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Effect of different drying methods on the degradation of selected flavonoids in *Centella asiatica*

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Abstract: The effect of different drying methods on the degradation of flavonoids in *Centella asiatica* was evaluated. *C. asiatica* leaf, root and petiole were dried using air-oven, vacuum oven and freeze drier. Flavonoid was determined utilizing reverse-phase high performance liquid chromatography (RP-HPLC). Results of the study revealed the presence of high concentration of flavonoids in *C. asiatica* leaf, root and petiole, which include, naringin (4688.8 ± 69 µg/100 g, 3561.3 ± 205 µg/ 100 g, and 978.3 ± 96 µg/ 100 g), rutin (905.6 ± 123 µg/ 100 g, 756.07 ± 95 µg/ 100 g, and 557.25 ± 58 µg/ 100 g), quercetin (3501.1 ± 107 µg/ 100 g, 1086.31 ± 90 µg/ 100 g, and 947.63 ± 83 µg/ 100 g) and catechin (915.87 ± 6.01 µg/ 100 g, 400.6 ± 67 µg/ 100 g, and 250.56 ± 18 µg/ 100g). Luteolin, kaempferol and apigenin on the other hand, were inconsistently present in some parts of *C. asiatica*. Air-oven treatment resulted in the highest total flavonoids degradation followed by vacuum oven and freeze dried with percent degradation of 97%, 87.6% and 73%, respectively. Catechin and rutin were found to be the most stable flavonoids with percent degradation up to 35%, 66% and 76% for freeze dried, vacuum oven and air oven, respectively.

Keywords: drying treatment, flavonoids, degradation, phenolic comp, antioxidant

Introduction

Epidemiological studies have shown that consumption of adequate fruits and vegetables is associated with a lower risk of degenerative diseases such as cancer and cardiovascular disease (Ames, 1995). Seinmetz and Potter (1991) published the first comprehensive review about the relationship between vegetables, fruits and cancer. They concluded that consumption of both vegetables and fruits were associated with reduced risks of various tumors, especially cancers of the respiratory and gastrointestinal tract (lung, esophagus, colon and stomach). The protection against mutagenicity and cytotoxicity provided for by vegetables and fruits were believed to be attributed to the presence of various phytochemicals, in particular, phenolic antioxidants that protect humans against oxidative damages by inhibiting or quenching free radicals and reactive oxygen species (Ikken *et al.*, 1999).

Flavonoids are diphenylpropanes found ubiquitously in plants (Harborne, 1994). It is one of the most important group of phenolic compounds and consists of mainly catechin, anthocyanidins, flavonols, flavones, flavonones and isoflavones (Ho, 1992). Interest on these bioactive compounds have arisen because of their ability to exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammation, antiallergic, antithrombotic and vasodilatory (Cook and Sammans, 1996). Flavonoids are very important for their ability to act as natural antioxidants in foods (Vinson *et al.*, 1999). They not only inhibit the autoxidation of lipids, but also retard lipid oxidation by inhibiting lipoxygenase activity (Ho, 1992). They act *in vitro* as scavengers of active oxygen species and electrophiles, and as chelators of metal ions, and they may, therefore, be beneficial *in vivo* to reduce the risk of cardiovascular diseases and related disorders (Hollman, 2001; Pietta, 2000). Many of the flavonoids and related compounds are known to possess strong antioxidative characteristics (Dziedzic and Hudson, 1983) and widely investigated as a new source of bioactive ingredient that can be incorporated into foods in the development of functional foods.

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C. asiatica (Linn) Urban or pegaga, a perennial herb from the family Umbelliferrae, has been reported to possess various physiological effects, including the ability to lower the blood pressure, antiallergic, anticancer, wound healing, bronchitis, asthma and often referred to as a rejuvenating medicament in Ayurvedic Pharmaphacoeia (Goh *et al*., 1995; Jaganath and Ng, 1999). Although *C. asiatica* was reported to contain various bioactive compounds and was used as a medicinal herb in various cultures, little investigation on its flavonoid content has been reported. Previous study has found that phenolic compounds contributed to the antioxidative activity demonstrated by *C. asiatica* (Zainol *et al*., 2003).

The problem associated with stress related disorders creates a pressing consumer need as well as an enormous business opportunity for successful development of food products with added benefits for the purpose. The potential of alternative treatments such as active compounds from plants in lowering oxidative stress and its complications, has become an interest of most researchers in recent years. Potential in application of *C. asiatica* that is rich in potent antioxidants such as flavonoids remains lucrative. However, it is important to evaluate the stability as well as degradation pattern of these bioactive compounds, before they can be used in the development of functional foods. The objective of the study is to determine the flavonoid content of different parts of *C. asiatica* and evaluate their stability upon treatment with different drying methods. Findings from this study is crucial in development of standardized products for positive identification of the extract of interest and as functional ingredient.

Materials and Methods

Raw materials and chemicals

C. asiatica was obtained from Malaysian Agriculture Research and Development Institute (MARDI), Selangor, Malaysia. CA 05 accession was selected for the study based on the previous study that showed CA 05 exhibited the highest antioxidative activity among the four accessions tested (Zainol *et al*., 2003). Flavonoid standards (flavanols *i.e* (+)-catechin, (-)- catechin (-)- epicatechin; flavonols *i.e* quercetin, myricetin, kaempferol and rutin; flavones *i.e* apigenin and luteolin; flavanones *i.e* naringin, hesperidin and hesperetin; isoflavone *i.e* daidzein), diethyldithiocarbamate acid (DEDTC) and trifluoroacetic acids (TFA) were obtained from Sigma Chemical. Co. (St. Louis, MO. USA), methanol (HPLC grade) was purchased from Merck (Germany), and hydrochloric acid was purchased from Malinckrodt Baker Inc. (Kentucky, USA).

Sample preparation

CA 05 was cleaned with running tap water and excess water removed using tissue paper. The whole samples were then separated into 3 parts, namely leaf, root and petiole and then divided into four portions ready for the drying treatments.

Freeze-drying method

A vertical freezer with 17.5 ft^2 of shelf space, designed and built by Labconco, MO, was used to freeze-dry the samples. Samples were placed in an aluminum foil and treated at –20°C for 24 hours. The samples were then placed in freeze drier for 3 days at -45°C. The samples were then ground and stored in air-tight containers at 4°C until further analysis.

Air-drying method

Samples were dried in an air-dryer (Memmert Inc. Germany) with a capacity of 22 ft^2 of shelf space at 45°C for 48 hours. Air-drying was carried out using horizontal air flow over samples that was placed in a single layer on anodized aluminum trays. *C. asiatica* was dried for 40-45 h to a moisture content of 4.0-5.2%. The samples were then ground and stored in air-tight containers at 4°C and ready for further analysis.

Vacuum oven-drying method

CA 05 was dried in a vacuum-dryer (Memmert Inc. Germany) at 45°C for 5 hours under vacuum condition (15 psi). The samples were then ground and stored in air-tight containers at 4°C and ready for further analysis.

Determination of flavonoids using HPLC

Thirteen flavonoid standards were used in determining flavonoid content of *C. asiatica*. The selection of standards was based on those commonly found in vegetables and herbs and also those widely investigated. In addition, they represented different flavonoid group such as flavonol (quercetin, myricetin, kaempferol), catechin, flavones (apigenin, luteolin) and flavanone (naringin). The method used was a slightly modified from that of Hertog *et al*. (1992) and Crozier *et al*. (1997). In brief, flavonoid glycosides were extracted and hydrolyzed with 6 M HCl at 90 °°C for 2 hrs in 60% (v/v) aqueous methanol. The RP-HPLC analysis was performed on a Waters (Milford, MA, USA) liquid chromatography comprising a Millennium 2010 Chromatography Manager, Waters 600 controller, 486 tunable absorbance detectors.

Reverse-phase separations was carried out using Symmetry C_{18} column (150 X 3.9 mm I.D) manufactured by Water (Milford, MA, USA). The flow rate was kept at 1.0 ml/min and the UV detector was set at 370 nm. Identification of flavonoids in the samples was carried out by comparing the retention times and spectral characteristics of their peaks with those of standards.

Statistical analysis

All measurements were conducted in triplicates and results obtained were expressed as mean ± std dev. and statistical analysis was done according to the SAS (1990) User's Guides. Duncan multiple range test (DMRT) was used to examine differences between groups. A p value < 0.05 was considered statistically significant.

Results and Discussion

Identification and quantification of flavonoids in fresh C. asiatica

In this study, the flavonoids analyzed include flavonols (myricetin, quercetin and kaempferol), flavones (luteolin and apigenin), flavanones (naringin) and flavanals (catechins). Results from the study revealed appreciable variations in the flavonoid content of leaf, root and petiole of *C. asiatica* tested (Table 1). Out of thirteen standards used, seven flavonoids were detected in the different parts of *C. asiatica* and they are identified as naringin (978-4688µg/100g), quercetin (947-3501 µg/100g), catechin (250-915 µg/100g), rutin (577-905 µg/100g), luteolin (357-677 µg/100g) kaempferol (72-329 µg/100g) and apigenin (45-164 µg/100g). The major flavonoids found in *C. asiatica* was naringin, followed by quercetin, catechin and rutin. Results of the study showed that *C. asiatica* consisted of high flavonols and flavanals content when compared with that of commonly consumed fruits and vegetables (Justesen *et al.,* 1998). The flavonoids found in C. *asiatica* are those commonly found in various fruits and vegetables. Results also showed that the concentration of the different flavonoids were found to be highest in the leaf of *C asiatica* (164-4688 µg/100g), followed by root (45-3561 µg/100g), and petiole (72-978 µg/100g), respectively. Similarly, Zainal *et al*. (2003) reported that highest phenolic compound and antioxidative activity was exhibited by leaf and root of *C. asiatica*.

It is interesting to note that *C. asiatica* consisted of excellent amount of potent antioxidants, quercetin, catechin and rutin. Hertog *et al.* (1992) and Hollman *et al*. (1996) reported that the major flavonol found in vegetables is quercetin, where brocolli, kale, french beans, celery, onions and cranberries contained >50mg/kg. Hertog *et al*. (1992) also reported the association between quercetin intake and relative risk of death from coronary heart diseases. Studies have shown that flavonoids which exhibit strong chelating ability, such as quercetin, inhibit lipid peroxidation much more efficiently (Deng *et al*., 1997). In addition, quercetin and rutin have been shown to be most effective in scavenging peroxyl radicals and this agrees with the well-established structure-activity relationship of flavonoids. Similarly, Meyer *et al.* (1998) reported that both catechin and quercetin showed high activity in inhibiting copper-catalysed oxidation of human LDL *in vitro*. The same finding was reported by Cao *et al*. (1997) who suggested that quercetin with five OH substitutions in its structures demonstrated high activity in ORAC assay (3.3 Trolox equivalents), which is consistent with its inhibitory effects on platelet aggregation. Results revealed that all parts of *C. asiatica*, in particular, the leaf consisted of high concentration of potent antioxidants and potentially a good source of bioactive ingredients to be used in development of functional foods that can alleviate oxidative stress and related disorders.

Effect of thermal treatments on flavonoid content of C. asiatica

The effect of different drying methods (freeze dried, vacuum-oven and air-oven) on the degradation of flavonoids in *C. asiatica* is shown in Table 1. Results of the study showed that destruction of flavonoids varied with the different drying treatments undergone by the samples. As expected, the loss of flavonoid was found to be the least in freeze dried samples (31 to 73%), followed by vacuum dried samples (63 to 87%) and finally oven dried samples (76 to 97%). The result is probably due to the temperature and time used in the drying techniques. According to Schieber (2001), the loss of macromolecules like flavonoid during heat treatment might be due to the harsh drying conditions, in particular, the temperature and duration used. Similarly, Davey *et al*. (2000) reported that wet thermal processing can affect the phytochemicals by thermal breakdown that affect the integrity of the cell structure which then resulted in the migration of components, leading to losses by leakage or breakdown by various chemical reactions involving enzymes, light and oxygen.

Various researchers have reported on the degradation of phytochemicals upon thermal treatment. Zhang and Hamauzu (2004) discovered significant reductions of 72%, 66% and 65% of total phenolics, ascorbic acid and antioxidant activity, respectively, when broccoli florets were boiled for 5

Table 1. Flavonoid content (µg/100g) of *C. asiatica* leaf, root and petiole undergone different drying methods

		Naringin	Rutin	Quercetin	Catechin	Luteolin	Keampferol	Apigenin	Total
F	L	4688.8 ± 69^{Aa}	905.6 ± 123^{Ac}	3501.1 ± 107^{Ab}	915.87 ±6.01^{Ac}	677.7±28.8^{Ad}	329.85±4.67^{Ade}	164.52 ± 14^{Ae}	11183.44
	R	3561.3 ±105^{Ba}	756.07 ± 95^{ABc}	1086.31 ± 90^{Bb}	400.6 ± 67^{Cd}	ND	136.4 ±3.31^{BCd}	45.25 ± 6.6^{Cd}	5985.95
	P	978.3 ± 96^{Ea}	557.25 ± 58^{Bc}	947.63 ± 83^{BCb}	250.56 ± 18^{Cd}	357.63 ± 32^{Bcd}	72.56 ± 9.6^{Ce}	111.84 ± 22.5 ^{Ae}	3275.77
	L	2652.7 ±137^{BCa}	621.16 ±107^{ABc}	926.16±64.3^{BCb}	593.35 ± 40^{Bc}	280.06±25^{Cd}	297.25 ± 77^{Bd}	34.68 ± 1.75^{Bd}	5405.36
FD	R	1664.53±79.5^{Ca}	556.07 ± 56.6^{Bb}	505.26 ±15.4^{Cb}	279.05 ±8.34^{Cc}	ND	210.84 ± 8.9^{Bc}	ND	3215.35
	P	380.82 ± 25.1^{Fa}	292.54 ± 10.6^{Db}	340.56±45.2^{CDab}	150.3 ±10^{Dc}	57.63 ±6.2^{Dc}	ND	103.79 ± 8.6^{Ac}	1325.69
	L	1204.51±69.8^{Ca}	332.82 ± 61.8^{Cb}	432.65 ± 4.53^{Cb}	319.2 ± 26.5^{Cb}	91.66±0.99^{Dc}	ND	109.04 ± 12.1^{Ac}	2489.88
VOD	R	786.27 ± 59.7^{Ea}	218.62 ± 17.9^{Dc}	366.63±19.12^{CDb}	253.68 ±31.7^{Cc}	ND	ND	ND	1625.16
	P	379.21 ± 17.5^{Fa}	287.15 ± 11.9^{Db}	119.54 ± 8.49^{Dc}	93.2 ± 10.2 ^{Dc}	ND	41.71 ± 12.5^{Cd}	87.68 ± 10.8^{Bd}	1008.49
	L	1095.32 ±10^{DEa}	209.26 ± 8.25^{Db}	102.65 ± 14.7^{Dc}	200.5 ± 9.0^{Db}	52.18±2.69^{Dc}	ND	ND	1659.91
AOD	R	456.07 ± 19.2^{Fa}	114.57±22.6^{DEc}	63.7 ± 16.52^{Dd}	56.64 ± 16.5^{Dd}	ND	46.28 ± 4.36^{Cd}	ND	737.26
	P	220.14 ± 15.6^{Fa}	103.03±15.5^{DEb}	61.95 ± 5.54^{Dc}	35.9 ± 12.0^{Dc}	ND	ND	ND	421.02

The data is expressed as µg/100g sample. Values are means ± standard deviation of triplicate analyses. Means within a column (A, B, C) and means within a row (a, b) marked with different letters are significantly different at (p<0.05). Abbreviations; FD = freeze dry, VOD = vacuum oven, AOD = air oven, F = fresh, L = leaf, R = root, P = petiole, ND = not detected.

mins. On the other hand, lower losses, 30% and 47.7%, for ascorbic acid was reported by Puupponen-Pimiä *et al.* (2003) and Volden *et al*. (2008) respectively, when cabbage was blanched.

All manners of processing were found to affect the flavonoid, anthocyanins significantly ($p < 0.05$) with reductions of 59%, 41% and 29% for blanching, boiling and steaming, respectively. The reductions observed in the processed cabbage were not fully recovered in the processing waters, fitting the notion that anthocyanins are degraded by heat (Markakis, 1982).

It is interesting to note that the different drying techniques used in the study affected the individual flavonoid differently. Results from the study revealed that among the flavonoid tested, catechin and rutin were found to be the least affected by the different drying treatments, with percent degradation of 35 to 78% and 31 to77% respectively (Table 2). hese results reveal that not all drying treatments affected the individual flavonoids in *C. asiatica* as deleterious as previously expected (Schieber *et al*., 2001) and that the loss of both catechin and rutin in *C. asiatica* is minimal upon freeze drying and are potential for successful incorporation in standardized herbal product in development of foods with health benefits.

The different effect upon thermal treatment observed between flavonoid types may be attributed to variation within each flavonoid as a result of the variation in number and arrangement of the hydroxyl groups, with the most commonly occurring being those with dihydroxylation in the 30 and 40 positions (Rice-Evans *et al*.,1996). Flavonoids, especially the flavan-3-ols catechin, epicatechin, gallocatechin, and epigallocatechin, are more thermostable (Herrmann, 1995; Bravo, 1998). As a consequence of that, they can be added to food products and represents a valuable resource and may act as functional ingredient or nutraceuticals to terminate free radical chain reactions in biological systems and therefore may play important role in alleviating risk in development of chronic diseases

Conclusion

Results from the study showed that leaf, root and petiole of *C. asiatica* accession CA 05 consisted of high concentration of flavonoids, in particular, potent antioxidants rutin, quercetin and catechin. Study on the effect of the different drying methods on flavonoids degradation found that freeze drying treatment resulted in the lowest degradation as compared to that of vacuum drying and air drying. Nevertheless appreciable level of the active compounds retained upon vacuum dried. The potent antioxidants catechin and rutin are found to be suitable to be used as markers for standardizing C. *asiatica* extract to be incorporated as ingredient in the development of functional foods, due to their stability upon the different drying treatments.

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Table 2. Flavonoids degradation (%) in *C. asiatica* leaf upon different drying methods

	Naringin	Rutin	Quercetin	Catechin
Freeze drying	43.4	31.4	73.5	34.9
Vacuum oven	74.3	63.3	87.6	65.2
Air Oven	76.7	76.8	97	78.1

References

Ames, B. N. 1998. Micronutrients prevent cancer and delay aging. Toxicology Letters, 102: 5-18.

Bravo, L. 1998. Polyphenol: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews 56: 317–33.

Cao, G., Sofic, E. and Prior, R. L. 1997. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Radical Biology and Medicine 22: 749–760.

Cook, N. C. and Samman, S. 1996. Flavonoids-chemistry, metabolism, cardioprotective effect and dietary sources. The Journal of Nutritional Biochemistry 7: 66-76.

Crozier, A., Jensen, E., Lean, M. E. J. and McDonald, M. S. 1997. Quantitative analysis of flavonoids by reverse phase high performance liquid chromatography. Journal of Chromatography A 761: 315 – 321.

Davey, M.W., Van-Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I. J. J., Strain, J. J., Favell, D. and Fletcher, J. 2000. Plant l-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. Journal of the Science of Food and Agriculture **80**: 825–860.

Dziedzic, S. Z. and Hudson, B. J. F. 1983. Hydroxy isoflavones as antioxidants for edible oils. Food Chemistry 11: 161-166.

Goh, S. H., Chuah, C. H., Moh, J. S. L. and Soepadmo, E. 1995. Malaysian plants for the treatment of cardiovascular diseases. Kuala Lumpur: Academe Art and Printing Services Sdn. Bhd.

Harborne, J. B. 1994. The flavonoids, advance in research since 1986. Chapman and Hall, London.

Heim, K. E., Tagliaferro, A. R. and Bobilya, D. J. 2002. Flavonoids antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry 13, 572-584.

Hertog, M. G. L., Hollman, P. C. H. and Vennema, D. P. 1992. Optimisation of the quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. Journal of Agriculture and Food Chemistry 40: 1591 – 1598.

Hollman, P. C. H. 2001. Evidence for health benefits of plant phenols: Local or systemic effects? Journal of the Science of Food and Agriculture **81**: 842–852.

Ikken, Y., Morales, P., Martinez, A. Marin, M. L., Haza, A. I. and Cambero, M. I. 1999. Antimutagenic effect of fruit and vegetable ethanolic extracts against N-nitrosamines evaluated by the Ames test. Journal of Agricultural Food Chemistry 47: 3257–3264.

Jaganath, I. B. and Ng, L. T. 1999. Herbs: The green pharmacy of Malaysia, Vinpress Sdn. Bhd., Kuala Lumpur, p. 1-3, 21-24.

Justesen, U., Knuthsen, P. and Leth, T. 1998. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. Journal of Chromatography A 799: 101–110.

Markakis, P. 1982. Stability of anthocyanins in foods. In: P. Markakis, Editor, Anthocyanins as food colors, Academic Press, New York, p. 163–180.

Monsoor, M.A. 2005. Effect of drying methods on the functional properties of soy hull pectin. Carbohydrate Polymers 61: 362-367.

Nijhuis, H. H., Torringa, H. M., Muresan, S., Yuksel, D., Leguijt C. and Kloek W. 1998. Approaches to improving the quality of dried fruit and vegetables. Trends in Food Science and Technology 9:13-20.

Pietta, P.G. 2000. Flavonoids as antioxidants, Journal of Natural Products **63**: p. 1035–1042.

Puupponen-Pimiä, R., Häkkinen, S.T., Aarni, M., Suortti, T., Lampi A.M. and Eurola M. 2003. Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. Journal of the Science of Food and Agriculture **83**: 1389–1402.

Rice-Evans, C., Miller, N. J. and Paganga, G. 1996. Structure-antioxidant activity relationship of flavonoids and phenolic acids. Free Radical Biology and Medicine 20: 933-956.

SAS Institute, Inc. SAS/STAT User's Guide, Version 6, 4th Ed. SAS Institute : 1990.

Schieber, A., Keller, P. and Carle, R. 2001. Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography Journal of Chromatography A 910: 265–273.

Steinmetz, K. A. and Potter, J. D. 1992. Vegetables, fruit, and cancer I. Epidemiology. Cancer Causes Control 2:325–357

Vinson, J. A., Jang, J., Yang, J., Dabbagh, Y., Liang, X., Serry, M., Proch, J. and Cai, S. (1999). Vitamins and especially flavonoids in common beverages are powerful In-Vitro antioxidants which enrich lower density lipoproteins and increase their oxidative resistance after ex-vivo spiking in human plasma. Journal of Agriculture and Food Chemistry 47: 2502-2504.

Volden, J., Borge, G. I. A., Bengtsson, G. B., Hansen, M., Thygesen, I. E. and Trude Wicklund, T. 2008. Effect of thermal treatment on glucosinolates and antioxidant-related parameters in red cabbage (*Brassica oleracea* L. ssp. *capitata f. rubra*). Food Chemistry 109: 595-605

Zainol, M. K., Abd-Hamid, A., Yusof, S. and Muse, R. 2003. Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) Urban, Food Chemistry 81: 575–581.

Zhang, D.L. and Hamauzu Y. 2004. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry **88**: 503–509.