DETERMINATION OF TOTAL POLYPHENOL CONTENT IN DIFFERENT BRANDS OF TEA

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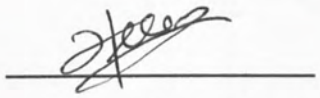

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ABSTRACT

Determination of Total Polyphenol Content in Different Brands of Tea

Polyphenol content are widely present in human diet as component of fruits, vegetables and is especially high in tea. Consumption of tea as a beverage exhibits significant health effects including antioxidative, anticarcinogenic, antimicrobial and protection against cardiovascular disease. Determination of total polyphenol concentration in seven brands (four types) of tea obtained from the local supermarket was carried out. The chosen brands were Boh tea, Sabah tea (Black tea), Shuang Long tea, Fairy tea (oo-long tea), China tea, Yu Xian Nu tea (green tea) and Borneo Rainforest (organic tea) respectively. Total polyphenol contents in each sample were analyzed by Folin Ciocalteu and Ferrous Tartarte method, following aqueous extraction and determination using UV-Spectrophotometer. The range and means of total polyphenol content (mg/g) using Folin Ciocalteu method with different parameters respectively are as follows; plain tea leaves (18.34–20.98; 19.7), addition of 2g sugar (21.48–20.06; 20.6), addition of 1ml lemon (18.96–21.25; 20.18) and addition of 5ml condensed milk (21.39–24.61; 22.6). In addition, the range and means of total polyphenol content (mg/g) using Ferrous Tartrate method for respective parameters is as follows; plain tea leaves (19.55–45.74; 30.5), addition of 2g sugar (50.94–24.75; 34.8), addition of 1ml lemon (22.05–46.81; 31.6) and addition of 5ml condensed milk (31.61–63.83; 41.1). The percentage increment of all the parameters analyzed with respect to the plain tea leaves samples using Folin Ciocalteu method range from 2.4–9.4 (addition of 2g sugar), 1.1–3.4 (addition of 1ml lemon) and 12.2–17.3 (addition of 5ml condensed milk) respectively. For Ferrous Tartrate method the percentage of increment is range as follows; 8.6–31.2 (addition of 2g sugar), 2.3–12.8 (addition of 1ml lemon) and 24.8–61.7 (addition of 5ml condensed milk). ANOVA one–way test on all the tea brands analyzed clearly showed that green tea brand (China tea) yields higher polyphenol content as compared to black tea brand (BOH tea). Independent t-test showed there was a significant difference between two methods analyzed ($p<0.05$), but from linear regression test, positive correlation could be achieved between both methods and total polyphenol content. Suggestion to use Ferrous Tartrate method would be a better choice for tea analysis. Overall, the variations of value for total polyphenol content in all tea analyzed depends mostly on solvent used, method of extraction, parameters analyzed and the most crucial part is the tea plant itself.

ABSTRAK

Penentuan Jumlah Kandungan Polifenol dalam Pelbagai Jenama Teh

Kandungan polifenol wujud secara meluas dalam diet manusia sebagai satu komponen buah, sayuran dan khususnya dalam teh. Pengambilan teh sebagai minuman menunjukkan kesan kesihatan yang ketara termasuk antioksida, antikarsinogenik, antimikrob dan perlindungan daripada penyakit kardiovaskular. Penentuan jumlah kandungan kepekatan polifenol dalam tujuh jenama teh (empat jenis) diperoleh daripada pasar raya tempatan telahpun dijalankan. Jenama yang dipilih adalah teh BOH, teh Sabah (teh hitam), teh Shuang Long, teh Fairy (teh oo-long) teh China, teh Yu Xian Nu (teh hijau) dan Borneo Rainforest (teh organik). Kandungan polifenol dalam setiap sampel telah dianalisis dengan menggunakan kaedah Folin Ciocalteu dan Ferrous Tartrate, diikuti pengekstrakan akueus dan penentuan dengan UV-Spectrophotometer. Julat dan min bagi jumlah kandungan polifenol (mg/g) menggunakan kaedah Folin Ciocalteu dengan parameter berlainan masing-masing adalah seperti berikut: daun teh biasa (18.34–20.98; 19.7), tambahan 2g gula (21.48–20.06; 20.6), tambahan 1ml limau (18.96–21.25; 20.18) dan tambahan 5ml susu pekat (21.39–24.61; 22.6). Tambahan pula, julat dan min bagi jumlah kandungan polifenol (mg/g) menggunakan kaedah Ferrous Tartrate mengikut parameter berasingan adalah seperti berikut; daun teh biasa (19.55–45.74; 30.5), tambahan 2g gula (50.94–24.75; 34.8), tambahan 1ml limau (22.05–46.81; 31.6) dan tambahan 5ml susu pekat (31.61–63.83; 41.1). Peratus peningkatan bagi semua parameter yang dikaji adalah berdasarkan kepada sampel daun teh biasa mengikut kaedah Folin Ciocalteu adalah seperti berikut; daun teh biasa, julat adalah di antara 2.4–9.4 (tambahan 2g gula), 1.1–3.4 (tambahan 1ml limau) dan 12.2–17.3 (tambahan 5ml susu pekat) masing-masing. Bagi kaedah Ferrous Tartrate pula, peratus peningkatan mengikut julat adalah seperti berikut; 8.6–31.2 (tambahan 2g gula), 2.3–12.8 (tambahan 1ml limau) dan 24.8–61.7 (tambahan 5ml susu pekat). Ujian ANOVA satu hala bagi kesemua analisis jenama teh jelas menunjukkan bahawa jenama teh hijau (teh China) mencatatkan kandungan polifenol tertinggi jika dibandingkan dengan jenama teh hitam (teh BOH). Ujian-t tidak bersandar menunjukkan terdapat perbezaan signifikan di antara dua kaedah yang dikaji, tetapi daripada ujian regresi linear, kolerasi positif dapat dicapai menerusi kaedah dan jumlah kandungan polifenol. Cadangan untuk menggunakan kaedah Ferrous Tartrate adalah lebih sesuai untuk analisis teh. Secara keseluruhannya, perbezaan nilai bagi jumlah kandungan polifenol dalam kesemua teh dianalisis bergantung kepada penggunaan bahan cecair, kaedah pengekstrakan, analisis parameter lain dan yang paling penting adalah pokok teh itu sendiri.

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SYMBOL / ABBREVIATION LIST

Symbol / Abbreviation	Meanings
%	percentage
µm	micrometer
<	less than
>	more than
±	plus or minus
&	and
p	value at significance 0.05 level
cm	centimeter
mm	milimeter
g/L	gram per liter
mg/L	milligram per liter
g	gram
°C	Celsius degree
ppm	part per million
g/kg	gram per kilogram
mg/kg	milligram per kilogram
m	meter
kg	kilogram
mg/d	milligram per day
ml	milliliter
β	beta
UV	ultraviolet
nm	nanometer
sec	seconds
min	minutes
AOAC	American Organization Analytical Chemist
s.d	standard deviation
n	no. of sample

APPENDIX

CHAPTER 1

INTRODUCTION

Tea is a very popular beverage especially in Malaysian community, when its popularity can be seen through varieties of tea brands that can be purchased in various places such as local supermarkets, convenience stores, vending machine and other retail outlet. This is due to the reason that tea is produce and manufacture locally, besides exporting to other countries. There are also many types and varieties of local tea brands that includes BOH tea, Sabah tea, Lipton tea, Chinese oo-long tea, green tea, jasmine tea and many others additional flavors of tea. People utilized the leaves or its infusion as drink for many purposes that include quenching of thirst, health, medicinal benefits or even leisure. Furthermore, with wider coverage and media exposure, many people are more aware on the benefits of tea and conscious towards promoting better health.

There is some truth in it as tea is a good stimulant. Tea contains caffeine like coffee but in lesser amount. Tea can be enjoyed in any time of the day, cold or hot, mild or strong, with or without sweetening, all depends on individual taste. Most people prefer consumed tea that usually served with mild sugar, ice and lemon or simply with addition of milk. YO-YO and Easy Way is two fast food outlets in Sabah, originated from Taiwan that emphasize and promoted tea beverages among youth in Malaysia. It is also common to see teenagers slurping milky tea while munching sago balls (known as pearls) in various shopping complexes.

1.1 Tea *(Camellia sinensis)*

Tea is considered one of the most widely consumed beverages in the world today although varies greatly in different amounts among consumer, with per capita consumption of approximately 120ml/day (Wang & Helliwell, 2000). It has accepted that, tea as secondary drinks only to water and with its medicinal properties being widely explored (Mukhtar & Ahmad, 2000). Tea is becoming more popular due to its attributed popularity of sensory properties, relatively low retail price, stimulating effect and potential health benefits (Baptista, Tavares & Carvalho, 1998). Annual production of about 1.8 million of dry tea leaf provides world per capita consumption of 40L beverages (Balentine, Harbowy & Tarka, 1998). Consumption of tea as beverages becomes larger and wider with more and more varieties and types of flavored brand tea that include instant tea, tea bags, pot bags, tin cans and plastic bottles being produced for the convenience of customer.

The tea plant is an evergreen laurel tree and is taxonomically classified as *Camellia sinensis* (L.) O. Kuntze of the family *Theaceae* with two different varieties namely; var. sinensis and var. assamica (Chu, 1997 & Hara, 2001). The leaves are dark green, alternate and oval, with serrated edges, and the blossoms are white, fragrant, and appear in clusters or singly (Thomson, 2000). There are three main types of tea, green, black and oo-long, defined by respective manufacturing (fermentation) techniques. Thus, tea is classified into green tea (unfermented), oo-long tea (semi-fermented) and black tea (fully fermented). Green teas leaves are produced by steamed or pan-fry lightly just after harvest and when the oxidative enzymes are denatured. Oo-long tea is prepared by allowing an intermediate period of auto-oxidation (Culter, 2000). Black tea is allow to oxidize even more by exposing rolled-up leaves to air until they are oxidized to black (Stengler, 2001). The degree of fermentation greatly affects the quality and type of tea (Chu, 1997). Instants tea are

prepared by extraction of tea solids from fermented but unfired tea leaves, black tea or green tea, followed by concentration of the extract and drying of the concentrate to a powder and then packed into smaller bags for convenience (Wickremasinghe, 1978).

Of the total amount of tea produced, 73%-78% is black, 20%-22% is green, and less than 2% is oolong tea represents the world tea consumption (Krishnan & Maru, 2004). Consumption of black tea is primarily in Western countries and in some Asian countries, whereas green tea mostly consumed in China, Japan, India, and a few countries in North Africa and the Middle East. On the other hand, oolong tea production and consumption are confining to southeastern China and Taiwan (Mukhtar & Ahmad, 2000). Consumption data is mostly estimate based on import, export and production statistics, therefore if determination of actual human consumption or ingestion must takes into account of method preparation and varying levels of tea leaves extraction into finished beverages (IARC, 1991).

The most important characteristic components of tea leaves are the polyphenols in the cell sap, which undergo series of chemical changes when the leaf is disintegrate during manufacture (Eden, 1976). The tea polyphenols also known as flavonols or catechins, comprising 30-40 percent of the extractable solids of dried tea leaves from which they can readily extracted into hot water infusions (Thomson, 2000 & Dreosti, 1996). The types of catechins occur in significant quantities of tea leaves are (+)-catechin, (–)-epicatechin, (+)-gallocatechin, (–)-epigallocatechin, (–)-epicatechin gallate and (–)-epigallocatechin gallate (Dreosti, 1996). A variety of methods have been developed for the measurement of polyphenols including ultraviolet, fluorescence, mass and electrochemical detection. A more selective and sensitive method is desired due to some drawbacks of interference by various compounds in the analytical samples (Li Ma *et al.*, 2002).

Polyphenols have significant antioxidant activity and numerous potentially beneficial medicinal properties. It is also important from the point of view of their possible adverse effects on biological systems including anti-inflammatory, antivirus and anticancer activity as well as prevention of certain cardiovascular disease (Li Ma *et al.*, 2002). Polyphenols acts as prevention of free radicals (unstable molecules) from damaging body tissue and genetic material inside the cells. These phytonutrients are even more powerful than the vitamins most commonly taken as supplements by consumer (Stengler, 2001). People nowadays tend to realize the positive and great effects of tea to maintain their health status. Therefore, consumption of tea has since been widely increased due to its great implications on human and mankind itself.

In addition to the direct consumption of tea either by brewing loose leaves or tea bags form, there have been more and more applications for tea extracts especially in the nutraceutical and food areas in recent years. Moreover, the amount of solid extracted is also affected by the nature of manufactured leaf, brewing variables such as leaf to water ratio, temperature and time. Thus, tea leaves, their extracts and consumer products themselves need to be standardized and routinely assayed for better performance as reference to further analysis in the future (Wang, Provan & Helliwell, 2002 & IARC, 1991).

1.2 Objectives

i) Extraction and isolation of total polyphenols in four types (seven different brands) of tea leaves

ii) Determination of total polyphenol by Folin – Ciocalteu and Ferrous tartrate method in tea leaves by using UV-Spectrophotometer

iii) Determination of total polyphenol content in tea infusions with addition of sugar, lemon and condensed milk from the tea leaves brands mentioned

iv) Comparison of method and total polyphenol content between different tea leaves brand mentioned and their infusions with addition of sugar, lemon and condensed milk

1.3 Justification of the project

Various countries have implemented or conducted studies in determining the polyphenol content in tea infusion from dried leaves or variety foodstuff. However, they are only few published records around Sabah concerning the relevant study about the polyphenol content especially in tea. Tea beverages are chosen because of wide consumption by people and due to its beneficial effects on their body. The tea sample is also easily obtained locally, as Sabah is the state producer of its own tea leaves and powder. The main aspect that has been emphasized in this study is the knowledge and confirmation of total polyphenols in different types of tea leaves brands includes leaves and bags that are available in Kota Kinabalu, Sabah.

Besides that, this study is conducted to determine and analyze the amount of these nutrient polyphenols that is being absorbed in the human body due to consuming of tea constantly. Most tea that is drunk is off 'black' variety that is made of crushed or partially fermented leaves of *Camellia sinensis*. However, some would prefer drink green tea instead, so the exact composition of tea as drink varies markedly and dependent on certain factors such as types and brands of tea, manufacturing, and the ratio of water to tea used before infusion is drunk, concentration as well as the temperature.

Nowadays, tea are being prepared by operators in such a way to enhance the flavor and taste by simply adding milk like for instances "*teh tarik*" and with a bit of lemon taste or better known as lemon tea. There might be some chemical reaction between substances like sugar, lemon or milk that will affect the overall content of total polyphenol in tea, therefore part of the analysis is to have a deeper insight on the various factors that will increase or decrease solubility of total polyphenol in water.

Tea is ordinarily prepared by the addition of boiling water at temperature, 100°c to tea leaves or tea bags in the ratio of 80:1 and allowing them to react together for 5 minutes or less. Therefore, the tea infusion sample is prepared by taking into account of people drinking habits. Some people like strong tea, while others dislike drinking tea due to the bitter taste of tannins. Thus, to estimate body absorption in human, brewing in water is an essential factor to evaluate the bioavailability of some antioxidant component especially polyphenols content in tea.

CHAPTER 2

LITERATURE REVIEW

2.1 Origins and history of tea

In ancient history, the origins of tea are not known although legend dates to as far as 2700B.C. However, the findings of reference and written accounts on tea was first found in Erh ya, an ancient Chinese dictionary of 356A.D. that stated the beverages is use as a drink or an act as a medicine to cure certain type of diseases (IARC, 1991 & Wickremasinghe, 1978). Lu Yu published the first monograph on tea regarding botany, cultivation, processing, utensils and proper way of tea drinking in 780A.D. Tea, *Camellia sinensis* is native to the southern regions of China and parts of India, Burma, Thailand, Laos and Vietnam (James, 1997). The historical and development flow of tea are followed by the occurrence and incidence of "Boston tea" party on year 1773 when the tax imposed was too high on tea which was imported to America that angered the colonist, led to dumping of tea in Boston harbor (McKim, 2003). After the British tea trade monopoly with China ended on 1833, tea begun to grown in India and Sri Lanka. Today, tea is widely grown with the main exporter and production of tea is India, China and Sri Lanka whereas the main importer of tea is United Kingdom, United States and Soviet Union (James, 1997).

2.1.1 The tea variety

Research has been conduct and proven that genus *Camellia* can be divide into more than 80 species (Chu, 1997). Tea plant from *Camellia sinensis* species that have a high economy value can be categorize into two varieties that are *assamica* and *sinensis* (Chu, 1997). The China type, var. *sinensis*, developed mostly at higher altitudes in forest whereas in southern forest that was more dense and wet were home for the Assam type, var. *assamica* (Willson, 1999). In this classification, small leaves (leaf length and width, 5.5-6.1 × 2.2-2.4cm) bush type trunk is simply known as var. *sinensis* whereas for var. *assamica* the leaves are larger (leaf length and width, 16-19 × 7-9cm) and the tree trunk is taller and bigger (Chu, 1997 & Wickremangsinghe, 1978).

Table 2.1: Differences between the two major tea varieties

Variety	Growth habitat	Leaf features	Leaf angle
China *Camellia sinensis* var. *sinensis* (L.)	Dwarf, slow growing, shrub like	Small, erect narrow, serrate, dark green	< 50°
Assam *Camellia sinensis* var. *assamica*	Tall, quick growing tree	Large, horizontal, broad, mostly non-serrated, light green	> 70°

Source: (Banerjee, 1992)

2.1.2 Tea cultivation and harvesting

Tea was first cultivated in China and then in Japan. Tea is now grown commercially, about 30 countries in tropical and subtropical regions of Asia, Africa and South America. All varieties and cultivars of tea belong to one single species that is

Camellia sinensis (L.) O. Kuntze, when in its natural form is a large tree but for commercial cultivated form is more like a bush (McKim, 2003). The tea plant is usually an evergreen bush that can grow to a height of 12-24m but the bushes are usually kept about 1m tall by pruning and harvesting of new growth of leaf, known as flush (IARC,1991). Teas grown in temperate zones such as China or Japan are made into green tea, while processing of black tea are those grown in tropical region such as India or Sri Lanka. Tea plant is well suited to a wide range of climate and soils with an abundant of rainfall (Macmillan, 1993).

Throughout many of the world tea growing regions, harvesting is done by hand for centuries ago and consists of nipping off the tender end leaves with bud and shoot (Macmillan, 1993). Mechanical harvesting is widely practiced by many countries if labor cost is high and may range from simple use of scissors to large self propeller that cut the tea hedge row and pluck to a uniform height. Mechanical harvesting is also not very practical on the steep slopes. Plucking cycle should correspond to flush development, which is regulated by climatic factors (Balentine, Harbowy and Tarka, 1998 & Macmillan, 1998). However, due to uniformity and quality of tea leaves, precise selection of leaf is only achievable by hand selection (IARC, 1991). A single worker will usually pluck 20 to 25 kg per day (Balentine, Harbowy and Tarka, 1998). In order to produce the best grade of good quality tea, only the bud and first two leaves from each twig are plucked, whereas for lower grade of tea quality, it is made from the third and fourth leaves with a bud that forms coarse plucking (McKim, 2003 & Macmillan, 1993).

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