

Effect of different drying methods on concentrations of several phytochemicals in herbal preparation of 8 medicinal plants leaves

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ABSTRACT

The effect of oven drying at $50^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 9 hour, $70^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 5 hour and freeze drying on retention of chlorophyll, riboflavin, niacin, ascorbic acid and carotenoids in herbal preparation consisting of 8 medicinal plants was evaluated. The medicinal plants selected were leaves of *Apium graveolens* (saderi), *Averrhoa bilimbi* (belimbing buluh), *Centella asiatica* (pegaga), *Mentha arvensis* (pudina), *Psidium guajava* (jambu batu), *Sauvagesia androgynous* (cekor manis), *Solanum nigrum* (terung meranti) and *Polygonum minus* (kesum). Results revealed that both type and conditions of the drying treatments affected retention of all phytochemicals analysed. Herbal preparation developed using oven drying was found to have inferior phytochemicals content compared to that obtained by freeze dryer. Nevertheless, the herbal preparation developed using all treatments still retain appreciable amount of phytochemicals studied, especially carotenoids, ascorbic acid, niacin and riboflavin and thus have potential for commercial purposes.

INTRODUCTION

Malaysia is rich in natural resources and a large segment of the local community use these natural products for medicinal purposes. Over the past decade, medical and scientific knowledge on the role of various nutrients in specific disease processes has advanced at an accelerating pace and create an exciting and explosive new area of research, resulting in increasing numbers of potential nutritional products with medical and health benefits. The majority of these health promoting food are from plants, hence the term

phytochemicals is often used to indicate the disease preventing compounds available from them (Mohamed, 1997). Examples of phytochemicals that have been reported to provide medical or health benefits and therefore why they were studied, are the use of beta carotene to prevent lung and skin cancer, niacin to prevent recurrent heart attacks, ascorbic acid in improving immune system, riboflavin for lesion treatment, lycopene in the prevention of cancer and chlorophyll to study the symptoms of senescence in

attempts to maintain the green colour for producing higher quality medicinal plants products. The current concept and trend of today is towards total health management, the emphasis being on prevention rather than cure and since the connection between diet and some chronic diseases like cancer and cardiovascular diseases are irrefutable, there is a real need for development of disease-fighting foods (Mohamed, 1997; Shukla, 1993). In view of these, aim of the study is to evaluate different drying methods in retaining several phytochemicals in herbal preparation consisting of 8 medicinal plants.

MATERIALS AND METHODS

Eight types of medicinal plants leaves were used in this study, namely *Apium graveolens* (saderi), *Averrhoa bilimbi* (belimbing buluh), *Hydrocotyle asiatica* (pegaga), *Mentha arvensis* (pudina), *Psidium guajava* (jambu batu), *Sauvagesia androgynous* (cekor manis), *Solanum nigrum* (terung meranti) and *Polygonum minus* (kesum). The plants were selected based on their long term usage in Malaysian traditional medicine. For example, *Apium graveolens* has been used for asthma, bronchitis and chest pain, *Averrhoa bilimbi* used for its antibacterial property, *hydrocotyle asiatica* for wound healing and memory, *Mentha arvensis* used in cough mixtures, *Psidium guajava* for curing stomach ache and gastroenteritis, *Sauvagesia androgynous* for treating nose ulceration and hypertension, *Solanum nigrum* as laxative and diuretic and *Polygonum minus* used for indigestion and after childbirth (Burkill, 1966; Goh et al., 1995; Zakaria and Mohd., 1994). The

plants were obtained from Sri Serdang wet market and "herbal unit" of Universiti Putra Malaysia.

Drying Treatments

Three drying methods used are oven drying at $50^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 9 hour, $70^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 5 hour and freeze drying. Portions of the leaves were dried to a final moisture content of below 10% and then ground in an electric grinder. Different types of the dried medicinal leaves were then mixed manually according to a specified ratio and stored in an airtight amber bottle at temperature of $4^{\circ} \pm 1^{\circ}\text{C}$, until analysis were performed.

Chemical Analysis

Chlorophyll

Total chlorophyll was determined by spectrophotometric method described by Amar Singh (1977). A small amount of CaCO_3 was added to 1g sample and then blended with 80% acetone for 3 minutes. The mixture was then filtered and chlorophyll extracted with 80% acetone. Absorbance reading was done at 645nm and 663nm using spectrophotometer.

Riboflavin

Riboflavin content of the sample was determined using AOAC method (1984). About 5g fresh or 2g dry sample was extracted with 65ml 0.1N sulphuric acid in boiling water bath for 30 minutes. Protein and other interfering substances from the cool extract are then precipitated

at pH 6.0. and 4.5. Potassium permanganate is then added to the extract to remove interfering fluorescent substances, followed by addition of hydrogen peroxide to remove excess permanganate. The fluorescence was measured with excitation wavelength of 440nm and emission wavelength of 565nm.

Niacin

Niacin was determined using cyanogen bromide colorimeter method (AOAC, 1984). Fresh sample (5g) or dry form (2g) was homogenized in sodium hydroxide and distilled water. The mixture was heated for 1 hour over a boiling water-bath, cooled and pH adjusted to 4.5. 17g of ammonium sulphate were added. Color development is achieved by reacting the extract with cyanogen bromide and then measured at 450nm.

Ascorbic acid

Ascorbic acid was determined using phenolindophenol dye method (AOAC, 1984). 10g of sample were blended with metaphosphoric acid-acetic acid extracting solution to a homogenous slurry. 5ml of the filtrate extract were then titrated with the standard indophenol to a pink end point.

Carotenoids

Total carotenoids were determined according to Tee, E.S. and Lim, C.L. (1992). 5g of sample was hydrolysed with 95% alcohol and 5ml of 100% KOH, by water-cooled refluxing apparatus for 30 minutes. The hydrolysate was extracted with 50ml hexane for 3 times and passed through

anhydrous sodium sulphate to dry. Hexane was then evaporated and transferred to a 10ml volumetric and make up to volume. Extracted samples were then filtered through 0.45 μ m nylon membrane filter and analyzed by a non aqueous reverse-phase HPLC using a μ Bondapak C₁₈ (3.9 x 300mm) column and a ternary mixture of acetonitrile, methanol and ethyl acetate (88 : 10 : 2, v/v) as the mobile phase. The standards used were α -carotene, β -carotene and lycopene. The carotenoids eluted detected and quantitated using a UV-Visible detector set at 450nm .

Statistical Analysis

Experimental data were analyzed using analysis of variance (ANOVA) and significant differences among means at p<0.05 were determined by Duncan's multiple range test (DMRT) using the Statistical Analysis System (SAS, 1987) computing programme.

RESULTS AND DISCUSSIONS

Overall Content of Phytochemicals

Table 1 showed the levels of phytochemicals analysed in fresh and dried herbal preparation. The drying treatments caused significant (p< 0.05) reduction in the levels of all phytochemicals analysed. Oven drying caused greater reduction in the levels of phytochemicals compared to freeze drying. This results confirmed previous reports in the literature and supports the advantages of freeze drying over oven drying (Salunkhe, 1974). Freeze drying resulted in the lowest % losses of all the phytochemicals analysed, followed by oven drying at 70°C ± 1°C

Table 1. Phytochemicals composition of fresh and dried herbal tea ^{1,2}

Phytochemicals	Drying Treatment			
	Fresh (control)	Oven drying 50 °C ± 1 °C, 9 hour	Oven drying 70 °C ± 1 °C, 5 hour	Freeze drying
Chlorophyll	1.230 ± 0.008 ^a	0.435 ± 0.013 ^c	0.963 ± 0.036 ^b	0.991 ± 0.010 ^b
Ascorbic Acid	58.200±1.760 ^a	14.200± 1.429 ^d	38.300± 0.942 ^c	45.900± 0.963 ^b
Niacin	0.359 ± 0.008 ^a	0.153 ± 0.003 ^d	0.198 ± 0.003 ^c	0.304 ± 0.003 ^b
Riboflavin	0.400 ± 0.000 ^a	0.191 ± 0.047 ^d	0.196 ± 0.002 ^c	0.200 ± 0.000 ^b
Carotenoids	3829.37 ± 58.10 ^a	2795.44 ± 22.5 ^d	3063.50 ± 22.1 ^c	3277.90 ± 22.40 ^b

¹Means ± standard deviation based on three observations, data reported on mg/100g samples except for carotenoids, data reported on µg/100g samples.

²Means within a row with different superscripts are significantly different using Duncan's Multiple Range Test at P<0.05

for 5 hour and oven drying at 50°C ± 1°C for 9 hour.

Effect of different drying treatments on phytochemicals content

Chlorophyll

In fresh herbal preparation, the total chlorophyll content was 1.230mg/100g and the value ranged from 0.435 - 0.991mg/100g after different drying treatments was applied. Drying treatment affect the chlorophyll content significantly (p< 0.05) in all treatments (Table 1). The loss of total chlorophyll was found to be greatest in oven drying at 50°C ± 1°C for 9 hour (64.63%), followed by oven drying at 70°C ± 1°C for 5 hour (21.70%) and least reduction in freeze drying samples (19.43%) (Table 2). This is reflected in the visual observations of the various samples. One of the most pleasing

attributes of foods is color, especially appearance of green in dried herbs leaves. Therefore, adverse changes in colour may alienate potential customer due to suggestion of possibility of poorly controlled processing and this must be prevented or minimized (Ramada et al., 1988).

Ascorbic acid

The different treatments resulted in a significant (p< 0.05) loss in ascorbic acid contents of the samples (Table 1). As expected, the loss of ascorbic acid was found to be highest in samples dried at 50°C ± 1°C for 9 hour (75.60%), followed by that of 70°C ± 1°C for 5 hour (34.19%) and minimum loss in freeze drying (21.13%) (Table 2). The losses of ascorbic acid maybe attributed to the thermal treatment applied (Yang and Atallah, 1985). In addition, oxidation to diketogulonic acid when

Table 2. Stability of Phytochemicals in relation to the drying treatment applied

Phytochemicals	Losses during processing (drying), (%)		
	Oven drying	Oven drying	Freeze drying
	50 °C ± 1 °C, 9 hour	70 °C ± 1 °C, 5 hour	
Chlorophyll	64.63	21.70	19.43
Ascorbic Acid	75.60	34.19	21.13
Niacin	57.38	44.85	15.32
Riboflavin	52.25	51.00	50.00
Carotenoids	27.00	20.00	14.40

samples were exposed to air, light, alkaline conditions and metal ions may also contribute to the losses encountered (Addo, 1981; Harris, 1975; IFT, 1986)

Niacin

Niacin content in fresh samples were 0.369mg/100g and ranged from 0.153 - 0.34mg/100g after different drying treatments were employed. There were significant ($p < 0.05$) differences amongst niacin contents of the various treatments (Table 1). Freeze dried herbal preparation retained the highest niacin (83.84-85.52%) followed by oven drying at 70°C ± 1°C for 5 hour (54.32-55.99%) and the lowest occurred in oven drying at 50°C ± 1°C for 9 hour (41.78-43.45%). Result revealed that herbal preparation obtained by freeze drying and oven drying at 70°C for 5 hours still retain appreciable amount of niacin.

Riboflavin

Analysis of fresh samples and samples after different drying at 50°C ± 1°C for 9 hour, 70°C ± 1°C for 5 hour

and freeze drying showed losses of riboflavin of 52.25%, 51.00% and 50.00% respectively (Table 2). Riboflavin in fresh herbal preparation were 0.4mg/100g and reduced to 0.20-0.19mg/100g after various drying treatments. Thermal processing by oven drying and low temperature processing by freeze drying resulted in significant ($p < 0.05$) decrease in riboflavin content (Table 1). Among the various drying treatments applied, the retention levels was found to be highest in freeze drying (50.00%) followed by oven drying at 70°C ± 1°C for 5 hour (43.50 - 44.50%) and lowest in oven drying at 50°C ± 1°C for 9 hour (36.00-59.50%).

Carotenoids

During the three methods of drying, a significant ($p < 0.05$) decrease in carotenoid content were observed (Table 1). Carotenoid content of fresh herbal preparation was 3829.37µg/100g and decrease due to oven drying at 50°C ± 1°C for 9 hour, 70°C ± 1°C for 5 hour and

freeze drying were $2795.44\mu\text{g}/100\text{g}$, $3063.50\mu\text{g}/100\text{g}$ and $3277.90\mu\text{g}/100\text{g}$ respectively. It was seen that dehydration at higher temperatures of $50^\circ\text{C}-70^\circ\text{C}$ led to greater destruction of carotenoid than losses encountered by dehydration by freeze drying (Table 2). Retention levels in carotenoid content for the various drying treatments were 72.41-3.59% (oven drying at $50^\circ\text{C} \pm 1^\circ\text{C}$ for 9 hour), 79.42 - 80.58% (oven drying at $70^\circ\text{C} \pm 1^\circ\text{C}$ for 5 hour) and 85.01-86.18% for freeze drying. The principle cause of deterioration of the carotenoid is oxidation, this being more severe once cellular integrity has been lost due to the high degree of unsaturation of the carotenoid (De la Mar and Francis, 1969; Kanner and Mandel, 1976; Carnevale et al., 1980; Malchev et al., 1982). Nevertheless, appreciable amount of carotenoid still remain in all the samples. Carotenoids, especially β -carotene has been found to be a potent antioxidant that can aid endogenous tocoferol in trapping free radicals produced during normal biological metabolism. Free radicals have been shown to be involved in etiology of various chronic diseases like coronary heart diseases, cancer, arthritis and premature aging.

Conclusion

In conclusion, the results indicated that freeze drying of leafy medicinal plants resulted in products that was superior to oven drying at $50^\circ\text{C} \pm 1^\circ\text{C}$ for 9 hour and $70^\circ\text{C} \pm 1^\circ\text{C}$ for 5 hour on the basis of nutrients retained namely chlorophyll, riboflavin, niacin, ascorbic acid and carotenoids. This study did not take into account the cost for drying the

plants because it is important to retain as much as possible the active ingredients in the dried herbal preparation. This is crucial in order to preserve the biological benefits that the herbs can do. Nevertheless, the study revealed that dried medicinal plants leaves still retain appreciable amount of chlorophyll, ascorbic acid, niacin, riboflavin and carotenoids in all the treatments except for the sample undergone oven drying at 50°C for 9 hours. Drying medicinal plants by oven at 70°C for 5 hours warrant further research based on level of phytochemicals remain in the treated samples and relatively low cost involved. Further study is required to carry out effectiveness of the herbal preparation in reducing risks for chronic diseases especially coronary heart disease, cancer and diabetes using rabbit or mouse before the product can be recommended to human.

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