# OPTIMIZATION OF EXTRACTION OF PHENOLIC ANTIOXIDANTS FROM TEA (*CAMELLIA SINENSIS* L.) FRUIT PEEL BIOMASS USING RESPONSE SURFACE METHODOLOGY

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Tea (*Camellia sinensis* L.) fruit peel, the main byproduct during the manufacture of tea seed oil, was used as raw material for the recovery of phenolic antioxidants. The effect of ethanol concentration, extraction time, and extraction temperature on total phenolic content (TPC) and ferric-reducing antioxidant power (FRAP) of the extracts from tea fruit peel was investigated. The maximum predicted TPC (47.5 mg GAE/g dry peel) was obtained under the optimum recovery conditions (43% ethanol, 60°C, and 33 min) given by using response surface methodology (RSM). A high correlation ($R^2$ = 0.929, $p$ < 0.01) between TPC and FRAP value was identified by linear regression analysis. Furthermore, gallocatechin (GC) and epigallocatechin (EGC) were found to be the major individual catechins in the extracts from tea fruit peel. Ethanol/aqueous extraction has been presented as an effective method for the recovery of phenolic antioxidants from tea fruit peel.

*Keywords: Tea fruit peel; Phenolic compounds; Antioxidants; Recovery; Optimization*

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## INTRODUCTION

Phenolic compounds are the primary bioactive constituents derived from plants and are commonly known as antioxidants, due to their redox properties. These properties allow them to act as free radical scavengers, hydrogen donors, reducing agents, and metal ion chelators (Javanmardi *et al.* 2003). In recent years, interest in the use of phenolic antioxidants from natural sources as food preservatives has increased greatly (Ghafoor *et al.* 2009; Shi *et al.* 2005), since the side effects of synthetic antioxidants represent a growing area of concern (Jayaprakasha *et al.* 2003). Moreover, phenolic antioxidants play an important role in protecting the human body against oxidative stress, induced by an imbalance between the generation and removal of reactive oxygen species and in retarding the progress of many chronic diseases (Ozsoy *et al.* 2008).

Currently, recovery of phenolic compounds from food processing waste biomass has attracted increasing attention as a potentially cheap and reliable source of new and efficient natural antioxidants (Ghafoor *et al.* 2009; Cam and Aaby 2010; Makris *et al.* 2007). Tea (*Camellia sinensis* L.) fruit peel is the main byproduct during the manufacture of tea seed oil, which has long been recognized as an edible oil of high quality. As a

consequence of the increase in popularity of tea seed oil, the amount of tea fruit peel produced has also increased. However, tea fruit peel is not typically utilized, and the majority is discarded as industrial waste. This results in the loss of a valuable resource and also in environmental pollution. Our previous work has revealed that phenolic-enriched extracts from tea fruit peel have remarkably high antioxidant activity and could potentially be developed as natural antioxidants (Wang *et al.* 2011). Thus, finding an effective way to recover phenolic antioxidants from tea fruit peel is critical for the commercial exploitation of this valuable resource.

Various novel techniques have been employed to recover phenolics from plant matrices, including ultrasound-assisted extraction, supercritical fluid extraction, and microwave-assisted extraction (Wang and Weller 2006). However, from the point of view of industrial production, solvent extraction is commonly chosen due to the simplicity and efficiency of the procedure and the low investment costs required in terms of equipment (Ballard *et al.* 2009; Gan and Latiff 2010; Pompeu *et al.* 2009). Although the polarity of the solvent plays an important role in the selective extraction of different bioactive compounds, ethanol and water are the preferred solvents as they are non-toxic and environmentally friendly. Previously, ethanol/aqueous extraction has been successfully used to recover phenolic compounds from food processing wastes, such as peel (Ma *et al.* 2008), shell (Contini *et al.* 2008), and pomace (Cam and Aaby 2010).

The objectives of this study were to: (i) use response surface methodology (RSM) to determine the optimal conditions for the recovery of phenolic antioxidants from tea fruit peel by ethanol/aqueous extraction, (ii) to measure the antioxidant activity of tea fruit peel extract using ferric-reducing antioxidant power (FRAP) assay, and (iii) to identify the individual catechins present in tea fruit peel extracts using high performance liquid chromatography (HPLC). The optimization parameters investigated were ethanol concentration, extraction temperature, and extraction time, respectively.

## EXPERIMENTAL

### Materials and Reagents

Tea fruit were collected from Panban tea garden (Zhejiang, China) and washed in distilled water three times prior to the peel being separated. After oven-drying at 40°C for 24 h, the dried peel was milled into powder by a pulverizer (XB-02, Xiaobao Machinery Co., Zhejiang, China) and passed through a 20-mesh sieve. The resulting tea fruit peel powder was stored in the refrigerator at -20°C until required.

Folin-Ciocalteu's phenol reagent, gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), and all catechin standards were purchased from Sigma Chemical Co. (Missouri, USA). Methanol and acetonitrile of HPLC grade were purchased from Tianjin Shield Co. (Tianjin, China). All other chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Co. (Shanghai, China).

### Extraction Procedure

The phenolic antioxidants were extracted using 30%, 60%, and 90% (v/v) ethanol in water. Ethanol (40 mL) was added to 1 g of tea fruit peel and placed in a reciprocal

shaking water bath at a speed of 150 rpm. Samples were heated to temperatures of 30, 50, and 70°C for 10, 25, and 40 min, respectively. The crude extracts were cooled to room temperature before centrifugation at 5,000×g for 10 to 15 min. The supernatant was collected and placed in a 50 mL volumetric flask for further analysis. Each solvent extraction was carried out in triplicate.

## Determination of Total Phenolic Compounds

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu method modified by Ranilla *et al.* (2010). A 50 μL sample of the solution was transferred into a 10 mL volumetric flask and mixed with 6 mL of distilled water. To each sample, 0.5 mL of Folin-Ciocalteu reagent (50%, v/v) was added and mixed. After 5 min, 1 mL of $Na_2CO_3$ (5%, m/v) was added to the mixture and adjusted to 10 mL with distilled water. After standing for 60 min at room temperature, the absorbance was measured at 760 nm. Gallic acid was used to construct the standard curve. TPC was expressed as mg gallic acid equivalents (GAE)/g dry peel.

## Determination of Antioxidant Activity

Antioxidant activity was determined using a ferric-reducing antioxidant power (FRAP) assay carried out according to a modified method of Benzie and Strain (1999). The working FRAP reagent was prepared by mixing 10 vol of 300 mM acetate buffer (pH 3.6) with 1 vol TPTZ (10 mM) in HCl (40 mM) and adding 1 vol of $FeCl_3$ (20 mM). The freshly prepared FRAP reagent was warmed at 37°C, and a reagent blank reading was taken at 593 nm. Subsequently, 20 μL of the sample was mixed with 480 μL of distilled water and added to the FRAP reagent (4.5 mL). A second reading at 593 nm was performed after 8 min. To determine the FRAP value of the sample, the initial blank reading with the FRAP reagent alone was subtracted from the final reading of the FRAP reagent plus the sample. A standard curve was prepared using a range of concentrations (25 to 1500 mM) of $FeSO_4$. The FRAP value of the extracts was expressed as mM $FeSO_4$/g of dry peel.

## Quantification of Catechins

Tea catechins, including C, (+)-catechin; CG, (+)-catechin gallate; EC, (-)-epicatechin; ECG, (-)-epicatechin gallate; EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin gallate; GC, (+)-gallocatechin; GCG, (+)-gallocatechin gallate, were determined according to the HPLC method described by Liang *et al.* (2007). The HPLC analysis conditions were as follows: injection volume, 10 μL; column, TC-$C_{18}$ 5 μm, 4.6 mm × 150 mm (Agilent Technologies Inc., CA, USA); oven temperature, 28°C; mobile phase A, acetonitrile/acetic acid/water (6/1/193); mobile phase B, acetonitrile/acetic acid/water (60/1/139); flow rate, 1 mL/min; and detecting wavelength, 280 nm. During gradient elution, mobile phase B increased from 30% to 85% by a linear gradient during the first 35 min and remained at 85% for a further 5 min. Catechins were identified and quantified by comparing their retention time and peak area with those of the authentic standards. The catechin content of the extracts was expressed as mg/g of dry peel.

## Statistical Analysis

RSM was used to determine the optimal conditions for extraction. RSM was performed using the Design-Expert software (Trial Version 7.1.6, Stat-Ease Inc., Minneapolis, USA). A central composition design (CCD) was used to investigate the effects of three independent variables (solvent concentration, extraction temperature, and extraction time) at three levels on the dependent variables (TPC and FRAP value). CCD uses the method of least-square regression to fit the data to a quadratic model. The quadratic model for each response was as follows:

$$Y = ß_0 + \sum ß_i X_i + \sum ß_{ii} X_i^2 + \sum\sum ß_{ij} X_i X_j$$

where Y represents the response variables and $ß_0$ is a constant. $ß_i$, $ß_{ii}$, and $ß_{ij}$ are the coefficients for linearity, square, and interaction, respectively. $X_i$ and $X_j$ are the independent variables. The model was constructed based on the variables at the 95% confidence level. The coded values of the experimental factors and factor levels used in the response surface analysis are given in Table 1.

**Table 1.** Code Levels of Independent Variables Used in the RSM Design

| Independent variables | Coded symbols | Levels | | |
|---|---|---|---|---|
| | | -1 | 0 | 1 |
| Extraction time (min) | $X_1$ | 10 | 25 | 40 |
| Extraction temperature (°C) | $X_2$ | 30 | 50 | 70 |
| Ethanol concentration (%, v/v) | $X_3$ | 30 | 60 | 90 |

The complete design consisted of 20 experimental points including six replicates of the center point. Coded values for the experiment designs and the corresponding TPC and FRAP values are given in Table 2.

**Table 2.** Experimental Design and Response Values

| Run number | Time (min) | Temperature (°C) | Solvent concentration (%, v/v) | TPC (mg GAE/g dry peel) | FRAP value (mM $FeSO_4$/g dry peel) |
|---|---|---|---|---|---|
| 1 | -1 | -1 | 1 | 37.1 | 843 |
| 2 | 0 | 0 | 0 | 43.1 | 1125 |
| 3 | 1 | -1 | -1 | 43.5 | 1033 |
| 4 | -1 | -1 | 1 | 17.8 | 499 |
| 5 | 0 | 1 | 0 | 43.3 | 1227 |
| 6 | 0 | 0 | -1 | 44.1 | 1306 |
| 7 | -1 | 1 | 1 | 22.5 | 530 |
| 8 | 1 | 1 | -1 | 43.6 | 1277 |
| 9 | 0 | 1 | 0 | 44.8 | 1273 |
| 10 | 0 | 0 | 0 | 45.3 | 1265 |
| 11 | 0 | 0 | 0 | 44.7 | 1305 |
| 12 | 0 | -1 | 0 | 37.5 | 990 |
| 13 | 1 | -1 | 1 | 19.8 | 543 |
| 14 | -1 | 1 | -1 | 40.6 | 1038 |
| 15 | 0 | 0 | 0 | 44.3 | 1266 |
| 16 | 1 | 0 | 0 | 43.6 | 1148 |
| 17 | 0 | 0 | 0 | 43.8 | 1176 |
| 18 | 1 | 1 | 1 | 27.8 | 794 |
| 19 | -1 | 0 | 0 | 37.7 | 988 |
| 20 | 0 | 0 | 1 | 25.0 | 658 |

## RESULTS AND DISCUSSION

### Optimum TPC Recovery Conditions

ANOVA analysis of the quadratic regression model for TPC demonstrated the model to be significant ($p < 0.01$) with an F-value of 92.84 (Table 3). The $R^2$ of the model was 0.9882, and no significance was found in the lack of fit ($p > 0.05$). This indicated that the accuracy of the polynomial model was adequate. The second-order polynomial model was expressed by the following quadratic equation:

$$Y = 43.59 + 2.26 X_1 + 2.36 X_2 - 9.60 X_3 - 0.012 X_1X_2 - 0.26 X_1X_3 + 1.14 X_2X_3 - 2.20 X_1^2 - 1.70 X_2^2 - 8.30 X_3^2$$

**Table 3.** ANOVA for the Effect of Time, Temperature, and Solvent Concentration on TPC, Using a Quadratic Response Surface Model

| Source | Sum of square | DF | F-value | *p*-value |
|---|---|---|---|---|
| Model | 1638.18 | 9 | 92.84 | < 0.0001 [b] |
| A | 18.96 | 1 | 9.67 | 0.0111 [a] |
| B | 7.35 | 1 | 3.75 | 0.0816 |
| C | 3.67 | 1 | 32.47 | 0.0002 [b] |
| AB | $1.25 \times 10^{-3}$ | 1 | $6.38 \times 10^{-4}$ | 0.9804 |
| AC | 0.55 | 1 | 0.28 | 0.6075 |
| BC | 10.35 | 1 | 5.28 | 0.0444 [a] |
| $A^2$ | 13.27 | 1 | 6.77 | 0.0264 [a] |
| $B^2$ | 7.92 | 1 | 4.04 | 0.0722 |
| $C^2$ | 189.30 | 1 | 96.55 | < 0.0001 [b] |
| Residual | 19.61 | 10 | | |
| Lack of Fit | 16.05 | 5 | 1.21 | 0.0617 |
| $R^2$ | 0.9882 | | | |

[a] $p < 0.05$; [b] $p < 0.01$; A, B, C were the variables of extraction time, extraction temperature, and extraction concentration, respectively.

To determine the optimum conditions for recovery of TPC from tea fruit peel, three-dimensional surface plots were constructed (Fig. 1). The influence of extraction time and temperature on TPC at a fixed ethanol concentration of 60% is shown in Fig. 1A. Although temperature was not shown to be a significant ($p > 0.05$) factor with respect to TPC (Table 3), it was found that at a relatively low extraction time (less than 33 min), TPC increased rapidly as the temperature increased from 30°C to 60°C, and moderately thereafter to higher temperatures of approximately 70°C. While at the range of a longer extraction time (33 to 40 min), decreased TPC was observed with increasing time after it reached the maximum. The impact on TPC of temperature was found to be similar to that of time.

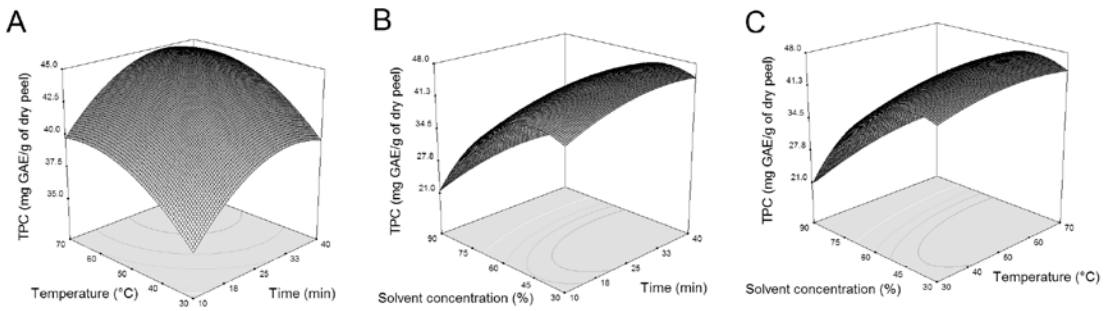


**Fig. 1.** Response surface plots of total phenolic contents (TPC) of tea fruit peel extracts as affected by extraction time, extraction temperature, and solvent concentration (ethanol/aqueous, v/v). (A) Extraction temperature and time (solvent concentration set to 60%); (B) solvent concentration and extraction time (extraction temperature set to 50°C); (C) solvent concentration and extraction temperature (extraction time set to 25 min)

The effect of extraction time and ethanol concentration on TPC at a fixed extraction temperature of 50°C is presented in Fig. 1B. Initially, TPC increased slowly with increasing ethanol concentration until a maximum was reached and subsequently decreased rapidly. A linear increase in TPC with increasing extraction temperature at a fixed ethanol concentration was observed (Fig. 1C). A clear quadratic effect on TPC of ethanol concentration at a fixed temperature was also observed (Fig. 1C). Based on the model, the maximum predicted yield of TPC of 47.5 mg GAE/g dry peel was obtained under the optimum recovery conditions of 43%, 60°C, and 33 min for ethanol concentration, temperature, and time, respectively. Validation experiments carried out under these optimal conditions found the yield to be 47.1 ± 0.6 mg GAE/g dry peel, which was not significantly different from the predicted value ($p > 0.05$). These results indicated that the quadratic model was reliable.

Solid-liquid extraction is a mass transport phenomenon in which solids contained in a matrix migrate into a solvent brought into contact with a matrix (Shi *et al.* 2005). In this study, ethanol concentration and extraction time were found to play a significant role in the extraction of phenolics from tea fruit peel (Table 3). The observation that extraction temperature had an insignificant ($p > 0.05$) effect on recovery of TPC from tea fruit peel was somewhat surprising, since it has been hypothesized that extraction temperature enhances the mass transport phenomenon by inducing changes in diffusion coefficients (Corrales *et al.* 2009). The influence of different factors on TPC recovery in different plant sources varies with the physical properties and chemical composition of the plant matrix and with the respective phenolic compounds present (Ghafoor *et al.* 2009; Pompeu *et al.* 2009; Silva *et al.* 2007). From the perspective of potential industrial applications, low cost and high-recovery efficiency are preferable criteria for large-scale production. Compared to other reported results, the TPC yield (47.5 mg GAE/g dry peel) obtained from tea fruit peel under the optimized conditions in the present study are considerably higher than those extracted previously from food processing wastes, such as grape seed (2.72 mg GAE/g dry material) (Ghafoor *et al.* 2009), citrus peel (19.12 mg GAE/g dry material) (Ma *et al.* 2008), potato peel (3.94 mg GAE/g dry material) and apple peel (35.22 mg GAE/g dry material) (Makris *et al.* 2007), and apple pomace (5.8 mg GAE/g dry material) (Cam and Aaby 2010). The yield in the current study is,

however, lower than that obtained from peanut skin (118 mg GAE/g dry material) (Ballard *et al.* 2009). From the obtained results, it is concluded that tea fruit peel can be treated as a phenolic-enriched resource for potential industrial development.

## Optimum FRAP Extraction Conditions

FRAP is a measure of the antioxidant effect of a substance in a reaction medium in terms of its reducing ability, which is the ability of a natural antioxidant to donate electrons (Shi *et al.* 2009). As shown in Table 2, the highest (1306 mM $FeSO_4$/g dry peel) and lowest (499 mM $FeSO_4$/g dry peel) FRAP values were observed in experimental runs 6 and 4, with the conditions of 30% ethanol concentration, 50°C and 25 min, and 90% ethanol concentration, 30°C and 10 min, respectively. ANOVA analysis revealed that the model was adequate, and that the antioxidant activity of the extracts was significantly ($p < 0.05$) affected by the linear terms of extraction time, extraction temperature, and ethanol concentration, and by the quadratic terms of extraction time and ethanol concentration (Table 4). The second-order polynomial model was expressed by the following quadratic equation:

$$Y = 1213.70 + 89.76\ X_1 + 100.40\ X_2 - 247.33\ X_3 + 33.63\ X_1X_2 - 15.12\ X_1X_3 - 19.70\ X_2X_3 - 125.77\ X_1^2 - 61.97\ X_2^2 - 211.32\ X_3^2$$

**Table 4.** ANOVA for the Effect of Time, Temperature, and Solvent Concentration on the FRAP Value, Using a Quadratic Response Surface Model

| Source | Sum of square | DF | F-value | *p*-value |
|---|---|---|---|---|
| Model | $1.41 \times 10^6$ | 9 | 32.24 | < 0.0001 [b] |
| A | $8.06 \times 10^5$ | 1 | 16.54 | 0.0023 [b] |
| B | $1.01 \times 10^5$ | 1 | 20.69 | 0.0011 [b] |
| C | $6.12 \times 10^5$ | 1 | 125.58 | < 0.0001 [b] |
| AB | $9.05 \times 10^3$ | 1 | 1.86 | 0.2029 |
| AC | $1.83 \times 10^3$ | 1 | 0.38 | 0.5536 |
| BC | $3.11 \times 10^3$ | 1 | 0.64 | 0.4432 |
| $A^2$ | $4.35 \times 10^5$ | 1 | 8.93 | 0.0136 [a] |
| $B^2$ | $1.06 \times 10^5$ | 1 | 2.17 | 0.1717 |
| $C^2$ | $1.23 \times 10^5$ | 1 | 25.21 | < 0.0001 [b] |
| Residual | $4.87 \times 10^5$ | 10 | | |
| Lack of Fit | $2.67 \times 10^5$ | 5 | 1.21 | 0.4188 |
| $R^2$ | 0.9667 | | | |

[a] $p < 0.05$; [b] $p < 0.01$

The three-dimensional plots for FRAP values are presented in Fig. 2. A linear increase in the FRAP value with increasing extraction time at a fixed temperature was observed (Fig. 2A). A similar increase in the FRAP value with increasing extraction temperature at a constant time was also observed (Fig. 2A). As shown in Fig. 2B, the FRAP value initially increased with increasing ethanol concentration and then declined rapidly when ethanol concentration increased further at a fixed extraction time. A similar

effect of ethanol concentration on the FRAP value at a fixed extraction temperature was observed (Fig. 2C). The influences of extraction time and extraction temperature on the FRAP value at fixed ethanol concentrations were also similar, as illustrated in Fig. 2B and 2C. Based on our results, the optimum recovery conditions for antioxidant activity were found to be 40%, 77°C, and 33 min for ethanol concentration, temperature, and time, respectively. It should be noted that these conditions, yielding a maximum predicted FRAP value of 1,372 mM $FeSO_4$/g dry peel, are very similar to the optimum conditions for TPC recovery. In addition, validation experiments carried out under these optimal conditions found the FRAP value was 1,336 ± 48 mM $FeSO_4$/g dry peel, which was not significantly different from the predicted value ($p > 0.05$).

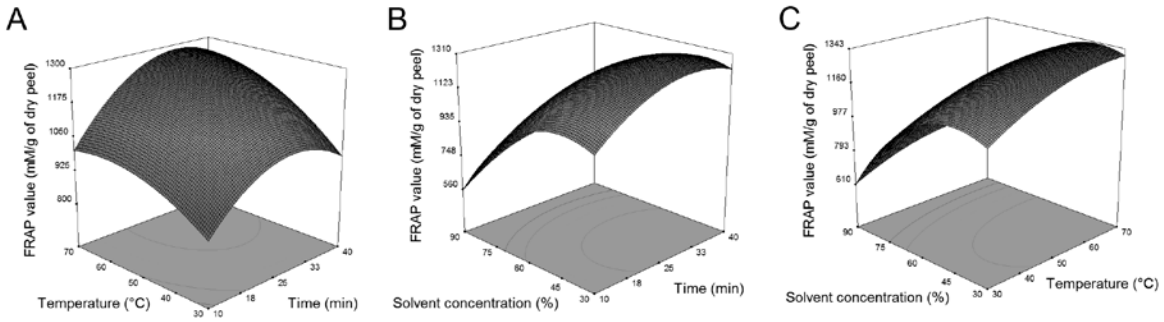


**Fig. 2.** Response surface plots of ferric-reducing antioxidant power (FRAP) of tea fruit peel extracts as affected by extraction time, extraction temperature, and solvent concentration (ethanol/aqueous, v/v). (A) Extraction temperature and time (solvent concentration set to 60%); (B) solvent concentration and extraction time (extraction temperature set to 50°C); (C) solvent concentration and extraction temperature (extraction time set to 25 min)

Phenolic compounds are well known to be important antioxidants, due to their contribution to the antioxidant activity of a wide range of plant extracts. This indicates that the phenolic-enriched extracts from tea fruit peel could potentially be used as natural antioxidants and utilized in a variety of food and pharmaceutical applications, by virtue of being extracted by non-toxic ethanol.

## Quantification of Catechins

Catechins are the primary type of polyphenols found in tea leaves. It has been proposed that they are responsible for the human benefits of teas, due to their outstanding antioxidant activity (Khan and Mukhtar 2007). Previous studies have revealed that catechins also exist in other parts of the tea plant, such as the flowers (Yang *et al.* 2009) and the seeds (Ravichandran 1993). The present study demonstrates that catechins can also be detected in tea fruit peel. The HPLC chromatograms of catechin standards and the sample were given in Fig. 3. The catechin content of the tea fruit peel extracts are given in Table 5.

Unlike the catechins found in tea leaves, GC and EGC were found to be the major individual catechins in fruit peel extract, and CG was not detected in all samples. Total catechin content (TCC) of the extracts ranged from 2.71 mg/g dry peel (experimental run 4) to 38.22 mg/g dry peel (experimental run 6). A significant association ($R^2 = 0.7731$, *p*

< 0.01) between TCC and TPC, as identified by linear regression analysis (Fig. 4), the proportion of TCC in TPC varied from 15% (experiment 4) to 87% (experiment 6) (Table 5), and the correlation between TCC and FRAP value was found to be 0.8204 ($p < 0.01$) (Fig. 5).

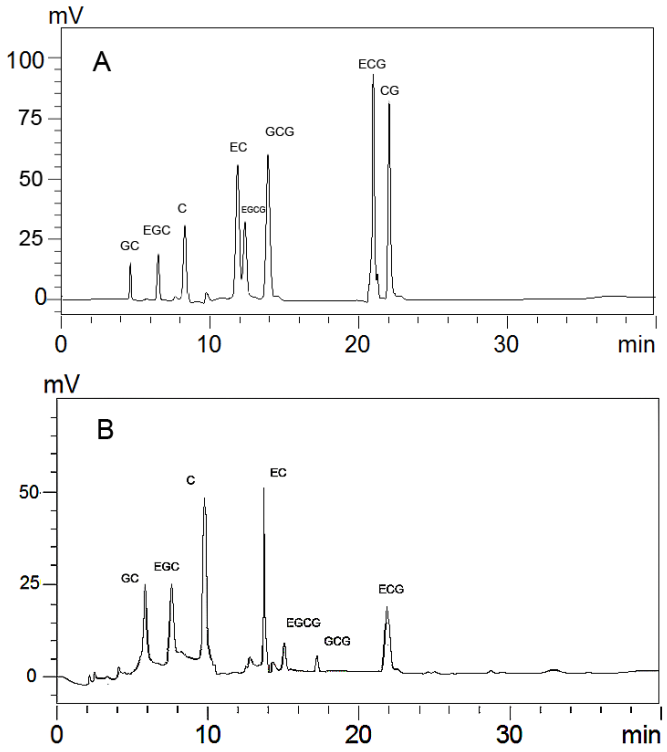


**Fig. 3.** HPLC chromatograms of catechin standards (A) and the extract (B, experimental run 6)

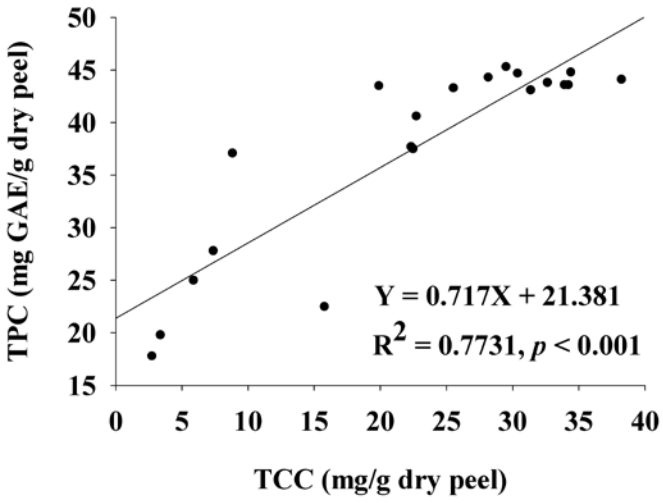


**Fig. 4.** Linear relationship between the total catechin content (TCC) and TPC of tea fruit peel extracts

**Table 5.** Quantification of Catechin Content Obtained from Tea Fruit Peel (mg/g dry peel)

| Run number | GC [a] | EGC [a] | C [a] | EGCG [a] | EC [a] | GCG [a] | ECG [a] | CG [a] | TCC [a] | TCC/TPC (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.79 ± 0.11 | 1.26 ± 0.05 | 2.66 ± 0.03 | 0.91 ± 0.04 | 1.81 ± 0.09 | 0.16 ± 0.00 | 0.52 ± 0.02 | 0.00 | 8.81 ± 0.43 | 24 |
| 2 | 14.13 ± 1.14 | 6.67 ± 0.16 | 4.03 ± 0.11 | 2.87 ± 0.16 | 2.58 ± 0.13 | 0.53 ± 0.03 | 0.92 ± 0.02 | 0.00 | 31.36 ± 2.31 | 73 |
| 3 | 7.45 ± 0.59 | 4.23 ± 0.25 | 3.00 ± 0.18 | 1.24 ± 0.03 | 2.25 ± 0.12 | 0.37 ± 0.02 | 0.91 ± 0.07 | 0.00 | 19.87 ± 1.20 | 46 |
| 4 | 0.37 ± 0.02 | 0.36 ± 0.03 | 0.13 ± 0.00 | 0.52 ± 0.01 | 0.63 ± 0.04 | 0.39 ± 0.02 | 0.24 ± 0.01 | 0.00 | 2.71 ± 0.13 | 15 |
| 5 | 4.82 ± 0.31 | 13.45 ± 0.71 | 2.92 ± 0.14 | 0.75 ± 0.02 | 1.42 ± 0.06 | 1.30 ± 0.03 | 0.54 ± 0.03 | 0.00 | 25.49 ± 2.02 | 59 |
| 6 | 13.48 ± 0.50 | 12.11 ± 0.65 | 5.26 ± 0.30 | 2.74 ± 0.11 | 3.12 ± 0.26 | 0.36 ± 0.02 | 0.90 ± 0.03 | 0.00 | 38.22 ± 2.21 | 87 |
| 7 | 5.02 ± 0.24 | 2.12 ± 0.11 | 2.84 ± 0.03 | 2.51 ± 0.14 | 2.12 ± 0.10 | 0.40 ± 0.02 | 0.79 ± 0.03 | 0.00 | 15.75 ± 0.09 | 70 |
| 8 | 13.78 ± 0.45 | 8.55 ± 0.62 | 3.90 ± 0.17 | 2.91 ± 0.25 | 2.81 ± 0.23 | 0.51 ± 0.04 | 0.95 ± 0.01 | 0.00 | 33.90 ± 1.72 | 78 |
| 9 | 14.71 ± 0.33 | 7.07 ± 0.23 | 4.59 ± 0.09 | 3.03 ± 0.06 | 2.63 ± 0.12 | 0.40 ± 0.01 | 1.01 ± 0.05 | 0.00 | 34.37 ± 1.57 | 77 |
| 10 | 10.51 ± 0.54 | 8.45 ± 0.24 | 4.60 ± 0.36 | 2.63 ± 0.10 | 2.45 ± 0.06 | 0.38 ± 0.01 | 0.96 ± 0.06 | 0.00 | 29.49 ± 1.49 | 65 |
| 11 | 6.20 ± 0.40 | 12.13 ± 0.51 | 3.72 ± 0.19 | 2.86 ± 0.07 | 3.05 ± 0.10 | 0.50 ± 0.03 | 0.94 ± 0.06 | 0.00 | 30.36 ± 1.73 | 68 |
| 12 | 6.86 ± 0.40 | 6.62 ± 0.29 | 3.61 ± 0.16 | 2.21 ± 0.08 | 2.22 ± 0.11 | 0.47 ± 0.03 | 0.80 ± 0.05 | 0.00 | 22.46 ± 0.93 | 60 |
| 13 | 0.61 ± 0.04 | 0.44 ± 0.03 | 0.23 ± 0.01 | 0.62 ± 0.04 | 0.80 ± 0.06 | 0.38 ± 0.02 | 0.30 ± 0.01 | 0.00 | 3.36 ± 0.15 | 17 |
| 14 | 5.74 ± 0.29 | 7.54 ± 0.51 | 5.03 ± 0.22 | 1.20 ± 0.03 | 2.24 ± 0.10 | 0.36 ± 0.03 | 0.63 ± 0.01 | 0.00 | 22.70 ± 1.36 | 56 |
| 15 | 8.00 ± 0.56 | 8.76 ± 0.31 | 3.95 ± 0.12 | 2.87 ± 0.07 | 2.86 ± 0.07 | 0.50 ± 0.03 | 0.92 ± 0.03 | 0.00 | 28.14 ± 0.70 | 64 |
| 16 | 13.91 ± 0.11 | 9.04 ± 0.36 | 3.93 ± 0.23 | 2.93 ± 0.13 | 2.92 ± 0.19 | 0.51 ± 0.02 | 0.98 ± 0.07 | 0.00 | 34.20 ± 0.61 | 78 |
| 17 | 13.34 ± 0.37 | 8.84 ± 0.37 | 3.66 ± 0.14 | 3.02 ± 0.20 | 2.88 ± 0.14 | 0.49 ± 0.02 | 0.95 ± 0.05 | 0.00 | 32.61 ± 2.07 | 74 |
| 18 | 1.25 ± 0.05 | 0.87 ± 0.02 | 1.53 ± 0.04 | 1.35 ± 0.06 | 1.54 ± 0.09 | 0.32 ± 0.02 | 0.63 ± 0.04 | 0.00 | 7.36 ± 0.39 | 26 |
| 19 | 8.96 ± 0.29 | 2.73 ± 0.16 | 3.46 ± 0.08 | 2.97 ± 0.15 | 2.65 ± 0.15 | 0.54 ± 0.03 | 0.95 ± 0.04 | 0.00 | 22.32 ± 1.72 | 59 |
| 20 | 0.84 ± 0.05 | 0.63 ± 0.03 | 1.60 ± 0.04 | 0.79 ± 0.03 | 1.22 ± 0.03 | 0.30 ± 0.01 | 0.48 ± 0.02 | 0.00 | 5.86 ± 0.50 | 23 |

[a] GC, (+)-gallocatechin; EGC, (-)-epigallocatechin; C, (+)-catechin; EGCG, (-)-epigallocatechin gallate; EC, (-)-epicatechin; GCG, (+)-gallocatechin gallate, ECG, (-)-epicatechin gallate; CG, (+)-catechin gallate; TCC, total catechin content

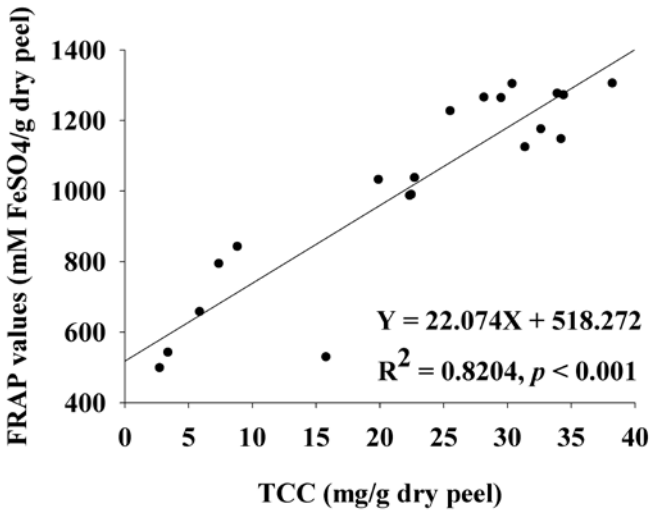


**Fig. 5.** Linear relationship between the TCC and FRAP values of tea fruit peel extracts

This can be attributed, at least partly, to the performance of colorimetry, which tends to be less accurate than that of HPLC. However, it still strongly suggests that in this study, phenolic compounds other than catechins were extracted from tea fruit peel using different concentrations of ethanol.

This indicates that the phenolics other than catechins also contribute to the antioxidant activity of tea fruit peel extract. Previously, phenolic compounds, such as gallic acid, quercetin, kaempferol, and myricetin have been found to be present in tea leaves (Wang *et al.* 2000) and flowers (Yang *et al.* 2009). While it is known that these phenolics also possess a remarkable antioxidant capacity, little is known about their presence in tea fruit peel. Therefore, the isolation and characterization of these phenolics in fruit peel warrants considerable further investigation.

## CONCLUSIONS

1. Tea fruit peel is a potentially valuable renewable bioresource for the development of phenolic antioxidants. TPC obtained from tea fruit peel under optimized conditions (43%, 60°C, and 33 min) was found to be higher than that from other food processing wastes previously reported in the literature.
2. From the point of view of industrial production, ethanol/aqueous extraction has been presented as an effective method for the recovery of phenolic antioxidants from tea fruit peel, although full characterization of the profile of different phenolics in tea fruit peel requires further study.

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