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Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species

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ABSTRACT

Effects of five different drying methods on the antioxidant properties (AOP) of leaves of *Alpinia zerumbet*, *Etlingera elatior*, *Curcuma longa*, and *Kaempferia galanga* were assessed. All methods of thermal drying (microwave-, oven-, and sun-drying) resulted in drastic declines in total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), and ferric-reducing power (FRP), with minimal effects on ferrous ion-chelating ability and lipid peroxidation inhibition activity. Of the non-thermal drying methods, significant losses were observed in air-dried leaves. Freeze-drying resulted in significant gains in TPC, AEAC, and FRP for *A. zerumbet* and *E. elatior* leaves. After one week storage, AOP of freeze-dried *E. elatior* leaves remained significantly higher than those of fresh control leaves. Freeze-dried tea of *A. zerumbet* was superior to the commercial tea for all AOP studied.

1. Introduction

Past studies on the antioxidant properties (AOP) of ginger species (Zingiberaceae) were confined to rhizomes. Although their leaves have been used for food flavouring and in traditional medicine (Larsen, Ibrahim, Khaw, & Saw, 1999), very little research has been done on their total phenolic content (TPC) and antioxidant activity (AOA).

Alpinia zerumbet, also known as Shell Ginger, is an ornamental plant with attractive fragrant flowers. In Japan, leaves of *A. zerumbet* (Getto) are sold as herbal tea, and are used to flavour noodles and wrap rice cakes. Its tea has hypotensive, diuretic, and anti-ulcerogenic properties (Mpalantinos, de Moura, Parente, & Kuster, 1998). Decoction of leaves has been used during bathing to alleviate fevers. From the leaves of *A. zerumbet*, flavonoids, kava pyrones, and phenolic acids have been isolated (Elzaawely, Xuan, & Tawata, 2007; Mpalantinos et al., 1998). Leaves of *A. zerumbet* had the highest TPC and AOA among five species of *Alpinia* studied (Chan et al., 2008). Leaves had higher inhibition of β-carotene oxidation and radical–scavenging activity than rhizomes (Elzaawely et al., 2007).

Etlingera elatior or Torch Ginger is widely cultivated throughout the tropics. Young inflorescences are commonly used as the ingredients of spicy dishes (Larsen et al., 1999). Post-partum women use *E. elatior* leaves together with other aromatic herbs for bathing to remove body odour. They are also used for cleaning wounds. Flavonoids in leaves of *E. elatior* have been identified as kaempferol 3-glucuronide, quercetin 3-glucuronide, quercetin 3-glucoside, and quercetin 3-rhamnoside (Williams & Harborne, 1977). Screening of leaves of 26 ginger species belonging to nine genera showed that species of *Etlingera* had the highest phenolic content and radical–scavenging activity (Chan et al., 2008). Leaves of *E. elatior* had the most outstanding AOP among five *Etlingera* species studied (Chan, Lim, & Omar, 2007).

Curcuma longa is a widely cultivated ginger plant with pungent rhizomes that produce turmeric, a popular spice for curries, food flavouring, and colouring. Curcumin, the active component of turmeric, is known to have a wide array of bioactivity including antioxidant, anti-inflammatory, anti-cancer, and cardio-protective properties. The aromatic leaves of *C. longa* are used for flavouring steamed and baked fish (Larsen et al., 1999). Phenolic content and radical–scavenging activity were significantly higher in rhizomes than in leaves of *C. longa*, but metal ion-chelating ability was higher in leaves (Chan et al., 2008).

Kaempferia galanga is a small, cultivated ginger plant with broadly ovate and pale green leaves. Its leaves and rhizomes are used in traditional medicine, perfumery, and food flavouring. Rhizomes of *K. galanga* are used as expectorants and carminatives. They are also used as ingredient for preparing 'Jamu', a local health tonic consumed by the Malays. Its mild spicy leaves are ingredients for savoury dishes. Its leaves and rhizomes are eaten fresh or cooked as a vegetable, and used in cosmetic powder and as a food flavouring agent. Phenolic content, radical–scavenging activity, and metal ion-chelating ability were significantly higher in leaves than in rhizomes of *K. galanga* (Chan et al., 2008).

In our present study, TPC and AOA of leaves of *A. zerumbet*, *E. elatior*, *C. longa*, and *K. galanga* as affected by three thermal drying methods (microwave-, oven-, and sun-drying) and two

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non-thermal drying methods (air- and freeze-drying) were assessed using five different antioxidant assays. These ginger species were selected for study because their leaves have been used for food flavouring and as traditional medicine. For leaves of *E. elatior*, the effects of microwave-drying for different durations and the effects of storage of freeze-dried leaves were studied. For *A. zerumbet*, AOP of tea from freeze-dried leaves were compared to those of the commercial Getto tea. This study represents the first systematic analysis of the effects of different drying methods on the AOP of ginger leaves. Aimed at developing protocols for producing herbal products with AOP comparable or superior to those of commercial ones, this study is probably the first to report that freeze-drying enhances the AOP of ginger leaves and tea.

2. Materials and methods

2.1. Chemicals and instruments

Folin–Ciocalteu's phenol reagent (Fluka, 2 N), gallic acid (Fluka, 98%), and anhydrous sodium carbonate (Fluka, 99%) were used for TPC analysis; 2,2-diphenyl-1-picrylhydrazyl (Sigma, 90%) for DPPH assay; ferric chloride hexa-hydrate (Fisher Scientific, 100%), potassium ferricyanide (Unilab, 99%), trichloroacetic acid (HmbG Chemicals, 99.8%), potassium dihydrogen orthophosphate (Fisher Scientific, 99.5%), and dipotassium hydrogen phosphate (Merck, 99%) for FRP assay; ferrozine (Acros Organics, 98%) and ferrous sulphate hepta-hydrate (HmbG Chemicals) for FIC assay; and β-carotene (Sigma, Type 1: synthetic), chloroform (Fisher Scientific, 100%), linoleic acid (Fluka), and Tween 40 (Fluka) for β-carotene bleaching (BCB) assay. Absorbance was measured with an Anthelie Advanced 5 Secoman UV–vis spectrophotometer. HPLC analysis was conducted using Agilent Technologies 1200 Series with Thermo Scientific BDS Hypersil Phenyl Column (4.6 × 100 mm).

2.2. Plant materials

Leaves of *A. zerumbet* and *E. elatior* were collected from Janda Baik in Pahang. The latter were also collected from Selayang and Kepong in Selangor. Leaves of *C. longa* were purchased from the supermarket and those of *K. galanga* were obtained from plants raised from rhizomes. Voucher specimens of these plants were deposited at the herbarium of Forest Research Institute Malaysia (FRIM). The commercial tea of *A. zerumbet* (Getto) was purchased from Okinawa, Japan.

2.3. Drying processes

Leaves were subject to five different drying methods, i.e., microwave-, oven-, sun-, freeze-, and air-drying. For each drying method, 1 g of fresh leaves was used. In microwave-drying, leaves were dried in a microwave oven (Sharp R-248E; 800 W) for 4 min. Oven-drying involved drying for 5 h in an oven (Memmert ULE 500) at 50 °C. Leaves were sun-dried in the greenhouse for three days with about 27 h of daylight. Mid-day temperature in the greenhouse can reach 35 °C. Leaves were air-dried for three days in the laboratory at ambient temperature of 25–30 °C and relative humidity of 33%. For each of the above drying methods, leaf pieces were spread out evenly on a Petri dish. In freeze-drying, leaf samples were lyophilised overnight in a vacuum flask at 0.125 mbar and −50 °C in a freeze-dryer (Christ Alpha 1–4).

2.4. Sample extraction

Extraction efficiencies of different solvents, namely, dichloromethane, ethyl acetate, methanol, and aqueous methanol (50%) were tested on *C. longa* and *E. elatior*. Leaves of the two species (1 g) were powdered with liquid nitrogen in a mortar and extracted with 50 ml of solvent, with continuous swirling for one hour at room temperature using an orbital shaker. Extracts were filtered under suction and stored at −20 °C for further use. Analysis of extracts was done in triplicate.

For subsequent analyses, fresh and dried leaves of *A. zerumbet*, *E. elatior*, *C. longa*, and *K. galanga* were extracted with methanol. For the analysis of AOP of tea extracts, 1 g of tea in powder form was extracted in 50 ml boiling water for 1 h with continuous swirling. The infusions were allowed to cool throughout extraction period. Extracts were filtered and stored at 4 °C for further analysis.

2.5. Total phenolic content

Total phenolic content (TPC) of extracts was determined using the Folin–Ciocalteu assay. Samples (300 μl) were introduced into test tubes followed by 1.5 ml of Folin–Ciocalteu's reagent (10 times dilution) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were allowed to stand for 30 min before absorbance at 765 nm was measured. TPC was expressed as gallic acid equivalent (GAE) in mg/100 g material. The calibration equation for gallic acid was $y = 0.0111x - 0.0148$ ($R^2 = 0.9998$) where y is the absorbance and x is the concentration of gallic acid in mg/l.

2.6. Determination of antioxidant activity

The methods described below were based on procedures previously described (Chan et al., 2008).

2.6.1. DPPH radical–scavenging activity

Different dilutions of extracts (1 ml) were added to 2 ml of 2,2-diphenyl-1-picrylhydrazyl (5.9 mg/100 ml methanol). Absorbance was measured at 517 nm after 30 min. Radical–scavenging ability was calculated as IC_{50} and expressed as expressed as AEAC in mg ascorbic acid/100 g as follows:

$$\text{AEAC (mg AA/100 g)} = \frac{IC_{50(\text{ascorbate})}}{IC_{50(\text{sample})}} \times 10^5$$

the IC_{50} of ascorbic acid used for calculation of AEAC was 0.00387 mg/ml.

2.6.2. Ferric-reducing power

Different dilutions of extracts (1 ml) were added to 2.5 ml phosphate buffer (0.2 M; pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v). The mixture was incubated at 50 °C for 20 min. Trichloroacetic acid solution (2.5 ml; 10% w/v) was added to stop the reaction. The mixture was then separated into aliquots of 2.5 ml and diluted with 2.5 ml of water. To each diluted aliquot, 500 ml of ferric chloride solution (0.1% w/v) were added. After 30 min, absorbance was measured at 700 nm. FRP was expressed as mg GAE/g. The calibration equation for gallic acid was $y = 16.767x$ ($R^2 = 0.9974$).

2.6.3. Ferrous ion-chelating ability

Solutions of 2 mM $FeSO_4$ and 5 mM ferrozine were diluted 20 times. $FeSO_4$ (1 ml) was mixed with different dilutions of extracts (1 ml), followed by ferrozine (1 ml). Absorbance was measured at 562 nm after 10 min. The ability of extracts to chelate ferrous ions was calculated as follows:

$$\text{Chelating effect}\% = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

where A_{sample} and A_{control} are absorbance of the sample and negative control, respectively.

2.6.4. Lipid peroxidation inhibition activity

Lipid peroxidation inhibition (LPI) activity was determined using the β-carotene bleaching (BCB) assay. β-Carotene/linoleic acid emulsion was prepared by adding 3 ml of β-carotene (5 mg in 50 ml chloroform) to 40 mg of linoleic acid and 400 mg of Tween 40. Chloroform was evaporated under reduced pressure and oxygenated ultra-pure water (100 ml) was added and mixed well. Initial absorbance of the emulsion was measured at 470 nm. Aliquots of the emulsion (3 ml) were mixed with 10 μl, 50 μl, and 100 μl of extracts and incubated in a water bath at 50 °C for 1 h. Bleaching rate of β-carotene was measured at 470 nm and 700 nm. Measurement at 700 nm is needed to correct for the presence of haze. LPI activity expressed as AOA (%) was calculated as follows:

$$\text{Bleaching rate (BR) of } \beta\text{-carotene} = \frac{\ln\left(A_{\text{initial}}/A_{\text{sample}}\right)}{60}$$

$$\text{AOA } (\%) = \left(1 - \frac{\text{BR}_{\text{sample}}}{\text{BR}_{\text{control}}}\right) \times 100$$

where A_{initial} and A_{sample} are absorbance of the emulsion before and 1 h after incubation, and $\text{BR}_{\text{sample}}$ and $\text{BR}_{\text{control}}$ are bleaching rates of the sample and negative control, respectively.

2.7. High performance liquid chromatography

Extracts of fresh and freeze-dried leaves of *E. elatior* were dissolved in 50% methanol and analysed using reverse-phase HPLC with a phenyl column. A 15-min linear gradient from 5% to 100% MeOH, was used to elute samples at 1 ml/min. Mobile phases were acidified with 0.1% trifluoroacetic acid for better resolution. Elution was monitored at 254 nm. Chromatograms of fresh and freeze-dried leaves were overlaid to display differences in constituents and their overall peak areas calculated.

3. Results and discussion

3.1. Description of plant species

Leaves of *A. zerumbet* are lanceolate, dark green, and emit an aromatic fragrance when crushed. Leaves of *E. elatior* are lanceolate, green, sometimes flushed pink when young, and have a pleasant sour scent. Leaves of *C. longa* are oblong or ovate, light green, and produce a pungent spicy aroma. The broadly ovate light green leaves of *K. galanga* have a mild spicy fragrance. *A. zerumbet* and *E. elatior* belong to the tribe Alpineae, and *C. longa* and *K. galanga* belong to the tribe Hedychieae. Alpineae species are medium- to large-sized forest plants of which *Etlingera* is the largest (Larsen et al., 1999). Hedychieae species are small- to medium-sized herbs.

3.2. Antioxidant properties of fresh leaves

3.2.1. Extraction with different solvents

TPC and AOA of leaf extracts of *C. longa* and *E. elatior* were studied using the Folin–Ciocalteu, DPPH radical–scavenging, and FRP assays, and expressed as mg GAE/100 g, mg AA/100 g, and mg GAE/g, respectively. Of the different solvents tested, methanol and 50% methanol were comparable and yielded the highest TPC and AOA. Ranking of extraction was of the following order: 50% methanol ≈ methanol > ethyl acetate > dichloromethane. The amount of antioxidative compounds extracted was reduced with solvents of decreasing polarity.

3.2.2. Extraction with methanol

Extraction efficiencies of methanol as measured by TPC values of first extraction of *A. zerumbet*, *E. elatior*, *C. longa*, and *K. galanga* leaves were comparable, being 78 ± 3%, 84 ± 1%, 83 ± 1%, and 84 ± 2%, respectively.

Of the four species, leaves of *E. elatior* had the highest TPC, AEAC, and FRP with values of 2420 mg GAE/100 g, 2960 mg AA/100 g, and 14 mg GAE/g, respectively. Leaves of *A. zerumbet* ranked second with values of 1990 mg GAE/100 g, 2180 mg AA/100 g, and 11 mg GAE/g, respectively. AOP of leaves of *E. elatior* and *A. zerumbet* had significantly higher values than those of *C. longa* and *K. galanga*. Results showed that larger plants of the tribe Alpineae growing in exposed forest sites have stronger AOP than those of smaller plants of the tribe Hedychieae growing in shaded sites (Chan et al., 2008).

3.3. Effects of thermal drying methods

Heat-treated leaves of *A. zerumbet* resulted in losses in TPC, AEAC, and FRP compared to those of fresh leaves. Losses were 50%, 58%, and 55% for microwave-drying; 43%, 49%, and 56% for oven-drying; and 47%, 57%, and 46% for sun-drying; respectively (Table 1). Losses in AOP were insignificant between the three drying methods. FIC values of heat-treated leaves were comparable or marginally lower (at higher concentration) than that of fresh leaves, with values, for example at 7 mg/ml concentration, falling in the range of 44–56% (cf, 57% for fresh sample).

AOP of heat-treated leaves of *E. elatior* were also adversely affected by microwave-, oven-, and sun-drying. Losses were 40%, 42%, and 70% for TPC; 59%, 58%, and 75% for AEAC; and 44%, 43%, and 76% for FRP; respectively (Table 1). Losses in AOP were highest

Table 1
Percentage loss in total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), and ferric-reducing power (FRP) of leaves of *A. zerumbet*, *E. elatior*, *C. longa*, and *K. galanga* following thermal drying (fresh weight)[a]

Species	Drying method	Water loss (%)	Percentage loss compared to fresh leaves		
			TPC (mg GAE/100 g)	AEAC (mg AA/100 g)	FRP (mg GAE/g)
A. zerumbet	Microwave-drying	60 ± 1	−50 ± 5a	−58 ± 2a	−55 ± 5a
	Oven-drying	60 ± 2	−43 ± 10a	−49 ± 6a	−56 ± 8a
	Sun-drying	59 ± 2	−47 ± 9a	−57 ± 9a	−46 ± 10a
E. elatior	Microwave-drying	75 ± 2	−40 ± 8a	−59 ± 9a	−44 ± 10a
	Oven-drying	68 ± 5	−42 ± 13a	−58 ± 16a	−43 ± 15a
	Sun-drying	76 ± 5	−70 ± 13b	−75 ± 13a	−76 ± 11b
C. longa	Microwave-drying	83 ± 1	−58 ± 14a	−59 ± 9a	−71 ± 5a
	Oven-drying	83 ± 2	−77 ± 2b	−77 ± 1b	−81 ± 1b
	Sun-drying	81 ± 1	−81 ± 1c	−84 ± 2c	−86 ± 2c
K. galanga	Microwave-drying	93 ± 2	−36 ± 10a	−27 ± 13a	−44 ± 3a
	Oven-drying	93 ± 2	−66 ± 1b	−66 ± 2b	−81 ± 2b
	Sun-drying	84 ± 1	−91 ± 1c	−86 ± 1c	−88 ± 2c

[a] Values of TPC, AEAC, and FRP of leaves are means ± SD ($n = 3$). For each column, values followed by the same letter (a–c) are not statistically different at $P < 0.05$ as measured by the Tukey HSD test. ANOVA applies between thermal drying methods of a species.

for sun-drying. FIC values of fresh leaves were slightly higher than those of microwave- and oven-dried leaves. Heat-treated leaves yielded slightly higher LPI activity. TPC and AOA of leaves of *E. elatior* microwave-dried for 2 min, 4 min, 6 min, and 8 min resulted in significant losses but their declines were comparable between different drying durations.

For leaves of *C. longa* and *K. galanga*, thermal drying also resulted in declines in TPC, AEAC, and FRP. Losses ranged from 58% to 81%, 59% to 84%, and 71% to 86% for *C. longa*; and from 36% to 91%, 27% to 86%, and 44% to 88% for *K. galanga*; respectively (Table 1). Losses in AOP were the least for microwave-drying and the greatest for sun-drying.

Processing methods are known to have variable effects on TPC and AOA of plant samples. Effects include little or no change, significant losses, or enhancement in AOP (Nicoli, Anese, & Parpinel, 1999). Food processing can improve the properties of naturally occurring antioxidants or induce the formation of new compounds with AOP, so that the overall AOA increases or remains unchanged (Tomaino et al., 2005).

Increase in AOA following thermal treatment has been reported in tomato (Dewanto, Wu, Adom, & Liu, 2002a), sweet corn (Dewanto, Wu, & Liu, 2002b), Shiitake mushroom (Choi, Lee, Chun, Lee, & Lee, 2006), and ginseng (Kang, Kim, Pyo, & Yokozawa, 2006). Increase in AOA following thermal treatment has been attributed to the release of bound phenolic compounds brought about by the breakdown of cellular constituents 350: 2 min, 4 min, 6 min, and 8 min, and the formation of new compounds with enhanced AOP (Dewanto et al., 2002a, 2002b; Tomaino et al., 2005).

Many studies have reported losses in TPC and AOA of plant samples following thermal treatments. Losses were mainly reported in vegetables (Ismail, Marjan, & Foong, 2004; Roy, Takenaka, Isobe, & Tsushida, 2007; Toor & Savage, 2006; Zhang & Hamauzu, 2004). Losses in AOP of heat-treated samples have been attributed to thermal degradation of phenolic compounds (Larrauri, Rupérez, & Saura-Calixto, 1997). Declines in AOP have been attributed to degradative enzymes, thermal degradation of phytochemicals, and to loss of antioxidant enzyme activities (Lim & Murtijaya, 2007). Declines in TPC and AOA are often accompanied by loss of other bioactive properties (Roy et al., 2007).

Results of this study showed that thermal drying methods had two major effects on the AOP of ginger leaves. TPC, AEAC, and FRP were adversely affected but not FIC ability and LPI activity. Compounds containing electron-donating atoms such as nitrogen could contribute substantially to the FIC ability and thus values were not affected by reduction in phenolic content. LPI activity depends more on the type of antioxidants present rather than their concentration. Some phenolic compounds such as quercetin are good inhibitors of lipid peroxidation while others such as catechin are poor inhibitors.

It would be presumptuous to infer that cooking and other thermal processing resulted in gains or losses in AOA without analysing a wide range of AOP and testing a variety of samples. A single treatment applied on a given sample could have variable effects on AOP. Gains in TPC and FIC ability, losses in FRP, but similarity in DPPH radical–scavenging activity have been reported for hot air-dried tomatoes (Chang, Lin, Chang, & Liu, 2006). TPC and oxygen radical absorbance capacity (ORAC) declined while DPPH radical–scavenging activity increased for heat-treated purple wheat bran (Li, Pickard, & Beta, 2007). TPC significantly declined in all vegetables studied while LPI ability was unchanged in some vegetables after thermal treatment (Ismail et al., 2004). TPC and DPPH radical–scavenging activity increased or remained unchanged depending on the type of vegetable and not on the type of cooking (Turkmen, Sari, & Velioglu, 2005). DPPH radical–scavenging activity increased in some cooked vegetables, while in others, the activity decreased (Yamaguchi et al., 2001).

An interesting finding from this study is the effect of microwave-drying on the AOP of leaves of *E. elatior*. Leaves microwave-dried for 2 min, 4 min, 6 min, and 8 min, which resulted in same weight loss (75 ± 2%), showed significant but comparable declines in TPC, AEAC, and FRP. A likely explanation is that microwave-drying for 2 min is sufficient to remove the moisture content and to decompose all heat-labile antioxidants, and subsequent heating would have no effect.

3.4. Effects of non-thermal drying methods

Air-drying of ginger leaves resulted in significant losses in TPC and AOA for all four species. Air-drying of *A. zerumbet* and *E. elatior* leaves significantly decreased TPC, AEAC, and FRP by 51%, 48%, and 50%; and by 49%, 51%, and 53%; respectively (Table 2). It resulted in drastic declines of 80%, 84%, and 80% for leaves of *C. longa*; and 70%, 77%, and 71% for leaves of *K. galanga*; respectively. Declines in AOP resulting from air-drying could be due to enzymatic degradation as the process was carried out at room temperature and takes several days for samples to dry. Contrary to results of this study, air-drying at 25–32 °C for 10 days of temperate herbs of lemon balm, oregano, and peppermint had variable effects on AOP which ranged from

Table 2
Total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), and ferric-reducing power (FRP) of fresh, air-, and freeze-dried leaves of *A. zerumbet*, *E. elatior*, *C. longa*, and *K. galanga* (fresh weight)[a]

Species	Drying method	Water loss (%)	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)	FRP (mg GAE/g)
A. zerumbet	Fresh		2470 ± 240a	3020 ± 428a	11 ± 2.0a
	Air-drying	55 ± 1	1220 ± 241b	1570 ± 358b	5.5 ± 1.2b
E. elatior	Fresh		2500 ± 554a	2990 ± 891a	17 ± 4.2a
	Air-drying	75 ± 5	1270 ± 341b	1460 ± 439b	8.0 ± 2.4b
C. longa	Fresh		391 ± 36a	251 ± 19a	2.9 ± 0.1a
	Air-drying	82 ± 2	78 ± 5b	41 ± 4b	0.5 ± 0.1b
K. galanga	Fresh		130 ± 7a	48 ± 3a	0.7 ± 0.1a
	Air-drying	81 ± 4	39 ± 3b	11 ± 1b	0.2 ± 0.0b
A. zerumbet	Fresh		1990 ± 62a	2180 ± 42a	11 ± 0.2a
	Freeze-drying	61 ± 2	2550 ± 55b	2530 ± 45b	12 ± 0.2b
E. elatior	Fresh		2420 ± 210a	2960 ± 362a	14 ± 0.7a
	Freeze-drying	76 ± 4	3050 ± 226b	4280 ± 55b	19 ± 1.3b
C. longa	Fresh		399 ± 15a	243 ± 28a	2.1 ± 0.1a
	Freeze-drying	82 ± 2	357 ± 20b	222 ± 12a	1.8 ± 0.1b
K. galanga	Fresh		133 ± 5a	42 ± 3a	0.7 ± 0.1a
	Freeze-drying	86 ± 3	112 ± 11b	38 ± 5a	0.6 ± 0.1a

[a] Values of TPC, AEAC, and FRP of leaves are means ± SD ($n = 3$). For each column, values followed by the same letter (a–b) are not statistically different at $P < 0.05$ as measured by the Tukey HSD test. ANOVA does not apply between species and between drying methods.

significant increase to significant decline (Capecka, Mareczeek, & Leja, 2005).

Freeze-drying of *A. zerumbet* leaves significantly increased TPC, AEAC, and FRP compared to those of fresh leaves. Values of freeze-dried leaves were 2550 mg GAE/100 g, 2530 mg AA/100 g, and 12 mg GAE/g while those of fresh leaves were 1990 mg GAE/100 g, 2180 mg AA/100 g, and 11 mg GAE/g, respectively (Table 2). This amounted to gains of 28%, 16%, and 9%, respectively. FIC values of freeze-dried leaves of *A. zerumbet* were comparable to those of fresh leaves.

Freeze-dried leaves of *E. elatior* similarly showed significantly higher values in TPC, AEAC, and FRP compared to those of fresh leaves. Percentage gains were 26%, 45%, and 36%, respectively (Table 2). FIC ability of freeze-dried leaves showed little change compared to that of fresh leaves.

HPLC chromatograms of extracts of fresh and freeze-dried leaves of *E. elatior* revealed some interesting results (Fig. 1). Apices of major compounds remained relatively unchanged. The chromatograms showed greater amounts of minor compounds in freeze-dried than fresh leaves. Between retention times of 4 and 10 min, the overall peak area of freeze-dried leaves was 11 930 mAU*s compared to 9050 mAU*s of fresh leaves. This represented an increase of 32%, which is comparable to the 26% increase in TPC.

An experiment was conducted to test the stability of freeze-dried leaves of *E. elatior*. Leaves were stored in sealed Petri dishes kept in the laboratory for a week at ambient temperature of 25–30 °C and relative humidity of 33%. After storage, control leaves showed a loss of 23%, 15%, and 21% in TPC, AEAC, and FRP while freeze-dried leaves showed minimal declines of only 7%, 2%, and 5%, respectively (Table 3). It should be noted that the stored freeze-dried leaves with TPC, AEAC, and FRP values of 2850 mg GAE/100 g, 4210 mg AA/100 g, and 18 mg GAE/g remained significantly higher than those of fresh control leaves with values of 2420 mg GAE/100 g, 2960 mg AA/100 g, and 14 mg GAE/g, respectively. In terms of FIC ability, control and freeze-dried leaves remained unchanged after storage for a week.

Unlike leaves of *A. zerumbet* and *E. elatior*, which showed significant gains in TPC, AEAC, and FRP, freeze-drying led to slight declines of 11%, 9%, and 14% for leaves of *C. longa*; and 16%, 10%, and 14% for leaves of *K. galanga*; respectively (Table 2). Declines in AEAC for *C. longa* and in AEAC and FRP for *K. galanga* were, however, insignificant. FIC values of freeze-dried leaves of *C. longa* were comparable to those of fresh leaves. Freeze-dried leaves of *K. galanga* showed slight decline in FIC values.

Freeze-drying of leaves of *A. zerumbet* and *E. elatior* resulted in significantly increased TPC, AEAC, and FRP but not FIC ability. There is no thermal degradation in freeze-drying and neither does the process allow degradative enzymes to function. Furthermore, freeze-drying is known to have high extraction efficiency because ice crystals formed within the plant matrix can rupture cell structure, which allows exit of cellular components and access of solvent, and consequently better extraction (Asami, Hong, Barrett, & Mitchell, 2003). The HPLC chromatogram of leaves of *E. elatior*, which showed greater amounts of minor compounds following freeze-drying, supported this inference.

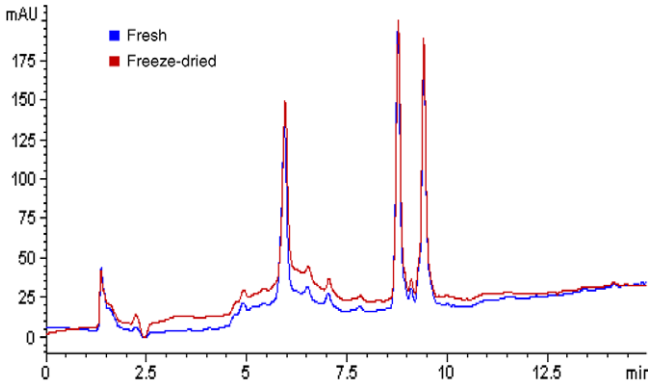

Fig. 1. Overlay of chromatograms (254 nm) showing greater amounts of minor compounds in freeze-dried than fresh leaves of *E. elatior*.

Table 3
The effect of one week of storage on total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), and ferric-reducing power (FRP) of control and freeze-dried leaves of *E. elatior* (fresh weight)[a]

Drying method	Storage (day)	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)	FRP (mg GAE/g)
Control	0	2420 ± 210a	2960 ± 362a	14 ± 0.7a
	7	1860 ± 180b	2510 ± 157a	11 ± 1.0b
Freeze-dried	0	3050 ± 226c	4280 ± 55b	19 ± 1.3c
	7	2850 ± 124c	4210 ± 227b	18 ± 1.7c

[a] Values of TPC, AEAC, and FRP of leaves are means ± SD (n = 3). For each column, values followed by the same letter (a–c) are not statistically different at $P < 0.05$ as measured by the Tukey HSD test.

Freeze-drying resulted in slight but significant declines in TPC and FRP for *C. longa* and in TPC for *K. galanga*. Freeze-dried leaves of *A. zerumbet* and *E. elatior* were thick, powdery, and easy to extract while those of *C. longa* and *K. galanga* were thin, papery, and difficult to extract. Variation in the ease of extractability due to modification of the matrix could explain why freeze-drying has different effects on these two groups of species.

Results of this study showed that freeze-drying had three major effects on the AOP of ginger leaves. Firstly, freeze-dried leaves of *C. longa* and *K. galanga* had the least decline in AOP compared with leaves dried using other drying methods (microwave-, oven-, sun-, and air-drying). Secondly, leaves of *A. zerumbet* and *E. elatior* showed enhancement in AOP following freeze-drying. Thirdly, freeze-dried leaves of *E. elatior* remained stable following one week of storage under sealed conditions and room temperature.

The first effect on the retention of AOP after freeze-drying has often been reported. Freeze-dried yam flours displayed the highest AOA compared to hot air- and drum-dried flours (Hsu, Chen, Weng, & Tseng, 2003). Freeze-dried marionberry, strawberry, and corn yielded higher TPC than air-dried samples (Asami et al., 2003). Freeze-dried water hyacinth leaves had higher AOA than sun- and oven-dried leaves (Bodo, Azzouz, & Hausler, 2004). Higher TPC and AOA have been reported in freeze-dried than hot air-dried daylily flowers (Mao, Pan, Que, & Fang, 2006).

The second effect on AOP enhancement and the third effect on the stability of AOP after freeze-drying have seldom been reported. Total AOA was 50% higher in freeze-dried asparagus (Nindo, Sun, Wang, Tang, & Powers, 2003). Chang et al. (2006) reported gains in FIC ability, but TPC, FRP, and DPPH radical–scavenging activity remained unchanged for freeze-dried tomatoes. Storage of freeze-dried extracts of potato peel waste for 15 days showed no degradation in phenolics and AOA (Rodríguez de Sotillo, Hadley, & Holm, 1994). The present study is probably the first to demonstrate AOP enhancement and stability following freeze-drying of ginger leaves.

3.5. Teas of *A. zerumbet*

Commercial and freeze-dried teas of *A. zerumbet* were extracted with hot water and methanol. Hot-water infusion of the freeze-dried tea was light yellow in colour and produced a mild aromatic fragrance. The commercial tea infusion was more aromatic and faint yellow in colour.

Table 4
Total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), and ferric-reducing power (FRP) of methanolic and hot-water extracts of freeze-dried and commercial teas of *A. zerumbet* (dry weight)[a]

A. zerumbet tea	Extraction	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)	FRP (mg GAE/g)
Freeze-dried	Methanol	6440 ± 154a	6410 ± 111a	31 ± 0.6a
	Hot-water	3970 ± 202b	2050 ± 574b	19 ± 2.7b
Commercial	Methanol	275 ± 54c	143 ± 43c	1.1 ± 0.3c
	Hot-water	649 ± 30d	430 ± 38d	3.1 ± 0.3d

[a] Values of TPC, AEAC, and FRP of teas are means ± SD ($n = 3$). For each column, values followed by the same letter (a–d) are not statistically different at $P < 0.05$ as measured by the Tukey HSD test.

For the commercial tea of *A. zerumbet*, TPC, AEAC, and FRP of hot-water extracts were 649 mg GAE/100 g, 430 mg AA/100 g, and 3.1 mg GAE/g, respectively (Table 4). Values of methanol extracts were 275 mg GAE/100 g, 143 mg AA/100 g, and 1.1 mg GAE/g, respectively. Hot-water extraction was more efficient than methanol extraction. For the freeze-dried tea, TPC, AEAC, and FRP of methanol extracts were 6440 mg GAE/100 g, 6410 mg AA/100 g, and 31 mg GAE/g while those of hot-water extraction were 3970 mg GAE/100 g, 2050 mg AA/100 g, and 19 mg GAE/g, respectively. Methanol extraction was more efficient than hot-water extraction.

Using the same solvent, the freeze-dried tea had stronger metal ion-chelating ability than that of the commercial tea (Fig. 2a). In terms of LPI, methanol extracts of the freeze-dried tea had the strongest activity followed by hot-water extracts of the commercial tea and hot water extracts of the freeze-dried tea, and by methanol extracts of the commercial tea (Fig. 2b). Values of both hot-water and methanol extracts of the freeze-dried tea were higher than those of the commercial tea in methanol.

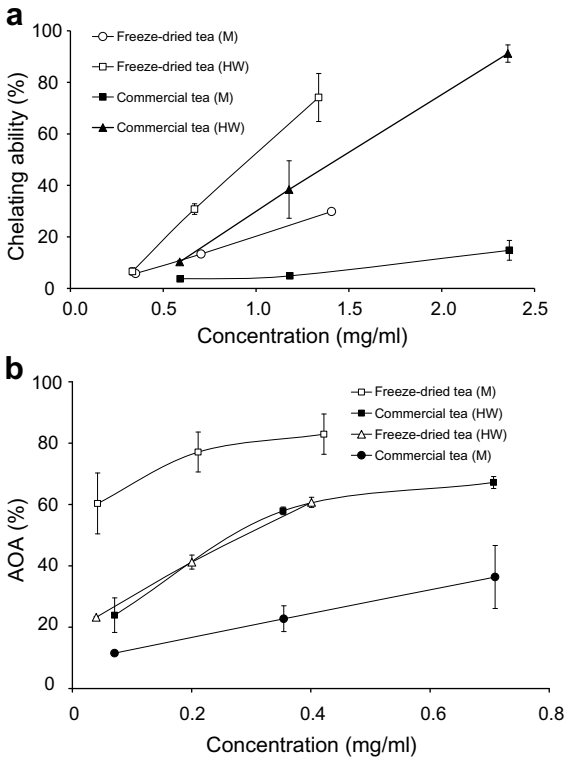

Fig. 2. Ferrous ion-chelating (FIC) ability (a) and lipid peroxidation inhibition (LPI) activity (b) of methanolic (M) and hot-water (HW) extracts of freeze-dried and commercial teas of *A. zerumbet* (dry weight).

Variability in AOP in the commercial and freeze-dried teas of *A. zerumbet* may be due to number of factors. They include drying methods, type of extraction solvents, and antioxidant assays used. The significantly lower TPC and AOA of the commercial tea could be due to the use of conventional drying methods where heat is applied during the manufacturing process. Consequently, much of the antioxidant compounds are lost through enzymatic degradation and/or heat decomposition. On the contrary, much of the AOP are retained in the freeze-dried tea as freeze-drying is non-thermal. Freeze-drying remains the best method of drying foods as the quality of freeze-dried products is comparable to fresh products (Ratti, 2001).

4. Conclusion

Results showed that freeze-drying is superior to other drying methods in preserving the AOP of ginger leaves. Thermal drying (microwave-, oven-, and sun-drying) resulted in significant declines in TPC, AEAC, and FRP with minimal effects on FIC ability and LPI activity. Microwave-drying of *E. elatior* leaves resulted in significant losses in TPC and AOA, but the declines were comparable between different drying durations. Of the two methods of non-thermal drying, air-dried leaves showed drastic losses in values for all species. Freeze-dried leaves had significant gains in TPC, AEAC, and FRP for *A. zerumbet* and *E. elatior*, but losses for *C. longa*, and *K. galanga*. Freeze-drying had minimal effect on the FIC ability of leaves of all four species. HPLC analysis showed the presence of greater amounts of minor compounds in freeze-dried than fresh *E. elatior* leaves. Values of freeze-dried leaves of *E. elatior*, after one week of storage, remained significantly higher than those of fresh control leaves. The freeze-dried tea of *A. zerumbet* was superior to the commercial tea for all AOP studied. Freeze-drying appears to be a sound method for producing tea and other herbal products from ginger species. Due to its high operation cost, freeze-drying can be applied to produce high-value speciality tea or spice powder from ginger leaves.

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