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Radical Scavenging Capacity of Rwandan CTC Tea Polyphenols Extracted Using Microwave Assisted Extraction

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Abstract: Extracts of Cutting, Tearing and Curling (CTC) black and green teas from Rwanda were extracted using decoction and Microwave Assisted Extraction (MAE). Green Tea Extract from Microwave Assisted Extraction (GTMAE) had higher concentration of total tea polyphenols than others; (GTMAE26%>GTD21%>BTMAE16%>BTD14%). The higher the concentration of the total tea polyphenols, the higher was free radical scavenging activity. Antioxidant activity of the teas extracts is expressed by the DPPH radical scavenging with the decreased absorbance in the order: green tea microwave assisted extraction > green tea decoction > black tea microwave assisted extraction > black tea decoction extraction. Consequently, MAE method was found to much more effective than decoction in total polyphenolics content, scavenging capacity, time and energy consumption for both CTC black and green tea.

Key words: Tea extracts, CTC tea, total polyphenols, decoction, microwave assisted extraction, scavenging capacity

INTRODUCTION

The tea plant *Camellia sinensis* (L.) Kuntze (family Theaceae) is native to the East Asia region and is grown in Rwanda and in many more countries worldwide. Tea is consumed worldwide, although in greatly different amounts; it is generally accepted that, next to water, tea is the most consumed beverage in the world (Hasan and Nihal, 2000).

Tea has been consumed for many thousands of years, but it is only in the last few decades that we are beginning to understand the full potential of this widely enjoyed beverage (Huafu Wang *et al*., 2000).

Tea Flavonoids, or tea extracts have been linked to benefits in reducing the risk of certain cancers and cardiovascular diseases in experimental animals (Huafu Wang *et al*., 2000).

Catechins dominate in green tea and theaflavins and thearubigins predominate in black tea. These kinds of tea flavonoids are thought to have the strongest chemopreventitive effects in animal models at the concentrations usually consumed by humans (Dreostic *et al*., 1997).

Tea polyphenols have a strong affinity for proteins and minerals and thus may affect nutritional status. The various phenolic groups of tea can bind to more than one place on a protein via hydrophobic interactions and hydrogen bonding. Polyphenols have a strong affinity for proteins with high proline content, such as milk caseins, gelatin and salivary proline-rich proteins. Whether tea consumption impairs protein absorption in humans remains to be investigated. Because of the strong binding affinity of tea polyphenols to metal ions, the possible effects of tea on the absorption of these nutrients is of importance (Chung and Janelle, 2000).

Most of the green tea polyphenols are flavonoids known as catechins. Catechins constitute about 25% of the dry weight of the tea leaf, although the total catechin content varies widely depending on clonal variation, growing location, seasonal/light variation and altitude (Balentine *et al*., 1998). In Fig. 1, are some major catechins: (-)-epigallocatechin-3- gallate (EGCG), (-) - epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), (-)-epicatechin (EC). In the manufacturing of black tea, the monomeric flavan-3-ols undergo polyphenol oxidase dependent oxidative polymerization leading to the formation of flavonols known as thearubigins and theaflavins and other oligomers in a process known as “oxidation”. Theaflavins possess benzotropolone rings with dihydroxy or trihydroxy substitution systems which give the characteristics color and taste of the black tea. About 10-20% of the dry weight of black tea is due to the thearubigins, which are even more extensively oxidized and polymerized, have a wide range of molecular weights and are less characterized (Lin *et al*., 2003).

The studies found that Total Phenolic Content (TPC) of green tea was higher than that of black and oolong tea due to the reduction of catechins during fermentation process and that also affected radical scavenging activity of the tea (Yokozawa *et al*., 1998) and confirmed by Atoui *et al*. (2005).

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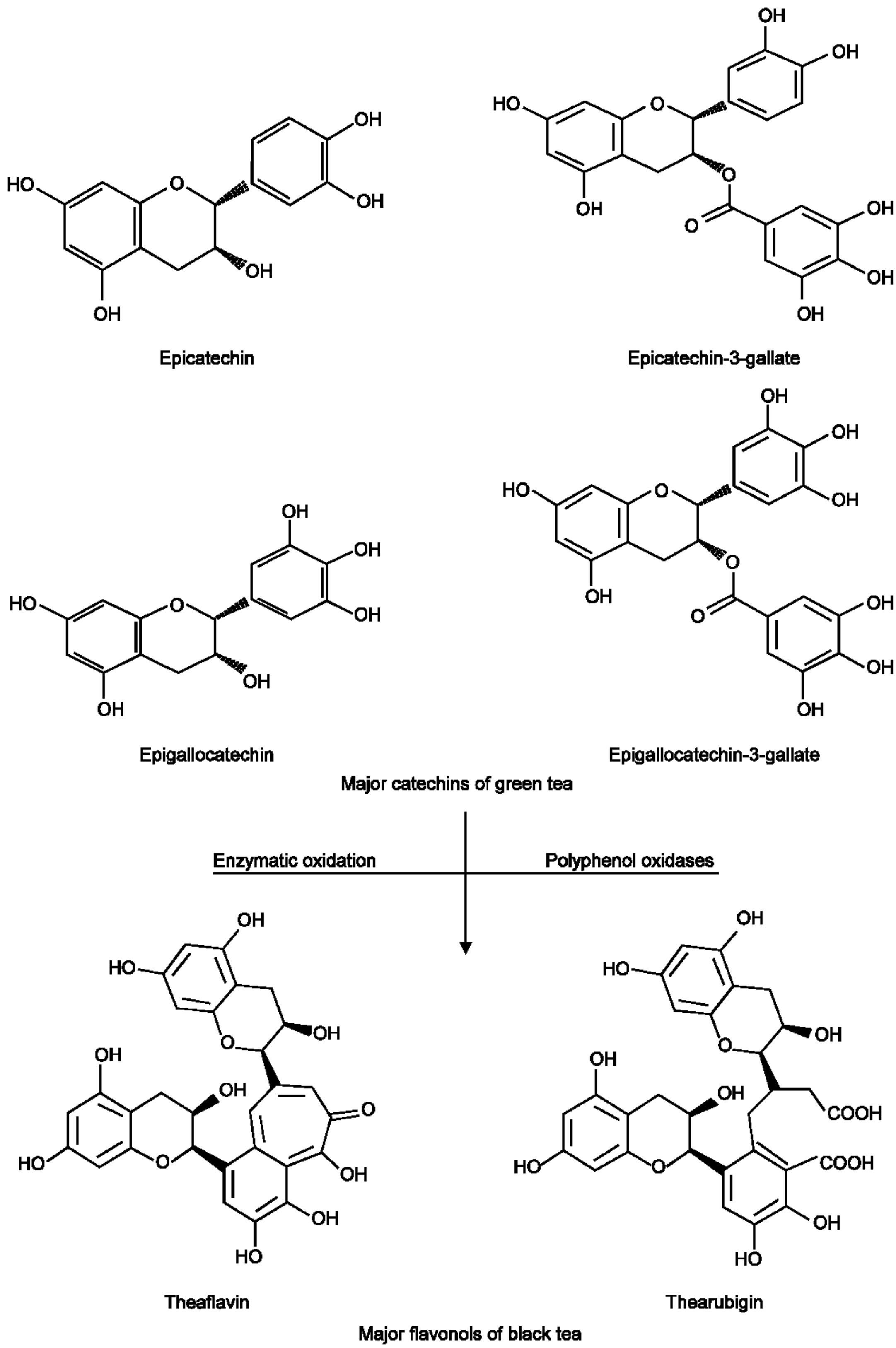

Fig. 1: Oxidation of catechins of green tea into flavonols of black tea

Microwave digestion of matrices for their eventual elemental analysis has been routinely used for several years (Kingston and Jassie, 1988). Recently, Microwave-assisted Extraction (MAE) has been used for the extraction of biologically active compounds, such as extraction of essential oils from the leaves of rosemary and peppermint (Chen and Spiro, 1994), extraction of taxanes from Taxus biomass (Mattina *et al.*, 1997), extraction of ergosterol and total fatty acids from fungal hyphae and spores, mushrooms, filtered air, artificially contaminated corn, naturally contaminated grain dust and soil (Young, 1995) and the extraction of azadirachtin-related limonoids from neem seed kernel (Dai *et al.*, 1999).

The aim of this study was to quantify the total polyphenolic content and the scavenging capacity of Rwandan CTC black and green teas, using new technology of extraction which uses the microwave and ultrasonic energies.

Results are reported here, on the scavenging capacity and contents of total phenolics in teas commonly processed and consumed in Rwanda.

MATERIALS AND METHODS

CTC (cutting, tearing and curling) black and green teas were purchased in Rwanda and brought to Jiangnan University and were processed and analyzed in food safety and quality control laboratory of the school of food science. Their moisture content was measured using a laboratory scale apparatus (SHANGPING DHS16-A). All chemicals used in the experiments were of analytical grade and available from the chemical department of Jiangnan University.

Tea extraction methods

Decoction or hot water extraction: In brief, 10 g of dried sample were extracted with 300 ml of distilled water at a temperature 77°C in a water bath for 30. Extracts were cooled at room temperature and filtered using whatman No.40 filter paper under vacuum. Samples are concentrated using rotary evaporator .final extracts were kept at 4°C for further use.

Microwave assisted extraction (MAE): A laboratory scale microwave extraction apparatus (CW-2000; Xintuo Technology Company. Shanghai, China) operated at atmospheric pressure with microwave frequency was used for the extraction purpose. The apparatus was equipped with a digital controlled system for temperature, time and power. The microwave power was of 450 W and the radiation was done for 120 sec to keep temperature not to rise above 70°C. 10 g of dried sample were extracted with 300 ml of distilled water as previous experiments. Final extracts were kept at 4°C for further use.

Total tea polyphenols determination: The amounts of total phenolic content in tea extracts were determined according to Yuanyuan *et al.* (2005).with modification. Triplicate mixtures of 2 ml extract filtrate, 8 ml distilled water and 10 ml iron tartrate solution were prepared in volumetric flask and toped to 50 ml with 0.05 mol/l sodium phosphate buffer solution at pH 7.5. The absorption values of the extracts were measured spectrophotometrically at a wavelength of 540 nm. Pure tea polyphenols were used as standard. The calibration equation for pure tea polyphenols was:

$$y = 0.356x + 0.010$$
$$R^2 = 0.998$$

DPPH free radical scavenging activity of tea extracts: The antioxidant activity of tea extracts were measured, as described by Yi *et al.* (2008) with some modifications, in terms of radical scavenging ability or hydrogen donating, by using the stable radical 2, 2-diphenyl-1-picrylhydryzyl (DPPH).

An aliquot of 1.5 ml of sample solution (2 mg/ml) was mixed with 1.5 ml of methanolic solution of DPPH (0.2 mM). The reaction mixture was incubated for 30 min in the darkness at room temperature. The absorbance of the resulting solution was measured with spectrophotometer at 517 nm. Methanol instead of sample solution was used as a control. DPPH scavenging capacity of the tested samples was measured as a decrease in the absorbance and calculated using the following equation:

$$\text{DPPH scavenging capacity (\%)} = (1 - A_{sample}/A_{control}) \times 100$$

Where A_{sample} and $A_{control}$ are the absorbance at 517 nm of the sample and control, respectively.

Statistical analysis: Determinations were carried out in three triplicate and data subjected to analysis of variance. Analysis of variance was performed using the ANOVA procedure. Statistical analyses performed according to SAS software. Significant differences between means were determined by Duncan's multiple range tests. p values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Moisture content was brought to 3% and this facilitates the pre processing of tea. Green tea was grinded into powder, this reached to breaking cells to facilitate the extraction.

Effect of cutting, tearing and curling (CTC) on extraction and black tea fermentation on total polyphenol content (TPC): Processing routes can be seen to result in a significant compositional of the green

Table 1: CTC black tea manufacturing and size fractions

Grade	Nominal classification	Particle size range
Broken Pekoe (BP)	Large	1.7-1.18 mm
Pekoe Fannings (PF)	Medium	1.18-500 µm
Pekoe Dust (PD)	Small	500-250 µm

From Conrad *et al.* (2001)

and black tea products. Differences in leaf size have an influence on the extraction efficiency of the tea component due to cell destruction during the CTC process and this result to exposure and extraction of tea components.

During green tea production, the main polyphenols (the catechins) remain relatively intact during the process, this is due to the enzymes, which catalyze their oxidative polymerization, are deactivated by heat treatment soon after plucking. Black tea production involves a leaf disruption step to promote the enzymatic oxidation of the flavonols (catechins) present in fresh green tea leaf to produce polymeric flavonoids (theaflavins and thearubigins), Robertson (1992) . The total polyphenol in green tea (26% GMAE and 21%GTD) are higher than in black tea(16%BMAE and 14% BTD); due to the different molecular properties of the green tea polyphenols (mainly catechins) and black tea polyphenols (mainly polymeric thearubigins), which have an influence on total polyphenols results. Fig. 2 shows the variation of total polyphenol level within the green and black tea samples using different methods of extraction.

DPPH free radical scavenging activity of tea extracts: DPPH radical scavenging show the antioxidant activity of different tea extracts. Figure 3 illustrates the decrease in absorbance of the DPPH radical due to the scavenging ability of soluble solids in the different tea extracts.

All tea extracts showed decrease in absorbance, where by Green Tea Extract Using Microwave Assisted Extraction (GTMAE) exhibited the fastest decrease compared to other extracts.

This shows the hydrogen donating ability as evaluated by DPPH radical scavenging method and the decreasing is in the order: GTMAE>GTD>BTMAE>BTD.

Green tea extracts (GTMAE and GTD) showed the fastest decreasing due to availability of tea polyphenols especially catechins.

Black tea extract (BTMAE and BTD) showed slow decreasing in absorbance due to less content in tea catechins compared to those in green tea extracts. Ki Won Lee and Hyong Joo Lee (2002) conclude that green tea has more health benefits than an equal volume of black tea in terms of antioxidant capacity. This can be explained by the fact that each tea is different in terms of composition and concentration of antioxidant compounds.

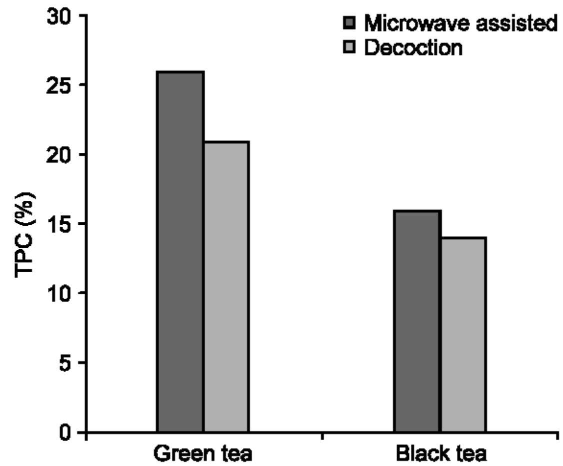

Fig. 2: The Total Polyphenolic Content (TPC) of tea extracts using different extraction methods

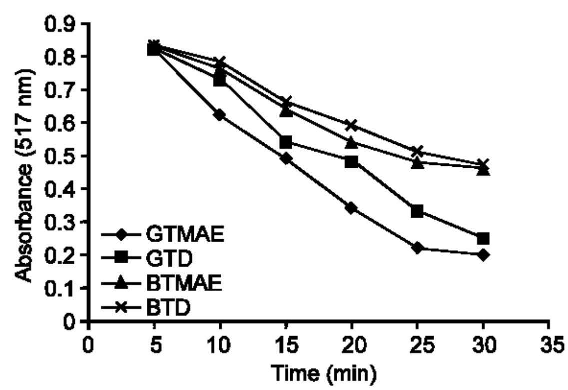

Fig. 3: The decrease in absorbance of the DPPH radical

During the fermentation process in black tea processing, catechins are oxided in thearubigins and theaflavins which leads to less scavenging capability.

Conclusion: Extracts obtained from Rwandan CTC black and green tea showed difference between green tea and black tea in total polyphenol content using different methods of extraction. Green tea extracts showed higher content in total polyphenols content compared to black tea extract for both methods of extraction; microwave assisted extraction and decoction with 26% and 21% for green tea and 16 and 14% for black tea respectively.

High content in polyphenols mainly catechins of the green tea extracts, showed fastest decreasing in absorbance of the DPPH radical, this was due to the scavenging capability of the catechins. therefore, MAE method was found to much more effective than decoction in total polyphenolics content ,scavenging capacity, time and energy consumption for both CTC black and green tea.

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REFERENCES

Atoui, A.K., A. Mansouri, G. Boskou and P. Kefalas, 2005. Tea and herbal infusions; their antioxidant activity and phenolic profile. Food Chem., 89: 27-36.

Balentine, D.A., M.E. Harbowy and H.N. Graham, 1998. Tea: The plant and its manufacture; chemistry and the consumption of the beverage in caffeine, (Ed.) G. Spiller.

Chen, S.S. and M. Spiro, 1994. Study of microwave extraction of essential oil constituents from plant materials. J. Microwave Power Electromagn. Energy, 29: 231-241.

Chung Yang, S. and M. Janelle Landau, 2000. Effects of tea consumption on nutrition and health. The J. Nutr., 0022-3166/00.

Conrad Astill, Mark R. Birch, Clive Dacombe, Philip G. Humphrey and Philip T. Martin, 2001. Factors affecting the caffeine and polyphenol contents of black and Green Tea infusions. J. Agric. Food Chem., 49: 5340-5347.

Dai, J., V.A. Yaylayan, G.S.V. Raghavan and J.R. Pare, 1999. Extraction and colorimetric determination of azadirachtin-related limonoids in neem seed kernel. J. Agric. Food Chem., 47: 3738-/3742.

Dreostic, I.E., M.J. Wargovich and C.S. Yang, 1997. Inhibition of carcinogenesis by tea: The evidence from experimental studies. Crit. Rev. Food Sci. Nutr., 37: 761-770.

Hasan Mukhtar and Nihal Ahmad, 2000. Tea polyphenols: Prevention of cancer and optizing health. Am. J. Clin. Nutr., 71: 1698S-1702S.

Huafu Wang, J. Goldon Provan and Keith Helliwell, 2000. Tea flavonoids: Their functions, utilisation and analysis. Trends Food Sci. Technol., 11: 152-160.

Kingston, H.M. and L.B. Jassie, 1988. Introduction to Microwave Sample Preparation: Theory and Practice, American Chemical Society, Washington, DC.

Ki Won Lee and Hyong Joo Lee, 2002. Antioxidant activity of black tea vs. Green tea. The J. Nutr., 132: 785.

Lin, Y.S., Y.J. Tsai, J.S. Tsay and J.K. Lin, 2003. Factor affecting the levels of tea polyphenols and caffeine in tea leaves. J. Agric. Food Chem., 51: 1864-1873.

Mattina, M.J.I., W.A.I. Berger and C.L. Denson, 1997. Microwave assisted extraction of taxanes from Taxus biomass. J. Agric. Food Chem., 45: 4691-4696.

Robertson, A., 1992. The chemistry and biochemistry of tea production-the non volatiles. In Tea Cultivation to Consumption, (Eds.) K.C. Wilson and M.N. Clifford. Chapman and Hall, London, UK., pp: 555-601.

Yi, Z., Y. Yu, Y. Liang and B. Zeng, 2008. *In vitro* antioxidant and antimicrobial activities of the extract of pericarpium citri reticulatae of a new citrus cultivar and its main flavonoids, LWT-Food Sci. Technol., 41: 597-603.

Yokozawa, T., E. Dong, T. Nakagawa, H. Kashiwagi, H. Nakagawa and S. Takeuchi, 1998. *In vitro* and *in vivo* studies on the radical scavenging activity of tea. J. Agric. Food Chem., 46: 2143-2150.

Yuanyuan Huang, Jianchun Sheng, Fangmei Yang and H.U. QiuHui, 2005. Effect of enzyme inactivation by microwave and oven heating on preservation quality of green tea. J. Food Eng., 78: 687-692.

Young, J.C., 1995. Microwave-assisted extraction of the fungal metabolite ergosterol and total fatty acids. J. Agric. Food Chem., 43: 2904-2910.