34.19^{aB} \pm 0.85$	$19.88^{bB} \pm 0.38$	$12.21^{cB} \pm 0.17$	$10.26^{dB} \pm 0.16$
18	$45.84^{aA} \pm 1.55$	$28.53^{bA} \pm 0.57$	$15.02^{cA} \pm 0.40$	$11.81^{dA} \pm 0.06$

Note: Mean ± SD (n = 3). Means followed by the same lowercase letter in a row and same uppercase letter in a column do not statistically differ.

Interestingly, catechins from the PTL were found to significantly inhibit peroxide formation in sesame oil as compared to the two controls (P <0.0001). After 18 days of oxidation, the peroxide values of the 200 ppm- and 100 ppm-catechin added samples increased to 11.81 and 15.02 $meqO_2$ /kg, respectively **(Table 5).** Moreover, both added catechin oils had a peroxide value under 15 $meqO_2$/kg, which is the recommended value for virgin oil of the Codex Alimentarius until the 18th day.

The protective effect of tea catechins against lipid oxidation has been reported in several publications. Tea polyphenols added to canola oil showed a higher protective capacity against lipid oxidation than butylated hydroxytoluene (BHT), an artificial preservative commonly used in the food industry (Chen *et al.*, 1996). Hara (2001) confirmed that green tea catechin extract had a stronger antioxidant effect than BHA and tocopherol. Toschi *et al.* (2000) demonstrated that green tea extract at concentrations of 0.02-0.04% had greater antioxidant activities against lipid oxidation in refined peanut oil than BHT. Old tea leaf extract (0.05-0.25%) was also effective in protecting rapeseed oil against oxidation (Zandi & Gordon, 1999).

The protective action of catechin-rich extract powder from PTL and the contribution of individual catechins could be proved by the correlation between the inhibition of peroxide formation and the reduction in catechin content over an accelerated oxidation time. **Table 6** shows the percentages of catechin reduction in the 200-ppm catechin added-sample over oxidation time and their Pearson correlations with the inhibition of peroxide formation (difference between the peroxide value of the negative control and that of the 200-ppm catechin added oil at a time of sampling).

Table 6 shows that the catechin content in oil reduced over storage time (P <0.0001) and the level of reduction was significantly different among individual catechins (P <0.0001). On day 0, the extracted catechins from the oil were considered 100%, which means 0% of reduction. During the time of storage, the extracted catechins from the oil decreased. The decrease of catechins suggested that these compounds progressively reacted with the lipid radicals formed by lipid oxidation leading to the inhibition of peroxide formation. This was reinforced by significant positive Pearson correlation coefficients between the decrease of catechins in the oil and the inhibition of peroxide formation (r = 0.91 to 0.97, P <0.01). In contrast, since caffeine has no antioxidant activity, its content did not change over the 18 days and no significant correlation between the caffeine content and the inhibition of peroxide formation was found (r = 0.08, P = 0.8672).

The antioxidant activity of catechins could be explained by their free radical scavenging capacity, which is dependent on their chemical structure. Catechins can donate hydrogen atoms or electrons from the hydroxyl groups in their

Table 6. Reduction of catechin and caffeine content during accelerated oxidation and Pearson correlations with the inhibition of peroxide formation

Catechins	Day of lipid accelerated oxidation							Pearson correlation
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	
EGC	0 e	9.94^{eA}	28.33^{dA}	41.31^{cA}	53.90^{bA}	68.50^{aA}	70.53^{aA}	0.95
C	0 c	0.0^{aB}	6.19^{bcB}	3.33^{cC}	3.33^{cBC}	14.88^{abC}	22.04^{aC}	0.91
Caffeine	0 a	0.82^{aB}	1.96^{aB}	-1.66^{aC}	-1.62^{aC}	-1.25^{aD}	1.46^{aD}	0.08
EC	0 d	-0.43^{dB}	7.60^{bcdB}	6.82^{cdC}	18.36^{abcB}	19.71^{abC}	25.05^{aC}	0.96
EGCG	0 f	12.46^{eA}	29.17^{dA}	43.19^{cA}	54.16^{bA}	62.89^{abA}	68.03^{aA}	0.94
ECG	0 f	10.30^{eA}	20.02^{dA}	29.92^{cB}	44.31^{bA}	55.04^{aB}	58.45^{aB}	0.97

Note: Means followed by the same lowercase letter in a row and same uppercase letter in a column do not statistically differ.

structure and become stable radicals. The stability of these antioxidant radicals was derived from the resonance delocalization of the unpaired electrons around phenolic nuclei (Shahidi, 2005). As shown in **Table 6**, after 18 days, EGC and EGCG seemed to be the most reactive in inhibiting the sesame oil oxidation with their reductions of around 70% followed by ECG with 58.45%. The rank of antioxidative activity of individual tea catechins in this study was as follows: EGC=EGCG>ECG>EC=C. Among the five main catechins in tea leaves, EGCG had the most antioxidant activity thanks to its high degree of OH substitution in the flavonoid backbone (Cao *et al.*, 1997; Roy *et al.*, 2010). However, it is interesting to note that although the number of OH groups in ECG (7 -OH substitutions) is higher than that in EGC (6 -OH groups), the lipid protective action of EGC was shown to be higher than that of ECG. This could be explained by the fact that EGC is more hydrophobic and more mobile than ECG (Chen *et al.*, 1996). Moreover, EGC has a free OH substitution at the 5'position that increases its antioxidative power (Hara, 2001).

Conclusions

In this study, the PTL catechin contents of ten popular tea varieties in Vietnam were quantified by HPLC-MWD and ranged from 65.57 to 136.88 mg/g DW. The optimized conditions for catechin extraction from PTL were found as follows: a liquid-to-solid ratio of 21.6, a temperature of 70°C, and a time of extraction of 31 minutes. The catechin-rich extract powder successfully inhibited lipid oxidation in sesame oil. This opens a new application for tea plants in the food industry and is also a solution to increase the economic value of tea plants.

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