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Separation of polyphenol compounds and caffeine from green tea extract

Sailom Sampanvejsobha¹, Suwit Siri wattanayotin², Natthakan Rungraeng^{3,} and Prinya Wongs³*

¹Learning Institute, King Monkut's University of Technology Thonburi, Bangkok, Thailand 10140

²Department of Food Engineering, King Monkut's University of Technology Thonburi, Bangkok, Thailand 10140

³School of Agro-Industry, Mae Fah Luang University, Chiang Rai, Thailand 57100

*Corresponding author: natthakan.run@mfu.ac.th

Abstract

The polyphenolic compounds in green tea extract i.e. tannins and polyphenols, especially catechins, attribute to organoleptic and health promoting functions. For tea caffeine, although it is known to stimulate nervous system; however, overuse of caffeine may induce a number of detrimental effects including headaches and migraines. Therefore, this research is aimed at optimizing the separation of caffeine and the major tea polyphenols namely catechins from green tea extract using adsorption/desorption technique. In this study, Assam green tea extract was prepared by boiling 5%w/v tea leaves in 100 mL of acidified deionized water at 95°C for 10 mins. The pH of the tea-hot water mixture in the extractor was controlled at pH 4 using citric acid. This was done to minimize oxidative degradation of active ingredients. Total polyphenol content (TPC) of the fresh extract measured using a spectrophotometer was 8.8%w/v. Thereafter, the extract was evaporated until the final volume was 10 mL. Subsequently, the concentrate was separately flown in different resin columns i.e. polyamide 6, amberlite XAD-7, nylon 6/6 and polyamine at a constant flow rate of 2.7 mL/min. Water-ethanol mixtures (0 to 95%v/v of ethanol) were used as eluting agents in desorption process. It was found that, caffeine and catechins were clearly isolated from each other when the polyamide column was initially desorbed with water (to elute caffeine) followed by 50%v/v ethanol in water (to elute catechins). The recovery percentages of the isolation process for caffeine and individual catechins were 36.4% and in the range of 28.5 to 98.7%, respectively. Therefore, the purified caffeine and catechins could be further used as ingredients in food, beverage and pharmaceutical industries.

Keywords : *Assam tea, polyphenols, separation, resin.*

1. Introduction

Consumption of functional foods has recently been increased, particularly antioxidant beverages from plant sources. Health benefits of antioxidant-rich foods include anti-inflammatory effects, preventions of cardiovascular disease, diabetes and cancer (1). Vitamin C, vitamin D, vitamin E, carotenoids and polyphenols are examples of the most commonly used natural antioxidants (2). Tea is the most widely consumed in the world. Black tea is fully fermented tea leaves. Whereas, oolong and green teas are semi- and non-fermented types. Recent research activities have shown that polyphenolic compounds found in tea, especially catechins, are effective scavengers of free radicals. Tea polyphenols have received considerable attention for their important bioactivities in recent times. The major disease-fighting compounds in tea are polyphenols i.e. catechins, especially epigallocatechin gallate (EGCG). Besides that, tea also contains a well known stimulant, caffeine. Therefore, to prevent the possible risk of caffeine, the consumption of tea among children and elderly people should be carefully monitored and limited. To overcome this problem, the separation of tea polyphenols and caffeine from the tea extract should be studied.

Traditional solvents used in extraction of polyphenols from tea leaves include methanol, ethanol, benzene and toluene because of their low-boiling point. Nowadays, food safety has become one of critical concerns for consumers. As a result, edible solvents such as water and ethanol have widely been selected to extract bioactive compounds from plants including

tea. The methods used to separate and purification of tea compounds are centrifugal separation, membrane filtrations i.e. ultrafiltration and reverse osmosis, and resin adsorptions. Polyamide resin possesses high polarity due to its hydrogen bonds which make it suitable for the separation of polymeric solutes such as phenolics and flavonoids (3). Therefore, this research is aimed at studying the effect of resins on the separation of caffeine and the major tea polyphenols namely catechins from green tea extract using adsorption/desorption technique.

2. Materials and Methods

2.1 Raw materials

Dried Assam green tea leaves (*Camellia sinensis* var. *assamica*) were purchased from local tea manufacturers in Chiang Rai, Thailand. The leaves were ground into fine powder using a hammer mill grinder. Thereafter, the powder was vacuum sealed before used.

2.2 Preparation of green tea extract

Assam green tea extract was prepared by brewing 5 g of tea powder in 100 mL deionized water at 95°C for 10 mins. During extraction, the pH values of the solution were varied into four levels (4, 5, 6 and 7) using citric acid. Fresh green tea extract was obtained by filtration using a filter paper (Whatman No.4). Subsequently, the extract was evaporated in a vacuum evaporator until the final volume reached 10 mL. The amounts of total polyphenols, tannin and caffeine in the extract and residual were determined using methods shown in literatures (4), (5) and (6), respectively.

2.3 Separation of polyphenols and caffeine

Four packed-bed separation columns were prepared by filling 20 g (or 40 g, depending on the experiment) of polyamide 6, amberlite XAD-7, nylon 6/6 and polyamine resins in glass cylinders. Adsorption and desorption processes were performed by pouring 10 mL of concentrate green tea extract into the prepared columns at a constant flow rate of 2.7 mL/min. Afterwards, water-ethanol mixtures (0, 10, 20, 30, 40, 50, 60 and 95%v/v of ethanol) were used as eluting agents to individually desorb tea polyphenols and caffeine from

each of the resin columns. The chemical compositions of eluents were analyzed by HPLC method (7).

2.4 Statistical analysis

Each of the experiments was performed in triplicate. The mean and standard deviations of the test values were determined and analyzed using analysis of variance (ANOVA) to a significance of 95% ($P < 0.05$).

3. Results and Discussions

3.1 Effect of pH on the chemical compounds

Table 1. Effect of pH on the chemical compositions of green tea extract

Sample	Total polyphenols (%)	Tannin (%)	Caffeine (%)
pH 4	8.82±0.04 ^a	10.74±0.13 ^c	3.53±0.05 ^c
pH 5	6.98±0.01 ^b	13.40±0.13 ^a	3.71±0.23 ^b
pH 6	6.83±0.05 ^b	12.26±0.05 ^b	3.90±0.16 ^{bc}
pH 7	6.80±0.10 ^c	12.01±0.05 ^b	4.37±0.19 ^a

Different superscript letters (a, b, and c) indicate significant differences in the same column ($P < 0.05$).

Table 1. shows that the concentration of total polyphenols in tea infusion decreased with

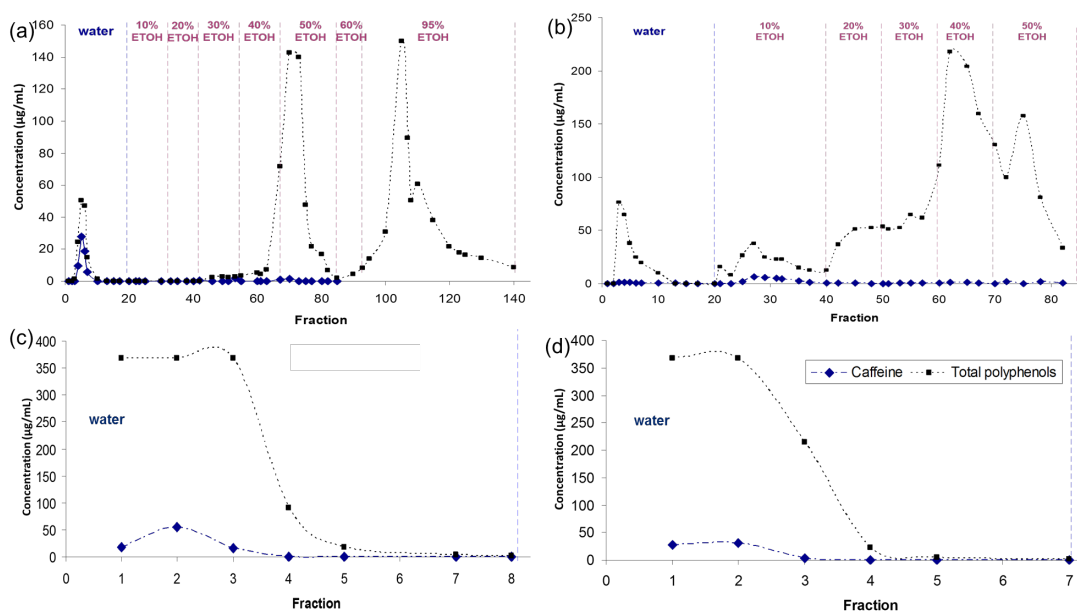


Figure 1. Concentration of total polyphenols and caffeine in eluted fractions using different resins (a) polyamide, (b) amberlite, (c) nylon, and (d) polyamine

increase of pH. The concentration of total concentration reached its highest at pH 4 (8.82%). However, this value continuously decreased to 6.98, 6.83, and 6.80% at pH 5, pH 6, and pH 7, respectively. On the other hand, the concentrations of tannin and caffeine were at their bottom at pH 4. The highest concentrations of tannin and caffeine were at pH 5 (13.40%) and pH 7 (4.37%), respectively. Since, polyphenols are considered ones of the strongest natural antioxidants. They could readily react with oxygen, especially at high temperature. Nonetheless, the antioxidative effect of tea extract could be stabilized in acidic solution i.e. pH < 4; thus, prevent decomposition tea polyphenols.

3.2 Effect of types of resin and ethanol concentration on the separation of polyphenols and caffeine

In this study, the pH of the tea-water mixture was controlled at pH 4. Results of adsorption and desorption of tea polyphenols and caffeine from packed bed resin columns are shown in figures 1 (a) to (d). It can be seen that the concentrations of total polyphenols and caffeine were significantly affected by types of resin and concentration of ethanol. Adsorbed caffeine

tended to be dissolved in strong polar solvent i.e. water because the polarity of caffeine is higher than polyphenols. However, both caffeine and polyphenols could be simultaneously dissolved in water when nylon and polyamine columns were used. Therefore, polyamide and amberlite resins were desirable for isolation of polyphenols from caffeine. Among these two resins, the separation effectiveness of polyamide was more pronounced than amberlite. Because caffeine also came off from amberlite when the resin was eluted by 10% ethanol; whereas, there was no significant amount of caffeine in all of the solvents used, except water. Therefore, polyamide and 50% vol. ethanol were further used as resin and eluent in subsequent study.

3.3 Effect of the weight of adsorption resin

The effect of the weight of polyamide used in the isolation of tea polyphenols and caffeine from green tea extract was studied in this section. Therefore, the optimal condition from previous sections was employed except

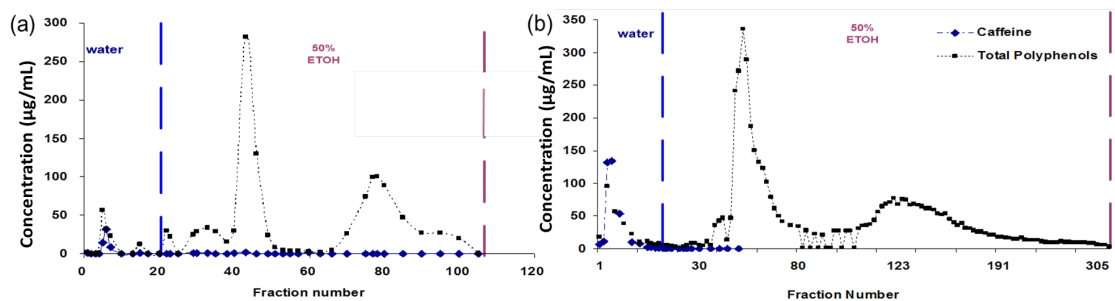


Figure 2. Concentration of total polyphenols and caffeine in eluted fractions from polyamide resins (a) 20 g-polyamide, and (b) 40 g-polyamide

the weight of polyamide resin which was varied into two sets i.e. 20 g and 40 g. The results are shown in figures 2 (a) and (b). It was found that the concentrations of polyphenols and caffeine in 40 g-polyamide was slightly better than 20 g-polyamide column because no caffeine in the eluent was detected after the its 50th fraction; therefore, it could be concluded that no further separation of caffeine from the eluent was required.

3.4 Separation yield

Nine catechins were found in tea extract before separation. These include major catechins namely epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). The results also indicated that tea

extract contained minor catechins namely gallocatechin gallate (GCG), gallocatechin (GC), catechin gallate (CG), catechin (C) and gallate (G). Recovery percentage of individual catechins and caffeine are present in table 2. According to the results, the separation yield of catechins was in the range of 28.53 to 98.65%, depending on types of catechin. In addition, separation yield for caffeine was 36.40%. The loss of components during the process was more pronounced in the separation of large compounds i.e. EGCG and EGC and caffeine than in small compounds including GC, C, and G. Because these large molecules tended to stick to the surface of polyamide resins; thus, could not be eluted by water.

Table 2. Yield of polyphenols and caffeine after separation

Compounds	Before separation	After separation	Yield (%)
Catechins			
EGCG	1.465	0.418	28.53
EGC	1.108	0.385	34.74
ECG	0.926	0.724	65.32
EC	0.818	0.549	67.14
GCG	0.308	ND	-
GC	0.861	0.726	84.40
CG	0.084	ND	-
C	0.533	0.407	76.42
G	0.547	0.54	98.65
Caffeine	1.642	0.598	36.40

ND = Non detectable

4. Conclusion

Separation of polyphenols and caffeine from green tea extract using different resins was performed in this study. Polyamide resins was capable of 1) binding to both tea polyphenols and caffeine in adsorption and 2) allowing the caffeine and tea catechins to be individually eluted by 50% vol. ethanol and water, respectively. These findings have significant applications in isolation of health-promoting substances i.e. catechins from other components.

5. References

- (1) Michael FH. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr.* 2004 March;79(3):362-371.
- (2) Karakaya S, El SN, Taş AA. Antioxidant activity of some foods containing phenolic compounds. *Int J Food Sci Nutr.* 2001 Nov;52(6):501-508.
- (3) Min G, Xiaolei W, Ming G, Zhiguo S, Ye W, Jan-Christer J. Separation of polyphenols using porous polyamide resin and assessment of mechanism of retention. *J Sep Sci.* 2011 Jun 28; 34(15):1853-1858.
- (4) Senji S, Yumi T, Yuki O. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chem.* 2005 March;89(4):569-575.
- (5) Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method Enzymol.* 1999;299: 152-178.
- (6) Lihu Y, Xu L, Yueming J, Nola C, Bruce D, Riantong S, Nivedita D, Ying X. Compositional analysis of teas from Australian supermarkets. *Food Chem.* 2006 January;94(1): 115-122.
- (7) Tetsuhisa G, Yuko Y, Masaaki K, Hitoshi N. Simultaneous analysis of individual catechins and caffeine in green tea. *J Chromatogr A* 1996 October 18;749(1-2):295-299.