Comparison of Antioxidant Activity in Tea drinks Using FRAP Assay

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ABSTRACT

Dangerous effects of free radicals and oxidative stress can be reduced by consistently taking food that produce antioxidant such as flavonoid and other polyphenol compounds. Hence this research was conducted in order to determine the antioxidant activity in tea drink served with sugar or milk as it was a routine drink for some people and cultures. The characterization was done using Attenuated Total Reflectance – Fourier Transform Infrared (ATR-FTIR) spectrometer to identify the antioxidant's presence in tea and antioxidant activity determination were carried out using Ferric Reducing Antioxidant Power (FRAP) assay. ATR-FTIR Spectrum shown the presence of varied numbers of hydroxyl group in several arrangements due to different polyphenols contained in tea powder has distinctive antioxidative properties. The estimation of antioxidant activity was done by using Ultraviolet Visible (UV-VIS) Spectrometer shown that black tea with addition of milk and sugar (BMS) contained the highest antioxidant activity which is 0.467 µg/L compared with black tea (B) and black tea with milk (BM) samples. It is proved that the addition of milk and sugar do not alter the antioxidant activity in tea samples.

Keywords: *black tea, antioxidant, polyphenol, FRAP Assay*

Background of Study

Tea plant is planted all over the world in 30 countries and the best condition for it to grow is adequate rainfall and semi acidic soil (Hajimahmoodi et al., 2008). Infusion from the Camellia senesis leaves and then after several processes, a different type of the tea drink such as oolong tea, green tea and black tea can be obtained. The beneficial effect of tea in our daily life is important and has becomes a habitual drink after water (Ryan & Petit, 2010).

Black tea is produced by complex processes of drying, maceration and fermentation. The taste of the black tea is slightly bitter due to these processes and usually the taste can be reduced by adding together milk or any optional items (Langley-Evans, 2001). In black tea, the oxidation of polyphenols during processing leads to the formation of catechins and gallic acid complexes such as theaflavins, theaflavinic acids, thearubigins or theasinensis, and proanthocyanidin polymers (Chaturvedula & Prakash, 2011). The major polyphenols or type of antioxidants that are contained in black tea is theaflavins and thearubigins. Theaflavins are also known as the contributor of the black tea properties for instant colour, 'mouth-feel', and the formation of tea cream. In accordance with the latest studies, with the existence of the hydrogen peroxide, the oxidation of tea catechins by the peroxidase (POD) produced eighteen theaflavins type components such as major theaflavins, theaflavates, and theaflavic acids. Thearubigins, in contrast with the theaflavins, their formation and structural information, as well as their contribution to the black tea quality is not very well known (Sang et al., 2011).

The antioxidants that are contained in the tea leaves are wide ranging from flavanols, flavandiols, flavonoids, and phenolic acids. From the tea leaves, these antioxidants have 30% of the dry weight (Hilal & Engelhardt, 2007). In 2011, Oboh and Omoregie, have studied the total phenolics content and antioxidant activity in Nigerian beverages which are cocoa, coffee and tea. Out of all samples, teas have high amount of total phenol and total flavonoids content. In the study of total phenol and total phenolic in different tea planted from Sabah Tea Plantation used black and green tea as their samples. The overall result pointed that

the green tea had better antioxidant than black tea. However, the total phenol and flavonoid content in both teas were going down with the increasing maturity and this means that the shoots had higher antioxidants compared to the matured leaves (Nor Qhairul Izzreen & Mohd Fadzelly, 2013).

Karakaya and El (2006) have been tested five types of tea for total phenols content which are sage, linden flower, fresh nettle, dried nettle leaves and black tea. Among all the types of tea, the fresh nettle has the highest total phenols content with 87.9 mg/l compared to others. The phenolic compounds were said to be the powerful antioxidant in teas when in low concentration. It will protect foods from oxidative deterioration. However at high concentrations, their oxidation products may interact with proteins, carbohydrates and minerals (Karakaya & El, 2006).

There are varieties of method used to evaluate the antioxidant activity, including spectrometry, chromatography and electrochemical techniques. The techniques below fall into categories of spectrometry method and this method were further divided into various ways of end product determination. The method or assay that used or employed colorimetry as end product determination are as follow : 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3 ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing antioxidant power (FRAP) method, potassium ferricyanide reducing power (PFRAP) method, and cupric reducing antioxidant power (CUPRAC) assay (Pisoschi & Negulescu, 2011).

The antioxidants are well known in preventing the diseases that maybe can cause harm to human health. The natural antioxidants (quercetin) bioavailability from onion and apple were firstly described then it followed by the availability of phenolic antioxidants in different foods like wine, onion, tea, berries or chocolate (Perez-Jimenez et al., 2009). Alteration in antioxidant contain in tea can be done by the introduction of milk. The addition of milk in tea is a question by some parties whether the antioxidant activity changed or not. Therefore, the objectives of this research are to find out the polyphenols contained in black tea and also to quantify and compare the antioxidant activity in sugared and milked tea using FRAP assay.

Methodology

There were three experiment procedures that have been done in this research including extraction of tea extracts, characterization of chemical structure of antioxidants and the evaluation of antioxidants concentration with three types of samples. The Attenuated Total Reflectance -Fourier Transform Infrared (ATR-FTIR) spectrometer (Perkin-Elmer, United States of America) was used to identify the functional groups and the structures contained the extracts of the tea samples.

Extraction of Antioxidants by Reflux Apparatus

An extraction as conducted by Cheong et al., (2005) via reflux apparatus were set up and 10 g tea powders transferred in the flask and the solution was continuously stirred during extraction process occurs at 80 °C. The samples then were washed with 2 mL chloroform in a separatory funnel to remove caffeine, pigments, and other nonpolar impurities. This step was repeated three times and negligible catechin compounds were found in the chloroform phase owing to their low solubility in chloroform. Next, the catechin compounds in the water phase were extracted to 2 mL ethyl acetate, and this step was also repeated three times. Now, negligible catechin compounds were found in the water phase.

Estimation of Antioxidant Capacity By FRAP Assay

This assay was developed using the experimental protocol described by Benzie and Strain (1996), but was modified in terms of time lapse; the reaction was evaluated at 30 min. The FRAP reagent was prepared freshly and contained 5 mL of 300 mM sodium acetate pH 3.6, 0.5 mL of 10 mM TPTZ , and 0.5 mL of 20 mM ferric chloride. The FRAP reagents were mixed with 200 µL aliquots of each extract. Then the mixture was incubated at 26 °C for 30 min; after this time, the absorbance, were read 470 nm using a UV-Vis spectrophotometer. The FRAP value was determined by plotting in a standard curve produced by the addition of ferrous sulphate to the FRAP reagent. Results were expressed as µ mol Fe^{+2} per gram of fresh weight (µ

mol Fe^{+2} g^{-1} FW) (Henríquez et al., 2010).

Results and Discussion

Characterization by using ATR-FTIR

All the three samples showed a similar trend of the infrared spectrums in the characterization of chemical

structures of antioxidants in black tea. There are three peaks that are clearly obvious in those spectrum which are the range of 3300 - 3310 cm^{-1}, 2109 –2116 cm^{-1} and 1637–1634 cm^{-1}. These proved that all three types of tea extracts have same absorption spectrums. Figure 1 showed the infrared spectrum for all the three samples B, BM and BMS were done by using ATR-FTIR technique.

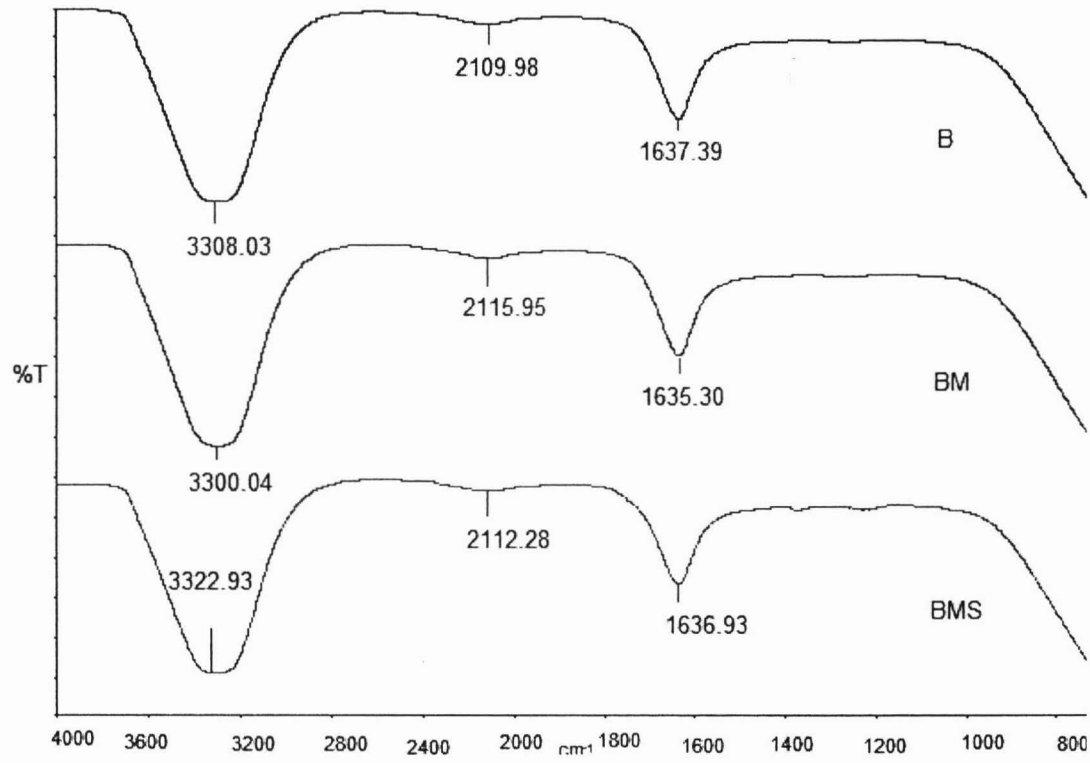

Figure 1: Infrared spectrum for black tea (B), black tea with milk (BM) and black tea with milk and sugar (BMS)

At the spectrum range between 1637–1634 cm^{-1} showed that the extracts have structure of C=O of ketone. The absorption spectrum of C=O in those extracts are lower than normally absorption of C=O of ketone which usually absorb at 1740-1660 cm^{-1}. This is due to the cyclic effect from the structures of antioxidants and the major antioxidants in black tea are theaflavins and thearubigins and theaflavins have cyclic structures of ketone. The absorption spectrum at 3300 – 3310 cm^{-1} is the O-H bond. There are two types of O-H bonds which are one for the water and the other for the alcohol. According to Menet et al., (2004), the spectrums obtained from this experiment contain some types of thé theaflavins. The theaflavins are divided into various types and some of them can be extracted by using ethyl acetate while the others remain in water phase. Since there were chances of interference of water in extracted samples, so the O-H band spectrum can be said as the O-H band for water (Lampman et al., 2010).

Another peak that is obvious is at the range of 2109 –2116 cm^{-1}. These peaks are in the range of

C≡C bonds, C≡N bonds and also the overtone peak for C-H. However, there are no C≡C and C≡N in the chemical structures of theaflavins. So, at the range of 2109 –2116 cm^{-1} there is an overtone peak for all the samples or some impurities during sample preparation (Lampman et al., 2010).

Determination of Antioxidant Activity by UV-Vis Spectrophotometer

Table 1 below shows the result of UV-Vis determination of antioxidants capacity using FRAP assay at wavelength of 470 nm in tea samples. The highest antioxidant activity contained in black tea with sugar and milk (BMS) and the lowest is in black tea (B) only.

Table 1: The Antioxidants Activity in Tea Samples

Samples	Concentration (µg/L)	Mean (µg/L)	Standard Deviation (µg/L)
B1	0.398		
B2	0.368	0.391	0.020
B3	0.407		
BM1	0.361		
BM2	0.407	0.409	0.049
BM3	0.459		
BMS1	0.410		
BMS2	0.503	0.467	0.050
BMS3	0.489		

The Ferric Reducing Ability of Plasma (FRAP) is the test used to measure the antioxidant power where the ferric (Fe^{3+}) was reduced to ferrous (Fe^{2+}) as shown in the figure 2 below. The test was done in low pH condition as the blue coloured ferrous-tripyridyltriazine complex formed. As the reaction is not specific, the Fe^{3+}- TPTZ reduction also can be achieved if other half reactions that have less positive redox potential than the Fe^{3+}/ Fe^{2+}-TPTZ react under reaction conditions (Benzie & Strain, 1996).

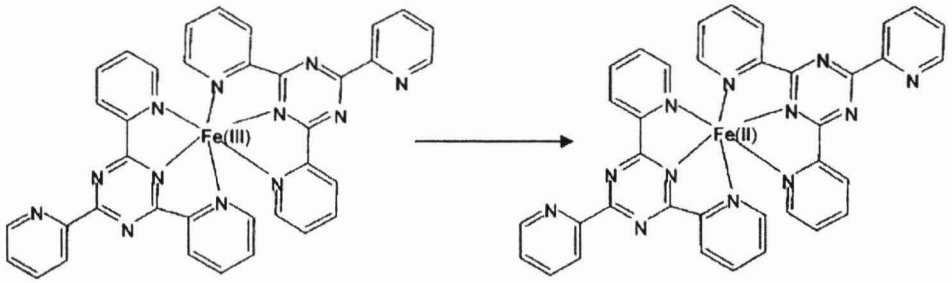

Figure 2: Reaction for FRAP assay

The FRAP assay was done by mixing sodium acetate, TPTZ and ferric chloride as the FRAP reagent in the ratio 10:1:1. The reagent then was mixed with ferrous sulphate to become standard of the spectrophotometric determination. The FRAP reagents were used on the day of preparation. The standard of FRAP Assay was prepared in a series of 0.1, 0.3, 0.5, 0.8 and 1.0 mmol and the wavelength was read at 593 nm and 596 nm. The colour of the standard was blue but the colour intensity is decrease as the molarity decrease. The blue colour exhibits between 400-490 nm but in the UV-Vis spectroscopy, the wavelength read by the machine is the absorb colour which is the complementary of the blue colour. This principle also applies in the samples (Lampman et al., 2010).The result showed that the BMS has higher antioxidant activity compared to the B and BM. At 470 nm, the

antioxidant activity of BMS was 0.467 µg/L while for B and BM was 0.391 µg/L and 0.409 µg/L respectively. These results are in the agreement with Sharma et al., (2008) which is the black tea with the milk did not only increase in antioxidant activity but the milk also stabilized the antioxidants. As studied by Kyle et al., in 2007 the formation of milk protein-polyphenol does not justify the antioxidant activity in tea samples and drinking the tea with full-fat milk did not affect changes in plasma antioxidant capacity. The increases in plasma antioxidant capacity proved that lack of suppressive effects of adding milk to tea on phenolic uptake. This means that some of the polyphenols do not only are bioavailable but also retain hydroxyl groups that capable to donate hydrogen in living things (Kyle et al., 2007).

Conclusion and Recommendation

This study has provided the information on the antioxidants contain in black tea and the comparison of antioxidant activity between sugared tea and milked tea. The experiment showed that all the three tea samples contained antioxidants which have been proved by the characterization of antioxidants in the samples by the aid of ATRFTIR spectrometer. The antioxidant activity was found the highest in the BMS samples by using FRAP assay. The addition of milk and sugar in tea did not alter the antioxidant activity in tea but on the other hand, stabilized it.

It is recommended that, the parameters of this research should be varied in the future in order to get better findings. The addition of various drinking samples in the samples will add up the number of parameters. It is also recommended that the extraction should be done at various temperatures by using different extraction solvents such as methanol and ethanol. The identification of antioxidants structural formula can be enhanced by using GC-MS. The usage of instrument like freeze dryer is a must to make sure that the samples are well homogenized.

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