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The effects of type and time of thermal processing on ginger (*Zingiber officinale* Roscoe) rhizome antioxidant compounds and its quality

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Abstract: The type of thermal processing either roasting or boiling gave an effect on the antioxidant quality of ginger (*Zingiber officinale Ross*) rhizome extracts.It was found that 6 minutes of either roasting or boiling gave the best effect of its antioxidant activity. This condition was shown by the free radical activity of extract of roasted samples: 84.21 ± 0.18% and 83.59 ± 0.52% for the boiled one. The total phenolic content of roasted samples was 29.94 ± 0.15 mg/g and for boiled samples was 30.87 ± 0.26%, whilst its gingerol content of roasted samples was 19.84 ± 0.19 mg/g and 20.78 ± 0.14 mg/g for boiled samples. The results of GC-MS analysis showed that methyl ester (0,17%), 9-octadecenoic (0.32%), nortrachelogenin (0.30%) were the compounds of antioxidant agent. It is interesting to note that zingerone compound total area increased from 5.68% to 6.32%. The FTIR analysis showed that C-O-C antisym strecht vibration at 1270.04 cm^{-1} wave was found for methyl ester compound and OH stretch H-bonded at 2870.84 and 2982.71 cm^{-1} wave were found for 9-octadecenoic compound.

Keywords: Ginger rhizome (*Zingiber officinale* Roscoe), antioxidant compounds, antioxidant quality

Introduction

Ginger (*Zingiber officinale* Roscoe) rhizome has become a very popular spice and used widely in Indonesian cuisine as well as in other countries. It is a common food additive in a number of foods and beverages and it is valued due to the volatile components especially the aromatic compounds which give a spicy, pungent and pleasant smell. Barley and Jacobs (2000) noted that these aroma compounds only partially contribute to the flavor of fresh ginger rhizome and the oleoresin content plays an important role for its pungency.

Beside fresh ginger rhizome, it is quite common to find dried sliced ginger rhizome or in its powdered form. Menon *et al*. (2007) had studied the effect of processing on the flavor compounds of Indian fresh ginger (*Zingiber officinale* Roscoe). They found that gerental (24.2%) and zingerone (14.2%) were the major components in the original aroma of fresh ginger and during processing it will be decreased. Furthermore they also reported that the hydrocarbon content of the oil increased and the oxygenated compounds decreased in the dry and oil products.

Jaya (2008) reported that in Indonesian beverages the ginger rhizome was boiled at temperature of around 100ºC for about 20 – 30 minutes and it is aimed to get a better ginger taste and less bitter taste on the traditional ginger drink. Puengphian and Sirichote (2007) studied the effect of drying ginger rhizome on its (6)-gingerol content and found that as drying time increased the amount of this compound decreased. While the study of Barley and Jacobs (2000) reported that the drying process also decreased the amount of the gingerol content of their samples, but they found an increase in terpene hydrocarbon and conversion of some monoterpene alcohols to their correponding acetates. Vankar *et al*. (2006) studied the stability of ginger components after heat treatment at 120º C and reported that the antioxidant activity of powdered form were stable.

In ginger rhizome extract, (6)-gingerol is the most abundant component found in fresh samples and the amount will decreasing during postharvest storage and processing especially thermal processing (Zang *et al.*, 1994; He *et al*., 1998). As noted by Menon *et al*. (

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2007) there are many reports available on the chemical composition of ginger as well as the composition of ginger oil which had been studied quite extensively. However there is very little information on the effect of thermal processing (roasting and boiling) on the antioxidant quality of Indonesian elephant ginger rhizome extract. The objectives of current study were to find out the effect of different type and time of thermal processing on local variety of ginger rhizome extract's antioxidant quality.

Materials and Methods

Sample preparation

The fresh samples of elephant ginger rhizome (Zingiber officiale Roscoe) of 10 months old were obtained from a ginger farmer in Tawang Argo village, Karang Ploso distric, Malang regency. After harvesting the ginger rhizomes were washed in running tap water and the skin were peeled manually, then it was weighed. For fresh samples the already peeled rhizomes were then sliced thinly 7-8 mm and blended at a speed of 1800 rpm for 15-20 seconds before the juice were squeezed out from the fine blended fresh ginger rhizomes.

The already peeled fresh ginger rhizomes were then roasted directly under the charcoal fire (temperature ±320^{o} C) for 2, 4, 6, 8 and 10 minutes with the distance from the fire sources about 1 cm. The roasted rhizome was sliced thinly around 7–8 mm and filled in the blender glass jar and blended with the speed of 1800 rpm until it was finely blended (around 15 – 20 seconds) and squeeze the juice out from the fine blended treated rhizome. A similar process was carried out for the boiling extract samples e.g. the fresh ginger rhizome (100 g) were boiled in 1 L tap water for 2, 4, 6, 8 and 10 minutes at ±100^{o} C, sliced thinly about 7-8 mm before blended in a blender with a speed of 1800 rpm for 15–20 seconds, then squeezed the juice out from the fine blended rhizome.

Determination of antioxidant activity

The fresh and treated ginger rhizome extracts were analyzed for its antioxidant activity using DPPH (1,1 diphenyl-2-picrylhydrazyl) radical scavenging assay as described by Khalaf *et al*. (2007), total phenol using the method of Miliauskas (2000) and the gingerol content following the method as described by Puengphian and Sirichote (2007). Whilst the best samples from the thermal treatments were analyzed for its antioxidant compounds using GC-MS and FTIR, as noted by Jaya (2008).

GC-MS and FTIR

The antioxidant compounds were analyzed using GC-MS QP2010S-Shimadzu under the following condition : column used were Rtx-5MS,30 m length and inner diameter of 0.25mm and the initial column temperature was 70^{o}C and final temperature was 280^{o}C (5^{o} C/minute), while the injector temperature was 300^{o} C with split mode injector and split ratio of 72.6 and pressure of 14.0 kPa. The flow rate was 40 ml/minute and the flow within the column was 0.50 ml/minute. The detector temperature was 300^{o} C and using Helium as the gas carrier with EI (Electron Impact); and the samples volume injected was 1µl. Compounds were identified by comparing retention indices/comparing mass spectra of each compound with those of authentic samples and library. While for functional unit determination the Shimadzu Fourier Transform Infrared Spectrophotometer - FTIR-8400S were used.

The IR spectra were recorded on FTIR-8400S (Shimadzu Deutchland GmbH) spectrophotometer in KBr and polyethylene pellets. Samples were weigh-in at 0.01 g and homogenized with 0.01 g KBr anhydrous by mortar agate. The mixture of sample and KBr were pressed by vacuum hydrolic (Graseby Specac) at 1.2 psi to obtained transparancy pellet. Scanned sample passed through infra red, where its continuing wave by detector that connected to computer and given described of tested sample spectrum. Samples were usually scanned in the absorption area of 600-4000 cm^{-1}. The results of analysis consisted of chemical structure, molecular binding form and certain functional group of tested sample as basic of spectrum type.

Statistical analysis

All statistical analyses were carried out using Microsoft Excel 2003. Analysis of variance (ANOVA) followed by Duncan Multiple Range Test at a level of $P<0.01$ if there was significant differences between samples. The best treatment was determined by effectivity index method as described by Susrini (2005). Identification and in order to elucidate its structure of antioxidant compounds in fresh and the best treated ginger rhizome extracts were accomplished by gas chromatography-mass spectrometry (GC-MS) and Fourier Transform Infrared Spectrophotometer (FTIR), respectively, with computerized integrated data processing and descriptively disscussed based on literature.

Results and Discussions

Fresh elephant ginger rhizome (Zingiber officiale Roscoe)

The antioxidant activity of fresh ginger rhizome extracts determined using DPPH assay (%), total phenol content (mg/g) and gingerol content (mg/g) are shown on Table 1. The antioxidant activity of fresh elephant ginger rhizome was 79.19 % and this result is in the range of antioxidant of ginger i.e 60 – 90% as reported by Kruawan and Kangsasalampai (2006). Whilst Kaur and Kapoor (2002) noted that the Indian elephant ginger rhizome had an antioxidant activity of 65% and they claimed that this herbal plant can be included into group of plants which has higher antioxidant activity.

Total phenol content of fresh ginger rhizome was found in the amount of 23.87 (mg/g), which is similar to the one reported by Puangphian and Sirichote (2007), who found the total phenol content of their samples was 24.63 ± 0.43 mg/g. Hinnenburg, Damien and Hiltman (2006) also noted a total phenol of 23.50 ± 1.26 mg/g in their samples, where the ginger rhizomes were extracted using hydro distillation method.

In regards of gingerol content, in this study, it was found that fresh elephant ginger rhizome of Indonesia contain 15.96 (mg/g). However, this result was less than the one reported by Puengphian and Siricothe (2007), where they found the gingerol content in their sample was 21.15 ± 0.13 mg/g. This difference figures might possibly due to different genetics, varieties, plantation techniques and its environments.

Identification antioxidant compounds of fresh elephant ginger rhizome (Zingiber officinale Roscoe)

A typical gas chromatogram of fresh elephant ginger rhizome is shown on Figure 1 and a list of the compounds identified appears on Table 2. While the infra red spectrum of fresh elephant ginger extract and its functional group analyzed using Fourier Transform Infra Red (FTIR) are shown on Figure 2 and Table 3. Thirty compounds of fresh elephant ginger extracts were identified using GC-MS and FTIR shown in Table 2 and after grouping those compounds, it could be grouped onto eight big groups namely terpene 77.75%; hydroxyl 6.56%; aldehyde 2.92%; alkene 0.40%; keton 0.31%; carboxylate acids 0.25%, alkyl 0.22% and ester 0.05%. The major compound identified was zingiberene (33.50%), followed by ß sesquiphellandrene (12.25%), ά-farnesene (7.82%), E-Citral (6.98%), zingerone (5.68%), ar-curcumen (4.91%), ß-bisabolene (4.41%), hexanal (2.55%), decanal (2.08%), (Z) ß-farnesene (1.48%), camphene (1.53%) and citral (1.07%). Sota *et al.* (2006) reported that the major compounds of ginger isolated by solvent extraction and analyzed by GC-MS were geranial zingiberene (35.3%), beta-sesquiphellandrane (19.7%), beta-bisabolene (6.7%), cis,trans-alpha-famesene dan geranial (5.2%) beta-bisabolene (8.6%), zingerone (7.7%) and geraniol (7,1%).

The principal constituent of this fresh elephant ginger was zingiberene, a kind of sesquiterpenes hydrocarbon and it had a woody-spicy and very tenacious. However, its content was higher than Chinese and Guinean ginger, that is, 31.1 and 19.89%, respectively as reported by Toure and Xiaoming (2007). While Iuresca *et al*. (1999) suggested that terpenes are important flavor and fragrance compounds widely distributed in nature. In particular, attention has been focused on the production of oxygenated derivatives of terpenes, commonly called terpenoids, which have a stronger odor.

The infra red spectrum of fresh elephant ginger rhizome extract as shown in Figure 2 was in the wave length range of 606.57 cm^{-1} to 3436.91 cm^{-1}, and there was 14 functional compounds found but only 13 compounds were identified as peak no 10 was not identified (Table 3). This unidentified peak was probably due to the relatively low energy vibration which was not identified by the infra red spectrophotometer.

The IR spectrum of the compound had been studied in order to elucidate its structure. In the spectrum, the presence of broad bands at 3406.05 and 3436.91 cm^{-1} can be attributed to (OH) stretching vibrations. The presence of bands with strong to medium intensities were also observed at 2983.67 and 2870.84 cm^{-1} which was confirmed as carboxylic acid group. Other strong to medium intensity bands were also observed at 1642.27 cm^{-1} due to the presence of alkena venyl group and at 1139.85 cm^{-1} due to the presence of the ether group. Some other bands appeared at 1514.98 and 1449.41 cm^{-1}; 1380.94 cm^{-1}; 1034.74 cm^{-1}; and 606.57 cm^{-1} which might be due to presence of C=C aromatic, alkena, alchohol primer and phenol group respectively.

In general, free radical scavenging and antioxidant activity of phenolics (e.g. flavonoids, phenolic acids) mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules, and is also affected by other factors, such as glycosylation of aglycones, other H-donating groups (-NH, -SH), etc (Cai *et al*., 2004).

Effect of different type and time of thermal processing on elephant ginger rhizome extract

Table 1. The average of DPPH assay, total phenol and gingerol content of fresh rhizome extracts

Parameters	Amount
DPPH (%)	79.19 ± 0.58
Total phenol (mg/g)	23.87 ± 0.51
Gingerol content (mg/g)	15.96 ± 0.32

Table 2. Identified compounds of fresh elephant ginger rhizome extract.

Peak	Fresh ginger rhizoma extract				Ginger rhizoma extract boiled at 6 minutes			
	Compounds	Molecule Structure	Retention time (min)	Amount (%)	Compounds	Molecule Structure	Retention time (min)	Amount (%)
1	Hexanal[a]	$C_6H_{12}O$	3.649	2.55	Hexanal[a]	$C_6H_{12}O$	3.641	2.43
2	Alpha-Pinene[b]	$C_{10}H_{16}$	6.142	0.34	Alpha-Pinene[b]	$C_{10}H_{16}$	6.131	0.69
3	Camphene[b]	$C_{10}H_{16}$	6.566	1.53	Camphene[b]	$C_{10}H_{16}$	6.558	3.39
4	Octanal[a]	$C_8H_{16}O$	8.157	0.84	Beta-myrcene[b]	$C_{10}H_{16}$	7.707	0.39
5	Beta-Phellandrene[b]	$C_{10}H_{16}$	8.994	0.89	Octanal[a]	$C_8H_{16}O$	8.152	0.78
6	1-Hexadecanol[a]	$C_{16}H_{34}O$	9.058	0.25	Beta-Phellandrene[b]	$C_{10}H_{16}$	8.991	1.99
7	Borneol[a]	$C_{10}H_{18}O$	3.628	0.53	Linalool[a]	$C_{10}H_{18}O$	11.336	0.20
8	Decanal[a]	$C_{10}H_{20}O$	14.810	2.08	Borneol[a]	$C_{10}H_{18}O$	13.633	0.57
9	Citral[a]	$C_{10}H_{16}O$	16.008	1.07	Decanal[a]	$C_{10}H_{20}O$	14.816	1.92
10	E-Citral[a]	$C_{10}H_{16}O$	16.950	6.98	Citral[a]	$C_{10}H_{16}O$	16.018	2.77
11	Isobornyl Acetate[a]	$C_{12}H_{20}O_2$	17.325	0.05	E-Citral[a]	$C_{10}H_{16}O$	16.957	6.04
12	2-Undecanone[a]	$C_{11}H_{14}O$	17.547	0.31	Isobornyl Acetate[a]	$C_{12}H_{20}O_2$	17.335	0.20
13	Propiconazole[a]	$C_{15}H_{17}C_{l2}N_3O_2$	20.133	0.37	2-Undecanone[a]	$C_{11}H_{14}O$	17.555	0.36
14	Beta-elemene[b]	$C_{15}H_{24}$	20.427	0.40	Geranyl Acetate[a]	$C_{12}H_{20}O_2$	20.155	0.33
15	Isocaryophyllen[b]	$C_{15}H_{24}$	21.585	0.14	beta-elemene[b]	$C_{15}H_{24}$	20.437	0.30
16	(Z) Beta-farnesene[b]	$C_{15}H_{24}$	22.116	1.48	Zingiberene[b]	$C_{15}H_{24}$	20.743	0.12
17	ar-curcumen[b]	$C_{15}H_{24}$	22.913	4.91	Alpha-farnesene[b]	$C_{15}H_{24}$	21.603	0.18
18	Zingiberene[b]	$C_{15}H_{24}$	23.297	33.50	(Z) Beta-farnesene[b]	$C_{15}H_{24}$	22.124	0.50
19	Alpha-farnesene[b]	$C_{15}H_{24}$	23.518	7.82	Isocaryophyllen[b]	$C_{15}H_{24}$	22.792	0.03
20	Beta-bisabolene[b]	$C_{15}H_{24}$	23.593	4.41	ar-curcumen[b]	$C_{15}H_{24}$	22.929	5.04
21	Beta-sesquiphellandrene[b]	$C_{15}H_{24}$	24.027	12.25	Zingiberene[b]	$C_{15}H_{24}$	23.298	27.63
22	Artemesia Triene[a]	$C_{10}H_{16}$	24.208	0.22	Alpha-farnesene[b]	$C_{15}H_{24}$	23.525	6.63
23	Elemol[a]	$C_{15}H_{26}O$	24.755	0.51	Beta-bisabolene[b]	$C_{15}H_{24}$	23.599	4.12
24	Nerolidol Z[a]	$C_{15}H_{26}O$	25.719	0.24	Epi-bicyclosesquiphellandrene[b]	$C_{15}H_{24}$	23.833	0.12
25	d-nerolidol[a]	$C_{15}H_{26}O$	26.296	0.62	Beta-sesquiphellandrene[b]	$C_{15}H_{24}$	24.035	10.47
26	Dehydrolinalool[a]	$C_{10}H_{16}O$	26.722	0.26	Carene[b]	$C_{10}H_{16}$	24.194	0.19
27	Zingerone[a]	$C_{11}H_{14}O_3$	27.409	5.68	Elemol[a]	$C_{15}H_{26}O$	24.773	0.30
28	Thujyl Alcohol[a]	$C_{10}H_{18}O$	27.675	0.88	Nerolidol[a]	$C_{15}H_{26}O$	24.992	0.11
29	Trans-Carveol[a]	$C_{10}H_{16}O$	28.124	0.40	Nerolidol[a]	$C_{15}H_{26}O$	26.313	0.38
30	1-Hexadecene[b]	$C_{16}H_{32}$	30.167	0.40	Zingerone[a]	$C_{11}H_{14}O_3$	27.433	5.12

Table 2. Identified compounds of fresh elephant ginger rhizome extract. (cont.)

31		Thujyl Alcohol[a]	$C_{10}H_{18}O$	27.676	0.90
32		Cis-Carveol[a]	$C_{10}H_{16}O$	28.136	0.37
33		Citral[a]	$C_{10}H_{16}O$	29.348	0.03
34		Octadecene[a]	$C_{18}H_{36}O$	30.183	0.23
35		Nerolidol[a]	$C_{15}H_{26}O$	30.975	0.27
36		Methyl ester[a]	$C_{14}H_{28}O_2$	33.117	0.17
37		2-Undecanethiol, 2-methyl-[c]	$C_{12}H_{26}S$	34.384	0.11
38		9-Octadecenoic[a] Acid[a]	$C_{19}H_{36}O_2$	36.602	0.32
39		(-)-Nortrachelogenin[a]	$C_{20}H_{22}O_7$	39.221	0.30
40		Decane, 5,6-bis (2,2-dimethylpropylidene)-, (E,Z)-[b]	$C_{20}H_{38}$	39.408	0.18
41		3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran[a]	$C_{10}H_{16}O$	40.473	4.46
42		Geranyl butyrate[a]	$C_{14}H_{24}O_2$	41.036	0.21
43		4-Ethylguaiacol[a]	$C_9H_{12}O_2$	41.183	1.07
44		Zingerone[a]	$C_{11}H_{14}O_3$	43.280	0.27
45		3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran[a]	$C_{10}H_{16}O$	43.959	1.58
46				44.188	1.63
47		Surfynol 104[a]	$C_{14}H_{26}O_2$	44.514	0.57
48		Edulan II[a]	$C_{13}H_{20}O$	45.592	0.89
49		3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran[a]	$C_{10}H_{16}O$	47.238	2.20
50		Zingerone[a]	$C_{11}H_{14}O_3$	47.950	0.93
Total area	91.91				99.99

[a] oxygenated compounds
[b] hydrocarbon compounds
[c] sulfurated compounds

Table 3. Functional compounds of fresh elephant ginger rhizome analyzed by using FTIR

No.	Wave length (cm^{-1})	Vibration type	Functional compound
1	606.57	O-H bond	Phenol
2	1034.74	C-OH stretch	Alcohol primer ($-CH_2OH$)
3	1077.17	C-O-C strech alkyl-aryl ether	Ether (R-O-R)
4	1139.85	C-O-C strech dialkyl ether	Ether (R-O-R)
5	1270.04	C-O-C strech venyl ether	Ether (R-O-R)
6	1380.94	CH_3 bond sym	Alkena, methyl $-CH_3-$
7	1449.41	Ring aromatic strech (4p)	C=C aromatic
8	1514.98	Ring aromatic strech (4p)	C=C aromatic
9	1642.27	C=C stretch	Alkena venyl ($-CH_2=CH_2$)
10	2093.59	--	--
11	2870.84	OH strech; H-bonded	Carboxylic acid (RCOOH)
12	2983.67	OH strech; H-bonded	Carboxylic acid (RCOOH)
13	3406.05	OH strech; H-bonded	OH
14	3436.91	OH strech; H-bonded	OH

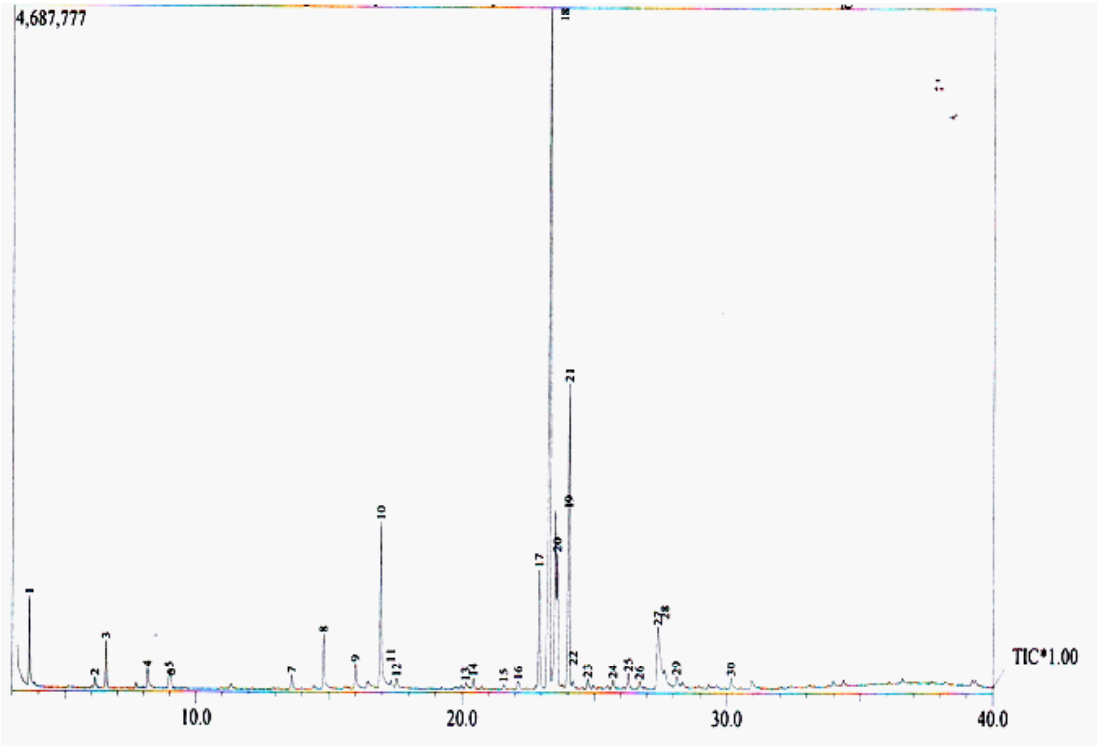

Figure 1. GC-MS chromatogram of fresh elephant ginger rhizome extract

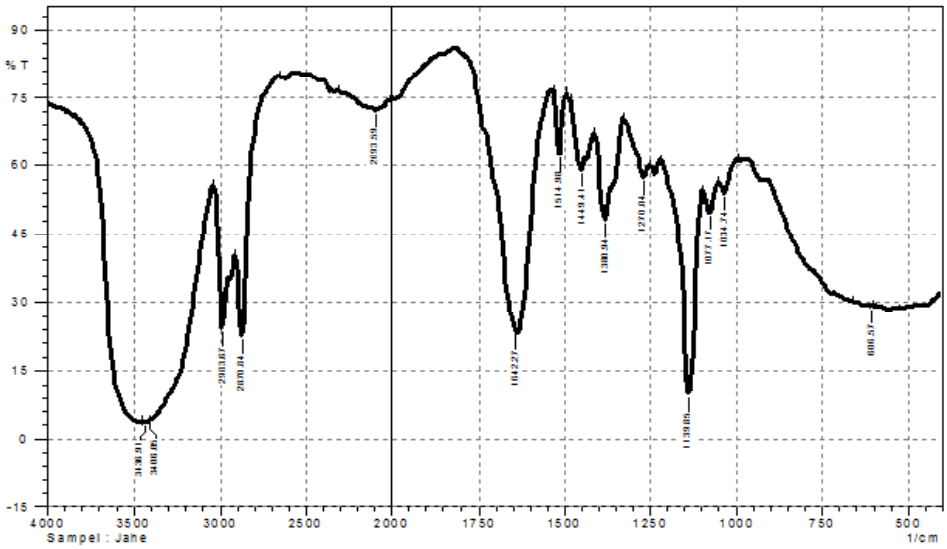

Figure 2. Infra red spectrum of fresh elephant ginger rhizome extract

The antioxidant activities of thermal processing on elephant ginger rhizome extracts determined using DPPH assay (%), total phenol content (mg/g) and gingerol content (mg/g) are shown on Table 4.

The range of free radical-scavenging activity of thermal processing on elephant ginger rhizome extracts by DPPH assay was 63.78 - 84.21%. As mention above in Table 4, several trends are occured. An increase in radical-scavenging activity was found in thermal processed elephant ginger rhizome extracts at 2 to 6 minutes. After heating, the solubilities of the active components probably increased because of decomposition of the cell wall and by passing of the solvent into the cell. Therefore, Shobana and Naidu (2000) suggested that the bound antioxidants might be released due to heat treatment, resulting in the higher antioxidant activity compared to fresh spices extract.

A significant decrease in radical-scavenging activity of thermal processing at 8 minutes might be occured as a decomposition of the active compounds, since the levels of these are quite low in the fresh product, it is more likely that cell damage during the heating process results in greater release of these compounds. Khatun *et al*. (2006) noted that coagulation of spices was observed after heating, therefore, the extraction ability might be decreased, resulting in a reduction in the radical-scavenging activities of these spices.

There were also similar trend occured in total phenol content of thermal processed samples. An increase of total phenol content of samples thermal procesed at 2-6 minutes is corresponding to glycosides which are hydrolyzed to form their aglycone. Khatun *et al*. (2006) noted that flavonoid present in living cells as glycosides may be breakdown by enzyme, acid or heat treatment to form their aglycone and sugar. Some aglycones are known to have a more active potential for antioxidant activity than their glycosides. However, it seems that some active components are degraded by heating to form less active components. The pH might also be a factor in reducing the antioxidant activities of spices. These results showed that a high correlation between phenolic content and DPPH, radical-scavenging activity had occured and such correlation were reported by Parejo *et al*. (2004).

In regards of gingerol content, an increase of gingerol content in thermal processing of elephant ginger rhizoma extract at 2 to 6 minutes were detected. As gingerol is part of phenol compound it could be influenced the antioxidant activity. Kaur and Kapoor (2002) suggested that the high antioxidant activity in ginger has been attributed to the active principal of gingerol. While Wohlmuth *et al*. (2005) also noted that the pungency of fresh ginger was due to a series of homologous phenolic ketones of which [6]-gingerol is the major one and could be increased its antioxidant activity.

Whereas, the significant decrease of gingerol content was detected in samples extracted at 8 minutes. The gingerols were thermally unstable and could be converted to their corresponding shogaols, which are present in dried ginger. Wohlmuth *et al*. (2005) reported that molecular structure of gingerol consisted of β-hydroxyl keto functional group which

was thermally labile. The thermal degradation products of [6]-gingerols including shogaols and aliphatic aldehydes possibly occurred during the drying process

The best treatment according to antioxidant activity parameter using effectivity index method (Susrini, 2005) was the elephant ginger rhizoma boiled at 6 minutes. It had the following properties: antioxidant activity as free radical-scavenging activiy was 84.21±0.18%; total phenol was 30±0.26 mg/g; and gingerol content was 20.78±0.14 mg/g.

Identification antioxidant compounds of the best elephant ginger rhizoma extracts sample

Identification antioxidant compounds by GC-MS

A typical gas chromatogram of extracts of elephant ginger rhizoma boiled at 6 minutes is shown in Figure 3 and a list of fifty compounds identified appears in Table 2. The major compound identified was zingiberene (27.63%), followed by ß-sesquiphellandrene (10.47%), ά-farnesene (7.31%), E-Citral (6.04%), zingerone (6.32) and ß-bisabolene (4.12%). Identification of elephant ginger rhizoma extratcs boiled at 6 minutes could be classes as 3 compounds, which are volatile compounds, non-volatile compounds and a newly compounds.

Volatile compounds

Total area of hydrocarbon groups of extracts of elephant ginger rhizoma boiled at 6 minutes had been decreased from 68.07% (fresh ginger rhizoma extract) to 61.97%. It indicates that total peak area of volatile compound which responsible to flavour the extracts of elephant ginger rhizoma boiled at 6 minutes had been decreased. Despite of this evident, the major of hydrocarbon groups that influnced the flavour such as α-pinene, camphene, β-phellandrene and ar-curcumene had been increased compared to the fresh ginger as shown in Figure 4. Bartley and Jacobs (2000) suggested that hydrocarbon compound has a significant influence on the flavour of the products and will give rise to shifts in flavour response during drying process. The major components in ginger essential oil are zingiberene, ar-curcumene, -farsene, -bisabolene, sesquiphellandrene, α-pinene, camphene and β-phellandrene. Some of these compounds could arise from dehydration of oxygenated compounds.

Non-volatile compounds

Non-volatile compounds of ginger rhizoma extract boiled at 6 minutes had been altered become derivative gingerol, such as zingerone. Bartley and Jacobs (2000) reported that approximately 50% of gingerol is converted by elimination of water, while the rest of it reconstituted as equimolar amounts of zingerone and hexanal. Jolad et al. (2005) also reported that commercially processed ginger dry of white ginger from chinese and yellow ginger varieties from Japan were detected of seven gingerol derivative compounds, as the following: 4-(4-hydroxyphenyl)-2-butanone, 4-hydroxy-3-methoxybenzenepropanal, 3,4-dimethoxybenzenepropanal, zingerone, methyl ether, gingerol and zingerol 2-methyl ether.

The amount of zingerone on ginger rhizoma boiled at 6 minutes had been increased of total peak areas from 5.68% on fresh to 6.32% as well as the free radical-scavenging determination using DPPH (79.19% on fresh to 84.21%). Tejasari (2007) reported that ginger's non volatile bioactive compounds could provide evidence of cellular immune response by increased the ratio of CD4+ CD8+ T-cells at 100 to 200 µg/mL concentrations using in vitro assay. The increase could be due to the increasing of ginger's non volatile bioactive compounds namely zingeron in fraction-3 which was separated by column vacuum chromatography method in enhancing the cellular and humoral immune response. However, Jolad *et al*. (2005) reported that zingeron as secondary products of gingerols decreases from 24.3% to 12.1% on drying at atmospheric pressure in a two-stage drum drier with a capacity of 1.5 tonne (80^{o}C in the first stage and 63^{o}C in the second stage).

Newly Compounds corresponding to antioxidant compounds

Fifty compounds were detected in the extracts of elephant ginger rhizoma boiled at 6 minutes (Table 2). After compared with fresh ginger rhizoma exract, there were 16 products of newly compounds detected which was at the retention time of 30.183 minutes, even β-myrecene, linaool, geranyl acetate and carene were detected at retention time of 7.707; 11.336; 20.155 and 24.194 minutes respectively.

Those compounds, then, were descriptively identified by comparing based on various literature which related to an antioxidant agent. Three compounds were identified as antioxidant compounds, namely: methyl ester, 9-octadecenoic and (-)-nortrachelogenin. Pino *et al*. (2005) noted that methyl ester was grouped in ester compound which had characteristics as volatile compound, less water soluble and completely soluble in alcohol and predominantly in organic solvent. These compounds had double bound and functional carboxyl group, therefore it could be referred as antioxidant agent.

The other compounds, 9-octadecenoic was a

Table 4. The average of DPPH assay, total phenol and gingerol content of thermal processed of elephant ginger rhizoma extracts

Different type of thermal processing	Time of thermal processing (minute)	DPPH assay (%)	Total phenol content (mg/g)	Gingerol content (mg/g)
Roasting (320°C±2°C)	2	81.80 ± 0.09 f	27.72 ± 0.26 e	17.95 ± 0.38 d
	4	82.97 ± 0.09 g	29.73 ± 0.27 g	19.43 ± 0.44 e
	6	83.59 ± 0.32 gh	29.94 ± 0.15 g	19.84 ± 0.19 e
	8	74.20 ± 0.43 d	19.89 ± 0.39 c	16.41 ± 0.17 c
	10	67.29 ± 0.55 b	9.82 ± 0.39 a	8.05 ± 0.11 a
Boiling (100°C±1 °C)	2	80.77 ± 0.15 e	26.54 ± 0.44 d	16.85 ± 0.41 c
	4	81.85 ± 0.16 f	28.42 ± 0.17 f	18.58 ± 0.45 d
	6	84.21 ± 0.18 gh	30.87 ± 0.26 h	20.78 ± 0.14 f
	8	73.08 ± 0.52 c	19.03 ± 0.09 b	13.81 ± 0.31 b
	10	63.78 ± 0.38 a	9.95 ± 0.28 a	7.87 ± 0.15 a

Means ± standard deviation in the same row with different accompanied letters are significantly different ($P \leq 0.01$)

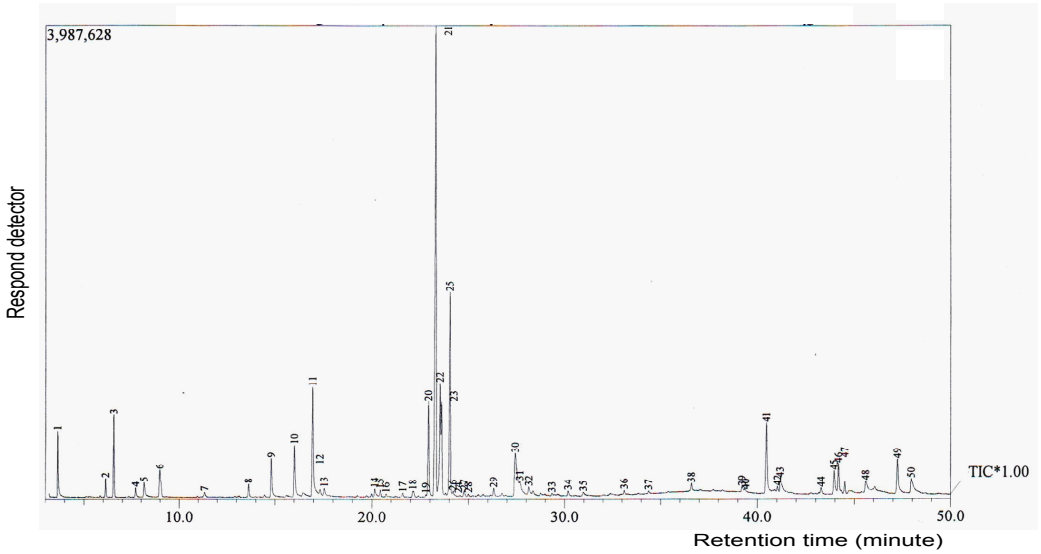

Figure 3. GC-MS chromatogram of extracts of elephant ginger rhizoma boiled at 6 minutes

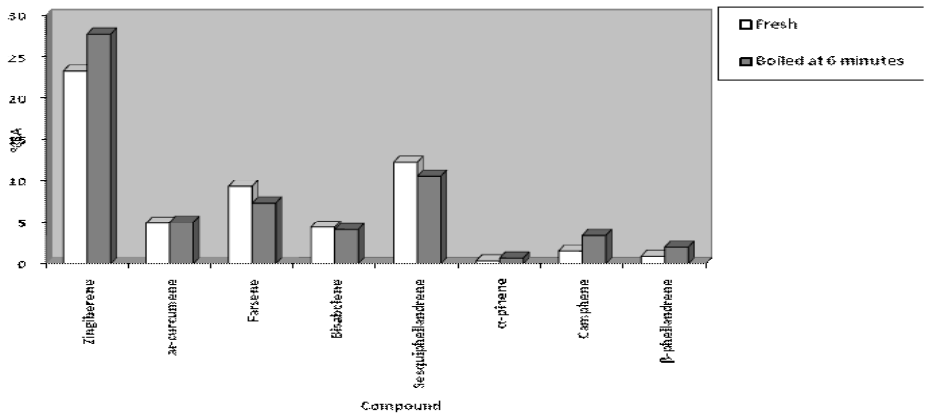

Figure 4. Hydrocarbon compounds contribute to the flavour ginger extracts sampel compared to the fresh ginger extract

Table 5. Functional compounds of elephant ginger rhizome extract boiled at 6 minutes analyzed by using FTIR

No.	Wave length (cm^{-1})	Vibration type	Functional compound
1	624.89	O-H bend	Phenol
2	1035.70	C-OH *stretch*	Alcohol Primer ($-CH_2OH$)
3	1081.03	C-O-C *stretch* alkl-aril eter	Ether (R-O-R)
4	1139.85	C-O-C *stretch* dialkil eter	Ether (R-O-R)
5	1236.29	C-O-C *stretch* vinil eter	Ether (R-O-R)
6	1270.04	C-O-C antisym stretch	Ester (RCOOR)
7	1379.01	CH_3 bend. sym	Alkane Metil $–CH_3$-
8	1451.33	Ring aromatic *stretch* (4p)	C=C Aromatic
9	1515.94	Ring aromatic *stretch* (4p)	C=C Aromatic
10	1643.24	C=C stretch	Alkene Vinil ($-CH_2=CH_2$)
11	2870.84	OH *stretch* H-*bonded*	Carboxilyc Acid (RCOOH)
12	2982.71	OH *stretch* H-*bonded*	Carboxilyc Acid (RCOOH)
13	3431.13	OH *stretch*; H *bonded*	OH

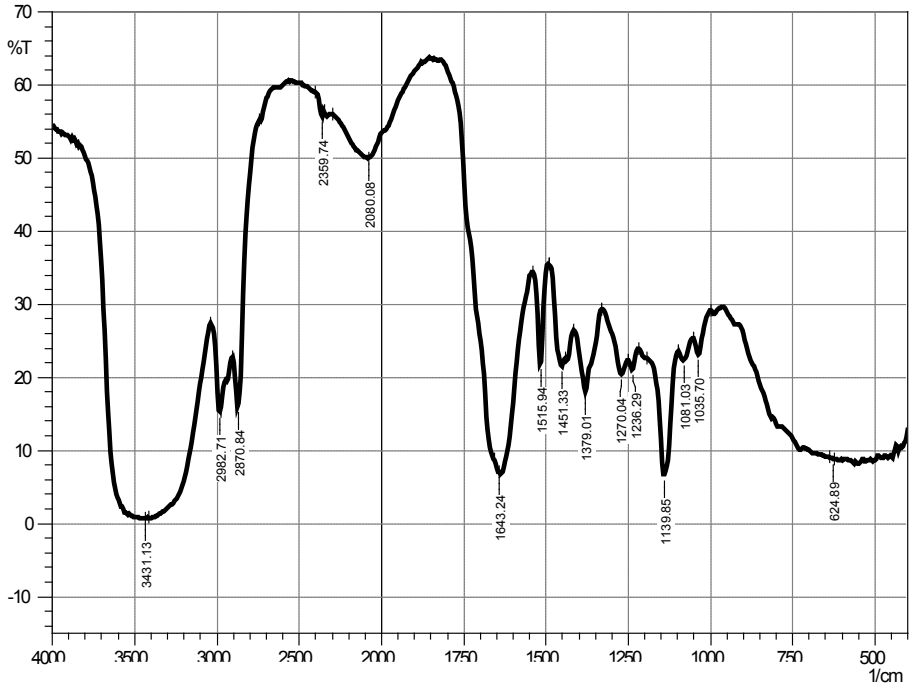

Figure 5. Infra Red Spectrum of elephant ginger rhizome extract boiled at 6 minutes

non-saturated fatty acid group which usually used as solvent corresponded to drugs. This compound could be as a regalutory of LDL receptor on the hamster if its structured incorruptible become cis isomer (Woollett *et al*., 1994). The last compounds, (-)-nortrachelogenin was included in lignin group which is the product of β-β-dimeritation enzimatic natural derivative of substituted phenilpropan. This compound was found in pine as an antioxidant and antitumor.

The noteworthy feature of volatile oil was the presence of geranial acetate, having a ginger-like odor and contribute to the most ginger flavour. This compounds included in oxygenated group, which had been increased from 23.47% on fresh samples to 36.28%. Bartley and Jacobs (2000) reported that thermal processed was sufficient to promote esterification with natural acetic acid (or acetic acid produced by thermal decomposition during drying), such as geranyl acetate which imparting important characteristis of ginger flavour. For example, it had been suggested that geranyl acetate content be used as one of determinants of appraisal to storage and to harvest time of Japanese gingers.

Identification antioxidant compounds by FTIR

The infra red spectrum of elephant ginger rhizome extract boiled at 6 minutes as shown in Figure 5 was in the wave length range of 624.89 cm^{-1} to 3431.13 cm^{-1}, and there was 13 functional compounds found (Table 5). The presence of broad bands at 3431.13 cm^{-1} can be attributed to (OH) stretching vibrations. The presence of strong to medium intensities bands were also observed at 2982.71 and 2870.84 cm^{-1} which confirms of carboxylic acid group. Other strong to medium intensity bands were also observed at 1643.24 cm^{-1} presence of alkena vinil group and 1139.85 cm^{-1} due to the presence of the ether group.

The infrared spectrum of elephant ginger rhizome extract boiled at 6 minutes had generated one functional compound namely ester (RCOOR) at 1270.04 cm^{-1} which methyl ester and geranyl butyrate as the compounds. Whereas methyl ester had been found in the extracts of elephant ginger rhizome boiled at 6 minutes which is known as antioxidant agent. Dahiya and Kaur (2007) reported that methyl ester at 1242, 1244 and 1245 cm^{-1} possess potent anthelmintic activity against *M. konkanensis*, *P. corethruses* and *Eudrilus* sp. and against gram negative bacteria *P. aeruginosa*. Sikora, Cieslik and Topolska (2008) noted that antioxidant related to the basic structure of composition of heteroxyclic chain where one of them was hydroxyl (OH), forming of metoxyl (OCH_3) and substitution of glycoside residues.

On the other hand, 1-hexadecanol compound in the extracts of ginger boiled at 6 minutes was not detected by GC-MS analysis, since functional group occured reduction of hydroxyl (OH) group. Jaafar *et al*. (2007) reported that 1-hexadecanol was found on starch oil of leave, stem, flower and rhizome of *Etlingera elatior*, an aromatic plant used widely in traditional therapy and also as a sensation of some foods. These starch oil were extracted by principle of hydro distilling using GC-MS.

Conclusion

Extracts of elephant ginger rhizome boiled at 6 minutes showed as the best thermal processed sample which were determined by GC-MS and FTIR. It has been shown to contain antioxidant compounds namely methyl ester, 9-octadecenoic and nortrachelogenin. It Is interesting to note that zingerone compound total area increased from 5.68% to 6.32%.

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