Extraction of Tea Catechins and the Possible Use as an Antioxidant in Lard

Rotsaman Chongcharoen* and Malinee Pinrungrot**

บทคัดย่อ

ในการสกัดสารคาเทชิน (catechins) จากชาเขียวญี่ปุ่น ชาเขียวจีน และชาเขียวใบหม่อน ด้วยน้ำกลั่นและอะซิโตน ที่ระดับความเข้มข้น 2% (w/v) พบว่าชาเขียวจีนมีปริมาณคาเทชินมากที่สุด เท่ากับ 4.11 ± 0.07 มิลลิกรัม/กรัมชาแห้ง (สกัดด้วย 60% อะซิโตน) เมื่อนำชาเขียวจีนมาสกัดโดยเพิ่มระดับความเข้มข้นของชา (2%, 5%, 10% และ 20% (w/v)) และอะซิโตน (50%, 60%, 70% และ 100%) เพื่อหาสภาวะที่เหมาะสมในการสกัด พบว่าการสกัดชาเขียวจีนเข้มข้น 5% (w/v) ด้วย 70% อะซิโตน ให้ปริมาณคาเทชินสูงที่สุดเท่ากับ 6.26 ± 0.06 มิลลิกรัม/กรัม จากนั้นศึกษาประสิทธิภาพของสารสกัดจากชาเขียวจีนในการยับยั้งกลิ่นหืนในน้ำมันหมู โดยใส่สารกันหืนสังเคราะห์บีเอชทีที่ระดับความเข้มข้น 0.02% (w/v) ซึ่งเป็นปริมาณที่อนุญาตให้ใส่ในไขมันสัตว์ เปรียบเทียบกับสารคาเทชินบริสุทธิ์ (d- (+) - catechin) และสารคาเทชินที่สกัดได้จากชาเขียวจีน ที่ระดับความเข้มข้นเดียวกัน พบว่าสารบีเอชทีที่เติมลงในน้ำมันหมู สามารถยับยั้งการเกิดกลิ่นหืนได้ดีที่สุดในวันที่ 8 ของการทดลอง โดยมีค่าเปอร์ออกไซด์เท่ากับ 7.75 ± 1.06 มิลลิอิควิวาเลนต์/กิโลกรัม รองลงมาคือสารสกัดจากชาเขียวจีนและสารคาเทชินบริสุทธิ์ ซึ่งมีค่าเปอร์ออกไซด์เท่ากับ 14.0 ± 2.12 และ 16.50 ± 2.12 มิลลิอิควิวาเลนต์/กิโลกรัม ตามลำดับ อย่างไรก็ตาม เมื่อเพิ่มความเข้มข้นของสารคาเทชินที่สกัดได้จากชาเขียวจีน (0.1%, 0.2% และ 1.0% (w/v)) ในน้ำมันหมู สามารถยับยั้งการเกิดเปอร์ออกไซด์ได้เพิ่มขึ้น โดยมีค่าเปอร์ออกไซด์ลดลงเท่ากับ 9.75 ± 1.06, 5.75 ± 0.35 และ 4.75 ± 1.06 มิลลิอิควิวาเลนต์/กิโลกรัม ตามลำดับ จากผลการทดลองนี้สรุปได้ว่า สามารถใช้สารคาเทชินจากชาเขียวจีนในการยับยั้งกลิ่นหืนในน้ำมันหมูแทนสารสังเคราะห์ได้

Abstract

Catechins were initially extracted from 2% (w/v) Japanese and Chinese green tea and mulberry *(Morusalba)* green tea. The Chinese green tea gave maximum catechins content of 4.11 ± 0.07 mg/g (60% acetone infusion). The optimization of acetone extraction was done with increasing amount of the Chinese green tea leaves. The maximum catechins content of 6.26 ± 0.06 mg/g was obtained from 5% (w/v) tea leaves extracted with 70% acetone. Tea catechins were tested for antioxidative effect in lard along with other synthetic antioxidants, butylated hydroxytoluene (BHT) and d-(+)-catechin. The peroxide values (meq/kg), measured after eight days of storage, were 7.75 ± 1.06 (0.02%, (w/v) BHT), 14.0 ± 2.12 (0.02% (w/v) tea catechins), and 16.5 ± 2.12 (0.02% (w/v) d-(+)-catechin), respectively. However, peroxide values decreased with increasing concentrations of tea catechins used as followed (meq/kg): 9.75 ± 1.06

* Lecturer, Department of Agro-Industrial Technology, Faculty of Applied Science, King Mongkut's Institue of Technology North Bangkok, Corresponding author's E-mail: rotsamanc@hotmail.com

** Student, Department of Agro-Industrial Technology, Faculty of Applied Science, King Mongkut's Institute of Technology North Bangkok.

(0.1% (w/v)); 5.75 ± 0.35 (0.2% (w/v)); 4.75 ± 1.06 (1.0% (w/v)). From this result, BHT could be replaced with tea catechins to prevent oxidative rancidity.

Keywords: Tea extract, Catechins, Infusion, Lard, Antioxidation

1. Introduction

Tea *(Camellia sinensis)* contains polyphenols as major constituents, comprising a group of active compounds known for multiple health benefits such as antioxigenicity [1], anticarcinogenicity [2], [3], antigenotoxicity [4]. Among these substances are catechins, flavonoids, which are composed of four major substances: epicatechin (EC); epicatechin gallate (ECG); epigallocatechin (EGC); epigallocatechin gallate (EGCG) and other four minor substances. Level of catechins varies with the types of tea, which are classified according to different manufacturing processes: green (non-fermented); Oolong (semi-fermented); black (fully fermented). Black tea extracts are generally less effective, in terms of oxidation potency, than green tea extracts, since phenolic substances in tea are oxidized during fermentation. Antioxidative activity of several natural extracts including green tea was reported to be comparable to that of conventional synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), commonly used in oil products [5], [6]. Therefore, this study was aimed to explore the possibility of using green tea extract as an alternative source for synthetic antioxidants, which were reported to be toxic to experimental animals [7]

2. Materials and Methods

2.1. Chemicals and Samples

d-(+)-catechin, sulfanilamide and BHT were purchased from Fluka (Buchs, Switzerland). Acetone was obtained from Labscan (Labscan limited, Dublin, Ireland). Three types of commercial tea leaves: Chinese green tea; Japanese green tea; mulberry green tea, used in this study were purchased from a local supermarket.

2.2. Extraction of Catechins from Green Tea Leaves

2% (w/v) of tea leaves were infused in hot water at 80°C. The mixtures were gently shaken for 20 minutes and were then filtered through Whatman no. 42 as described by Yoshida, Kiso and Goto [8]. For acetone infusion, 2%, 5%, 10% and 20% (w/v) of tea leaves were soaked in various concentrations of acetone: 50%; 60%; 70%; 100%. The mixtures were shaken at 200 rpm for 60 min and filtered through Whatman no. 42 [9].

2.3. Estimation of Total Catechins in Green Tea Extracts

The concentrations of catechins in green tea extracts were determined spectrophotometrically at 425 nm (UV-160A, Shimadzu, Japan) for yellow color complexes formed as a result of electrophilic substitution reaction of diazo compound with falvan-3-ol molecules in acid medium. [9].

2.4. Preparation of Tea Catechins

The bulk preparation of Chinese green tea extract was conducted using 70% acetone. The extract was evaporated on hot plate at 50°C until all solvent disappeared and then dried in hot air oven at 50°C for 12 h. The dried extract powder was kept in amber vial and in a desiccator at room temperature. Catechins content was estimated from the dried extract prior to the determination of antioxidant activity in lard (the amount of dried extract added was based on the quantity of catechins present in the extract).

2.5. Determination of Peroxide Value

Various amounts of tea catechins (0.02, 0.1, 0.2 and 1.0% (w/v)) were incorporated in lard as a source of natural antioxidant. Lard without the supplement of antioxidant was used as control. Lipid oxidation of lard was initiated by shaking at 100 rpm for 20 min and subsequently incubated at 70°C. Peroxide values (PV) were evaluated using AOAC official method 965.33 [10] and expressed as milliequivalent peroxide/kg sample (meq/kg). The PVs were measured on alternate day until the value

of control reached 20 meq/kg substrate, indicating the occurrence of oxidative rancidity in animal fats. Tea catechins were also replaced with synthetic antioxidants, 0.02% (w/v) BHT, and 0.02% (w/v) d-(+)-catechin. Antioxidant activities of the tea catechins were compared with those of BHT and d-(+)-catechin.

2.6 Statistic Analysis

Statistic analyses were processed by SPSS. Two-factor with interaction ANOVA was determined. Subsequently, comparison of mean differences, among the levels of each factor, was performed using either multiple comparisons by least significant difference (LSD) or independent t-test.

3. Results and Discussion

3.1. Extraction of Tea Catechins by Hot Water and 60% Acetone Infusion

From preliminary study of the extraction of tea catechins by hot water infusion, it was found that Chinese green tea yielded maximum concentration of catechins, followed by Japanese green tea and mulberry green tea, respectively (data not shown). For the former two, the increase in catechins concentrations were observed with increasing amount of tea leaves added while the latter yielded relatively low concentration of catechins, regardless of the amount of tea leaves added. Maximum concentrations of catechins were obtained when using 2% (w/v) of green tea leaves (data not shown).

Two-factor analysis of variance with interaction between the two factors was conducted to compare the amount of total catechins extracted (mg/g dry leaves) from various types of tea using a particular solvent. When catechins were extracted with either hot water of 60% acetone infusion, maximum catechins content was obtained from Chinese green tea leaves, followed by Japanese and mulberry green tea, respectively (Table 1).

Table 1 Amount of total catechins extracted from 2% (w/v) Chinese, Japanese and mulberry green tea

Types of tea	Total catechins (mg/g)[a]	
	Hot water infusion	60% acetone infusion
Chinese green tea	3.17 ± 0.10	4.11 ± 0.07
Japanese green tea	2.47 ± 0.37	3.18 ± 0.09
Mulberry green tea	0.02 ± 0.006	0.03 ± 0.006

[a] All analyses were performed in triplicate; values are given as mean ± standard deviation.

The amounts of catechins extracted were significantly different among all types of tea at the level of 0.05, regardless of the solvent system used for extraction.

Independent t-test was then used to compare mean differences between the two extraction methods. It was found that 30% increase in catechins levels were observed when hot water was replaced with 60% acetone infusion, in the case of Chinese and Japanese green tea (both with significant differences at the level of 0.05). This may be attributed to the extraction power of aqueous acetone solution, the type of tea leaves as well as the procedures to which they were subjected (since harvested till displayed on shelves). However, the catechins level of mulberry green tea obtained in this study was very low and no significant mean differences between the two methods were observed. It was possible that mulberry leaves contained only minimum amount of catechins. The Chinese green tea leaves were then chosen to undergo further study.

3.2. Extraction of Catechins from Chinese Green Tea with Various Concentrations of Acetone

In this experiment, Chinese green tea leaves were infused by various concentrations of acetone: 50%; 60%; 70%; 100% as well as with the varying amount of dry leaves (2%, 5%, 10% and 20% (w/v)). Two-factor analysis of variance with interaction between the two factors was conducted and multiple comparisons of mean differences on the levels of each factor was done by LSD to compare total catechins contents extracted (mg/g dry leaves) from constant amount of tea leaves with varying concentrations of solvent and vice versa.

Table 2 Amount of total catechins extracted from 2%, 5%, 10% and 20% (w/v) Chinese green tea with various concentrations of acetone

Concentration of tea leaves (% w/v)	Total catechins (mg/g)[a] extracted by acetone infusion (% v/v)			
	50	60	70	100
2	3.21 ± 0.10	4.11 ± 0.07	2.86 ± 0.04	0.04 ± 0.01
5	5.31 ± 0.06	5.43 ± 0.07	6.26 ± 0.06	0.04 ± 0.01
10	4.15 ± 0.02	4.25 ± 0.06	4.45 ± 0.03	0.05 ± 0.01
20	2.54 ± 0.03	2.60 ± 0.01	2.97 ± 0.02	0.04 ± 0.01

[a] All analyses were performed in triplicate; values are given as mean ± standard deviation.

From Table 2, considering any constant amount of tea leaves used for extraction, the amount of catechins extracted increased with increasing concentration of acetone used to a certain degree (all showed significant differences among differing acetone concentrations at the level of 0.05). However, it appeared that at lower amount of tea leaves (2% (w/v)), maximum catechins levels was obtained from 60% acetone infusion while optimum concentration of acetone increased (70% acetone) as the amount of tea leaves increased.

Considering the efficient recovery of total catechins from various amount of dry leaves using the same concentration of acetone, means of total catechins levels obtained were significantly different at all levels of dry weight when 50%, 60% and 70% acetone were used). However, as the concentration of acetone increased to 100%, no significant mean differences in catechins levels were observed among all levels of dry weight of tea leaves used at the level of 0.05. This finding was in agreement with that reported by Perva-Uzunalić, Škerget, knez, Weinreich, Otto and Grüner [11].

It was also found that maximum catechins levels were obtained from 5% (w/v) dry leaves when 50%, 60% and 70% acetone were applied. In addition, low power of extraction was observed with the increasing amount of tea leaves. It was suggested that the amount of active ingredients obtained was dependent on extraction solvent and amount of sample used. From this study, optimum conditions for maximum catechins recovery was obtained from the extraction of 5% (w/v) Chinese tea leaves with 70% acetone (6.26± 0.06 mg/g).

3.3. Determination of Antioxidative Effect of Green Tea Extract in Lard

The evaluation of PV is useful to determine the early stages of fat oxidation and the product is considered rancid when PV of 20 – 40 meq/kg was reached [12]. In this study, the amount of BHT added in lard was 0.02% (w/v), complying with the maximum allowable limit (200 mg/kg). The same amount of d-(+)-catechin and tea catechins was also added to lard in relation to BHT along with other increased amounts of tea catechins.

Also, in this experiment, two-factor analysis of variance with interaction between two factors was conducted and multiple comparisons of mean differences on the levels of each factor was done by LSD to compare PV from different kinds of antioxidant at various time courses. Among treatment used, control (without the addition of any antioxidant) had greatest degree of rancidity and had significant mean differences among other treatments, at all specified time points throughout the experiment.

It was found that in the absence of antioxidant, lard became rancid after 6 days of storage with PV of 22.5 ± 2.83 meq/kg (Table 3). However, PVs of lard supplemented with 0.02% (w/v) antioxidants did not exceed 20 meq/kg after 8 days of storage, with the antioxidant activity in the following order: BHT > tea catechins > d-(+)-catechin. PVs of the latter two showed no significant mean differences between each other but were significantly different from BHT at the level of 0.05. Obviously, the amount of peroxide formed was inversely proportional to the increase in level of tea catechins added.

Throughout the entire storage period, 0.2% and 1.0% (w/v) tea catechins were shown to be more effective in inhibiting the formation of peroxide as compared to 0.02% (w/v) BHT. Oxidative rancidity was most effectively retarded with the addition of 1.0% tea catechins, with PV of 4.75 ± 1.06 meq/kg f ollowed by that with 0.2% (w/v) tea catechins (5.75 ± 0.35 meq/kg) and 0.02% (w/v) BHT (7.75 ± 1.06 meq/kg), respectively. However, no significant mean differences were found among these treatments at the level of 0.05.

Table 3 Peroxide values of lard supplemented with various antioxidants measured over the time course

Antioxidants	Peroxide values (meq/kg) measured[a]			
	Day 2	Day 4	Day 6	Day 8
1) Control	5.00 ± 0.00	19.00 ± 1.41	22.50 ± 2.12	34.00 ± 2.83
2) 0.02% BHT	1.25 ± 0.35	3.50 ± 0.71	5.75 ± 1.06	7.75 ± 1.06
3) 0.02% d-(+)-catechin	2.50 ± 0.71	9.00 ± 0.71	11.75 ± 1.06	16.50 ± 2.12
4) 0.02% tea catechins[b]	2.75 ± 0.35	8.25 ± 1.06	10.00 ± 1.41	14.00 ± 2.12
5) 0.1% tea catechins[b]	2.50 ± 0.71	3.25 ± 0.35	4.75 ± 0.35	9.75 ± 1.06
6) 0.2% tea catechins[b]	1.50 ± 0.71	2.25 ± 0.35	3.75 ± 0.35	5.75 ± 0.35
7) 2.0% tea catechins[b]	1.00 ± 0.00	1.75 ± 0.35	3.50 ± 0.71	4.75 ± 1.06

[a] All analyses were performed in duplicate; values are given as mean ± standard deviation.

[b] The values shown above represented % (w/w) of tea catechin in lard. Tea catechins were added in forms of dried extract of tea leaves. The amount of catechins added was calculated from the actual quantity of catechins present in the dried extract.

According to Gramza & Korczak [13] tea catechins can act as antioxidants by donating hydrogen atom to free radicals, inhibiting chain reaction or by chelating metal ions. The agreeable antioxidant activities of tea catechins may result from EGCG, a major catechin substance which structurally contains several hydroxyl groups that act to chelate most of the metal ions required for catalyzing oxidative reactions. This is in agreement with the finding of Cao, Sofic, and Prior [14] that antioxidant activity of flavonoids was generally determined by the number and location of hydroxyl groups on the flavonoid ring structure. However, the efficiency of dried acetone extract against lipid oxidation may also result from the presence of non-polyphenolic substances, which were reported to have antioxidative activity [15], [16].

4. Conclusions

The results of this study indicated that acetone was more effective in extracting catechins from tea leaves than hot water, with 70% acetone being the most effective extraction solvent. Among the tea samples studied, highest extractable catechins were obtained from 5% (w/v) Chinese green tea under the specified conditions. Although the resulting dried extract powder contained merely 4.4% (w/w) catechins, at the same amount present, commercial standard and tea catechins had comparable, though with slightly greater, activity against lipid oxidation, for the latter. In addition, 0.2% and 1.0% tea catechins also showed greater antioxidative activities than 0.02% BHT, though with no significant mean differences as determined by statistic analyses. The results implied that tea catechins, in sufficient amount, could be used as a substitute for BHT to prevent oxidative rancidity.

In addition, the antioxidative activities achieved could be attributed to the presence of other polyphenolic and nonpolyphenolic compounds that also function as antioxidants. Further study on the contribution of individual substance in tea extract to the lowering peroxide value as well as optimizing extraction yield and solubility of such active ingredients may prove useful for its future application in food products.

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