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THESIS

FISHY ODOUR DEODORISATION AND NATURAL ANTIOXIDANT PROPERTIES OF THE EXTRACTS FROM RHIZOME OF *Alpinia galanga* (L.) Sw.

NOPPARAT MAHAE

A Thesis Submitted in Partial Fulfillment of
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Nopparat Mahae 2009: Fishy Odour Deodorisation and Natural Antioxidant Properties of the Extracts from Rhizome of *Alpinia galanga* (L.) Sw. Doctor of Philosophy (Food Science), Major Field: Food Science, Department of Food Science and Technology. Thesis Advisor: Associate Professor Siree Chaiseri, Ph.D. 131 pages.

This study investigated fishy odour deodorisation ability and antioxidant activity of the extracts from galangal rhizomes (*Alpinia galanga*). The extracts were obtained by three processes: water extraction, ethanol extraction and essential oil extraction. In fishy odour deodorisation study, the crude extract from fresh rhizomes was also used. Volatile compounds were evaluated using gas chromatography-mass spectrometry (GC-MS). The most abundant volatile component in the essential oil was 1,8-cineol (18.91%). The most abundant volatile compound in crude extract (32.65%) and ethanol extract (61.03%) was tentatively identified as 1′-acetoxychavicol acetate. The crude extract also contained high 1,8 -cineol (17.33%). Water extract had the unknown ($[M]^+$at *m/z*=192) that comprised 76.04% of the volatiles. The content of phenolic compounds and flavonoids in the extracts were also evaluated by high performance liquid chromatography (HPLC). Ethanol extract had the highest total phenolic compounds (31.49 mg GAE/g extract) and flavonoids (13.78 mg CE/g extract). Fishy odour reduction abilities of the extracts were studied by sensory evaluation of the model systems containing trimethylamine (TMA) and (*Z*)-4-heptenal. All extracts were more effective in reducing fishy odour from TMA than that from (*Z*)-4-heptenal. Among all galangal extracts, the crude extract was the most effective in deodorisation of fishy odour from TMA. This was followed by ethanol extract. Essential oil and water extract were less efficient in reducing the TMA fishy odour. The Principal Component Analysis (PCA) and the correlation indicated that fishy odour deodorisation was correlated with the concentrations of alcohols, acids, phenolic compounds and unknown compounds. Water extracted, ethanol extracted and essential oil samples were evaluated for their antioxidant activities by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ORAC methods. The results showed that the ethanol extract had the highest antioxidant activity. The IC_{50} of water extract, ethanol extract, essential oil, α-tocopherol and BHA were 55.48, 10.66, 455.43, 1.45 and 0.41 mg/mL, respectively. This study showed that ethanol extract of galangal could be used to reduce fishy odour and oxidation in food products.

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Nopparat Mahae

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LIST OF ABBREVIATIONS

AAPH	=	2,2′-Azobis(2-methylpropionamidine) dihydrochloride
AUC	=	Area under curve
BHA	=	Butylated hydroxyanisole
CE	=	Catechin equivalent
CT	=	Cycle time
DI	=	Deionised
DM basis	=	Dry matter basis
DPPH	=	2,2-diphenyl-1-picryhydrazyl
EE	=	Ethanol extract
ESO	=	Essential oil
ET	=	Electron transfer
FRAP	=	Ferric ion reducing antioxidant power
GAE	=	Gallic acid equivalent
HAT	=	Hydrogen atom transfer
HPLC	=	High performance liquid chromatography
IOU	=	Inhibited oxygen uptake
GC-MS	=	Gas chromatography-mass spectrometry
m/z	=	Mass-to-charge
OAV	=	Odour activity value
ORAC	=	Oxygen radical absorbance capacity
PCA	=	Principal component analysis
r	=	Correlation coefficient
RI	=	Retention index
RMCD	=	Randomly methylated β-cyclodextrin
TE	=	Trolox equivalents
TEAC	=	Trolox equivalent antioxidant capacity
TMA	=	Trimethylamine
TOSC	=	Total oxidation scavenging capacity
TRAP	=	Total radical trapping antioxidant parameter

LIST OF ABBREVIATIONS (continued)

WE	=	Water extract
WM basis	=	Wet matter basis (fresh weight)

FISHY ODOUR DEODORISATION AND NATURAL ANTIOXIDANT PROPERTIES OF THE EXTRACTS FROM RHIZOME OF *Alpinia galanga* (L.) Sw.

INTRODUCTION

Galangal (*Alpinia galanga*), a rhizome closely related to ginger family, is used to flavour Thai food. It pairs well with many ingredients of Thai food that compose of coconut, garlic, chili peppers, kaffir lime leaves, turmeric, fish sauce, tamarind and shallots. In Thailand, galangal is used in curry pastes, Tom-Yam (Thai soup) and many curries. Galangal rhizome has a wide range of application in traditional medicine (Scheffer and Jansen, 1999; Yang and Eilerman, 1999). The traditional medicine from galangal can be used to cure skin diseases, indigestion, colic, dysentery, enlarge spleen, respiratory diseases, cancer of mouth and stomach, and systemic infections and cholera. It can also be used as an expectorant and after childbirth (Scheffer and Jansen, 1999). 1´-Acetoxy-chavicol acetate and 1´-acetoxyeugenol acetate from galangal were reported as anti-tumor substance (Itokawa *et al.*, 1987; Kondo *et al.*, 1993). Essential oil from galangal was reported as a potential anti-carcinogen (Lam and Zheng, 1991; Zheng *et al.*, 1993). The essential oil from both fresh and dried rhizomes of galangal exhibited the antimicrobial activities against gram-positive bacteria, yeast and some dermatophytes. The most active compound was terpinen-4-ol (Janssen and Scheffer, 1985).

Like ginger, galangal is a ‘de-fisher’ and so appears frequently in fish and shellfish recipes. It has a unique flavour profile and is described as having more woody, minty and floral aroma than ginger. The potent odourants of galangal are 1,8-cineole, linalool, geranyl acetate, eugenol and chavicol acetate (Mori *et al*., 1995). Compounds causing fishy odour have been extensively reviewed. These compounds are trimethylamine (Cadwallader *et al*., 1995; Prost *et al*., 1998 and Fukami *et al*., 2002), 2,4,7-decatrienal (Meijboom and Stroink, 1972; Karahadian and Lindsay, 1989; Karahadian and Lindsay, 1990), (*Z*)-4-heptenal (Karahadian and Lindsay, 1990;

Cha and Cadwallader, 1998; Hartvigsen *et al*., 2000), *trans,cis*-2-4-heptadienal (Hartvigsen *et al*., 2000), dimethyl trisulfide, 4-ethyl-6-hepten-3-one (Fukami *et al*., 2002) and (*E,Z*)-2,6-nonadienal and 1-penten-3-one (Venkateshwarlu *et al*., 2004). Several methods to reduce fishy odour have been investigated. Such methods are the extraction of fishy compounds with supercritical carbon dioxide (Spinelli *et al*., 1987), use of acidic steam source for deodorisation (Karahadian and Lindsay, 1990), treatment with lipoxygenase (Hu and Pan, 2000), depression with mirin containing soy sauce (Kasahara *et al*., 1990) and masking with perilla, Yuzu peel and young leaf of Japanese pepper (Kasahara and Osawa, 1998).

Galangal is a good source of natural antioxidants. The search for natural replacements for synthetic antioxidants has been increased because of the requirement to meet safety standards. Then natural antioxidants presented in food and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects. Potent sources of antioxidants have been searched in several plant materials. Herbs and spices are harmless sources for natural antioxidants (Gorden and Weng, 1992; Kim *et al.*, 1994) and show high antioxidant activity (Wu *et al.*, 2004). Natural antioxidants from spices and herbs are generally classified as vitamin, phenolic compounds including flavonoids and phenolic acids, and volatile compounds (Carrubba and Calabrese, 1998; Nakatani, 1996; Namiki, 1990). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. Additionally, they have a metal chelating potential (Rice-Evans *et al*., 1995). Antioxidant activity of galangal extract (4 mg in 4 mL of 99.5% ethanol) was stronger than α-tocopherol. Such extract was prepared from crushed fresh rhizome soaked in acetone for 18 days and then removed the solvent (Jitoe *et al*., 1992). Few researches have found that ethanolic extract of galangal can reduce lipid oxidation in meat and meat products (Cheah and Abu Hasim, 2000; Juntachote *et al.,* 2006b). Ethanolic extract of galangal acted as radical scavenger. It exhibited strong superoxide anion scavenging activity, Fe^{2+} chelating activity, and reducing power in a concentration-dependent manner (Juntachote and Berghofer, 2005). However, dried galangal is more effective as an antioxidant than

its ethanol extract when used in cooked ground pork (Juntachote *et al.,* 2006b). The best condition to extract phenolic compounds from dried galangal is 75% ethanol at 75 °C for 90 min (Juntachote *et al.,* 2006a). Zaeoung *et al.* (2005) studied the antioxidant activities of the extracts from fresh galangal but used methanol instead of ethanol for extraction. Extract of galangal using 50% alcohol in water did not show acute toxicity at 10 g/kg of test animal (Mokkhasmit *et al*., 1971).

In Thailand, herbs and spices including galangal are widely used. Fresh galangal has been used to flavour foods and reduce fishy odour. Because the flavour of galangal is not as strong as other herbs and spices, galangal is therefore more suitable to be used in a wide range of food products. This study scoped the investigation on the use of galangal extract as an antioxidant and to reduce fishy odour.

OBJECTIVES

1. To identify volatile compounds and aroma active compounds from crude extract, water extract, ethanol extract and essential oil from rhizome of galangal.

2. To investigate the fishy odour deodorisation property of the extracts from rhizome of galangal.

3. To study on antioxidant activity of the extracts from rhizome of galangal.

LITERATURE REVIEW

Alpinia galanga

Galanga or galangal (*Alpinia galanga* or *Languas galanga*) is used in foods in Thailand and throughout South Asia and South-East Asia. It is used as flavouring agent in Thai curry paste. Air-dried galangal rhizome composition per 100 g is: moisture 14 g, total ash 9 g, matter soluble in 80% ethanol 49 g, matter soluble in water 19 g, total sugar 9 g, total nitrogen 3 g, total protein 16 g (Scheffer and Jansen, 1999). There are two different species of galangal: smaller galangal (*Alpinia officinarum* Hamce) and greater galangal (*Alpinia galanga* Wild). This dissertation will focus on the greater galangal or 'Kha' that usually used in Thai food. Fresh greater galangal root has yellowish brown to pale brownish skin with reddish brown rings. The fresh galangal has different flavour profile from that of the dried one (Raghavan, 2007).

Plants of ginger family have some volatile compounds glycosidically bound with sugars. Free volatile compounds can be released from such compounds by enzymatic or acidic hydrolysis (Ly *et al.*, 2002; Wu *et al*., 1990). Forty compounds from galangal were identified. Most of them were monoterpenes, monoterpene alcohols, monoterpene esters, and sesquiterpenes. There were also methyleugenol, eugenol acetate, chavicol (4-allylphenol) and chavicol acetate reported (De Pooter *et al*., 1985). The oxygenated fraction of galangal oil was identified as 1,8-cineol (49.6%), 2-acetoxy-1,8-cineol (17.3%) and 1′-acetoxychavicol acetate (14.0%) (Mori *et al*., 1995). The compositions of galangal essential oil from different reports are displayed in Table 1

Table 1 Compositions of essential oil from rhizome of galangal.

compound	Composition (%)		
	De Pooter *et al.* (1985)	Jantan *et al.* (2004)	Arambewela and Arawwawala (2007)
α-Thujene	-	0.1	-
α-Pinene	10.2	2.0	1.9
α-Fenchene	-	-	-
Camphene	0.5	0.1	6.4
Verbenene (Pinadinene)	-	-	-
Sabinene	tr	0.5	-
β-Pinene	1.6	0.6	0.8
β-Myrcene(Myrcene)	0.7	0.1	0.1
α-Phellandrene	-	-	+
δ-3-Carene	-	0.1	-
α-Terpinene	-	tr	+
p-Cymene	0.8	0.1	6.5
Limonene	1.6	-	-
1,8-Cineol	5.5	40.5	6.3
(*E*)- β-Ocimene	-	tr	-
γ-Terpinene	tr	0.3	-
trans-Sabinene hydrate	-	tr	-
Terpinolene	tr	0.1	-
Linalool	tr	0.1	-
α-Fenchol	-	tr	-
cis-*p*-Menth-2-en-1-ol	-	0.1	-
trans-p-Menth-2-en-1-ol	-	0.1	-
Camphor	-	tr	4.9
Citronellal	-	tr	-
cis-Verbenol	-	-	-

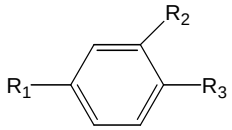
Table 1 (continued)

Compound	Composition (%)		
	De Pooter *et al.* (1985)	Jantan *et al.* (2004)	Arambewela and Arawwawala (2007)
p-Menth-1,5 diene-8-ol	-	-	-
Borneol	tr	0.4	0.9
Terpinene-4-ol	0.3	1.3	3.5
p-Cymenol	tr	-	-
p-Cymene-8-ol	-	0.1	-
α-Terpineol	0.2	1.1	+
cis-Piperitol	-	0.1	-
trans-Piperitol	-	0.1	-
Fenchyl acetate	-	0.1	4.5
Citronellol	-	tr	-
Chavicol	0.2	2.0	-
Bornyl acetate	2.5	0.1	0.6
Carvacrol	-	0.1	-
δ-Elemene	-	0.1	-
Chavicol acetate	1.0	2.5	-
Citronellyl acetate	1.6	-	-
Eugenol	-	0.7	-
α-Copaene	0.7	0.4	-
Geranyl acetate	5.1	0.3	-
β-Elemene	-	0.3	-
Tridecane	tr	-	-
Tetradecane	-	0.2	-
Methyl eugenol	-	1.5	-
β-Caryophyllene	0.9	3.6	-
β-Gurjunene	-	0.2	-

Table 1 (continued)

Compound	Composition (%)		
	De Pooter *et al.* (1985)	Jantan *et al.* (2004)	Arambewela and Arawwawala (2007)
α-Bergamotene	0.7	2.0	-
(*Z*)- β-Farnesene	-	0.4	-
α-Humulene	0.6	0.7	6.0
(*E*)-β-Farnesene	18.2	3.2	-
ar-Curcumene	1.9	0.5	-
β-Selinene	-	0.7	-
Eugenyl acetate	1.5	-	-
Pentadecane	1.9	2.9	-
β-Bisabolene	16.2	8.4	-
β-Sesquiphellandrene	1.6	2.6	-
(*Z*)-Nerolidol	-	0.1	-
(*E*)-Nerolidol	-	0.2	-
Caryophyllene oxide	2.5	0.8	-
γ-Eudesmol	-	0.7	-
β-Eudesmol	-	0.7	-
β-Bisabolol	-	1.1	-
(*Z*,*E*)-Farnesol	-	3.8	-
1-Heptadecene	-	1.6	-
Heptadecane	-	0.2	-
(*Z*,*Z*)-Farnesol	-	0.2	-
(*E*,*E*)-Farnesol	-	0.2	-
(*E*,*E*)-Farnesyl acetate	-	1.7	-
1-Nonadecene	-	0.1	-
Zerumbone	-	-	44.8

1′-Acetoxychavicol acetate (galangal acetate) from galangal has many benefits. It exhibits a unique pungent sensation, which is less intense than that of capsaicin and without a lingering effect. Galangal acetate can be used as alcohol enhancer or and alcohol replacer in alcoholic and alcohol-free beverages (Yang and Eilerman, 1999). 1′-Acetoxychavicol acetate demonstrated anti-tumour effect (Itokawa *et al.*, 1987; Kondo *et al.*, 1993). The other compounds from methanol extract of galangal are shown in Figure 1. 1′-Acetoxyeugenol acetate is an anti-tumour agent similar to 1′-acetoxychavicol acetate, (Itokawa *et al.*, 1987). The aqueous solution of galangal acetate was no longer pungent after reflux. The products from the reaction were 1′-hydroxychavicol acetate, *p*-acetoxycinnamic alcohol and *p*-coumaryl diacetate, as shown in Figure 2 (Yang and Eilerman, 1999).



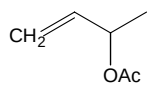
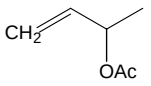
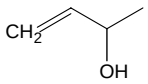
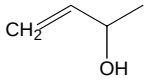
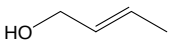
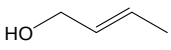
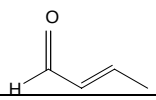
compound	R1	R2	R3	
1		H	OAc	← 1′-acetoxychavicol acetate
2		OCH_3	OAc	1′-acetoxyeugenol ← acetate
3		H	OAc	
4		H	OH	
5		OCH_3	OCH_3	
6		H	OCH_3	
7		H	OH	

Figure 1 Structures of phenylpropanoids from galangal.

Source: Itokawa *et al.* (1987)

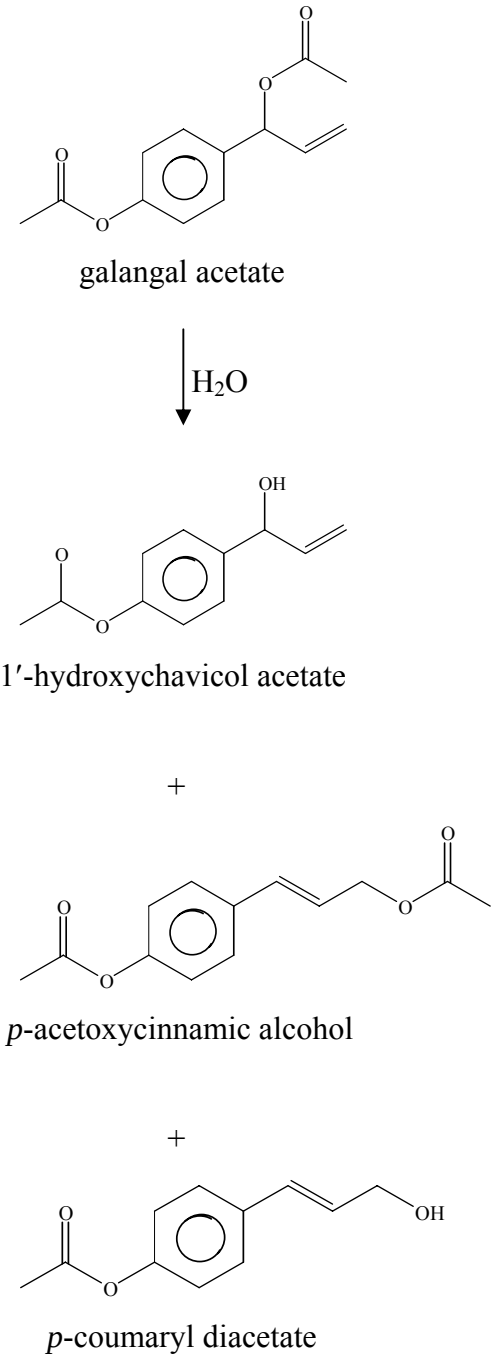


Figure 2 Hydrolysis of galangal acetate.

Source: Yang and Eilerman (1999)

Besides galangal root, galangal seed also contains 1′-acetoxychavicol acetate and 1′-acetoxyeugenol acetate. These compounds are principal anti-ulcer agents (Mitsui *et al*., 1976). Mass spectra of 1′-acetoxychavicol acetate and 1′-acetoxyeugenol acetate are shown in Figure 3.

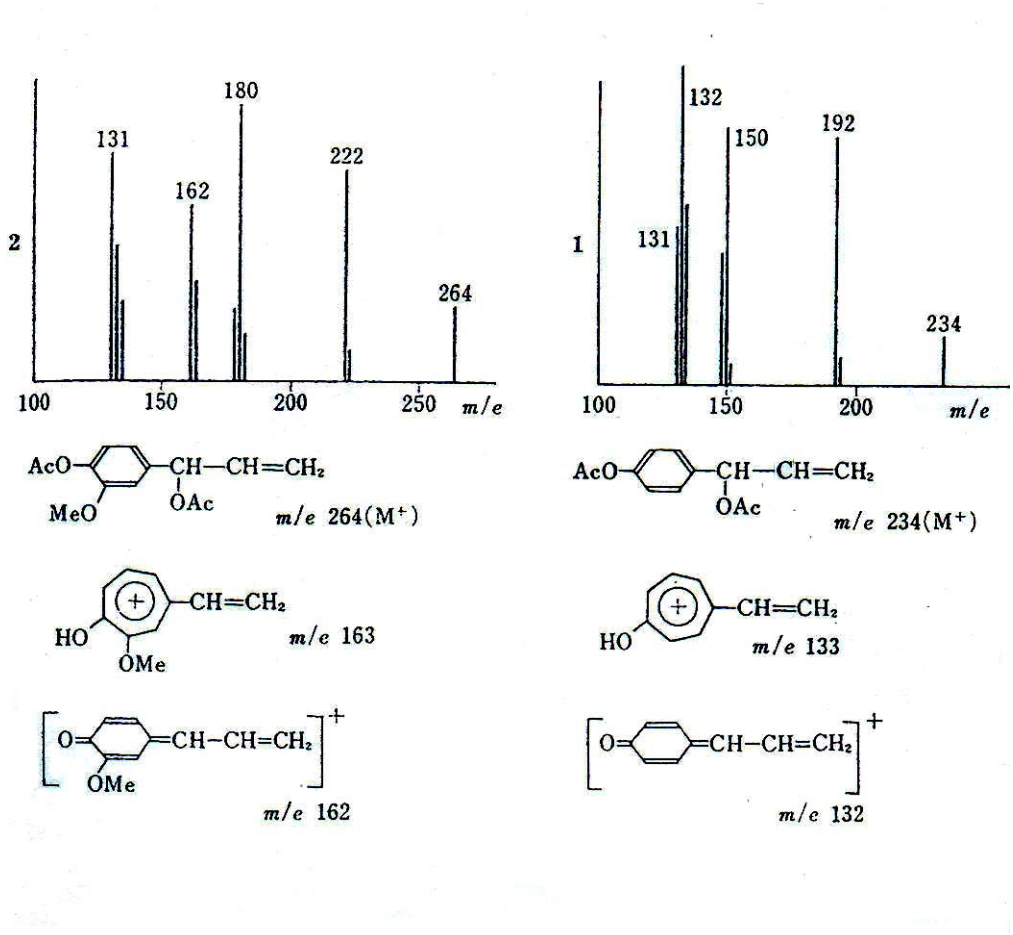


Figure 3 Mass spectra of 1′-acetoxychavicol acetate (1) and 1′-acetoxyeugenol acetate (2).

Source: Mitsui *et al*. (1976)

Compounds contributed to fishy odour

Many compounds that caused fishy odour have been investigated. These compounds are trimethylamine, 2,4,7-decatrienal, (*Z*)-4-heptenal and other compounds.

Trimethylamine (TMA)

TMA is obtained from the trimethylammonium group of choline. The accumulation of TMA in rotting fish is responsible for the 'fishy' odour characteristic. Choline is taken up by mitochondria and converts to glycine betaine via betaine aldehyde. Then, betaine may convert to TMA (Seibel and Walsh, 2002). The diagram of the formation of TMA is as follows (Figure 4):

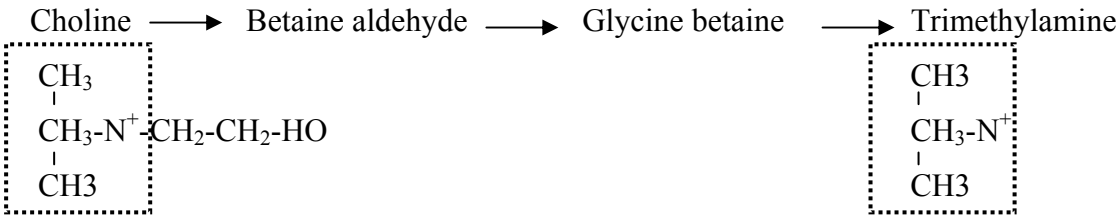


Figure 4 Formation of TMA from choline.
Source: Seibel and Walsh (2002)

Considering the fact that choline is presented and it generally decreases as TMA increases, then the precurser of TMA could be choline (Hebard *et al*., 1982). The odour characteristic of TMA is pungent ammoniacal fishy odour and taste (Leffingwell, 2004). TMA have been viewed as an important aroma compound in many marine fish, cooked spiny lobster tail meat (Cadwallader *et al*., 1995), cook Turbot (Prost *et al*., 1998) and fish sauce (Fukami *et al*., 2002).

2,4,7- Decatrienal

Meijboom and Stroink (1972) first proposed that two 2,4,7-decatrienal isomers (*trans,cis,cis*- and *trans,trans,cis*-) were the most important contributors to the objectionable fishy odour in oxidised fish oils and other oils containing linolenic acid or long chain *n*-3 fatty acids. They described this fishy odour as whale oil-like and noted that the isomer exhibited slightly different fishy odour qualities.

Autoxidation of long chain polyunsaturated ω-3 fatty acids that are abundant in fish lipid has been reported to produce 2,4,7-decatrienal (Figure 5). Besides 2,4,7-decatrienal, other aldehydes are also produced. These products were generated via β-scission of alkoxy radicals. The alkoxy radicals are generated by the hemolytic cleavage of each isomer of hydroperoxides (Fujimoto, 1989).

H-donating character of tocopherol-type compounds is possible to cause a preferential of *cis-trans* rather than *trans-trans* monohydroperoxide. This provides the direct precursors of 2,4,7-decatrienals (Durnford and Shahidi, 1998) (Figure 6).

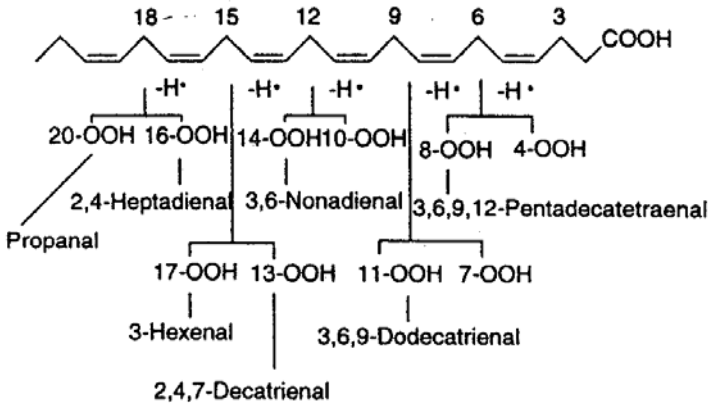


Figure 5 Formation of 2,4,7-decatrienal and other aldehydes and autoxidation of docosahexaenoic acid.

Source: Durnford and Shahidi (1998)

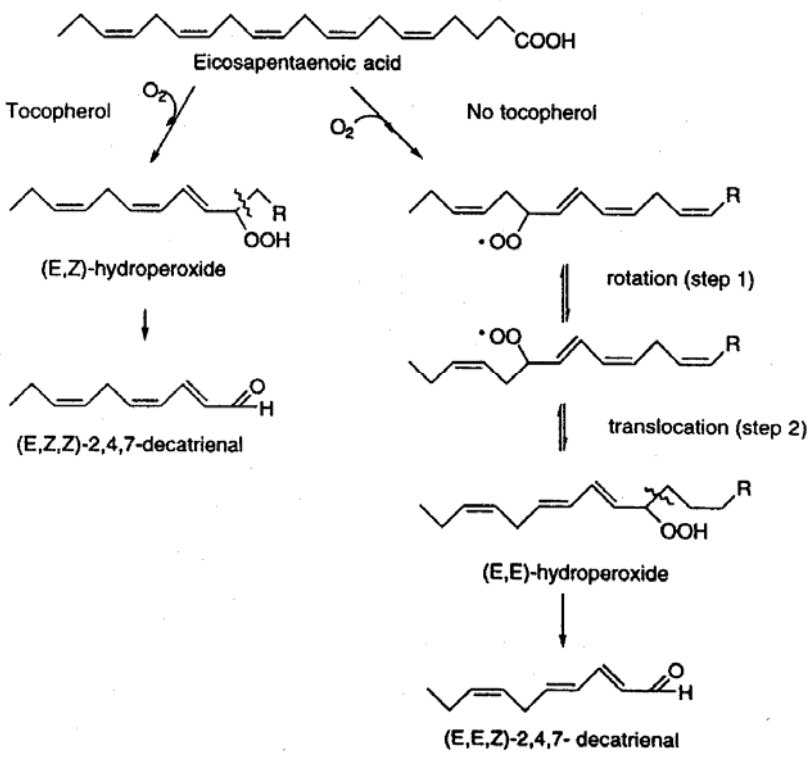


Figure 6 Formation of isomeric hydroxides of polyunsaturated fatty acid.

Source: Durnford and Shahidi (1998)

(*Z*)-4-Heptenal

(*Z*)-4-Heptenal is not recognised as fishiness when evaluated by individual. It appears to complement the fishy, green, and burnt odour of decatrienal when presents at high concentrations (Karahadian and Lindsay, 1989). (*Z*)-4-Heptenal is produced by the water-mediated retro-aldol condensation of (*E,Z*)-2,6-nonadienal. This compound is derived from eicosapentaenoic acid (C20:5 n-3) through the action of endogenous lipoxygenases (Figure 7). The products from this mechanism reveal the pleasant green, planty, and melon like aromas of the fresh fish (Cadwallader, 2000).

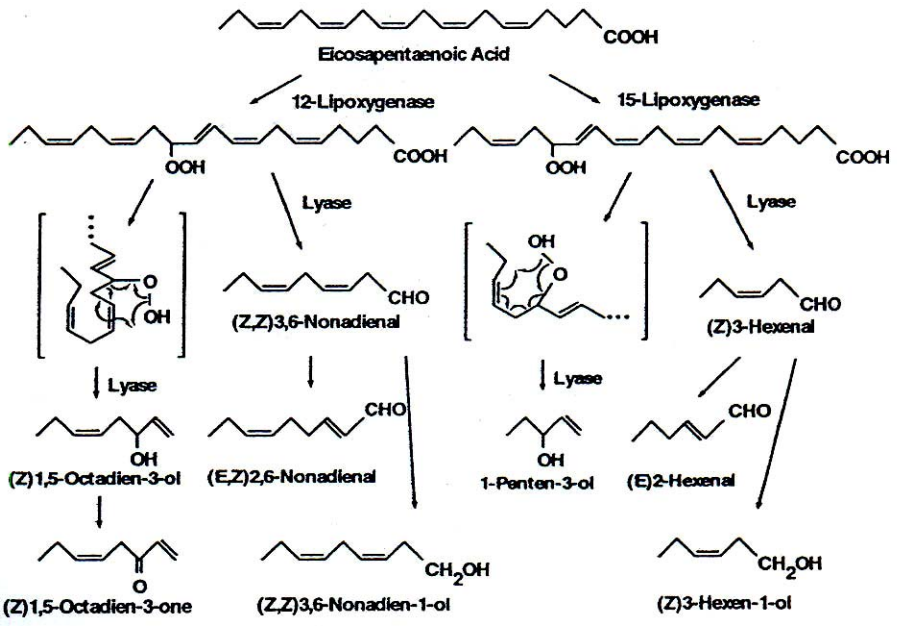


Figure 7 Proposed mechanisms for the biogenesis of selected aroma components of freshly harvested fish.

Source: Josephson and Lindsay (1986)

For (*Z*)-4-heptenal formation, the data indicated that 3-hydroxy-*Z*-6-nonenal was formed first by the addition of water to the alpha/beta double bond of (*E,Z*)-2,6-nonadienal. This was followed by retro-aldol condensation of 3-hydroxy-*Z*-6-nonenal to yield (*Z*)-4-heptenal as show in Figure 8 (Josephson and Lindsay, 1987).

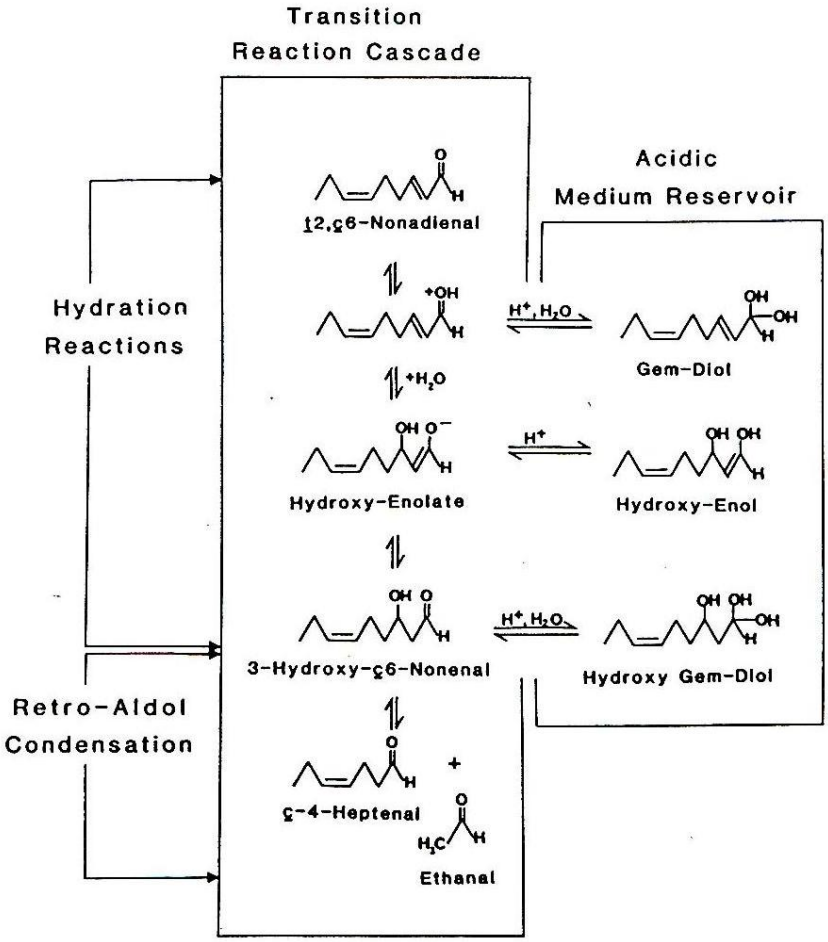


Figure 8 Proposed mechanism for the formation of (*Z*)-4-heptenal.
Source: Josephson and Lindsay (1987)

The formation of (*Z*)-4-heptenal from (*E,Z*)-2,6-nonadienal in cod liver oil is shown in Figure 9. After 12 days of holding, (*E,Z*)-2,6-nonadienal was decreased, the flavour of oil change into fishy/burnt. At this time, (*Z*)-4-heptenal increased because it was formed from retro-aldol reaction of (*E,Z*)-2,6-nonadienal (Karahadian and Lindsay, 1990).

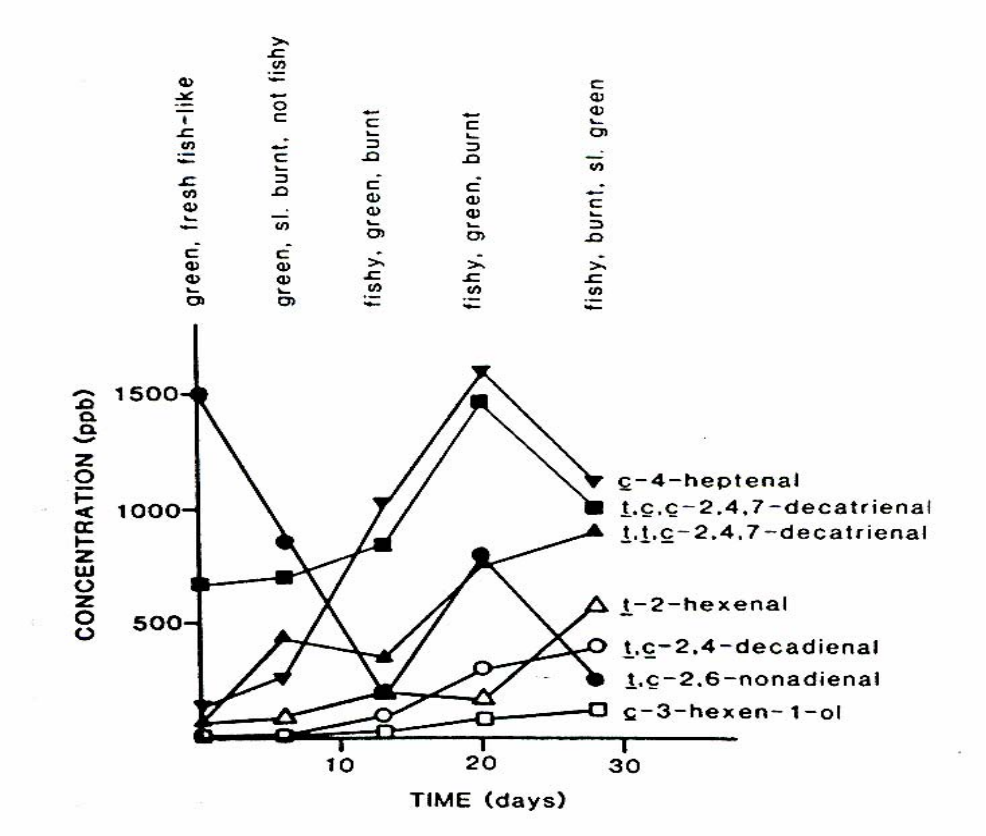


Figure 9 Concentration of compounds associated with fishy development in oxidising low-temperature (100 °C for 2 h) acetic acid (0.01 N) steam deodorised cod liver oil containing 200 ppm dilauryl thiodipropionate.

Source: Karahadian and Lindsay (1990)

The products that (*Z*)-4-heptenal was characterised as fishy odour are fish oil enriched mayonnaise (Hartvigsen *et al*., 2000) and skipjack tuna sauce (Cha and Cadwallader, 1998).

Other compounds

The other compounds related to fishy odour are (*E,Z*)-2-4-heptadienal (Hartvigsen *et al*., 2000), 4-ethyl-6-hepten-3-one (Fukami *et al*., 2002) and (*E, Z*)-2,6-nonadienal and 1-penten-3-one (Venkateshwarlu *et al*., 2004).

Flavour of herbs and spices

Spices are whole or ground seeds, fruits, barks or roots of plants. Herbs are the leafy parts of annual or perennial shrubs or plants. There are many definitions of spice. The Food and Drug Administration (FDA) has defined spice as any "aromatic vegetable substance in the whole, broken or ground from, except for those substances which have been traditionally regarded as foods, such as onion, garlic and celery; whose significant function in food is seasoning rather than nutritional; that is true to name; and from which no portion of any volatile oil or other flavouring principle has been remove" (Francis, 2000).

Properties and uses of spices are: 1) flavouring properties 2) antioxidant properties 3) preservative action 4) antimicrobial activity 5) physiological and medical aspects 6) use in perfumery and cosmetics and etc. (Pruthi, 1980). For flavouring properties, herbs and spices have been used to season foods. The component of spice that imparts to its particular flavour is essential oil. The major chemical components of essential oil are terpenes. Terpenes that have 10, 15 and 20 carbons are call monoterpenes, sesquiterpenes and diterpenes, respectively (Reineccius, 1994; Hirasa and Takemasa, 1998). Table 2 shows the major components of the essential oil of some spices.

Table 2 Chemical compounds contained in essential oil of spices.

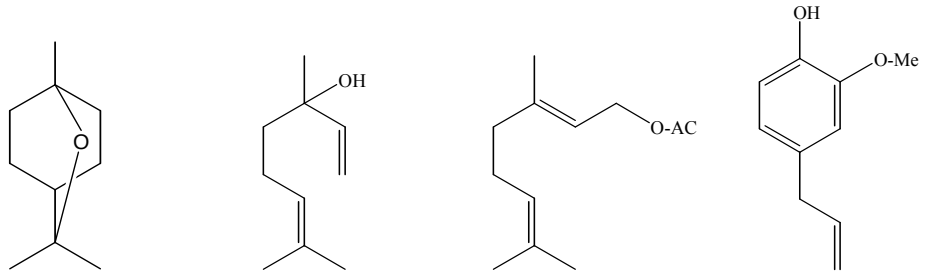
Spice	Chemical compounds in essential oil
Allspice	Eugenol, thymol, phellandrene, caryophyllene, cineol, methyl eugenol
Anise	Anethole, methyl chavicol, anise aldehyde, limonene
Basil	Methyl chavicol, linalool, cineol, anethole
Bay leaf	Cineol, α-pinene, phellandrene, eugenol, limonene, borneol
Caraway	d-Carvone, limonene, carveol

Table 2 (continued)

Spice	Chemical compounds in essential oil
Cardamom	Cineol, α-terpinyl acetate, limonene, sabinene, myrcene
Celery	Limonene, selinene, sesquiterpene alcohol
Cinnamon	Cinnamaldehyde, eugenol, caryophyllene, pinene
Clove	Eugenol, caryophyllene, acetyl-eugenol
Coriander	Linalool, α,β-pinene, *p*-cymene
Cumin	Cuminaldehyde, phellandrene, limonene
Fennel	Anethole, limonene, fenchone, α-pinene, camphene
Garlic	Diallyl disulfide, diallyl trisulfide, ally propyl disulfide
Ginger	Gingiberene, phellandrene, borneol, linalool, shogaol, gingeroene
Marjoram	α-Terpineol, terpinene, terpinene-4-ol, α-pinene, limonene, borneol chavicol
Nutmeg/Mace	Myristicin, α-pinene, eugenol, geraniol, limonene, terpineol
Oregano	Thymol, carvacrol, α-pinene
Pepper	Piperine, caryophyllene, α-pinene, phellandrene, camphene, myrcene
Rosemary	Cineol, borneol, camphor, terpineol, linalool
Sage	Cineol, linalool, camphor, borneol, α-pinene, thujone
Star anise	Anethole, methyl chavicol, α-pinene, limonene, phellandrene
Tarragon	Methyl chavicol, ocimene, myrcene, phellandrene
Thyme	Thymol, carbachol, linalool, α-pinene, borneol
Dill	Carvone, α-pinene, limonene, phellandrene

Source: Hirasa and Takemasa (1998)

For galangal, the fresh form has different flavour profile from the dried form. The fresh galangal is aromatic, spicy, peppery, ginger-like and has a slightly sour note while dried galangal is spicier with a cinnamon-like taste. Young rhizome is pink in color and is more tender and flavourful (Raghavan, 2007). The main compounds of galangal are 1,8-cineole, linalool, geranyl acetate, eugenol and chavicol acetate (Mori *et al*., 1995). The chemical structures of certained compounds are shown in Figure 10.



(1) (2) (3) (4)

Figure 10 1,8-Cineole (1), linalool (2), geranyl acetate (3) and eugenol (4).
Source: Adams (1995)

Mechanism of deodorising/odour masking

Spices are sometimes used for deodorising or masking the smell of raw materials. Deodorising/masking can be divided into three types: chemical, physical and sensational (Figure 11). The mechanisms are as fallowed (Hirasa and Takemasa, 1998; Kawai *et al.*, 2003).

1. Chemical deodorisation. For this mechanism, smelling compounds are changed to non-volatile compounds or to odourless substances through some chemical reactions such as neutralisation, addition, condensation, oxidation or reduction. For example, a fish smell can be deodorised by soaking it in lemon juice or vinegar.

2. Physical deodorisation. The unpleasant smelling compounds are absorbed by porous active carbon or zeolite, include them in cyclodextrin and ventilation or diffusion

3. Sensational deodorisation. This method is theoretically divided into two types: “masking” in a narrow sense, in which a strong spice flavour spice flavour covers an unpleasant smell, and “offset deodorising” in which two compounds having different odours become odourless when mixed. In this technique, the actual odour dose not decrease in intensity or disappear – it is simply not perceived as readily.

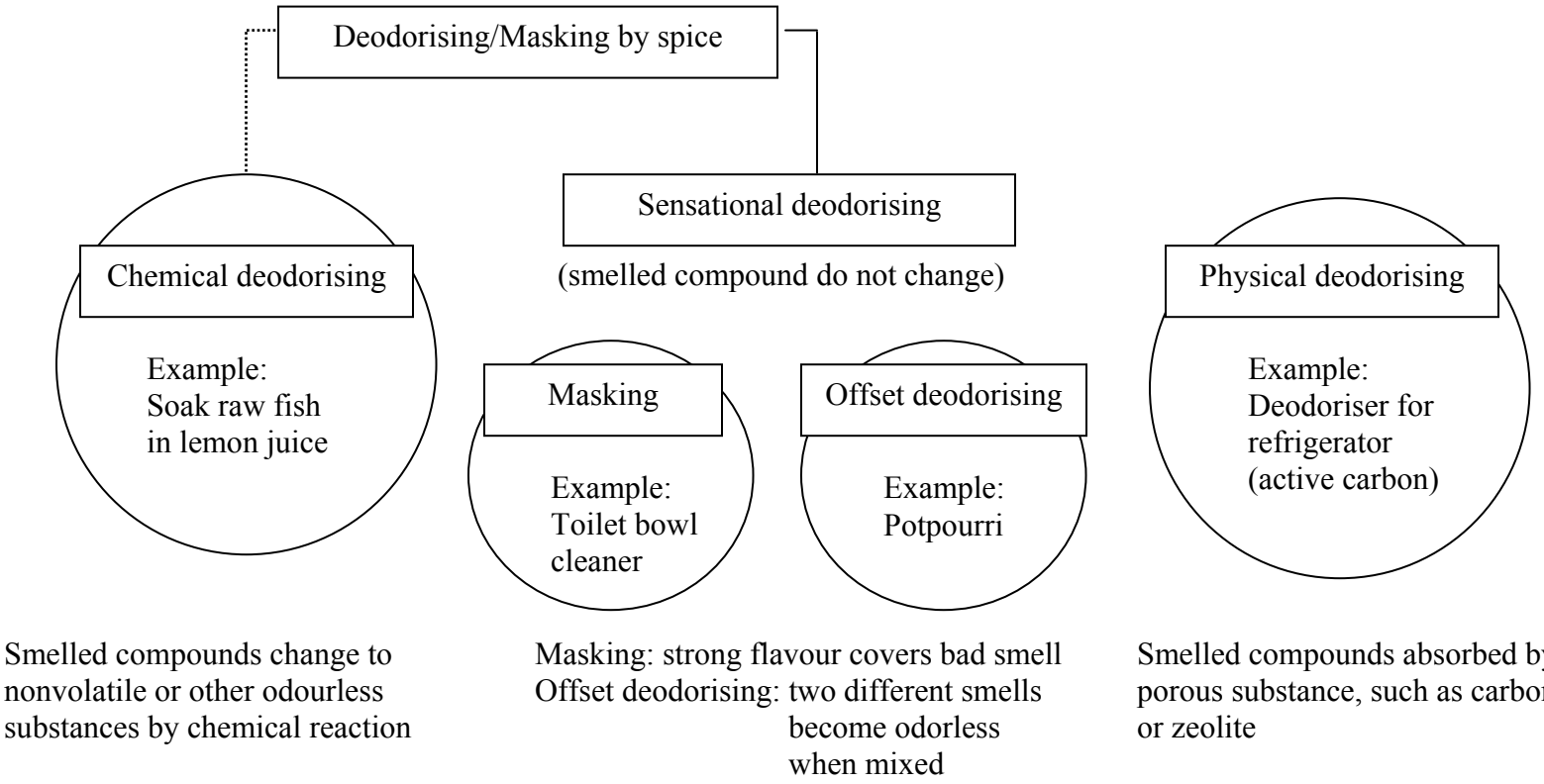


Figure 11 Deodorising/masking mechanisms of spices.

Source: Hirasa and Takemasa (1998)

Chemical deodorisation

Karahadian and Lindsay (1990) used acetic acid as the steam source for the deodorisation of cod liver oil. Burnt/fishy odour produced by high concentration of 2,4,7-decatrienal was reduced when fish oil was incorporated into mayonnaise. The acidic medium (pH=3.4) provided by acetic acid probably facilitated the hydration of the 2-3 double bonds of 2,4,7-decatrienal. Such reaction would result in loss of significant volatiles as well as caused an alteration of the molecular features of the parent molecules (Karahadian and Lindsay, 1989).

Sensational deodorisation

Sensational deodorising of fishy odour has been studied using many natural ingredients. Perilla, Yuzu peel and young leaf of Japanese pepper were used to mask fishy odour in boiled sardine. The main characteristic compounds of perilla and Yuzu peel were perillaldehyde and γ-terpinen, respectively. Young leaf of Japanese pepper had three major compounds, α-pinene, citronellal and 2-undecanone. Perillaldehyde greatly contributed to the masking of fishy odour of the boiled sardines when perilla was added independently (Kasahara and Osawa, 1998). Mirin containing soy sauce could suppress fishy odour in roasted salted-dried sardine. It seems that phenethyl alcohol, 4-ethylguaiacol and 2-acetylpyrrole greatly contributed to mask the odour of sardine (Kasahara *et al*., 1990). Marine green macro algae lipoxygenase could also reduce the undesirable odour of fish oil by the production of desirable volatile compounds via position-specific cleavage of hydroperoxides (Hu and Pan, 2000). Mirin containing soy sauce was used in salt-dried sardine for suppressing fishy odour. The chromatograms of each treatment and identified volatiles are shown in Figure 12 and Table 3. Fishy odour was suppressed for sample C. The chromatogram of fishy volatile was not change after the addition of mirin and soy sauce. Then the suppressing effect was a result from the specific compound in sample C (phenethyl alcohol, 4-ethylguaiacol and 2-acetypyrrole greatly) that contributed to mask fishy odour of salt-dried sardine (Kasahara *et al*., 1990).

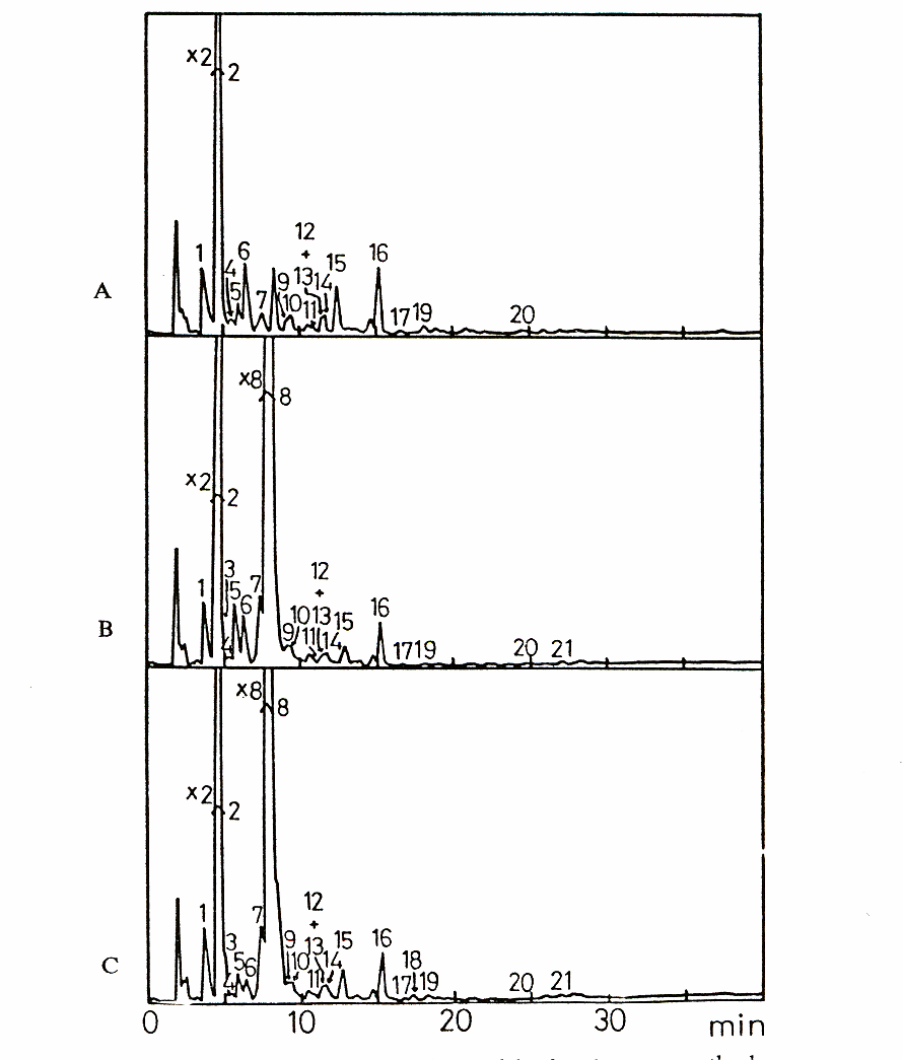


Figure 12 Gas chromatogram of whole flavour obtained with the head space method

A: roasted salt-dried sardine

B: roasted "mirin-boshi"

C: roasted "mirin added soysauce-boshi"

Source: Kasahara *et al*. (1990)

Table 3 Identified volatile in head space vapor of three treated sardine.

Peak No. Figure 12	Name	Sample[1]		
		A	B	C
1	Cyclohexane	+	+	+
2	Acetaldehyde	+	+	+
3	Propionaldehyde	+	+	+
4	Isobutyraldehyde	-	+	+
5	Acetone	+	+	+[2]
6	*N*-Butyraldehyde	+	+	+
7	Ethyl acetate	-	-	+
8	Isovaleraldehyde	+	+	+
9	Ethyl alcohol	-	+	+
10	Diacetyl	+	+	+
11	*n*-Valeraldehyde	+	+	+
12	*n*-Propyl alcohol	+	+	+
13	Toluene	+	+	+
14	Crotonaldehyde	+	+	+
15	2,3-Pentanedione	+	+	+
16	Caproaldehyde	+	+	+
17	Isobutyl alcohol	-	-	+
18	*n*-Butyl alcohol	-	-	+
19	1-Penten-3-ol	+	+	+
20	*n*-Heptanal	+	+	+
21	Isoamyl alcohol	-	-	+
22	2-Hexenal	+	+	+
23	Styrene	+	+	+
24	*N*-Nonanal	+	+	+

Table 3 (continued)

Peak No. Figure 12	Name	Sample[1]		
		A	B	C
25	Furfural	-	+	+
26	Phenylacetaldehyde	-	-	+

[1] Signs of sample: the same as shown in Figure 12.

[2] Tentatively identified by t_R of GC.

+ identified, - unidentified

Source: Kasahara *et al.* (1990)

Physical deodorisation

Supercritical carbon dioxide is a superior substance for purifying fish oil. The unique properties of supercritical carbon dioxide provide a selective system for separating deleterious and undesirable substances from fish oil. Odour, pigments and products of autoxidation that contribute to the unattractive and toxic properties of fish oil are readily separated from the major and desired polyunsaturated triacylglycerols (Spinelli *et al*., 1987).

Deodorants for food

There are many substances using as deodorants, both synthesis and from natural extracts. The deodorised substances must be safe for consumption. Then the deodorised substances are usually obtained from plants. Most important active compounds are polyphenolic compounds (Kida *et al*., 2002; Yamaguchi *et al*., 2007). Besides phenolic compound, the other compounds that using as deodorants for foods are shown in Table 4.

Table 4 Deodorant using in food application.

Deodorant substance	Source
cyclodextrin	Japanese Patent Application Laid-Open No.Sho-55-122700/1980
Organic acid such as L-ascorbic acid benzoic acid, gluconic acid folic acid and nicotinic acid	Japanese Patent Application Laid-Open No.Sho-60-136506/1985
Extract of green tea	Japanese Patent Application Laid-Open No.Sho-60-185558/1985
Extract of red beet, cacao, coffee and parsley	Japanese Patent Application Laid-Open No.Sho-60-207664/1985
Perilla extract	Japanese Patent Application Laid-Open No.Sho-60-214726/1985
Persimmon extract	Japanese Patent Application Laid-Open No.Sho-61-87562/1986
Butterbur extract	Japanese Patent Application Laid-Open No.Sho-61-206448/1986
Seaweed extract	Japanese Patent Application Laid-Open No.Sho-62-152463/1987
Finely divided Ganoderma "Mannentake"	Japanese Patent Application Laid-Open No.Sho-62-181048/1987
Tannin fraction of tea leaf extract	Japanese Patent Application Laid-Open No.Hei-2-284997/1990
Mushroom extract	Japanese Patent Application Laid-Open No.Hei-2-277456/1990 and Hei-5-38358/1993
Mugwort extract	Japanese Patent Application Laid-Open No.Hei-7-33636/1995

Source: Kawai *et al*. (2003)

Food antioxidant

Antioxidants are food additives that retard atmospheric oxidation and its degrading effect thus extending the shelf life of food (Somogyi, 2006). They can be classified as primary, secondary and synergists, depending on their particular function. Primary antioxidants donate hydrogen atoms to free lipid radicals to terminate free radicals by forming stable products. Secondary antioxidants function by decomposing lipid peroxides into more stable end products. Synergistic antioxidants are primary oxygen scavenger and metal chelators (Rajalakshmi and Narasimhan, 1996; Eskin and Przybylski, 2001). The examples of these compounds are listed in Figure 13.

Antioxidant capacity assays

The determination of free radical scavenging is important. It is because free radical generation is directly related to the oxidation in food and biological system. The mechanism by which antioxidants can play their protective role can roughly be classified into two types. First mechanism is referred to as hydrogen atom transfer (HAT), the free radical remove a hydrogen atom from the antioxidant (ArOH) that itself becomes a radical. The second mechanism is based on electron transfer (ET), the antioxidant can give an electron to the free radical become itself a radical cation. For this mechanism, the radical cation arising from the electron transfer must be stable, then it does not react with substrate molecule.(Leopoldini *et al*., 2004; Huang *et al*., 2005). The assay based on these two mechanisms are shown in Table 5.

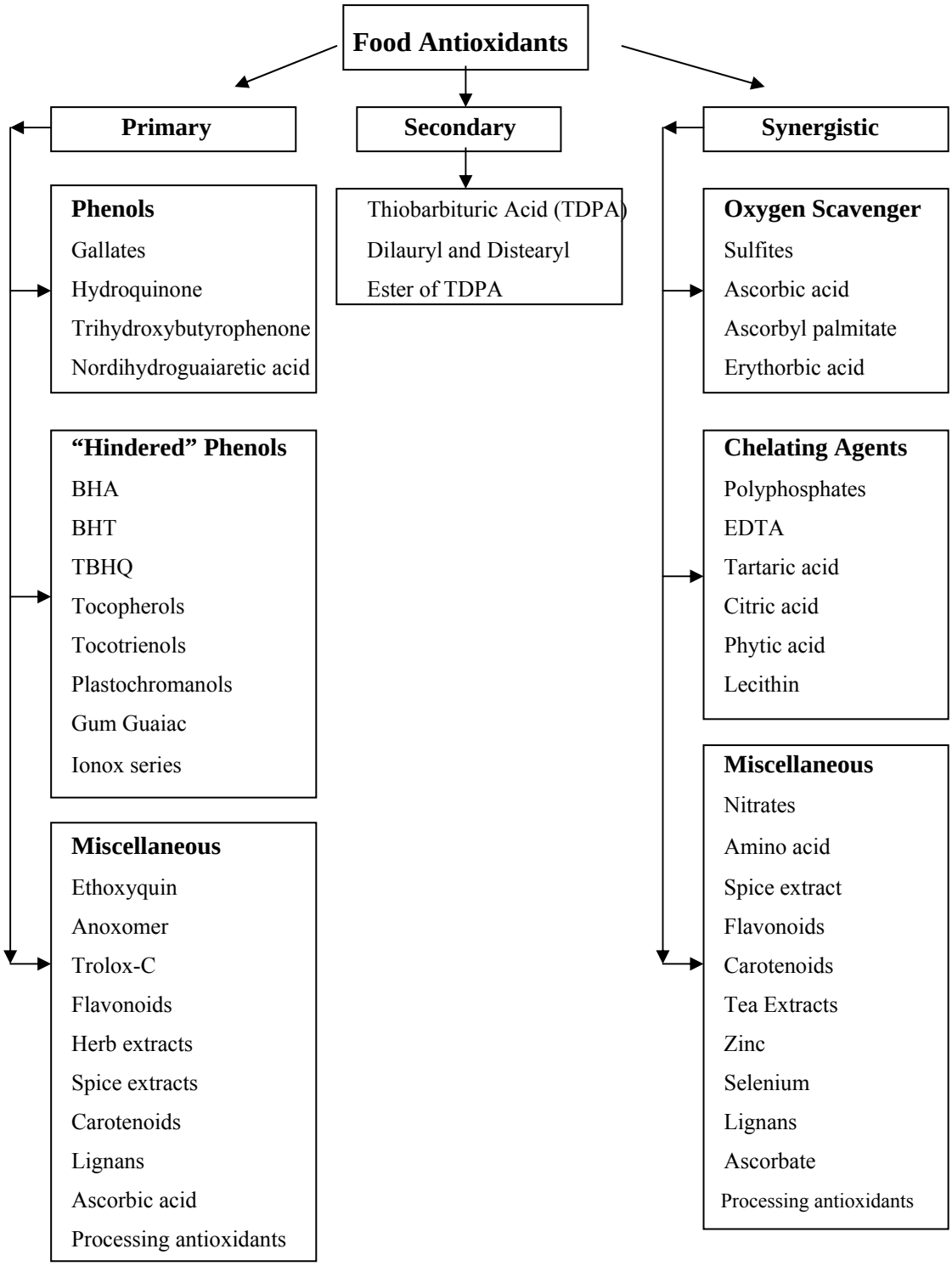


Figure 13 Type of food antioxidants.

Source: Eskin and Przybylski (2001)

Table 5 In vitro antioxidant capacity assays.

Mechanism	Assay
Hydrogen atom transfer (HAT)	ORAC (oxygen radical absorbance capacity)
$R^{\bullet} + ArOH \longrightarrow RH + ArO^{\bullet}$	TRAP(total radical trapping antioxidant parameter)
	Crocin bleaching assay
	IOU (inhibited oxygen uptake)
	inhibition of linoleic acid oxidation
	Inhibition of LDL oxidation
Electron transfer (ET)	TEAC (Trolox equivalent antioxidant capacity)
$R^{\bullet} + ArOH \longrightarrow R^{-} + ArOH^{\bullet+}$	FRAP (ferric ion reducing antioxidant parameter)
	DPPH (diphenyl-1-picrythydrazyl)
	Cupper(II) reduction capacity
	Total phenolic assay by Folin-Ciocalteu reagent
Other assay	TOSC (total oxidation scavenging capacity
	Inhibition of Briggs-Rauscher oscillation reaction
	Chemiluminescence
	electronchemiluminescence

Source: Leopoldini *et al*. (2004); Huang *et al*. (2005)

Oxygen Radical Absorbance Capacity Assay (ORAC). The ORAC method is used to quantify peroxy radical scavenging capacity. This assay base on hydrogen atom transfer (HAT) reaction (Huang *et al*., 2005). The original method work on R-phycoerythrin (R-PE) and β-phycoerythrin (β-PE), a phycobiliprotein which is a target of free radicals, as the probe. The fluorescence decay of these phycobiliprotein is an indication of damage from its reaction with the peroxy radical. The developed method replaces R-PE and β-PE with fluorescein (FL). AAPH {2,2′-azobis(2-amidinopropane) dihydrochloride} was used as peroxyl radical generator ($ORAC_{ROO\cdot}$). As the reaction of FL and peroxyl radical progresses, FL was consumed and FL intensity decrease. If the antioxidant presence, the FL decay was inhibited.

The results were expressed as ORAC units or Trolox equivalent (Delange and Glazer, 1989; Cao *et al*., 1993; Huang *et al*., 2005).

DPPH assay. DPPH assay analyses the hydrogen atoms or electrons' donation ability of the corresponding extracts and the pure standard compounds by measuring from the bleaching of a purple methanol solution of DPPH. As reported elsewhere (Burits and Bucar, 2000; Cuendet *et al.*, 1997), this spectrophotometric assay uses the stable radical DPPH as a reagent.

Total Phenols Assay by Folin-Ciocalteu Reagent. The mechanism of total phenol assay by Folin-Ciocalteu reagent (FCR) base on electron transfer (ET) reaction. The reaction of phenolic compound and FCR occur only under basic condition. Then the sample was adjusted to pH ~10 by the addition of sodium carbonate solution (Huang *et al*., 2005).

Natural antioxidants of herbs and spices

Addition of herbs and spices to food is an established procedure in most cultures. Herbs and spices provide foods with flavouring and preserving agents, including antimicrobial substances and antioxidants. Natural antioxidants become more attractive because of the safety of synthetic antioxidants. Then natural antioxidant present in food and other biological material have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effect. Many compounds in herbs and spices that act as antioxidants are listed in Table 6.

Table 6 Chemical components of spices that act as antioxidants.

Spice	Chemical component
Rosemary	Carsonol, cenozoic acid, rosmanol
Sage	Rosmanol, epirosmanol
Turmeric	Curcumin, 4-hydroxycinnmoyl methane
Clove	Eugenol
Oregano	Phenolic glucoside, caffeic acid, rosmarinic acid, protocatechuic acid
Mace and nutmeg	Myrist phenone
Sesame seed	Sesaminol, δ-tocopherol, sesamol
Ginger	Shogoal, gingerol

Source: Raghavan (2007)

The structure-activity relationship

The major of natural antioxidants are phenolic compounds and the most important groups of natural antioxidant are tocopherol, flavonoids and phenolic acid. Flavonoids constitute a large group of naturally occurring plant phenolic compounds. The position and the degree of hydroxylation are primary important for antioxidant activity of the compounds. Polyphenols were more effective than monophenol. The occurrence of the secondary hydroxyl group in the *ortho* or *papa* position enhances antioxidant activity (Yanishlieva-Maslarova, 2001). Generally, antioxidant activity of flavonoid depended on chemical structure: (a) a catechol (*ortho* diphenolic) moiety of the B-ring, (b) the 2,3 double bond in conjugation with a 4-oxofunction of a carbonyl group in the C-ring and (c) occurrence of hydroxyl group at the 3 and 5 position (Figure 14) (Shi *et al*., 2001).

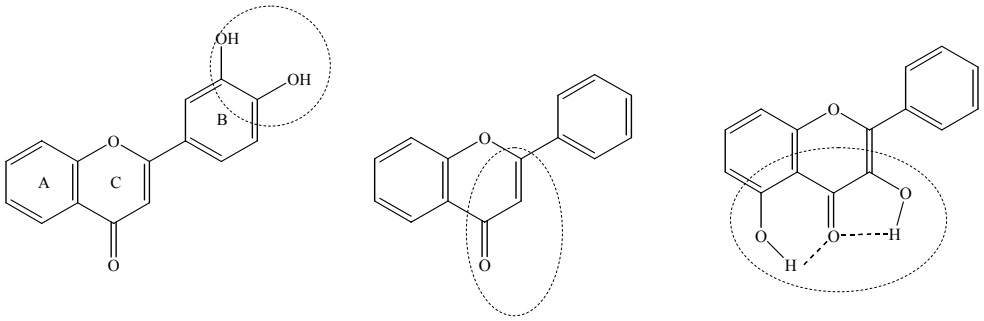


Figure 14 Antioxidant activity-structure relationships of flavonoids.
Source: Shi *et al*. (2001)

Antioxidant property of galangal

The smaller galangal (*Alpinia officinarum* Hance) contained 1,8-cineol, eugenol, chavicol and other phenylpropanoids as the major components of its essential oil (Ly *et al*., 2001). Antioxidative compounds were isolated from the methanol extract of the fresh rhizome of smaller galangal. Seven phenylpropanoids (1-7) were finally obtained by reversed-phase HPLC, and their structures were elucidated by MS and NMR analysis. The structure of compounds 1-7 are characterised as follows (Figure 15).

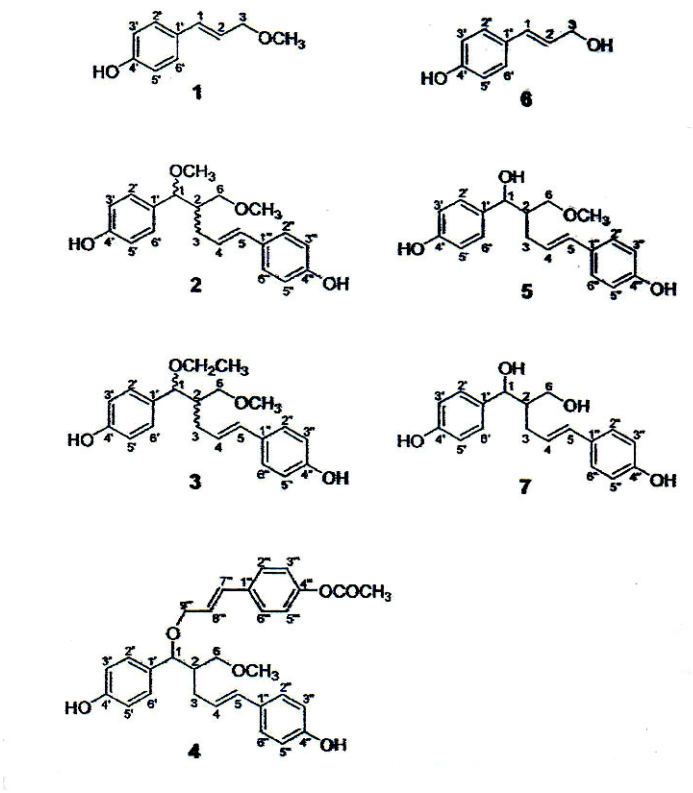


Figure 15 phenylpropanoids (1-7) isolated from smaller galangal (*Alpinia officinarum* Hance).

Source: Ly *et al*. (2003)

Compounds 1-7 are expected to act as antioxidants because they have one or two phenolic hydroxyl groups in the molecule. The differences in antioxidative behavior of the isolated compounds were observed in their inhibition of methyl linoleate autoxidation. Compound 1 and 6 each had one phenolic hydroxyl group, the antioxidative activity of 6 was higher than that of 1. Compounds 2-5 exhibited lower antioxidative activity than that of α-tocopherol, a well-known chain-breaking antioxidant. Compound 7 had almost the same activity as α-tocopherol. These results indicated that the number of hydroxyl group in the phenylpropanoid molecule might influence its antioxidative activity (Ly *et al*., 2003).

For greater galangal (*Alpinia galanga*), Two phenolic compounds were isolated from chloroform extract of the rhizome. Chemical analysis of these

compound yield *p*-hydroxy cinnamaldehyde and [di-(*p*-hydroxy-cis-styryl)], as shown in Figure 16 (Barik *et al*., 1987).

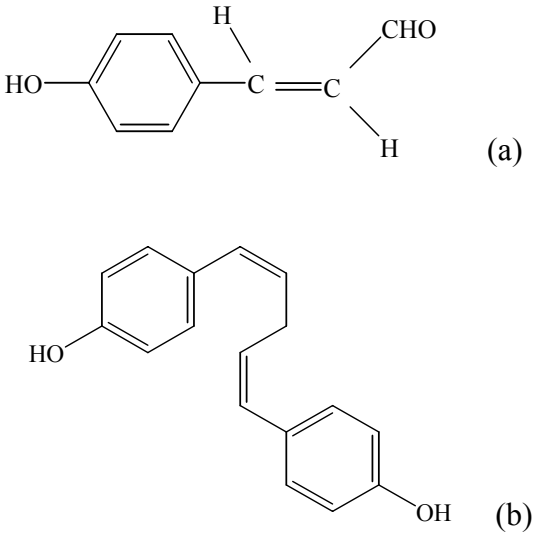


Figure 16 Structure of *p*-hydroxy cinnamaldehyde (a) and [di-(*p*-hydroxy-cis-styryl)] (b).

Source: Barik *et al*. (1987)

Antioxidant activity of galangal extract (4 mg in 4 mL of 99.5% ethanol) was stronger than α-tocopherol (4 mg in 4 mL of 99.5% ethanol) when fresh rhizome was crushed and soaked for 18 days in acetone (2 L/kg) at 23 °C and remove solvent (Jitoe *et al*.,1992), the result was shown in Figure 17. Ethanolic extract of galangal acted as radical scavenger. It exhibited strong superoxide anion scavenging activity, Fe^{2+} chelating activity, and reducing power in a concentration-dependent manner (Berghofer and Juntachote, 2005).

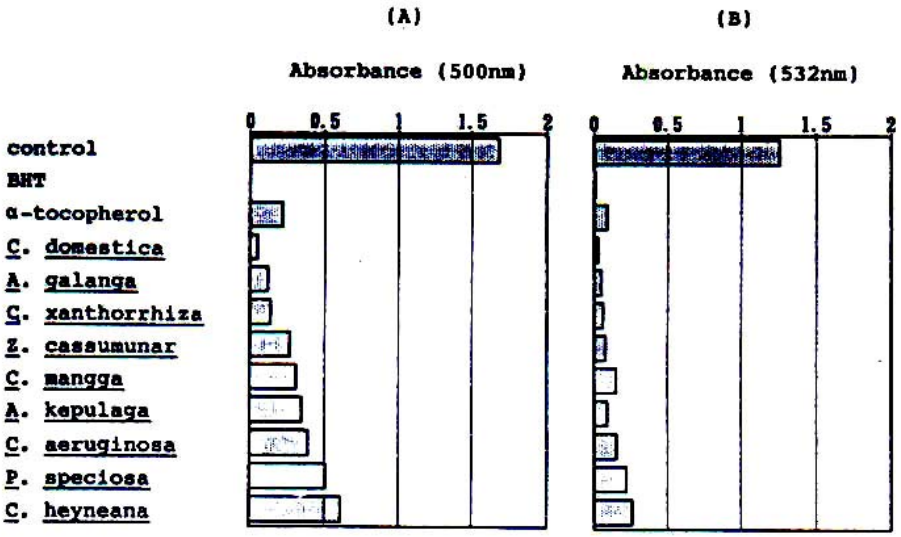


Figure 17 Antioxidant activity of acetone extracts (4 mg) of nine tropical gingers: (A) thiocyanate method; (B) TBA method. As reference sample, BHT (4 mg) and α-tocopherol (4 mg) were used.

Source: Jitoe *et al.*(1992)

The applications of galangal as natural antioxidant were performed in minced beef (Cheah and Abu Hasim, 2000) and cooked ground pork (Juntachote *et al.*, 2006b). Compare ethanol extract of galangal with dried powder of galangal, dried powder of galangal showed high efficiency to minimise lipid oxidation (Juntachote *et al.*, 2006b).

MATERIALS AND METHODS

Materials

1. Raw materials

Fresh rhizome of galangal (*Alpinia galanga*) was purchased from local market in Bangkok, Thailand.

2. Chemical Reagents

1. Acetone, ethanol and methanol (Merck KgaA, Germany)
2. Dichloromethane, HPLC grade (Merck KgaA, Germany)
3. Methanol, HPLC grade (LAB-SCAN, Thailand)
4. Trimethylamine (Sigma-Aldrich, USA)
5. (*Z*)-4-Heptenal (Fluka, Switzerland)
6. 2-undecanol (Sigma-Aldrich, USA)
7. Flavour reference standards (Sigma-Aldrich, USA and Fluka, Switzerland)
8. *n*-Alkane standards (C6-C30) (Aldrich Chemical Co. Ltd., USA.)
9. *p*-coumaric acid (Sigma-Aldrich, Germany)
10. Myricetin (Sigma-Aldrich, France)
11. (+)-Catechin (Sigma-Aldrich, Japan)
12. Gallic acid (Fluka, Spain)
13. Folin-Ciocalteu's phenol reagent (Fluka, Switzerland)
14. 2,2-Diphenyl-1-picrylhydrazyl, DPPH (Sigma-Aldrich, USA)
15. Fluorescein sodium salt, FL (Sigma-Aldrich, USA)
16. (+)-∝-Tocopherol (Sigma-Aldrich, USA)
17. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Trolox (Sigma-Aldrich Chemie GMbH, Germany)
18. 2,2′-azobis (2-methylpropionamidine) dihydrochloride, AAPH (Sigma-Aldrich Chemie GMbH, Germany)

19. Randomly methylated beta cyclodextrin (RMCD), technical grade, (Cyclodextrin Technologies Development, Inc., USA)
20. Butylated hydroxyanisole (Fluka, Switzerland)
21. Sodium sulphate anhydrous (Ajax Finechem, Australia)
22. Sodium chloride (Ajax Finechem, Australia)
23. Sodium carbonate (BDH Laboratory Supplies, England)
24. Sodium nitrite (Ajax Finechem, Australia)
25. Aluminium chloride (Ajax Finechem, Australia)
26. Sodium hydroxide (Merck, Germany)
27. Deodorised distilled water
28. Nitrogen gas
29. Helium carrier gas (ultra high purity)
30. Liquid nitrogen

3. Equipments and Instruments

1. Essential oil extractor (Becthai Bangkok Equipment and Chemical Co., Ltd., Thailand)
2. Gas chromatography, HP 6890 (Agilent Technologies Inc., USA)
3. Mass spectrometer, HP5973 (Agilent Technologies Inc., USA)
4. Agilent 1100 HPLC Series system (Agilent Technologies Inc., USA).
5. Freezer -40˚C, MDF-435 (Sanyo, Japan)
6. High vacuum distillation unit, B62426952 (Edwards, England)
7. Spectrophotometer, Spectro 23 (LaboMed, Inc., USA)
8. Fluorescent microplate reader, FLUOstar OPTIMA (BMG LABTECH, Germany)
9. 96 Well black microplates, (BMG LABTECH, Germany)
10. Freeze dryer, Heto FD 2.5 (Heto Lab Equipment, Denmark)
11. Rotary evaporator, Rotavapor R-114 (Buchi, Switzerland)
12. Laboratory test sieve 60 mesh (Endecotts Ltd., England)
13. Refrigerated circulator, EYELA COOL ACE CA-1100 (Tokyo Rikakikai Co. Ltd., Japan)

14. Water bath (Memmert, Germany)
15. Distillation unit

Methods

Part 1: The study on composition of galangal extracts

1. Sample preparation and extraction

Fresh rhizome of galangal was purchased from local market in Bangkok, Thailand. Galangal was cleaned with tap water before used. Four types of the extracts; crude extract, water extract, ethanol extract and essential oil, were obtained. The crude extract and the essential oil samples were obtained from fresh rhizome of galangal (Figure 18a) whereas the water extract and the ethanol extract samples were obtained from dry powder of galangal, received from lyophilised fresh rhizome (Figure 18b).

(a) (b)

Figure 18 Fresh rhizome (a) and dry powder of galangal (b)

1.1 Crude extract

To prepare for the crude extract, rhizomes were cut into small pieces and disintegrated using a blender to form paste. Galangal paste was squeezed through two

layers of muslin cloth to obtain the liquid crude extract. The liquid crude extract was immediately isolated for volatile compounds.

1.2 Water extract

Fresh galangal rhizomes were lyophilised using a freeze dryer (Heto FD 2.5, Heto Lab Equipment, Denmark). The fine powder (60 meshes) of dried galangal was obtained using a blender and passed through a 60 meshed sieve. Ten grams of dried powder was refluxed with 100 mL distilled water for 3 h at 80˚C. The mixture was cooled to room temperature and filtered through a sinter glass funnel (No.1). The residue was then re-refluxed with 100 mL distilled water under the same condition. The two extracts were combined and concentrated in a rotary evaporator at 60 °C. The concentrated extract was freezed dried and kept in a glass container at – 40 °C.

1.3 Ethanol extract

Fresh galangal rhizomes were lyophilised using a freeze dryer. The fine powder (60 mesh) of dried galangal was obtained using a blender and passed through a 60 meshed sieve. Galangal powder was extracted using 50 % ethanol (v/v) in water. This condition based on the preliminary study and Liyana-Pathirana and Shahidi (2005). The extraction was performed for 1 h in a shaking waterbath at 50 °C. The solid-to-solvent ratio was 1:10. The extract was filtered through a sinter glass funnel (No.1). The residue was re-extracted under the same condition. The two extracts were combined and concentrated in a rotary evaporator at 40 °C. The dried extract was obtained using a freeze dryer. The extract was then kept in a glass container at – 40 °C.

1.4 Essential oil

Essential oil was obtained from the fresh galangal by steam distillation using a glass essential oil extractor. The fresh rhizomes were cut into small pieces.

Then, 320 g of galangal was comminuted with 400 mL water. The slurry was prepared in a 1-L round bottom flask and steam-distilled in a glass steam distillation extractor for 4 h (Figure 19). The essential oil was filtered through anhydrous sodium sulphate and kept in a glass container at – 40 °C.

Figure 19 Essential oil extraction of galangal rhizomes.

2. Volatile compounds analysis

2.1 Crude extract

Six grams of the crude extract and 6 g of NaCl were dissolved in 14 mL deodorised water and added with 20 µL of the internal standard (10 µg/mL of 2-undecanol in dichloromethane) in a 250 mL glass bottle. Then, the solution was

extracted with 20 mL dichloromethane. The extraction time was 30 min on a stirring plate. The sample was then transferred to a 250 mL round bottom flask. The round bottom flask was immersed in liquid nitrogen until the sample was frozen. The flask was equipped with the high vacuum apparatus (Figure 20). The vacuum was applied (ca.10^{-5} Torr) to the system. After the process continued for 1 h, the flask was warmed in the 50 °C water for 1 h. The volatile extract was recovered from the first receiving tube and concentrated to 5 mL under a gentle stream of nitrogen gas. The concentrated extract was dried over anhydrous sodium sulphate and concentrated to 1 mL under a gentle nitrogen stream and stored in a glass vial at -40 °C until analyses.

Figure 20 High vacuum distillation unit.

Qualitative and quantitative analyses of the extracts were performed using an HP 6890 gas chromatograph equipped with an HP 5973 Mass Selective Detector (Figure 21). Mass Selective Detector was operated at 70 eV, scanning speed 1 s over 40-300 amu range and ion source temperature of 180 °C. A non-polar column, HP-5 capillary column (60 m x 0.25 mm x 0.25 µm film thickness), was used under the following conditions: inlet temperature, 200 °C; detector temperature, 280 °C; oven temperature programme, initial temperature 35 °C rate 10 °C/ min to 250, hold for 10

min. Helium was used as carrier gas at the flow rate of 1.5 mL/min. The split ratio was 1:5. A polar-column, an HP-FFAP (60 m x 0.25 mm x 0.25 µm film thickness), was used under the following conditions: split-splitless inlet at 200 °C; detector temperature, 280 °C; oven temperature programme, initial temperature 40 °C, rate 6 °C/ min to 210, and hold for 10 min. Helium was used as carrier gas at the flow rate of 1.3 mL/min. The split ratio was 1:5.

Figure 21 Gas chromatography-mass spectrometry (GC-MS).

2.2 Water extract and ethanol extract

One gram of each extract (water extract and ethanol extract) was dissolved in 25 mL of deodorised water and was added with 20 µL of the internal standard (10 µg/mL of 2-undecanol in dichloromethane) in a 250 mL glass bottle. Then, the solution was extracted with 25 mL dichloromethane for 30 min on a stirring plate. The extraction was consecutively transferred to a 250 mL round bottom flask. The extract was immersed in liquid nitrogen until it was frozen. Volatile compounds and the solvent were vacuum-distilled at about 10^{-5} Torr. After the process continued for

1 h, the flask was warmed in the 50 °C water for 1 h. The volatile extract was recovered from the first receiving tube and concentrate to 5 mL under a gentle stream of nitrogen gas. The concentrated extract was dried over anhydrous sodium sulphate and was concentrated to 1 mL under a gentle stream of nitrogen gas and stored in a glass vial at -40 °C until analyses.

Volatile compounds were analysed using a GC-MS. For nonpolar column, HP-5 capillary column (60 m x 0.25 mm x 0.25 µm film thickness) was used under the same conditions as 2.1. For polar-column, HP-FFAP capillary column (60 m x 0.25 mm x 0.25 µm film thickness), modified previous condition (2.1) to gain better separation under the following conditions: inlet temperature, 200 °C; detector temperature, 280 °C; oven temperature programme, initial temperature 40 °C rate 10 °C/ min to 210, and hold for 30 min. Helium was used as carrier gas at the flow rate of 1.5 mL/min. The split ratio was 1:5.

2.3 Essential oil

Qualitative and quantitative analysis of the essential oil were performed using GC-MS. Diluted samples (1/100, v/v, in dichloromethane) of 1.0 µL were injected manually to GC. 2-Undecanol (10 mg/mL dichloromethane) was used as an internal standard. The internal standard was added to the sample at the concentration of 10 µL/mL. Non-polar and polar-columns were used under the same condition as 2.1.

2.4 Volatile compounds identification and quantification

Compounds were identified based on matching mass spectra of the unknowns with mass spectra from the Wiley275 Mass Spectra Database and by comparison of the retention indices related to the retention times of *n*-alkane series. Some compounds were also confirmed using authentic standards that were

commercially available. The linear retention indices (LRI) or retention index (RI) was calculated as follows (Bot and Schrijvers, 1994):

$$LRI = 100n + 100(t_{R,i}-t_{R,n})/ (t_{R,n+1}-t_{R,n})$$

Where: n = number of carbon atoms of *n*-alkane

$t_{R,i}$ = retention time of component i

$t_{R,n}$ = retention time of *n*-alkane with n carbon atoms

$t_{R,n+1}$ = retention time of *n*-alkane with n+1 carbon atoms

Relative concentrations of the positively identified compounds were determined using the MS response factors for each component related to the internal standard. Flavour reference standards were used to confirm some compound, as following: linalool, geranyl acetate, α-terpineol, 1,8-cineol, α-pinene, δ-3-carene, *p*-cymene, limonene, β-caryophyllene.

2.5 Odour activity value (OAV)

Odour activity value (OAV) was calculated by dividing the concentration observed in the sample by the published aroma threshold for that compound (Rouseff and Naim, 2000).

3. Phenolic compound analysis

3.1. Determination of total phenolic compounds

Total phenolic content of galangal extracts were determined by a modification of the Folin-Ciocalteu method, according to Wolfe *et al*. (2003). Folin-Ciocalteu reagent (0.125 mL) was added to the test tube containing 0.5 mL of deionised water and 0.125 mL of a known dilution of the extract. The reaction was allowed for 6 min. After that, 1.25 mL of 7% sodium carbonate solution was added to the test tube and the mixture was diluted to 3 mL with deionised water. The mixture

was allowed to stand for 90 min. The absorbance was then measured at 760 nm. The content of phenolic compounds was expressed as gallic acid equivalent (GAE).

3.2. Determination of flavonoid content

The determination of flavonoids was performed according to the colorimetric assay described by Wolfe *et al*. (2003). A volume of 0.25 mL of known dilution extract (50%, 5%, 1% and 12.5% for crude extract, water extract, ethanol extract and essential oil, respectively) and 1.25 mL of distilled water were added to a test tube. Then 0.075 mL of 5% sodium nitrite solution was added to the mixture. The reaction was allowed to stand for 5 min. After that, 0.15 mL of 10 % aluminium chloride was added. After 6 min, 0.5 mL of 1 M sodium hydroxide was added and the mixture was diluted with 0.275 mL of distilled water. The absorbance was read immediately at 510 nm. A calibration curve was prepared with (+)-catechin, and the flavonoid content was expressed as (+)-catechin equivalents.

3.3. HPLC analysis

Identification and quantification of non-volatile phenolic compounds were achieved using an Agilent 1100 HPLC Series system (Figure 22). The ethanol extract was dissolve in methanol and filtered through a 0.45 μm regenerated cellulose membrane filter. Samples were separated on a reversed-phase Zorbax SB-C18 analytical column (100 x 3.0 nm i.d., 3.5 μm particle). The column was operated at 48 °C. The detection of the compounds used the variable UV-VIS detector, performed at 280 nm. The mobile phase was methanol (solvent A) and a buffer solution (solvent B). The buffer solution was prepared by dissolved potassium dihydrogen phosphate (40 mM) in DI water. The pH of the solution was adjusted to 2.3 with 85% orthophosphoric acid. The binary gradient started with a linear gradient from 5% to 42% of solvent A in the first 35 min, followed by the isocratic elution with 42% solvent A for the next 3 min. The flow rate was 1 mL/min and the injection volume was 10 μL. The solutions were filtered through a 0.45 μm nylon membrane filter and

degassed in an ultrasonic bath before used. Retention times and spectra were compared to those of the pure standards.

Figure 22 High performance liquid chromatography (HPLC).

Part 2 Fishy odour deodorisation property of the extract.

1. Preparation of fishy odour deodorised solution

Four galangal extracts (crude extract, water extract, ethanol extract and essential oil) were used to study on their fishy odour masking property. Two authentic compounds which have been reported as fishy odour compounds, TMA (Cadwallader *et al*., 1995; Prost *et al*., 1998; Fukami *et al*., 2002) and (*Z*)-4-heptenal (Cha and Cadwallader, 1998; Hartvigsen, *et al*., 2000), were used. The concentrations of TMA and (*Z*)-4-heptenal solutions used were 200 ppm and 40 ppm, respectively. These concentrations chosen were based on the concentrations reported in fish by Cha and Cadwallader (1998) and Miller *et al.* (1972). The concentrations of crude extract, water extract, ethanol extract and essential oil (10 % essential oil in ethanol) in fishy odour solutions were 0.0992 mL/mL, 10.21 mg/mL, 9.35 mg/mL and 0.006 mL/mL,

respectively. These concentrations of the extracts used were equal to 3.2 g of fresh galangal rhizome that previously shown the ability to reduce fishy odour in the preliminary study.

2 Sensory evaluation of the fishy deodorised solution

The difference from control test was used to determine the difference of the samples (Meilgaard *et al.*, 1999). Ten milliliters of the fishy odour deodorised solution in a small glass was coded with three numeric system. TMA (200 ppm) and (*Z*)-4-heptenal (40 ppm) were used as control. Thirty panelists evaluated the differences. The scale of the difference was as follows:

0	=	No difference
1	=	Very slight difference
2	=	Slight/moderate difference
3	=	Moderate difference
4	=	Moderate/large difference
5	=	Large difference
6	=	Very large difference

First, serve an idientified control sample. Then serve test samples, one of which is the blind control. Ask the panelists to evaluate the size of the difference between each sample and the control and rate the difference with scale. The mean difference from the control for each sample and for the blind control was calculated. The results were evaluated by the analysis of variance. The Dunnett's test was used for multiple comparisons with the control.

3. Effect of volatile compounds on fishy odour deodorisation property

Principal component analysis (PCA) was used. Factors with Eigen values greater than 1 were selected for grouping. The varimax rotation method was applied. PCA transformed the original data matrix (volatile compound) into approximation of

volatile compound groups. Correlation coefficients (*r*) were calculated to identify the possible association between groups of volatile compound and fishy odour reduction data.

4. Effect of phenolic compounds on fishy odour deodorisation

The correlation of deodorisation and phenolic compounds included flavonoid was measured. The effect of phenolic compound and flavonoid on fishy odour deodorisation was evaluated.

Part 3: Antioxidants property of galangal extracts

1. Antioxidant activity assay of the extracts

Antioxidant activities of three extracts (water extract, ethanol extract and essential oil) from galangal rhizomes were studied. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and ORAC methods were used to determine the possible antioxidant activities. For essential oil sample, the solution was prepared by dissolving essential oil in ethanol (at various concentrations, 0-25% w/v). The diluted samples were then analysed for their antioxidant activities. For ethanol extract and water extract, the sample solutions were prepared by dissolving the extracts at various concentrations in distilled water before the antioxidant activity testes. The evaluated concentrations were 0-1% w/v for ethanol extract and 0-5% w/v for water extract.

1.1 $ORAC_{FL}$ method

The ORAC assay in this study was followed the method described by Huang *et al.* (2002) and Prior *et al.* (2003).

1.1.1 ORAC assay on plate reader

ORAC assay of hydrophilic and lipophilic were carried out on a FLUOstar plate reader that was equipped with an incubator and an injection pump. The temperature of the incubator was set at 37 °C. AAPH was used as a peroxyl generator and Trolox was used as a standard.

A stock fluorescein solution (stock #1) was prepared by dissolving 0.0225 g of fluorescein (FL) in 50 mL 0.075 M phosphate buffer (pH 7.0). A second stock solution was prepared by diluting 50 μL of stock solution #1 in 10 mL of phosphate buffer. A 320-μL portion of solution #2 was added to 20 mL of phosphate buffer, of which 200 μL was added to each well. This provided 7.5 nmole of fluorescein per well, or a final concentration of 14 μM.

A stock standard of Trolox (500 μM) was aliquoted into small vials for storage at – 40 °C until use. In the standard assay, 20 μL Trolox calibration solution (6.25, 12.5, 25, 50 μM) in phosphate buffer (0.075 M, pH 7.0) were pipetted into appropriate wells. A new set of stock Trolox vials were removed from the freezer daily for use. This study used a 96-well microplate.

Fresh AAPH solution was prepared for each run. Old AAPH solution was flushed from the syringe, and the syringe was primed with new AAPH before starting the run. In addition, to optimise the signal amplification to give the maximum sensitivity, a gain adjustment was performed by manually pipetting 200 μL of FL into a designation well before starting the programme, also the other plates. During cycle 4, the pump was programmed to inject 75 μL of AAPH (17.2 mg/mL) into the respective well to give a final AAPH concentration of 9.4 μmole/well. The plate contents were mixed and shaked for 8 s followed each injection and/or reading.

1.1.2 Lipophilic $ORAC_{FL}$

For the lipophilic antioxidant assay, the extract was dissolved in 250 μL of acetone and then diluted with 750 μL of a 7% RMCD solution (50% acetone /

50% water, v/v) and was shaken for 1 h at room temperature on an orbital shaker at 400 rpm. Any further dilution was prepared with 7% RMCD solution. The 7% RMCD solution was used as a blank and to dissolve the Trolox standard for the lipophilic antioxidant assay. The sample (20 μL) and of the standard (20 μL) were added to the 96-well microplate. Fluorescein solution (200 μL) was added by manually pipetting into the microplate. Seventy five microlitres of AAPH solution (17.2 mg/mL, 9.4 μmole/well) was added by injectors in the microplate reader. The reading was initiated immediately.

1.1.3 Hydrophilic $ORAC_{FL}$

In hydrophilic antioxidant assay, the extract was dissolved and further diluted with phosphate buffer. A 20-μL portion of the sample was added to a well in the 96-well microplate. The fluorescein solution and AAPH solution were added in the same manner as the lipophilic antioxidant assay, except that only 37.5 μL of the AAPH solution was added to the assay mixture.

1.1.4 Calculation

The final $ORAC_{FL}$ values were calculated by using a regression equation ($Y = a + bX$, linear; or $Y = a + bX + cX^2$, quadratic) between Trolox concentration (Y, μM) and the net area under the FL decay curve (X). Linear regression was used in the range of 6.25-50 μM Trolox. Data were expressed as micromole of Trolox equivalents (TE) per liter or per gram of sample (μmol TE/g μmole TE/L). The area under curve (AUC) was calculated as

$$AUC = (0.5 + f5/f4 + f6/f4 + f7/f4 + \ldots + fi/f4) \times CT \qquad (1)$$

Where $f4$ = initial fluorescence reading at cycle 4, fi = fluorescence reading at cycle i, and CT = cycle time in minutes. The net AUC was obtained by subtracting the AUC

of the blank from that of a sample. The data were analysed by Microsoft Excel to apply equation 1 and calculated the AUC.

1.2 DPPH Assay

The DPPH assay was carried out as described by Tepe *et al*. (2005). Samples were diluted to various concentrations (essential oil was diluted in ethanol, water extract was diluted in water and ethanol extract was diluted in water). For BHA, the solution was dissolved in methanol. Fifty-microliter aliquots of various concentrations of the samples were added to 5 mL of 0.004 % methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The purple color bleaching of the DPPH reagent was evaluated as positive antioxidant activity. Inhibition of free radicals by DPPH in percent (*I* %) was calculated in the following way:

$$I\,\% = [(A_B - A_A)/A_B] \times 100$$

Where $I\,\%$ = Inhibition percentage

A_B = absorbance value of blank after 30 min

A_A = absorbance value of sample after 30 min

Extraction concentration that had 50% inhibition (IC_{50}) was calculated from the plot of the inhibition percentage against the extraction concentration. The assay was carried out in triplicate.

2. Antioxidant activity of water extract, ethanol extract and essential oil incorporated with BHA

Antioxidant activity of three extracts (water extract, ethanol extract and essential oil) from galangal rhizome incorporated in butylated hydroxyanisole (BHA) was studied. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used to determined the antioxidant activity. For essential oil, sample solution was prepared by dissolved various concentrations of essential oil in 0.02% BHA solution (w/v methanol). The

concentrations of essential oil ranged from 0 to 25% (w/v). For ethanol extract and water extract, the sample solutions were prepared by dissolving various concentrations of the extract in 0.01% BHA solution (w/v methanol). The concentrations of the ethanol extract and the water extract ranged from 0 to1.0% (w/v) and from 0 to 5.0% (w/v), respectively.

3. Effect of phenolic compounds on antioxidant activity

The correlation between antioxidant activity and phenolic compounds included flavonoids was studied. The effect of phenolic compounds and flavonoids on antioxidant activity was evaluated.

4. Statistical analysis

The data were subjected to analysis of variance (ANOVA), and the significant of the difference between means was determined by Duncan's multiple-range test ($p \leq 0.05$) using statistical analysis software (SPSS version 12.00, SPSS Inc., Chicago, IL). In addition, Pearson correlation was also performed.

RESULTS AND DISCUSSION

Part 1: The study on composition of galangal extracts

Four types of galangal extract in this study were crude extract, water extract, ethanol extract and essential oil. Crude extract, water extract and ethanol extract were hydrophilic whereas essential oil was lipophilic. The picture of water extract, ethanol extract and essential oil is shown in Figure 23. Crude extract and essential oil were obtained from fresh rhizome of galangal where as water extract and ethanol obtained from dry powder of galangal. Fresh galangal rhizomes were lyophilised using a freeze dryer to obtained dry powder. Freeze drying was performed to minimize the reaction and easy to handle sample while it was storage, before analysed.

Figure 23 Water extract (WE), ethanol extract (EE) and essential oil (ESO).

1. Volatile compounds of galangal extracts

1.1 Crude extract

The volatile composition of crude galangal extract was analysed by GC/MS. The results are summarised in Table 7. Volatiles of the crude extract constituted of 60.92 and 55.61 μg/g fresh galangal for cyclic monoterpenes and cyclic sesquiterpenes. The most abundant volatile compound in the crude extract was the compound that had M^+ at *m/z* = 234 (32.65%, Appendix Table 1), followed by 1,8-cineol (17.33%, Appendix Table 1) . The overall odour characteristic of the crude extract was the same as that of fresh galangal. This odour characteristic of crude extract was similar to the odour of 1,8-cineol that was strong, camphoraceous, cool and fresh. 1,8-Cineol displayed the high odour active value (OAV) in Table 8. 1,8-Cineole, linalool, geranyl acetate, eugenol and chavicol acetate that had been reported as galangal potent odorants (Mori *et al*.,1995) were found in this extract

It should be noted that compound that had M^+ at *m/z* = 234 was tentatively identified as 1′-acetoxychavicol acetate. This compound was found only in crude extract and ethanol extract sample. The mass spectrum identification of this compound (Figure 24) was based on the pattern of 1′-acetoxychavicol acetate (Figure 25) isolated from methanol extract of galangal seed (Mitsui, *et al*., 1976). 1′-Acetoxychavicol acetate from *n*-pentane/diethyl ether extract of dried slice rhizome of *A. galanga* showed mass spectra [*m/z* (relative intensity %)] as following: 150 (100), 132 (96), 43 (93), 40 (65), 133 (51), 192 (38), 131 (28), 77 (24), 149 (19) and 105 (19) (Janssen and Scheffer, 1985). 1′-Acetoxychavicol acetate was also isolated from galangal oleoresin extracted with pentane (Yang and Eilerman, 1999). The most abundant mass spectra peaks of this compound were 192, 150, 132 and 43. The molecular weight was estimated as 234. The characteristic fragments include CH_3-$C{\equiv}O^+$ at *m/z* 43, from methyl ketones $C_6H_5^+$ at *m/z* 77, from phenyl derivative and loss mass 42 (234-192, 192-150) due to the formation of $CH_2{=}C{=}O$ by McLafferty rearrangement (Yang and Eilerman, 1999).

Table 7 Concentrations of volatile compounds from the extracts of galangal rhizome.

No.	Compound	RI		ID	Concentration (μg/g fresh galangal)			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
	Acyclic monoterpenes and derivatives							
	Alcohols							
1	Linalool	1102	1540	a, b, c9, d	0.04 ± 0.01	-	0.05 ± 0.00	1.36 ± 0.12
2	Geraniol	1277	-	a, b, c8	-	-	-	0.06 ± 0.00
	Esters							
3	Neryl acetate	1365		a, b, c2	-	-	-	1.50 ± 0.39
4	Geranyl acetate	1383	1755	a, b, c9, d	0.39 ± 0.01	-	0.69 ± 0.23	8.24 ± 2.47
	Hydrocarbons							
5	Myrcene	992	1162	a, b, c9	-	-	-	12.88 ± 1.22
6	(*E*)-β-Ocimene	1050	1248	a, b, c9	-	-	0.25 ± 0.35	0.76 ± 0.12
	Cyclic monoterpenes and derivatives							
	Alcohols							
7	*trans*-Sabinene hydrate	1076	1457	a, b, c1	0.46 ± 0.17	-	0.16 ± 0.01	0.15 ± 0.03
8	*cis*-Sabinene hydrate	1107	1546	a, b, c1	0.32 ± 0.12	-	0.18 ± 0.01	-
9	*trans*-*p*-Menth-2-en-1-ol	1131	1559	a, b, c9	-	-	-	0.36 ± 0.00

Table 7 (continued)

No.	Compound	RI		ID	Concentration (μg/g fresh galangal)			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
10	*cis*-Verbenol	1153	-	a, b	0.21 ± 0.06	-	-	0.78 ± 0.21
11	*trans*-Verbenol	1159	-	a, b	-	-	0.18 ± 0.00	-
12	*p*-Menth-1,5 diene-8-ol	1161	-	a, b, c2	-	-	-	0.19 ± 0.00
13	δ-Terpineol	1181	1678	a, b, c6	-	-	0.63 ± 0.03	-
14	Terpinene-4-ol	1190	-	a, b, c9	2.03 ± 0.66	-	0.46 ± 0.06	16.11 ± 1.49
15	*p*-Cymene-8-ol	1194	1851	a, b	0.71 ± 0.22	-	0.48 ± 0.07	1.02 ± 0.42
16	α-Terpineol	1202	-	a, b, c9, d	2.44 ± 0.76	-	2.03 ± 0.10	13.81 ± 3.76
17	*cis*-Piperitol	1207	-	a,b	-	-	-	0.36 ± 0.23
18	*trans*-Carveol	1230	-	a, b	-	-	-	0.98 ± 0.43
	Aldehydes							
19	α-Campholene aldehyde	1137	-	a, c6	-	-	-	0.37 ± 0.06
	Ketones							
20	Camphor	1164	1537	a,b	-	-	0.07 ± 0.09	
	Esters							
21	Myrtenyl acetate	1337	-	a, b	-	-	-	0.37 ± 0.02

Table 7 (continued)

No.	Compound	RI		ID	Concentration (μg/g fresh galangal)			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
22	*trans*-Carvyl acetate	1343	-	a, b	0.09 ± 0.01	-	-	2.29 ± 0.91
23	Thymyl acetate	1361	1856	a, b, c7	0.064 ± 0.02	-	0.12 ± 0.06	1.09 ± 0.01
24	*cis*-Carvyl acetate	1369	-	a, b, c2	-	-	-	3.82 ± 0.22
25	Exo-2-hydroxycineole acetate	1375	-	a, c6	1.76 ± 0.46	0.19 ± 0.01	1.01 ± 0.39	1.10 ± 0.18
26	Bornyl acetate	-	1581	a, b, c4	0.18 ± 0.02	-	0.22 ± 0.00	2.94 ± 0.02
27	Carvacryl acetate	-	1881	a, c6	0.05 ± 0.00	-	0.26 ± 0.01	-
	Ethers							
28	1,8-Cineol	1041	1216	a, b, c9, d	45.63 ± 19.23	0.06 ± 0.02	0.86 ± 0.14	239.77 ± 34.65
29	Exo-2-hydroxycineol	1235	1862	a, c6	0.45 ± 0.10	0.38 ± 0.03	0.50 ± 0.05	-
30	Dehydro-1,8-cineol	-	1190	a, b	0.26 ± 0.09	0.04 ± 0.00	0.05 ± 0.01	-
	Hydrocarbons							
31	α-Thujene	932	1034	a, b, c9	0.21 ± 0.08	-	-	5.57 ± 1.66
32	α-Pinene	942	1032	a, b, c9, d	1.62 ± 0.69	-	-	35.69 ± 3.85
33	α-Fenchene	956	1063	a, b, c2	-	-	-	0.20 ± 0.02
34	Camphene	958	1071	a, b, c9	-	-	0.04 ± 0.03	0.97 ± 0.09
35	Verbenene (Pinadinene)	963	-	a, b, c2	-	-	-	0.20 ± 0.04

Table 7 (continued)

No.	Compound	RI		ID	Concentration (μg/g fresh galangal)			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
36	Sabinene	981	1124	a, b, c9	1.48 ± 0.61	-	-	23.32 ± 2.98
37	β-Pinene	986	1112	a, b, c9	0.55 ± 0.22	-	-	12.78 ± 1.37
38	α-Phellandrene	1011	1165	a, b, c9	-	-	-	0.74 ± 0.12
39	δ-3-Carene	1018	1149	a, b, c1, d	-	-	-	0.04 ± 0.00
40	α-Terpinene	1024	1180	a, b, c9	0.11 ± 0.04	-	-	5.77 ± 0.67
41	*p*-Cymene	1032	1270	a, b, c9, d	0.17 ± 0.06	-	-	3.81 ± 0.33
42	Limonene	1038	1203	a, b, c9, d	1.75 ± 0.74	-	0.03 ± 0.01	24.35 ± 2.72
43	γ-Terpinene	1066	1244	a, b, c9	0.35 ± 0.16	-	0.01 ± 0.00	9.91 ± 0.99
44	Terpinolene	1096	1280	a, b, c9	0.03 ± 0.01	-	-	2.35 ± 0.22
45	1,3,8-*p*-Menthatriene	1121	-	a, b, c2	-	-	-	0.52 ± 0.06
	Phenol							
46	Thymol	1289	1903	a, b, c6	-	-	-	0.09 ± 0.01
	Acyclic sesquiterpenes and derivatives							
	Alcohol							
47	(*E*,*E*)-Farnesol	1727	-	a, b	-	-	-	1.98 ± 0.36

Table 7 (continued)

No.	Compound	RI		ID	Concentration (μg/g fresh galangal)			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
48	(*E*)-Nerolidol	-	2037	a, b	-	-	0.25 ± 0.15	-
	Aldehyde							
49	Farnesal	1754	-	a, b	-	-	0.19 ± 0.10	4.58 ± 0.62
	Ester							
50	(*E*,*E*)-Farnesyl acetate	1847	-	a, b, c2	0.10 ± 0.00	-	3.51 ± 0.61	18.60 ± 2.67
	Hydrocarbons							
51	(*E*)-β-Farnesene	1462	1666	a, b, c2	9.74 ± 1.02	0.64 ± 0.12	8.71 ± 0.26	101.91 ± 32.59
52	α-Bergamotene	-	1584	a, b	-	-	-	0.70 ± 0.13
53	(*E*,*E*)-α-Farnesene	-	1745	a, b	0.48 ± 0.00	-	-	-
	Cyclic sesquiterpenes and derivatives							
	Alcohols							
54	*cis*-Sesquisabinene hydrate	-	2004	a, b	-	-	0.20 ± 0.02	-
55	Elemol	-	2090	a, b	-	-	0.10 ± 0.01	-
56	Zingiberenol	-	2112	a, b	-	-	-	0.46 ± 0.07
57	α-Cadinol	-	2210	a, b	-	-	-	1.24 ± 0.08

Table 7 (continued)

No.	Compound	RI		ID	Concentration (ug/g raw galangal)			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
58	α-Bisabolol	-	2222	a, b	-	-	0.27 ± 0.06	1.86 ± 0.03
59	Juniper camphor	-	2310	a, b	-	-	-	1.34 ± 0.01
	Hydrocarbons							
60	α-Copaene	1395	1489	a, b	0.30 ± 0.03	-	0.07 ± 0.01	4.53 ± 0.89
61	β-Elemene	1408	1589	a, b	1.92 ± 0.24	0.02 ± 0.01	0.07 ± 0.05	25.44 ± 6.69
62	β-Caryophyllene	1447	1600	a, b, c6, d	12.09 ± 2.73	0.52 ± 0.12	2.95 ± 1.36	99.04 ± 11.24
63	γ-Gurjunene	1469	-	a, b, c2	-	-	-	0.64 ± 0.02
64	allo-Aromadendrene	1473	-	a, b, c8	-	-	-	0.48 ± 0.04
65	β-Selinene	1482	-	a, b, c2	6.03 ± 1.70	0.52 ± 0.12	1.74 ± 0.65	48.25 ± 4.13
66	α-Amorphene	1508	-	a, b	-	-	-	11.32 ± 1.68
67	Valencene	1514	-	a, b, c8	7.92 ± 1.33	0.48 ± 0.12	5.84 ± 1.80	45.26 ± 15.68
68	β-Bisabolene	1523	1729	a, b	15.87 ± 2.25	1.22 ± 0.28	11.17 ± 1.00	97.47 ± 19.84
69	δ-Cadinene	1530	1759	a, b, c1	-	-	0.06 ± 0.02	1.33 ± 0.09
70	β-Sesquiphellandrene	1539	1770	a, b	0.93 ± 0.05	0.09 ± 0.04	0.95 ± 0.35	8.53 ± 2.16
71	Selina-3,7(11)-diene	1544	-	a, b, c2	0.90 ± 0.12	-	-	9.49 ± 5.50
72	Germacene B	1589	1811	a, b, c6	0.71 ± 0.02	0.10 ± 0.02	0.71 ± 0.33	8.63 ± 0.36

Table 7 (continued)

No.	Compound	RI		ID	Concentration (μg/g fresh galangal)			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
73	δ-Elemene	-	1467	a, b	0.22 ± 0.00	-	0.25 ± 0.00	3.27 ± 0.07
74	γ-Elemene	-	1635	a, b	0.09 ± 0.01	-	0.37 ± 0.00	1.97 ± 0.23
75	Humulene	-	1672	a, b, c4	3.85 ± 0.82	0.07 ± 0.05	0.23 ± 0.07	41.04 ± 13.25
76	γ-Selinene	-	1679	a, b	-	-	-	0.74 ± 0.17
77	Germacene D	-	1712	a, b	1.48 ± 0.22	0.02 ± 0.01	0.62 ± 0.30	29.51 ± 5.17
78	δ-Guaiene	-	1716	a, b	0.02 ± 0.01	-	-	-
79	α-Selinene	-	1727	a, b	2.50 ± 0.52		3.49 ± 1.57	-
80	γ-Cadinene	-	1767	a, b	0.78 ± 0.09	-	-	-
	Oxide							
81	Caryophyllene oxide	1616	-	a, c5	-	-	-	0.73 ± 0.04
	Others and derivatives							
	alcohols							
82	4-Methyl-2-pentanol	821	-	a, c10	-	0.07 ± 0.03	-	-
83	Coumaryl alcohol	1734	-	a	-	14. 29 ± 2.66	1.93 ± 1.02	-
84	1-Hexadecanol	1885	-	a, b	-	0.03 ± 0.02	-	-
85	*trans-p*-2,8-Menthadienol-1	1130	1625	a, b	-		-	2.27 ± 0.33

Table 7 (continued)

No.	Compound	RI		ID	Concentration (μg/g fresh galangal)			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
	Aldehyde							
86	Vanillin	1417	-	a, b	-	0.09 ± 0.02	-	-
	Esters							
87	Chavicol acetate	1354	1967	a, c6	2.45 ± 0.25	-	1.83 ± 0.53	21.55 ± 0.04
88	Cuminyl acetate	1430	1975	a, c6	-	-	0.10 ± 0.02	19.70 ± 14.8
89	Eugenyl acetate	1535	2271	a, b, c2	0.16 ± 0.04	0.18 ± 0.03	1.52 ± 0.21	3.72 ± 1.02
90	Diethyl phthalate	1604	-	a, c3	-	-	-	0.24 ± 0.18
	Hydrocarbons							
91	Pentadecane	1499	1495	a, b, c2	3.13 ± 0.19	0.12 ± 0.00	1.17 ± 0.04	21.63 ± 1.02
92	Hexadecane	1597	-	a, b, c2	-	-	-	0.26 ± 0.02
93	6(*Z*),9(*E*)-Heptadecadiene	1673	-	a, c6	0.42 ± 0.02	-	0.74 ± 0.76	3.67 ± 0.44
94	1-Heptadecene	1679	-	a, b	0.78 ± 0.07	0.01 ± 0.01	0.34 ± 0.04	4.84 ± 0.16
95	Heptadecane	1697	1699	a, b, c2	0.18 ± 0.03	-	-	2.74 ± 0.17
96	Tridecane	-	1295	a, b	-	-	-	0.54 ± 0.01
97	Tetradecane	-	1392	a, b	0.09 ± 0.02	-	-	1.29 ± 0.08

Table 7 (continued)

No.	Compound	RI		ID	Concentration (μg/g fresh galangal)			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
	Phenols							
98	Chavicol (4-allylphenol)	1256	2350	a, b, c2	3.01 ± 0.70	0.68 ± 0.06	7.51 ± 1.26	4.41 ± 2.56
99	Methyl eugenol	-	2022	a, b	0.30 ± 0.00	-	2.56 ± 0.39	7.62 ± 0.26
100	Eugenol	-	2183	a, b	0.11 ± 0.01	-	0.39 ± 0.08	1.44 ± 0.07
	Acids							
101	Myristic acid	1827	2034	a	-	0.45 ± 0.47	-	-
102	Acetic acid	-	1447	a, c12	-	2.69 ± 0.15	0.33 ± 0.15	-
103	Propanoic acid	-	1536	a, c11	-	0.01 ± 0.00	-	-
	Other							
104	1′-Acetoxychavicol acetate	1655	2505	c13	84.62 ± 27.45	-	308.42 ± 94.57	-
	$[M]^+$ at *m/z* 234							
	Unknowns							
105	$[M]^+$ at *m/z* 212	1351	1741		35.63 ± 11.52	1.45 ± 0.17	25.08 ± 1.19	118.22 ± 12.92
106	$[M]^+$ at *m/z* 220	1555	2212		-	-	4.35 ± 0.64	-
107	$[M]^+$ at *m/z* 192	1575	2649		4.63 ± 1.58	135.76 ± 28.88	-	-
108	$[M]^+$ at *m/z* 236	1661	2445		0.91 ± 0.21	2.54 ± 0.65	9.82 ± 2.00	8.18 ± 1.27

Remark: ID = Identification

a = comparison of our MS data with Wiley 275 library data.

b= comparison of our RI data with ESO data base of essential oil (Boelens Aroma Chemical Information Service, 1999).

c= comparison of our RI data with literature data, c1= Le Quere and Latrasse (1990), c2 = Adams (1995), c3 = Ramarathnam *et al.* (1993), c4 = Tucker *et al.* (2002), c5 = Bouzouita *et al*. (2003), c6 = Lorjaroenphon (2004), c7= Letchamo *et al*. (2005), c8 = Martínez *et al.* (2005), c9 = Figuérdoé *et al*. (2005), c10 = Timón *et al*. (1998), c11 = Münch *et al*. (1997), c12 = Tairu *et al*. (2000), c13 = Mitsui *et al*. (1976)

d= comparison of our RI data with the authentic compound.

Table 8 Odour activity values (OAV) of volatile compounds in the extract from rhizome of galangal.

No.	Compound	Odour threshold (ppm)[d]	OAV[e]				Odour description[f]
			CE	WE	EE	ESO	
	Acyclic monoterpenes and derivatives						
	Alcohols						
1	Linalool	0.001[1]	40	-	50	1,360	Floral-woody odour with faint citrus note
2	Geraniol	0.1[3]	-	-	-	1	Sweet, floral, rose-like
	Esters						
3	Neryl acetate	8.5[3]	-	-	-	<1	Very sweet fruity-floral
4	Geranyl acetate	0.46[3]	1	-	2	18	Sweet, fruity, floral, rosy odour; apple-like in dilution
	Hydrocarbons						
5	Myrcene	0.1[1]	-	-	-	129	Resinous terpene
6	(*E*)-β-Ocimene	0.34[1]	-	-	1	2	Sweet, herbaceous terpene
	Cyclic monoterpenes and derivatives						
	Alcohols						
7	*trans*-Sabinene hydrate	55.0[3]	<1	-	<1	<1	Sweet minty, camphoraceous odour reminiscent of terpineol

Table 8 (continued)

No.	Compound	Odour threshold (ppm)[d]	OAV[e]				Odour description[f]
			CE	WE	EE	ESO	
8	Terpinene-4-ol	1.2[1]	2	-	<1	13	Sweet, earthy, musty, slight peppery, pleasantly herbaceous, terpeny, spicy
9	α-Terpineol	5.0[1]	<1	-	<1	3	Sweet, floral (lilac), lime odour
10	*trans*-Carveol	0.25[1]	-	-	-	4	Caraway-like, sweet spearmint-like odour
	Ketones						
11	Camphor	4.6[3]	-	-	<1	-	Sweet, herbaceous, anise (artificial Licorice) odour
	Esters						
12	Bornyl acetate	1.38[3]	<1	-	<1	2	Sweet, herbaceous, piney odour reminiscent of pine needles
	Ethers						
13	1,8-Cineol	0.064[3]	713	1	13	3,746	Strong, camphoraceous, cool, fresh odour
	Hydrocarbons						
14	α-Pinene	0.19[1]	9	-	-	188	Resinous, pine
15	Sabinene	0.98[2]	2	-	-	24	Spicy terpenic citrusy
16	β-Pinene	1.5[1]	<1	-	-	9	Dry, woody, resinous-piney
17	α-Phellandrene	0.16[2]	-	-	-	5	Fresh, spicy, citrus, peppery, woody-minty
18	δ-3-Carene	0.77[5]	-	-	-	<1	Fresh, harsh, terpentine note
19	α-Terpinene	0.085[2]	1	-	-	68	Refreshing, lemony-citrusy terpene odour

Table 8 (continued)

No.	Compound	Odour threshold (ppm)[d]	OAV[e]				Odour description[f]
			CE	WE	EE	ESO	
20	*p*-Cymene	0.12[1]	1	-	-	32	Strong, characteristic, terpene odour; oxidised lemon note
21	Limonene	0.2[1]	9	-	<1	122	Fresh, sweet, hydrocarbon and orange citrus odour
22	γ-Terpinene	0.26[2]	1	-	<1	38	Refreshing herbaceous-citrus like terpene odour
23	Terpinolene	0.041[1]	1	-	-	57	Sweet, piney, slightly anisic, somewhat pleasant odour
24	1,3,8-*p*-Menthatriene	0.015[6]	-	-	-	35	Herbaceous, green, parsley like.
	Phenol						
25	Thymol	0.79[3]	-	-	-	<1	Strong, sweet, medicinal-herbaceous, spicy-phenolic
	Cyclic sesquiterpenes and derivatives						
	Alcohols						
26	Elemol	0.1[3]	-	-	1	-	Sweet woody odour with floral undertone
	Hydrocarbons						
27	β-Caryophyllene	0.15[1]	81	4	20	660	Woody, spice, dry odour
28	Humulene	0.39[1]	10	<1	1	105	Mild woody with earthy weak spice notes
	Oxide						
29	Caryophyllene oxide	0.41[1]	-	-	-	2	Dry, woody, faint cedar, tobacco like notes

Table 8 (continued)

No.	Compound	Odour threshold (ppm)[d]	OAV[e]				Odour description[f]
			CE	WE	EE	ESO	
	Others						
	Aldehyde						
30	Vanillin	0.058[7]	-	2	-	-	Powerful, creamy, vanilla-like odour
	Hydrocarbons						
31	1-Heptadecene	8.0[4]	<1	<1	<1	1	
32	Tetradecane	1.0[4]	<1	-	-	1	Mild hydrocarbon
	Phenols						
33	Methyl eugenol	0.775[3]	<1	-	3	10	Musty, tea-like, mild-spicy, clove-like odour
34	Eugenol	0.1[3]	1	-	4	14	Strong, spicy, dry, pungent, smoky, clove-like
	Acids						
35	Myristic acid	10.0[8]	-	<1	-	-	Very faint, waxy-oily; nearly odourless
36	Acetic acid	26.0[1]	-	<1	<1	-	Strong, pungent, sour, vinegar odour with sour, acid
37	Propanoic acid	2.0[7]	-	<1		-	Pungent, sour milk odour

Remark: d= Threshold in water (ppm) from: 1 = Tamura *et al*.(2001), 2 = Boonbumrung *et al*.(2001), 3 = Tamura *et al*.(1993), 4 = Tamura *et.al*. (1995), 5 = Tamura *et.al*.(1996), 6 = Masanetz and Grosch (1998), 7 = Buttery *et al*.(1999), 8 = Cherchinski (1961).

e= Odour activity value (OAV) was calculated by dividing the concentration observed in the sample by the published aroma threshold for that compound.

f= odour description from Flavor-Base' 2004 (Leffingwell, 2004), Pino *et al*.(2002).

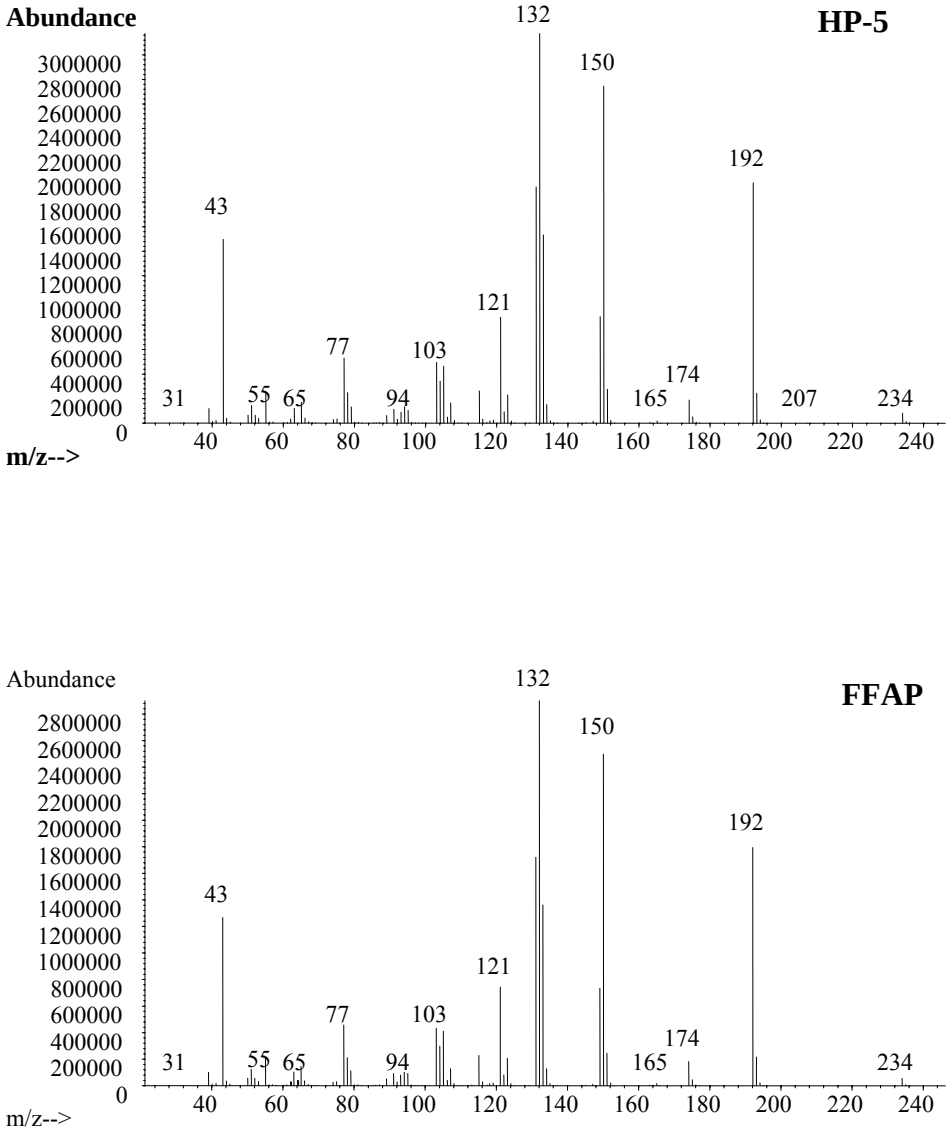


Figure 24 Mass spectra of 1′-acetoxychavicol acetate from crude extract of rhizome of *A. galanga* on HP-5 and FFAP columns.

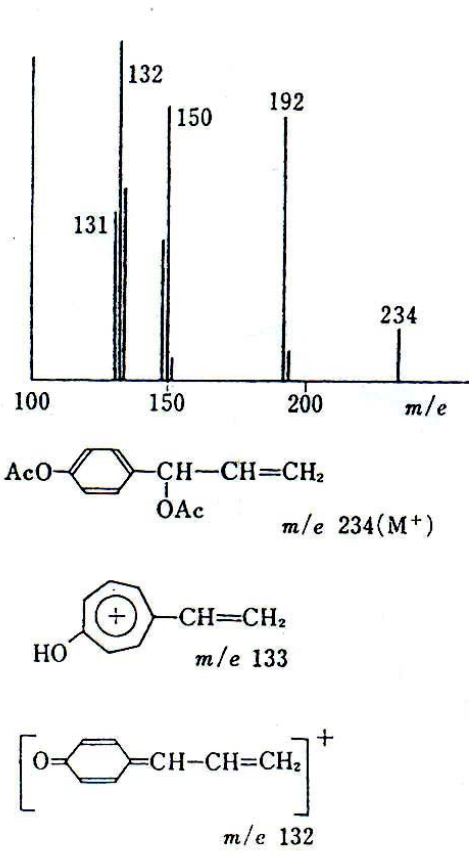


Figure 25 Mass spectra of 1′-acetoxychavicol acetate from seed of *A. galanga*.

Source: Mitsui *et al*. (1976)

1.2 Water extract

The appearance of the water extract sample was yellow-brown solid. The yields of the extract were 35.65% (dry basis) and 3.19% (wet basis). Volatile compounds identified in water extract sample are listed in Tables 7. The main constituent was the unknown, $[M]^+$ at *m/z* 192 (76.04%, Appendix Table 1) and composed of acid. The galangal potent odourant, 1,8-cineol and other terpenes were found at low concentrations. The odour characteristic of the water extract was not the same as fresh galangal. There was not virtually appeared any odour in this extract. Then OAV value of 1,8-cineol in this extract was low, compared with other extracts (Table 8).

1.3 Ethanol extract

The appearance of the ethanol extract was yellow-brown solid. The yields of the ethanol extract were 32.65% (dry basis) and 2.92% (wet basis). The results of ethanol extract sample analysis are given in Table 7. Ethanol extract sample consisted of 7.29 μg/g fresh galangal for cyclic monoterpenes and 29.09 μg/g fresh galangal for cyclic sesquiterpenes that were lower than those of essential oil. The major volatile compounds in the ethanol extract was the compound that had M^+ at *m/*z = 234 (61.03%, Appendix Table 1). The potent odourants of galangal such as 1,8-cineole, linalool, geranyl acetate, eugenol and chavicol acetate reported by Mori *et al*. (1995) were found in this extract in low quantity, but higher than water extract. The odour characteristic of the extract was similar to that of fresh galangal rhizome but at lower intensity when compared to that of essential oil as indicated by OAVs in Table 8. The compound that had M^+ at *m/*z = 234 was tentatively identified as 1′-acetoxychavicol acetate as previously mentioned.

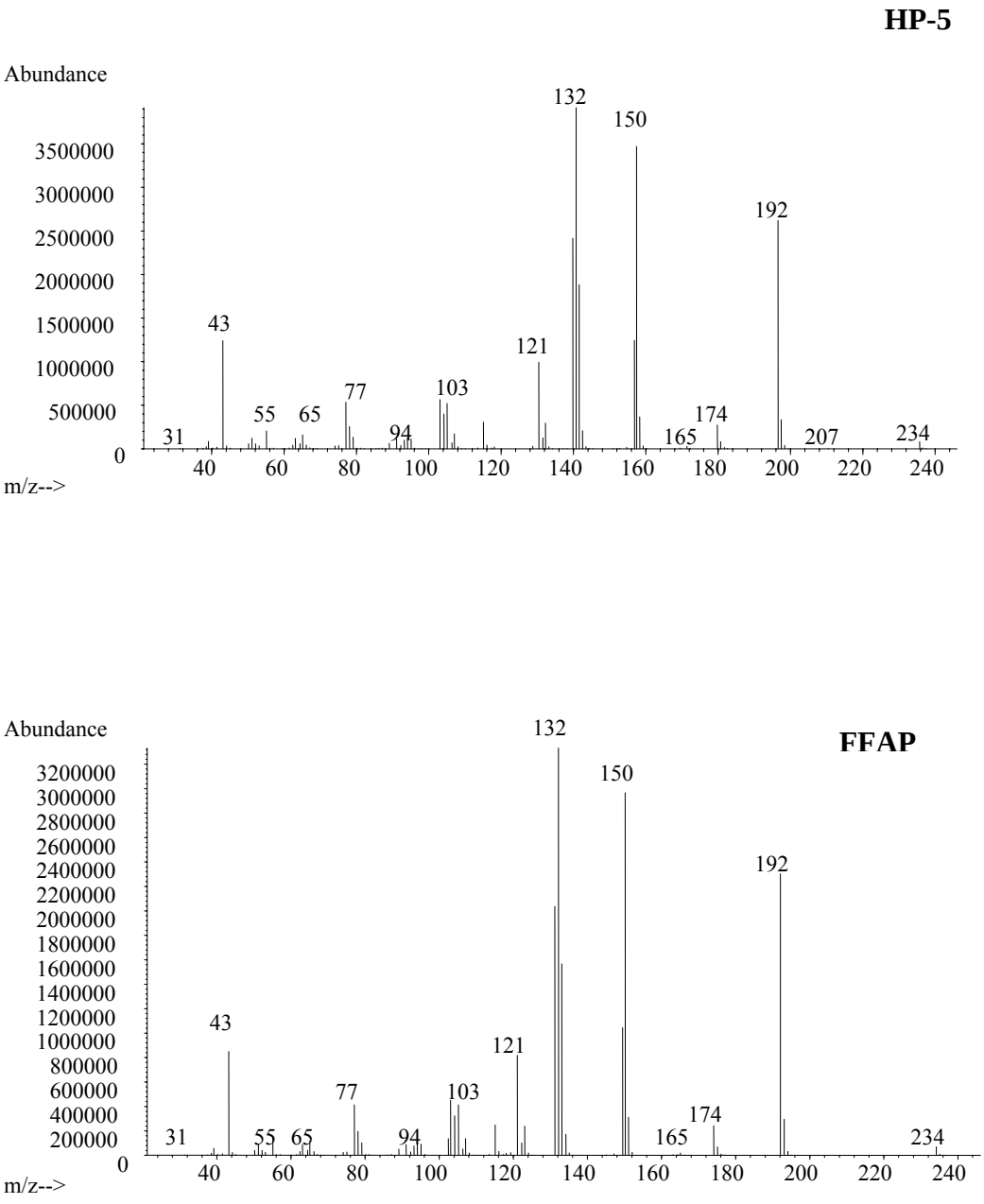


Figure 26 Mass spectra of 1′-acetoxychavicol acetate from the ethanol extract of rhizome of *A. galanga* on HP-5 and FFAP columns.

1.4 Essential oil

The hydro-distillation of galangal fresh rhizome gave clear oil with 0.184 % yield (wet basis). The specific gravity was 0.9811 (g/mL). The chemical compositions of essential oil were obtained by GC/MS analysis. Table 7 summarises the qualitative and quantitative analyses of the volatiles according to the order of elution on HP-5 and FFAP columns. The identified constituents are listed according to their chemical classes. Essential oil had high concentrations of cyclic monoterpenes (411.82 μg/g fresh galangal) and cyclic sesquiterpenes (441.84 μg/g fresh galangal) but had low concentrations of acyclic monoterpenes (24.80 μg/g fresh galangal) and acyclic sesquiterpenes (127.77 μg/g fresh galangal). Acyclic terpenes are relatively unstable and some have a slightly aggressive odour due to their highly unstable structures (Bauer *et al*., 2001). Among cyclic terpenes, terpene hydrocarbons possess little flavour value but serve as starting materials for the synthesis of fragrance and flavour materials. Oxygenated derivatives of terpene hydrocarbons, i.e., alcohols, aldehydes, ketones and esters, are major contributors to the distinctive odours and flavour (Reineccius, 1994; Bauer *et al*., 2001). The formation of oxygenated compound from terpene hydrocarbon is shown in Figure 27.

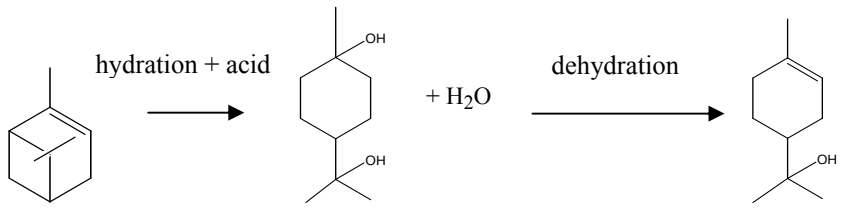


Figure 27 The formation of α-terpineol from α-pinene.
Source: adapted from Bauer *et al*. (2001).

The most abundant component in the rhizome oil was 1,8-cineol (18.91%, Appendix Table 1). The percentage was greater than 5.5% reported in galangal oil from Malaysia (De Pooter *et al.*, 1985) but lower than 40.5% of galangal oil from the same country reported by Jantan *et al*. (2004). The reason for the differences in the

percentage of 1,8-cineol might be due to the differences in growing conditions as well as extraction conditions. All of the potent odourants of galangal (1,8-cineole, linalool, geranyl acetate, eugenol and chavicol acetate) reported by Mori *et al*. (1995) were also founded in the essential oil in this study. The odour characteristic of the galangal oil was similar to fresh galangal rhizome. Galangal odour of essential oil was the strongest in comparison to the other extracts as indicated by, the highest OAV of 1,8-cineol (Table 8). Essential oil contained the highest concentrations of terpenes when compared with the other extracts (Table 9). 1′-Acetoxychavicol acetate, found in crude and ethanol extract, was not found in essential oil. This compound was not stable in aqueous solution and was lost through hydrolysis and isomerisation during steam distillation (Yang and Eilerman, 1999).

Table 9 Comparison of chemical compositions of crude extract (CE), water extract (WE) ethanol extract (EE) and essential oil (ESO) of galangal.

Chemical classes	Concentration (μg/g fresh galangal)			
	CE	**WE**	**EE**	**ESO**
Acyclic monoterpenes and derivatives	**0.43**	**-**	**0.74**	**24.80**
- Alcohols	0.04	-	0.05	1.42
- Esters	0.39	-	0.69	9.74
- Hydrocarbons	-	-	-	13.64
Cyclic monoterpenes and derivatives	**60.92**	**0.67**	**7.29**	**411.82**
- Alcohols	6.17	-	4.12	33.76
- Aldehydes	-	-	-	0.37
- Ketones	-	-	0.07	-
- Esters	2.14	0.19	1.61	11.61
- Ethers	46.34	0.48	1.41	239.77
- Hydrocarbons	6.27	-	0.08	126.22
- Phenols	-	-	-	0.09

Table 9 (continued)

Chemical classes	Concentration (μg/g fresh galangal)			
	CE	**WE**	**EE**	**ESO**
Acyclic sesquiterpenes and derivatives	**10.32**	**0.64**	**12.66**	**127.77**
- Alcohols	-	-	0.25	1.98
- Aldehydes	-	-	0.19	4.58
- Esters	0.10	-	3.51	18.60
- Hydrocarbons	10.22	0.64	8.71	102.61
Cyclic sesquiterpenes and derivatives	**55.61**	**3.04**	**29.09**	**441.84**
- Alcohols	-	-	0.57	4.90
- Hydrocarbons	55.61	3.04	28.52	436.94
Oxides	**-**	**-**	**-**	**0.73**
Acids	**-**	**3.15**	**0.33**	**-**
Others	**95.25**	**21.83**	**326.51**	**95.92**
- Alcohols	-	14.39	1.93	2.27
- Aldehydes	-	0.09	-	-
- Esters	2.61	0.18	3.45	45.21
- Hydrocarbons	4.60	0.13	2.25	34.97
- Phenols	3.42	0.68	10.46	13.47
- Others	84.62	6.36	308.42	-
Unknown	41.17	139.75	39.25	126.40

2. Phenolic compounds of galangal extracts

Total phenolic compound of crude extract, water extract, ethanol extract and essential oil were 14.38 ± 0.48, 26.31 ± 2.47, 91.99± 11.94 and 0.92± 0.03, mg GAE/ 100g WM, respectively (Table 10). While the flavonoid content of crude extract, water extract, ethanol extract and essential oil were 6.07 ± 0.07, 4.71 ± 0.08 and 40.25 ± 1.74 and 0.04 ± 0.00 mg CE/100g WM, respectively (Table 11).

Table 10 Total phenolic content of crude extract, water extract, ethanol extract and essential oil of galangal. (data expressed as milligrams of gallic acid equivalent, GAE).

Sample	Total phenolic content		
	mg GAE/ g extract	mg GAE/ g DM	mg GAE/ 100g WM
Crude extract	0.56 ± 0.02^{a}	-	14.38 ± 0.48^{b}
Water extract	8.25 ± 0.78^{b}	2.94 ± 0.28^{a}	26.31 ± 2.47^{c}
Ethanol extract	31.49 ± 4.09^{c}	10.28 ± 1.33^{b}	91.99 ± 11.94^{d}
Essential oil	5.01 ± 0.14^{b}	-	0.92 ± 0.03^{a}

Remark - DM = dry matter basis, WM = fresh weight basis
- The different letters within the same column indicate significant differences between treatments after one-way ANOVA ($p \leq 0.05$).

Table 11 Flavonoid contents of crude extract, water extract, ethanol extract and essential oil of Galangal (data expressed as milligrams of catechin equivalent,CE).

Sample	Flavonoid content		
	mg CE/ g extract[A]	mg CE/g DM[B]	mg CE/100g WM[C]
Crude extract	0.24 ± 0.00^{a}	-	6.07 ± 0.07^{b}
Water extract	1.48 ± 0.02^{b}	0.53 ± 0.01^{a}	4.71 ± 0.08^{b}
Ethanol extract	13.78 ± 0.60^{c}	4.50 ± 0.19^{b}	40.25 ± 1.74^{c}
Essential oil	0.20 ± 0.01^{a}	-	0.04 ± 0.00^{a}

Remark - DM = dry matter basis, WM = fresh weight basis
- The different letters within the same column indicate significant differences between treatments after one-way ANOVA ($p \leq 0.05$).

Type of phenolic compounds were analysed by HPLC. Catechin, *p*-coumaric acid and myricetin were found in ethanol extract. For crude extract, all of those compounds were found except myricetin (Table 12)

Table 12 Phenolic compounds in crude extract and ethanol extract, analysed by HPLC.

compound	concentration		
	mg/g extract	mg/g DM	mg/100g WM
Crude extract			
Catechin	0.06 ± 0.00	-	1.40 ± 0.03
p-Coumaric acid	0.003 ± 0.000	-	0.08 ± 0.00
Ethanol extract			
Catechin	1.74 ± 0.26	0.57 ± 0.08	5.08 ± 0.75
p-Coumaric acid	0.07 ± 0.00	0.02 ± 0.00	0.19 ± 0.00
Myricetin	0.45 ± 0.03	0.15 ± 0.01	1.32 ± 0.10

Remark - DM = dry matter basis, WM = fresh weight basis

Part 2 Fishy odour deodorisation property of the extracts.

1. Fishy odour deodorised property of the extract

Two types of authentic compound that were reported as fishy odour compounds, TMA (Cadwallader *et al*., 1995; Prost *et al*., 1998; Fukami *et al*., 2002) and (*Z*)-4-heptenal (Cha and Cadwallader, 1998; Hartvigsen, *et al*., 2000), were used for fishy odour deodorisation study. Four extracts, crude extract, water extract, ethanol extract and essential oil were tested for their deodorisation property. The difference from control test was used to determine the difference of fishy odour in each sample with 30 panelists. The results are shown in Table 13.

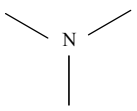
The differences between TMA and (*Z*)-4-heptenal (Figure 28) are the odour characteristics, molecular weight and chemical structure. The odour characteristic of TMA is pungent ammoniacal fishy odour and taste (Leffingwell, 2004), while odour characteristic of (*Z*)-4-heptenal is fishy, green, burnt (Karahadian and Lindsay, 1989). The molecular weight of TMA and (*Z*)-4-heptenal are 59 and 112 respectively. TMA showed the dominant of deodourised property from all extracts. It is hard to explain why the fishy odour deodourisation over TMA was more efficient over than (*Z*)-4-heptenal. The result from Table 13 can be concluded that crude extract of galangal is the most effective in fishy odour deodourisation. The deodourised property from high to low was crude extract sample > ethanol extract sample > essential oil > water extract sample.

Table 13 Mean difference from control of fishy odour deodorisation property of galangal extract.

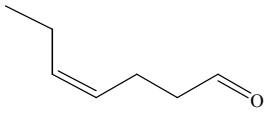
Extract	Mean difference	
	TMA	(*Z*)-4-Heptenal
Essential oil	3.60[a]	2.77[b]
Crude extract	4.47[b]	2.47[b]
Ethanol extract	4.40[b]	2.90[b]
Water extract	3.40[a]	1.10[a]

Remark: - The differences of letters within the same column indicate significant differences between treatments after one-way ANOVA ($p \leq 0.05$).

0 = No difference, 1 = Very slight difference, 2 = Slight/moderate difference, 3 = Moderate difference, 4 = Moderate/large difference, 5 = Large difference, 6 = Very large difference



TMA



(*Z*)-4-heptenal

Figure 28 Chemical structure of TMA and (*Z*)-4-heptenal.
Source: NIST (2005)

2. Effect of volatile compounds on fishy odour reduction

All of volatile compound from crude extract, water extract, ethanol extract and essential oil were reduced to three principle component, which accounted for 100 % of variation (Table 14).

Table 14 Factor loading value for volatile compounds of galangal extracts.

No.	compound	component		
		1	2	3
1	Linalool	.998		
2	Geraniol	.997		
3	Neryl acetate	.997		
4	Geranyl acetate	.999		
5	β-Myrcene (Myrcene)	.997		
6	(*E*)-β-Ocimene	.968		
7	*trans*-Sabinene hydrate			.966
8	*cis*-Sabinene hydrate			.841
9	*trans*-*p*-menth-2-en-1-ol	.997		
10	*cis*-Verbenol	.947		
11	*trans*-Verbenol		.966	
12	*p*-menth-1,5 diene-8-ol	.997		
13	δ-Terpineol		.966	

Table 14 (continued)

No.	compound	component		
		1	2	3
14	Terpinene-4-ol	.987		
15	*p*-Cymene-8-ol	.729		
16	α-Terpineol	.986		
17	*cis*-Piperitol	.997		
18	*trans*-Carveol	.997		
19	α-Campholene aldehyde	.997		
20	Camphor		.966	
21	Myrtenyl acetate	.997		
22	*trans*-Carvyl acetate	.994		
23	Thymyl acetate	.998		
24	*cis*-Carvyl acetate	.997		
25	Exo-2-hydroxycineole acetate			.996
26	Bornyl acetate	.997		
27	Carvacryl acetate		.918	
28	1,8-Cineol	.970		
29	Exo-2-hydroxycineol	-.959		
30	Dehydro-1,8-cineol			.749
31	α-Thujene	.994		
32	α-Pinene	.994		
33	α-Fenchene	.997		
34	Camphene	.999		
35	Verbenene (Pinadinene)	.997		
36	Sabinene	.992		
37	β-Pinene	.994		
38	α-Phellandrene	.997		
39	δ-3-Carene	.997		

Table 14 (continued)

No.	compound	component		
		1	2	3
40	α-Terpinene	.996		
41	*p*-Cymene	.994		
42	Limonene	.991		
43	γ-Terpinene	.995		
44	Terpinolene	.996		
45	1,3,8-*p*-Menthatriene	.997		
46	Thymol	.997		
47	(*E*,*E*)-Farnesol	.997		
48	(*E*)-Nerolidol		.966	
49	Farnesal	.999		
50	(*E*,*E*)-Farnesyl acetate	.995		
51	(*E*)-β-Farnesene	.995		
52	α-Bergamotene	.997		
53	(*E*,*E*)-α-Farnesene			.795
54	*cis*-Sesquisabinene hydrate		.966	
55	Elemol		.966	
56	Zingiberenol	.997		
57	α-Cadinol	.997		
58	α-Bisabolol	.998		
59	Juniper camphor	.997		
60	α-Copaene	.993		
61	β-Elemene	.991		
62	β-Caryophyllene	.987		
63	γ-Gurjunene	.997		
64	allo-Aromadendrene	.997		
65	β-Selinene	.988		
66	α-Amorphene	.997		

Table 14 (continued)

No.	compound	component		
		1	2	3
67	Valencene	.987		
68	β-Bisabolene	.988		
69	δ-Cadinene	.999		
70	β-Sesquiphellandrene	.995		
71	Selina-3,7(11)-diene	.988		
72	Germacene B	.997		
73	δ-Elemene	.997		
74	γ-Elemene	.999		
75	α-Humulene	.989		
76	γ-Selinene	.997		
77	Germacene D	.995		
78	δ-Guaiene			.795
79	α-Selinene		.616	
80	γ-Cadinene			.795
81	Caryophyllene oxide	.997		
82	4-Methyl-2-pentanol			-.836
83	Coumaryl alcohol			-.863
84	1-Hexadecanol			-.836
85	*trans*-*p*-2,8-Menthadienol-1	.997		
86	Vanillin			-.836
87	Chavicol acetate	.993		
88	Cuminyl acetate	.997		
89	Eugenyl acetate	.953		
90	Diethyl phthalate	.997		
91	Pentadecane	.986		
92	Hexadecane	.997		
93	6(*Z*),9(*E*)-Heptadecadiene	.992		

Table 14 (continued)

No.	compound	component		
		1	2	3
94	1-Heptadecene	.985		
95	Heptadecane	.992		
96	Tridecane	.997		
97	Tetradecane	.991		
98	Chavicol (4-allylphenol)		.909	
99	Methyl eugenol	.969		
100	Eugenol	.984		
101	Myristic acid			-.836
102	Acetic acid			-.861
103	Propanoic acid			-.836
104	1′-Acetoxychavicol acetate		.885	
	$[M]^+$ at *m/z* 234			
105	$[M]^+$ at *m/z* 212	.959		
106	$[M]^+$ at *m/z* 220		.966	
107	$[M]^+$ at *m/z* 192			-.818
108	$[M]^+$ at *m/z* 236		.853	
% explained variance		77.101	13.666	9.232
% cumulative variance		77.101	90.768	100.000

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser normalisation.

The correlation between volatile compound component from PCA and fishy odour reduction was analysed (Table 15). Well positive correlation was showed between component III and fishy odour deodorisation. Most of volatile compounds in component III were alcohols (*trans*-sabinene hydrate, *cis*-sabinene hydrate, 4-methyl-2-pentanol, coumaryl alcohol and 1-hexadecanol) and acids (myristic acid, acetic acid and propanoic acid). Component III also composed of a phenolic compound

(vanillin). The deodourisation of TMA by acid had been reported (Schieberle and Grosch, 1988; Freeburg *et al*., 1994; Schmidhauser *et al*., 1995; Sinki, 1997; Leão Lana, *et al*., 2006). For alcohol, the study on effect of mirin flavourings on improvement of sardine odour showed that isobutyl alcohol, phenethyl alcohol, 4-ethylguaiacol and 2-acetypyrrole greatly derived from mirin contributed to mask fishy odour of salt-dried sardine (Kasahara *et al*., 1990). For vanillin, it demonstrated the deodorising effect on TMA, to salt formation between the phenolic compound and amine (Shiraishi *et al*., 1982). Besides alcohol, acid and phenolic compound, some unknown compound in component III showed well correlation with fishy odour deodorisation too. In addition, Component II also showed trend of positive correlation with fishy odour deodorisation.

Table 15 Correlation of volatile compound groups from PCA and fishy odour deodorisation.

	FAC1	FAC2	FAC3	Deodorisation
FAC 1	1			
FAC 2	0.000	1		
FAC 3	0.000	0.000	1	
Deodorisation	-0.420	0.405	0.812	1

Deodourising/odour masking can be divided into three types: chemical, physical and sensational deodorisation as reported by Hirasa and Takemasa (1998). Sensational deodorisation is theoretically divided into two types: masking and offset deodourising. All of galangal extracts exhibited fishy odour deodorisation. The deodorising effects from alcohol, acid and phenolic compound were chemical deodorisation. Essential oil, that composed most of galangal potent odorant compounds, also showed this effect. Then sensational deodorisation, masking, may be one effect of fishy odour deodorisation by galangal extracts. In conclusion, the mechanisms of fishy odour reduction in TMA were two ways: (1) the masking property of the fishy odour with the unique flavour of galangal potent odorant compound and (2) the reduction of TMA by acid, alcohol and phenolic compound.

The fishy odour deodorising effect in essential oil may be caused by the first mechanism. Water extract exhibit the second mechanism. For crude extract and ethanol extract, it showed both of these two mechanisms. Because crude extract and ethanol extract also composed of galangal potent odorant compound, with the less concentration than essential oil.

3. Effect of phenolic compounds on fishy odour deodorisation

Phenolic compounds may also assist in the reduction of fishy odour. Kida *et al*. (2002) demonstrated that tea catechin showed deodorising effect on ethylamine and trimethylamine (TMA) via a neutralisation reaction. Tea catechin acted as acid, thus causing a decreased in the volatile amine. Catechins were also reported as deodorants for food products, pharmaceutical tablets and air purification (Yamaguchi *et al*., 2007). The amount of total phenolic compounds and flavonoid content of crude extract, water extract ethanol extract and essential oil were determined (Table 10 and 11). The correlations of phenolic compounds and flavonoid content and the fishy odour intensity of TMA and (*Z*)-4-heptenal were reported in Table 16. The result showed that TMA (r = 0.479) and (*Z*)-4-heptenal (r =0.251) contents were not well correlated with fishy odour reduction. Flavonoid content was not well correlated with fishy odour reduction of TMA (r = 0.586) and (*Z*)-4-heptenal (r = 0.415). However, the correlation of fishy odour reduction with flavonoid content was higher than with phenolic content. This may be related to the amount of (+)-catechin (Table 12). (+)-Catechin, in this study, showed the same effect on deodorisation of TMA as described by Kida *et al.* (2002).

Table 16 Correlation of TMA and (*Z*)-4-heptenal deodorisation with phenolic content and flavonoid content of galangal.

	TMA deodorisation	(*Z*)-4-heptenal deodorisation	Phenolic content	Flavonoid content
TMA deodorisation	1			
(*Z*)-4-heptenal deodorisation	0.629	1		
Phenolic content	0.479	0.251	1	
Flavonoid content	0.586	0.415	0.984*	1

* - correlation is significant at the 0.05 level.

Part 3: Antioxidants property of galangal extracts

1. Antioxidant activity of the extract from rhizome of galangal

1.1 Antioxidant activity of water extract

Although ethanol extraction is commonly used to extract plant antioxidants extraction, it was reported that ethanol extract possess immunological properties in animal and patients (Frankiewicz and Scheller, 1984). When the extract was used as food antioxidant, alcohol should be substituted with less harmful solvent. Though water is not as effective as organic solvent to extract useful compounds from plant, it can be a good candidate.

Two types of free radical scavenging method were used, DPPH and ORAC, to test the ability of antioxidant activity of water extract. According to these two methods, the result showed antioxidant effect in the same way. For DPPH test, the reaction following a concentration-dependent pattern, as the concentration increased the antioxidant activity increased (Table 17). The water extract of galangal in this study was more effective as an antioxidant than the essential oil sample but was less effective than the ethanol extract sample (Table 17, 18, 19). The examples of

water extract from plants, that showed antioxidant activity were fresh propolis (Nagai *et al*., 2003), *Aralia elata* (Chung *et al*., 2005) and peanut hull (Lee *et al*., 2006)

Table 17 Antioxidant activities of galangal water extract sample at different concentrations.

Water extract concentration (%,w/v)	Radical scavenging activity (*I*%)
1.0	11.667 ± 0.242 [a]
2.0	20.705 ± 0.364 [b]
3.0	28.942 ± 0.535 [c]
4.0	36.635 ± 0.693 [d]
5.0	45.513 ± 0.801 [e]

Note: the differences of letters within the same column indicate significant differences between treatments after one-way ANOVA ($p \leq 0.05$).

1.2 Antioxidant activity of ethanol extract

Ethanol extract sample showed the highest antioxidant activity compared with water extract sample and essential oil (Table 17, 18, 19). Although Jitoe *et al*. (1992) showed that antioxidant activity of *A. galanga* extract (4 mg in 4 mL of 99.5% ethanol) was stronger than α-tocopherol (4 mg in 4 mL of 99.5% ethanol) when fresh rhizome was crushed and soaked for 18 days in acetone (2 L/kg) at 23 °C and remove solvent, the antioxidant activity of essential oil, water extract sample and ethanol extract sample in this study were lower than that of α-tocopherol.

Table 18 Antioxidant activities of galangal ethanol extract sample at different concentrations.

Ethanol extract concentration (%,w/v)	Radical scavenging activity (*I*%)
0.2	11.433 ± 0.480 [a]
0.4	20.031 ± 0.594 [b]
0.6	30.405 ± 0.714 [c]
0.8	40.062 ± 1.220 [d]
1.0	45.483 ± 0.286 [e]

Note : the differences of letters within the same column indicate significant differences between treatments after one-way ANOVA ($p \leq 0.05$).

1.3 Antioxidant activity of essential oil

Antioxidant activity of essential oil exhibited the concentration-dependent pattern. As the concentration increased the antioxidant activity increased (Table 19). The compounds, namely α-pinene, camphene, sabinene, β-pinene, myrcene, ρ-cymene, 1,8-cineole, limonene, γ-terpinene, linalool, borneol, terpinene-4-ol, α-terpineol, geranyl acetate, methyl eugenol, β-caryophyllene were reported to show antioxidant property (Dorman *et al*., 1995). Our preliminary study showed that 1,8-cineol, linalool and geranyl acetate exhibited low antioxidant activity. Antioxidant activity of galangal essential oil was not so high, though many compounds showed antioxidant activity. Tomaino *et al.* (2005) studied the antioxidant activities of herbs and spices. The effectiveness order was clove » cinnamon >nutmeg > basil » oregano » thyme. The most effective essential oil seemed to be those contained high content of eugenol (Tomaino *et al*., 2005)**.**

Table 19 Antioxidant activities of galangal essential oil at different concentrations.

Essential oil concentration (%,v/v)	Radical scavenging activity (*I*%)
5.0	6.647 ± 0.638 [a]
10.0	12.656 ± 0.439 [b]
15.0	18.057 ± 0.055 [c]
20.0	21.029 ± 0.221 [d]
25.0	26.366 ± 0.192 [e]

Note: the differences of letters within the same column indicate significant differences between treatments after one-way ANOVA ($p \leq 0.05$).

1.4 IC_{50} of the extract and ORAC value

The IC_{50} of the extracts are shown in Table 20. The ethanol extract sample was the most effective radical scavenger, compared with water extract sample and the essential oil. ORAC value of essential oil, water extract sample and ethanol extract sample were 1730.32, 6845.40 and 8114.43 μmol TE/g, respectively (Table 21). Though essential oil was the least effective antioxidant, it might exhibit high biological activity because of the best accumulation in the body compared with other water soluble antioxidants (Burton and Ingold, 1986). Nevertheless, antioxidant activity of the water extract was lower than that of the ethanol extract.

Table 20 The IC_{50} of essential oil, water extract sample, ethanol extract sample of galangal, α-tocopherol and BHA.

Sample	IC_{50} (mg/mL)
Essential oil	431.69
Water extract	54.39
Ethanol extract	10.50
α-Tocopherol	1.45
BHA	0.41

IC_{50} = Extract concentration of sample provide 50% inhibition.

Table 21 The ORAC values of essential oil, water extract and ethanol extract of galangal.

Sample	ORAC value (μmol TE/g extract)
Essential oil	1730.32 ± 80.73^{a}
Water extract	6845.40 ± 89.19^{b}
Ethanol extract	8114.43 ± 718.48^{c}

Note: the differences of letters within the same column indicate significant differences between treatments after one-way ANOVA ($p \leq 0.05$).

2. Antioxidant activity of essential oil, water extract sample and ethanol extract sample incorporated with BHA

2.1 Antioxidant activity of galangal water extract sample incorporated with BHA

The antioxidant activity of water extract that incorporated with BHA was studied. The objective of this incorporation is to observe the synergistic effect. The result did not show synergistic effect. The reaction was the concentration-dependent.

Water extract sample can reduce dose of synthetic antioxidant (BHA) when BHA was using as an antioxidant. The inhibition of 0.01% BHA + 0% water extract sample was 15.980 ± 0.492 %, and increased to 53.500 ± 1.837 % when incorporated with 5% water extract sample (Table 22).

Table 22 Antioxidant activities of 0.01% BHA added with different concentration of water extract of galangal.

Solution	Radical scavenging activity (*I*%)
0.01 % BHA+ 0% WE	15.980 ± 0.492 [a]
0.01 % BHA+ 1% WE	24.800 ± 0.638 [b]
0.01 % BHA+ 2% WE	33.301 ± 0.528 [c]
0.01 % BHA+ 3% WE	40.205 ± 1.815 [d]
0.01 % BHA+ 4% WE	49.601 ± 1.650 [e]
0.01 % BHA+ 5% WE	53.500 ± 1.837 [f]

Note - solution was prepared by dissolved various concentrations of water extract in 0.01 % BHA (w/v methanol), WE = water extract sample

- the differences of letters within the same column indicate significant differences between treatments after one-way ANOVA ($p \leq 0.05$).

2.2 Antioxidant activity of galangal ethanol extract sample incorporated with BHA

Ethanol extract sample could increase antioxidant activity of BHA when mixed together. The inhibition of 0.01% BHA+ 0% ethanol extract sample was 15.851 ± 0.732%, and increased to 58.921 ± 0.424% when used with 1.0% water extract sample (Table 23).

Table 23 Antioxidant activities of 0.01 % BHA added with different concentrations of ethanol extract of galangal.

Solution	Radical scavenging activity (*I*%)
0.01 % BHA+ 0% EE	15.851 ± 0.732 [a]
0.01 % BHA+ 0.2% EE	25.259 ± 1.131 [b]
0.01 % BHA+ 0.4% EE	35.591 ± 0.237 [c]
0.01 % BHA+ 0.6% EE	45.845 ± 0.272 [d]
0.01 % BHA+ 0.8% EE	53.308 ± 1.006 [e]
0.01 % BHA+ 1.0% EE	58.921 ± 0.424 [f]

Note - Solution was prepared by dissolved various concentrations of ethanol extract in 0.01% BHA (w/v methanol), EE = ethanol extract sample.

- The differences of letters within the same column indicate significant differences between treatments after one-way ANOVA ($p \leq 0.05$).

2.3 Antioxidant activity of galangal essential oil incorporated with BHA

Antioxidant activity of essential oil incorporated with BHA was also concentration-dependent. The inhibition of 0.02% BHA+ 0% essential oil was 29.852 ± 1.121%, and increased to 41.199 ± 0.256% when incorporated with 25% essential oil (Table 24). It could reduce the use of BHA in oil soluble food and reduce the side effect of synthetic antioxidants.

Table 24 Antioxidant activities of 0.02% BHA added with different concentrations of essential oil of galangal.

Solution*	Radical scavenging activity (*I*%)
0.02% BHA+ 0% ESO	29.852 ± 1.121 [a]
0.02% BHA+ 5% ESO	32.108 ± 0.588 [b]
0.02% BHA+ 10% ESO	34.720 ± 0.634 [c]
0.02% BHA+ 15% ESO	36.460 ± 0.588 [d]
0.02% BHA+ 20% ESO	38.620 ± 0.436 [e]
0.02% BHA+ 25% ESO	41.199 ± 0.256 [f]

Notes - Solution was prepared by dissolved various concentrations of essential oil in 0.02% BHA (w/v methanol), ESO = essential oil.

- The differences of letters within the same column indicate significant differences between treatments after one-way ANOVA ($p \leq 0.05$).

3. Total phenolic compound and flavonoid content

The amounts of total phenolic compound and flavonoid of water extract, ethanol and extract essential oil were determined (Table 10 and 11). The correlation of total phenolic compound and flavonoid content and antioxidant activity were calculated (Table 25). Total phenolic compounds of water extract, ethanol extract and essential oil were 26.31 ± 2.47, 91.99± 11.94 and 0.92± 0.03 mg GAE/100g WM, respectively. Antioxidant activity was well correlated with total phenolic content (R^2 = 0.800, $p \leq 0.01$). The flavonoid content of water extract, ethanol extract and essential oil were 0.04 ± 0.00, 4.71 ± 0.08 and 40.25 ± 1.74, respectively. There was the significant correlation between antioxidant activity and flavonoid content (R^2 = 0.720, $p \leq 0.05$). It could be noticed that about 50% of phenolic compounds in ethanol extract were flavonoids. The antioxidant activity may be related with the amount of flavonoids.

Table 25 Correlation between galangal antioxidant activity, phenolic compound and flavonoid content.

	Activity	Phenolic content	Flavonoid content
Activity	1		
Phenolic content	.800**	1	
Flavonoid content	.720*	.978**	1

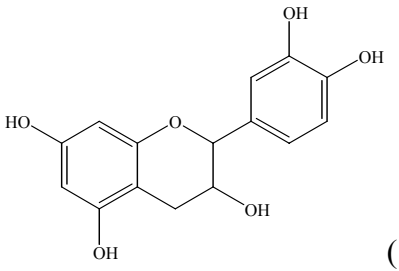
* - correlation is significant at the 0.05 level

**- correlation is significant at the 0.01 level

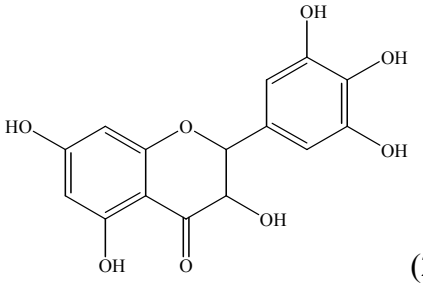
4. Active compound of lipophilic and hydrophilic extracts

The volatile compounds of the extract were analysed by GC-MS (Table 28). The volatile phenolic compounds and derivative of lipophilic extract (essential oil) were thymol, chavicol, methyl eugenol and eugenol. All of volatile phenolic compounds and derivative in lipophilic extract except thymol were also detected in hydrophilic extract (water extract and ethanol extract). Most of phenolic compounds in lipophilic extract were volatile and presented in low concentrations leading to low antioxidant activity compared to the hydrophilic extracts. Unlike lipophilic extracts, hydrophilic extract also contained non-volatile phenolic compounds.

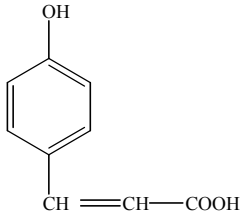
Ethanol extract that showed the highest antioxidant activity was analysed for non-volatile phenolic compounds by HPLC (Table 12). Three phenolic compounds were identified and UV-VIS spectra were compared with those of standards: (+)-catechin, *p*-coumaric acid and myricetin. Their chemical structures are shown in Figure 29. The concentrations of (+)-catechin, *p*-coumaric acid and myricetin were 5.08 ± 0.75, 0.19 ± 0.00 and 1.32 ± 0.10 mg/100 g WM, respectively. Barik ***et al.*** (1987) identified hydroxy cinnamaldehyde and [di-(*p*-hydroxy-cis-styryl)], in the chloroform extract of galangal. (+)-Catechin and myricetin in ethanol extract may be one reason of the correlation between flavonoid and antioxidant activity



(1)



(2)



(3)

Figure 29 Chemical structures of catechin (1), myricetin (2) and *p*-coumaric acid (3).

Source: Hall (2001).

Table 26 Concentrations of volatile-phenolic compounds and derivatives in the extracts from rhizome of galangal, analysed by GC-MS.

No.	Compound	RI		Identification	Concentration		
		HP-5	FFAP		μg/g extract	μg/g dry weight	μg/g fresh weight
	Essential oil						
1	Thymol	1289	1903	a, b, c2	48.56 ± 7.16	-	0.09 ± 0.01
2	Chavicol (4-allylphenol)	1256	2350	a, b, c1	2,390.45 ± 1,387.35	-	4.41 ± 2.56
3	Methyl eugenol	-	2022	a, b	4,130.38 ± 139.07	-	7.62 ± 0.26
4	Eugenol	-	2183	a, b	782.30 ± 36.65	-	1.44 ± 0.07
	Water extract						
1	Chavicol (4-allylphenol)	1259	2359	a, b, c1	21.24 ± 1.85	7.56 ± 0.66	0.68 ± 0.06
2	Vanillin	1417	-	a, b	-	0.09 ± 0.02	-
	Ethanol extract						
1	Chavicol (4-allylphenol)	1260	2359	a, b, c1	257.39 ± 43.25	84.11 ± 14.13	7.51 ± 1.26
2	Methyl eugenol	1411	2022	a, b, c1	87.63 ± 13.37	28.64 ± 4.37	2.56 ± 0.39
3	Eugenol	-	2195	a, b	13.32 ± 2.92	4.35 ± 0.95	0.39 ± 0.08

Remark: a = comparison of our MS data with Wiley 275 library data.

b= comparison of our RI data with ESO data base of essential oil (Boelens Aroma Chemical Information Service, 1999).

c= comparison of our RI data with literature data, c1 = Adams (1995), c2 = Lorjaroenphon (2004).

The other possible antioxidative compound of ethanol extract, which was hydrophilic extract, was 1′-acetoxychavicol acetate. The concentration of 1′-acetoxychavicol acetate in ethanol extract was showed in Table 27. Antioxidant activity of 1′-acetoxychavicol acetate and its related compounds was studied by Kubota *et al*. (2001). The result indicated that 1′-acetoxychavicol acetate showed the highest activity compared with its related compound. From this study, 1′-acetoxychavicol acetate was the main peak of volatile compound in ethanol extract. Then 1 ′-acetoxychavicol acetate from this study may be another major compound for antioxidant activity of ethanol extract.

Table 27 Concentrations of 1′-acetoxychavicol acetate in galangal ethanol extract.

Compound	Concentration		
	μg/g extract	μg/g dry weight	μg/g fresh weight
1′-acetoxychavicol acetate	10563.30 ± 3238.97	3452.06 ± 1058.49	308.42 ± 94.57

CONCLUSIONS

The main volatile compounds of the extracts are terpenes, especially in crude extract and essential oil. They were characterised by their high concentrations of cyclic monoterpenes and cyclic sesquiterpenes and low concentrations of acyclic monoterpenes and acyclic sesquiterpenes. Ethanol extract and water extract also composed of acids.

The extracts from rhizome of galangal showed both fishy odour deodorisation property and antioxidant activity. For fishy odour deodorisation of TMA, crude extract showed the most effective and ethanol extract was next below. This effect could be caused by galangal potent odourants and neutralization effect from acid and phenolic compound in the extracts. For antioxidant activity, ethanol extract exhibited the highest activity. Though essential oil was the lowest effective antioxidant, it might exhibit high biological activity because of its best accumulation in the body. There are many types of herbs and spices that show high antioxidant activity, but their strong flavour limits the application. In contrast to other herbs and spices, galangal gives the weak and pleasant flavour allowing it to be used in various food products.

Both fishy odour deodorisation and antioxidant activity possessed galangal extract to be a useful natural food additive. In addition, many traditional medicine property of galangal will let the extract of this plant have attracted considerable interest.

RECOMMENDATIONS

1. Phenolic compounds that are different in chemical structure should be further studied for the effect on fishy odour deodorisation. Then it can be concluded the effect of chemical structure on fishy odour deodorisation.

2. The other kinds of plant that composes of high phenolic compounds should be further studied for the effect on fishy odour deodorisation.

3. The extraction process influence the purity of the antioxidant extract, then the phenolic compound extraction process should eliminate all of the others compound.

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APPENDIX

APPENDIX A

Appendix Table A1 Percent peak area of volatile compounds from the extracts of galangal rhizome.

No.	Compound	RI		ID	% Peak area			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
	Acyclic monoterpenes and derivatives							
	Alcohols							
1	Linalool	1102	1540	a, b, c9, d	0.014	-	0.011	0.108
2	Geraniol	1277	-	a, b, c8	-	-	-	0.005
	Esters							
3	Neryl acetate	1365		a, b, c2	-	-	-	0.122
4	Geranyl acetate	1383	1755	a, b, c9, d	0.159	-	0.132	0.637
	Hydrocarbons							
5	β-Myrcene (Myrcene)	992	1162	a, b, c9	-	-	-	1.020
6	(*E*)-β-Ocimene	1050	1248	a, b, c9	-	-	0.041	0.060
	Cyclic monoterpenes and derivatives							
	Alcohols							
7	*trans*-Sabinene hydrate	1076	1457	a, b, c1	0.176	-	0.034	0.012

Appendix Table A1 (continued)

No.	Compound	RI		ID	% Peak area			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
8	*cis*-Sabinene hydrate	1107	1546	a, b, c1	0.120	-	0.036	-
9	*trans*-*p*-menth-2-en-1-ol	1131	1559	a, b, c9	-	-	-	0.028
10	*cis*-Verbenol	1153	-	a, b	0.083	-	-	0.060
11	*trans*-Verbenol	1159	-	a, b	-	-	0.038	-
12	*p*-menth-1,5 diene-8-ol	1161	-	a, b, c2	-	-	-	0.015
13	δ-Terpineol	1181	1678	a, b, c6	-	-	0.130	-
14	Terpinene-4-ol	1190	-	a, b, c9	0.787	-	0.092	1.277
15	*p*-Cymene-8-ol	1194	1851	a, b	0.276	-	0.096	0.079
16	α-Terpineol	1202	-	a, b, c9, d	0.948	-	0.417	1.083
17	*cis*-Piperitol	1207	-	a,b	-	-	-	0.027
18	*trans*-Carveol	1230	-	a, b	-	-	-	0.076
	Aldehydes							
19	α-Campholene aldehyde	1137	-	a, c6	-	-	-	0.029
	Ketones							
20	Camphor	1164	1537	a,b	-	-	0.011	

Appendix Table A1 (continued)

No.	Compound	RI		ID	% Peak area			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
	Esters							
21	Myrtenyl acetate	1337	-	a, b	-	-	-	0.028
22	*trans*-Carvyl acetate	1343	-	a, b	0.037	-	-	0.174
23	Thymyl acetate	1361	1856	a, b, c7	0.028	-	0.023	0.084
24	*cis*-Carvyl acetate	1369	-	a, b, c2	-	-	-	0.300
25	exo-2-hydroxycineole acetate	1375	-	a, c6	0.690	0.112	0.194	0.086
26	Bornyl acetate	-	1581	a, b, c4	0.082	-	0.026	0.229
27	Carvacryl acetate	-	1881	a, c6	0.025	-	0.032	-
	Ethers							
28	1,8-Cineol	1041	1216	a, b, c9, d	17.331	0.032	0.173	18.907
29	Exo-2-hydroxycineol	1235	1862	a, c6	0.178	0.217	0.103	-
30	Dehydro-1,8-cineol	-	1190	a, b	0.114	0.016	0.010	-
	Hydrocarbons							
31	α-Thujene	932	1034	a, b, c9	0.080	-	-	0.438
32	α-Pinene	942	1032	a, b, c9, d	0.616	-	-	2.814
33	α-Fenchene	956	1063	a, b, c2	-	-	-	0.016

Appendix Table A1 (continued)

No.	Compound	RI		ID	% Peak area			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
34	Camphene	958	1071	a, b, c9	-	-	0.008	0.076
35	Verbenene (Pinadinene)	963	-	a, b, c2	-	-	-	0.016
36	Sabinene	981	1124	a, b, c9	0.567	-	-	1.841
37	β-Pinene	986	1112	a, b, c9	0.212	-	-	1.010
38	α-Phellandrene	1011	1165	a, b, c9	-	-	-	0.058
39	δ-3-Carene	1018	1149	a, b, c1, d	-	-	-	0.003
40	α-Terpinene	1024	1180	a, b, c9	0.041	-	-	0.457
41	*p*-Cymene	1032	1270	a, b, c9, d	0.067	-	-	0.294
42	Limonene	1038	1203	a, b, c9, d	0.667	-	0.006	1.899
43	γ-Terpinene	1066	1244	a, b, c9	0.133	-	0.003	0.784
44	Terpinolene	1096	1280	a, b, c9	0.013	-	-	0.186
45	1,3,8-*p*-menthatriene	1121	-	a, b, c2	-	-	-	0.041
	Phenol							
46	Thymol	1289	1903	a, b, c6	-	-	-	0.007

Appendix Table A1 (continued)

No.	Compound	RI		ID	% Peak area			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
	Acyclic sesquiterpenes and derivatives							
	Alcohol							
47	(*E*,*E*)-Farnesol	1727	-	a, b	-	-	-	0.157
48	(*E*)-Nerolidol	-	2037	a, b	-	-	0.030	-
	Aldehyde							
49	Farnesal	1754	-	a, b	-	-	0.023	0.363
	Ester							
50	(*E*,*E*)-Farnesyl acetate	1847	-	a, b, c2	0.042	-	0.708	1.470
	Hydrocarbons							
51	(*E*)-β-Farnesene	1462	1666	a, b, c2	3.920	0.364	1.819	7.901
52	α-Bergamotene	-	1584	a, b	-	-	-	0.052
53	(*E*,*E*)-α-Farnesene	-	1745	a, b	0.229	-	-	-

Appendix Table A1 (continued)

No.	Compound	RI		ID	% Peak area			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
	Cyclic sesquiterpenes and derivatives							
	Alcohols							
54	*cis*-Sesquisabinene hydrate	-	2004	a, b	-	-	0.024	-
55	Elemol	-	2090	a, b	-	-	0.012	-
56	Zingiberenol	-	2112	a, b	-	-	-	0.036
57	α-Cadinol	-	2210	a, b	-	-	-	0.097
58	α-Bisabolol	-	2222	a, b	-	-	0.033	0.146
59	Juniper camphor	-	2310	a, b	-	-	-	0.106
	Hydrocarbons							
60	α-Copaene	1395	1489	a, b	0.119	-	0.009	0.375
61	β-Elemene	1408	1589	a, b	0.771	0.010	0.012	1.980
62	β-Caryophyllene	1447	1600	a, b, c6, d	4.765	0.294	0.559	7.841
63	γ-Gurjunene	1469	-	a, b, c2	-	-	-	0.050
64	allo-Aromadendrene	1473	-	a, b, c8	-	-	-	0.036
65	β-Selinene	1482	-	a, b, c2	2.712	0.195	0.334	3.818

Appendix Table A1 (continued)

No.	Compound	RI		ID	% Peak area			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
66	α-Amorphene	1508	-	a, b	-	-	-	0.897
67	Valencene	1514	-	a, b, c8	3.152	0.269	1.148	3.547
68	β-Bisabolene	1523	1729	a, b	6.350	0.683	2.267	7.537
69	δ-Cadinene	1530	1759	a, b, c1	-	-	0.012	0.107
70	β-Sesquiphellandrene	1539	1770	a, b	0.377	0.049	0.183	0.686
71	Selina-3,7(11)-diene	1544	-	a, b, c2	0.364	-	-	0.738
72	Germacene B	1589	1811	a, b, c6	0.288	0.039	0.137	0.687
73	δ-Elemene	-	1467	a, b	0.104	-	0.030	0.257
74	γ-Elemene	-	1635	a, b	0.035	-	0.045	0.152
75	α-Humulene	-	1672	a, b, c4	1.520	0.037	0.051	3.200
76	γ-Selinene	-	1679	a, b	-	-	-	0.058
77	Germacene D	-	1712	a, b	0.591	0.011	0.118	2.341
78	δ-Guaiene	-	1716	a, b	0.009	-	-	-
79	α-Selinene	-	1727	a, b	1.139		0.425	-
80	γ-Cadinene	-	1767	a, b	0.362	-	-	-

Appendix Table A1 (continued)

No.	Compound	RI		ID	% Peak area			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
	Oxide							
81	Caryophyllene oxide	1616	-	a, c5	-	-	-	0.058
	Others							
	Alcohols							
82	4-methyl-2-pentanol	821	-	a, c10	-	0.036	-	-
83	Coumaryl alcohol	1734	-	a	-	8.036	0.367	-
84	1-Hexadecanol	1885	-	a, b	-	0.017	-	-
85	*trans-p*-2,8-menthadienol-1	1130	1625	a, b	-		-	0.180
	Aldehyde							
86	Vanillin	1417	-	a, b	-	0.057	-	-
	Esters							
87	Chavicol acetate	1354	1967	a, c6	1.027	-	0.359	1.699
88	Cuminyl acetate	1430	1975	a, c6	-	-	0.021	1.509
89	Eugenyl acetate	1535	2271	a, b, c2	0.062	0.100	0.306	0.302
90	Diethyl phthalate	1604	-	a, c3	-	-	-	0.019

Appendix Table A1 (continued)

No.	Compound	RI		ID	% Peak area			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
	Hydrocarbons							
91	Pentadecane	1499	1495	a, b, c2	1.270	0.070	0.242	1.709
92	Hexadecane	1597	-	a, b, c2	-	-	-	0.021
93	6(*Z*),9(*E*)-Heptadecadiene	1673	-	a, c6	0.173	-	0.129	0.290
94	1-Heptadeccene	1679	-	a, b	0.326	0.008	0.068	0.381
95	Heptadecane	1697	1699	a, b, c2	0.074	-	-	0.219
96	Tridecane	-	1295	a, b	-	-	-	0.043
97	Tetradecane	-	1392	a, b	0.042	-	-	0.101
	Phenols							
98	Chavicol (4-allylphenol)	1256	2350	a, b, c2	1.188	0.390	1.590	0.334
99	Methyl eugenol	-	2022	a, b	0.142	-	0.514	0.599
100	Eugenol	-	2183	a, b	0.051	-	0.047	0.114
	Acids							
101	Myristic acid	1827	2034	a	-	0.314	-	-
102	Acetic acid	-	1447	a, c12	-	1.047	0.041	-
103	Propanoic acid	-	1536	a, c11	-	0.005	-	-

Appendix Table A1 (continued)

No.	Compound	RI		ID	% Peak area			
		HP-5	FFAP		Crude extract	Water extract	Ethanol extract	Essential oil
	Other							
104	1′-Acetoxychavicol acetate	1655	2505	c13	32.651	-	61.032	-
	$[M]^+$ at *m/z* 234							
	Unknown							
105	$[M]^+$ at *m/z* 212	1351	1741		13.783	0.828	5.186	9.476
106	$[M]^+$ at *m/z* 220	1555	2212		-	-	0.876	-
107	$[M]^+$ at *m/z* 192	1575	2649		-	76.041	-	-
108	$[M]^+$ at *m/z* 236	1661	2445		0.356	1.403	1.198	0.653

Remark: ID = Identification.

a = comparison of our MS data with Wiley 275 library data.

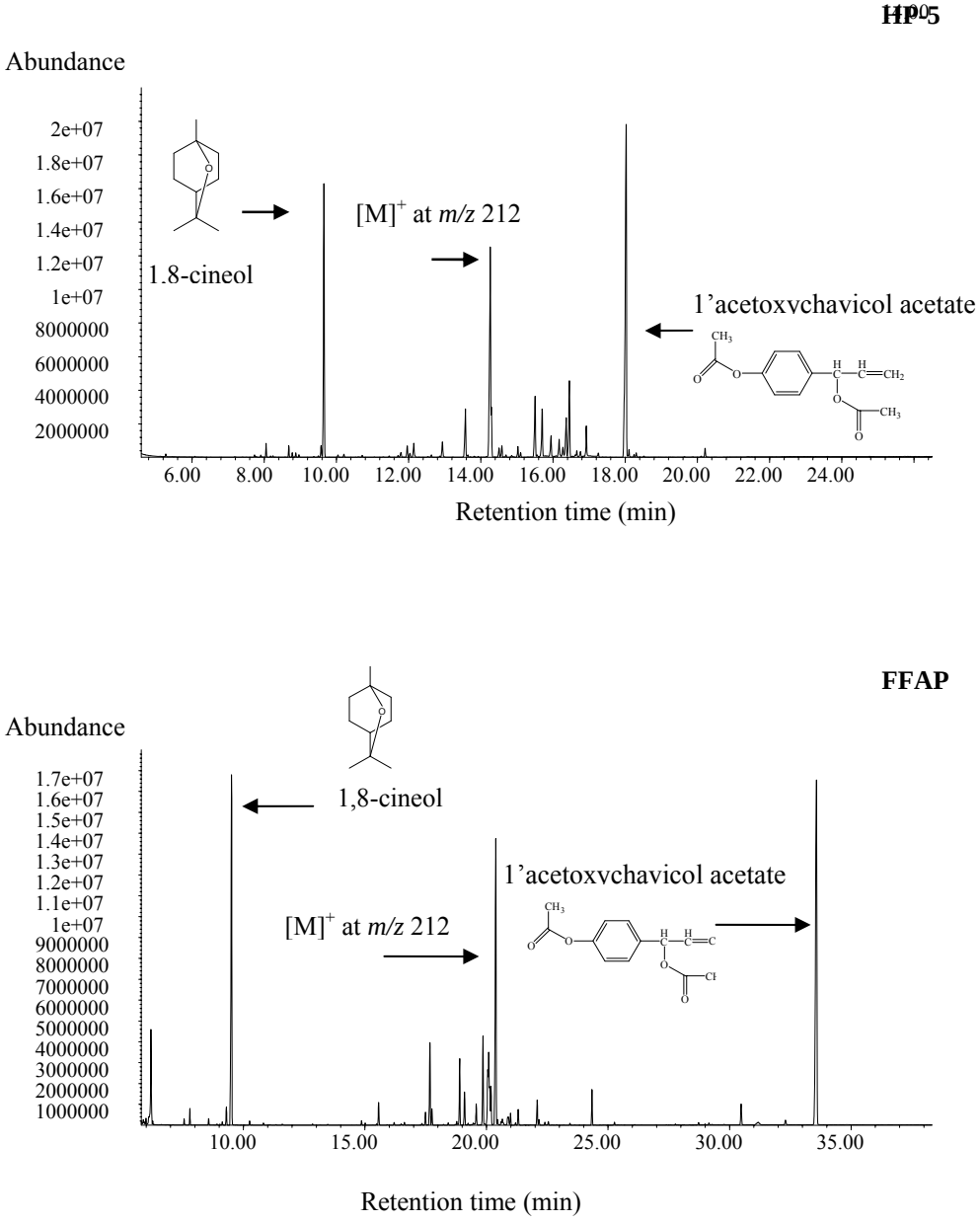
b= comparison of our RI data with ESO data base of essential oil (Boelens Aroma Chemical Information Service, 1999).

c= comparison of our RI data with literature data, c1= Le Quere and Latrasse (1990), c2 = Adams (1995), c3 = Ramarathnam *et al.* (1993), c4 = Tucker *et al.* (2002), c5 = Bouzouita *et al.*(2003), c6 = Lorjaroenphon (2004), c7= Letchamo *et al*. (2005), c8 = Martínez *et al.* (2005), c9 = Figuérdoé *et al*. (2005), c10 = Timón *et al*. (1998), c11 = Münch *et al*. (1997), c12 = Tairu *et al*. (2000), c13 = Mitsui *et al*. (1976).

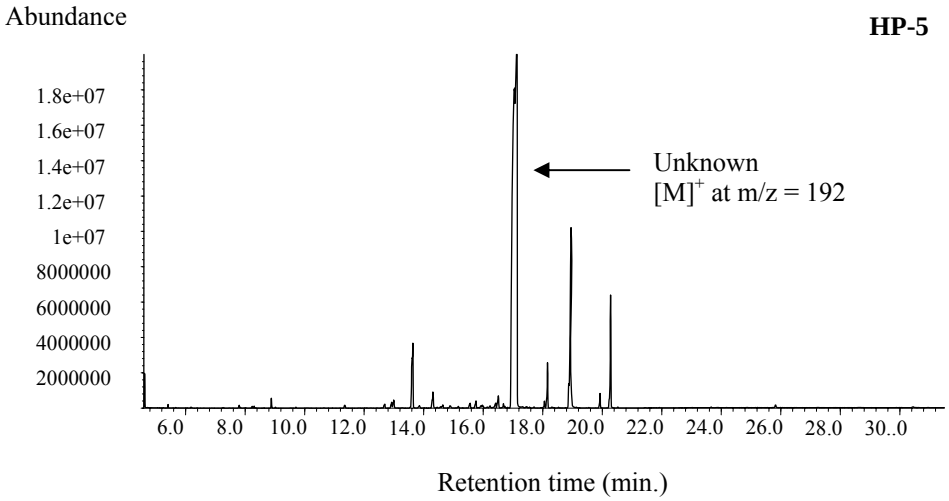
d= comparison of our RI data with the authentic compound.

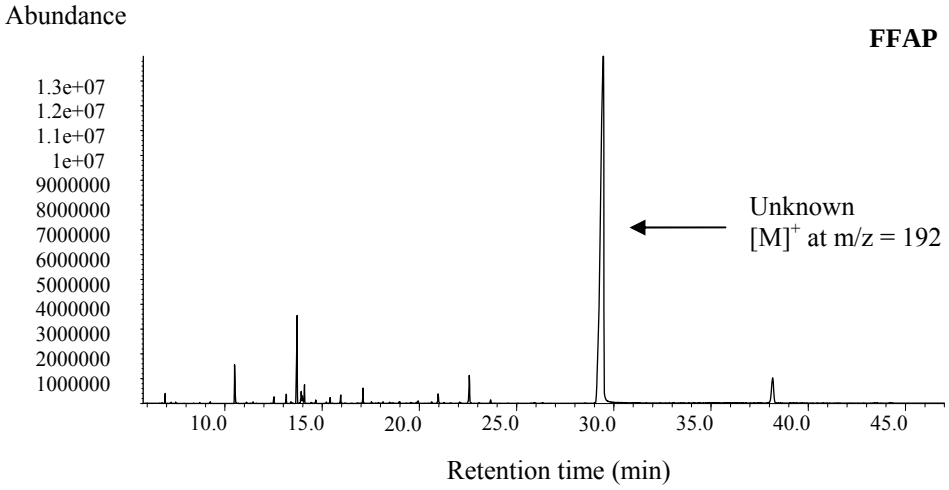
APPENDIX B

Chromatogram of galangal extract from GC-MS

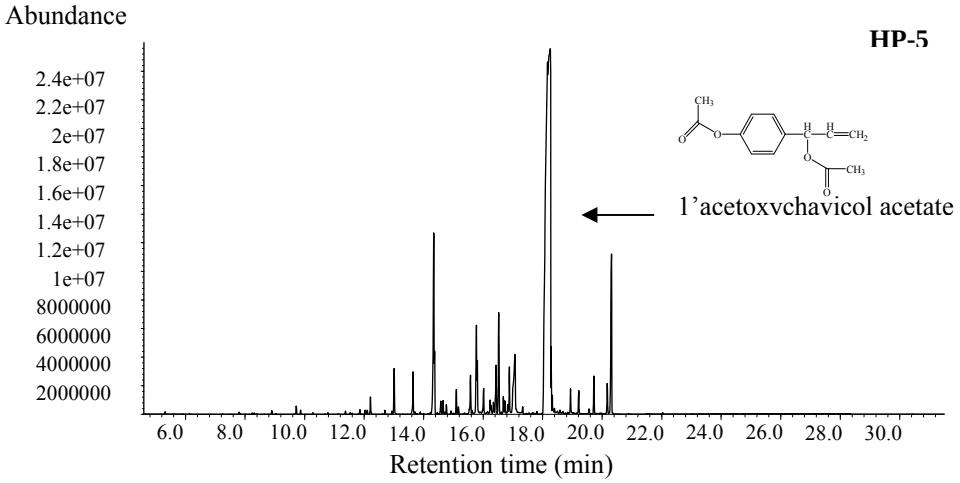


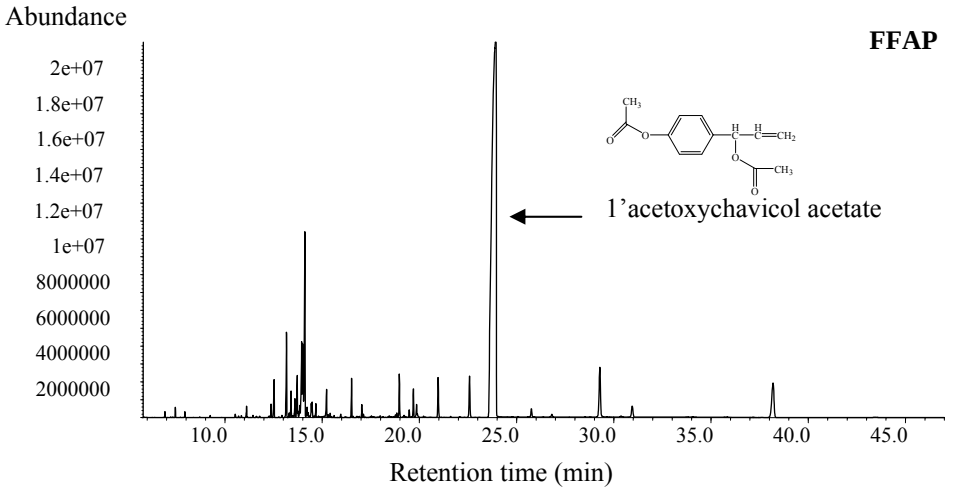
Appendix Figure B 1 Chromatograms of crude extract from the rhizome of galangal.



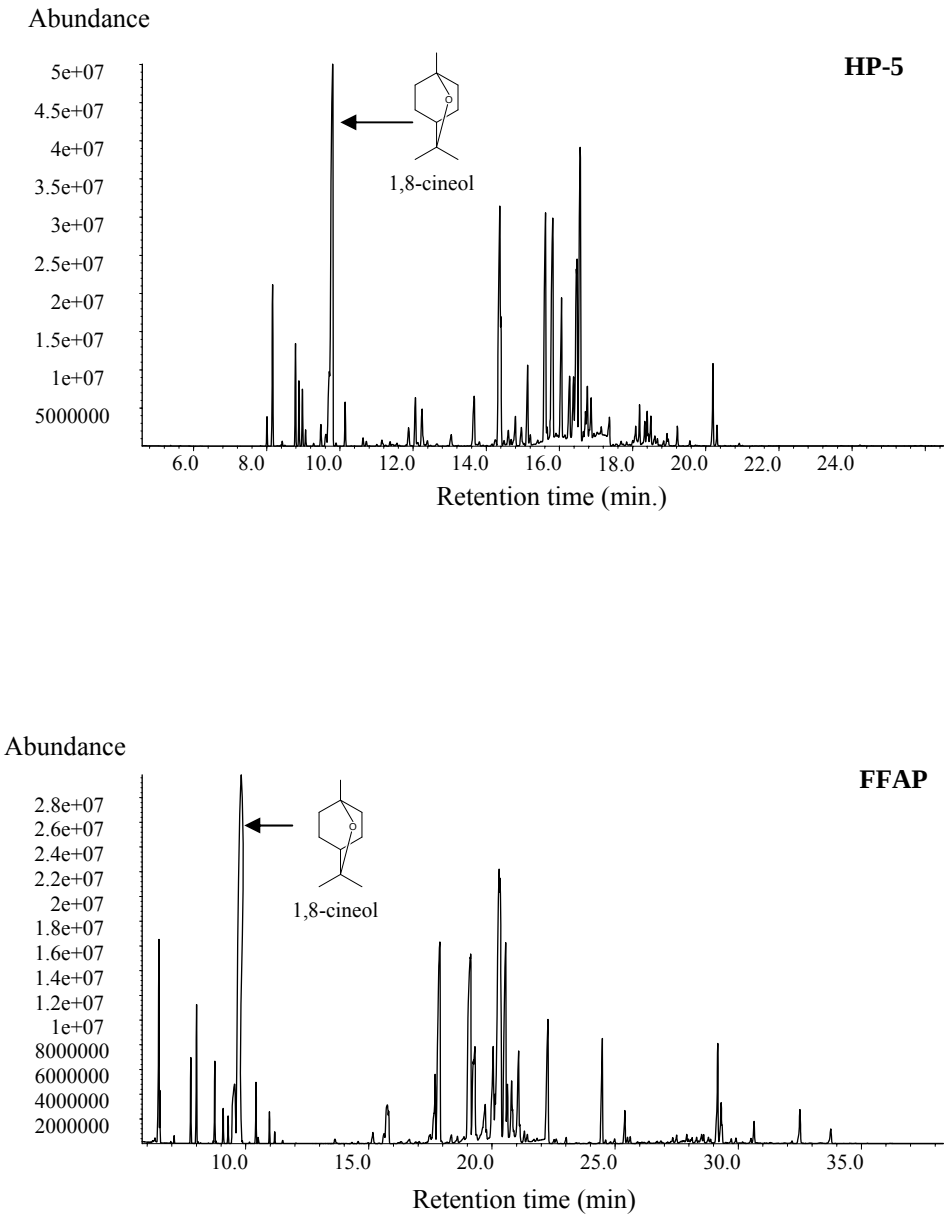


Appendix Figure B 2 Chromatograms of water extract from the rhizome of galangal.





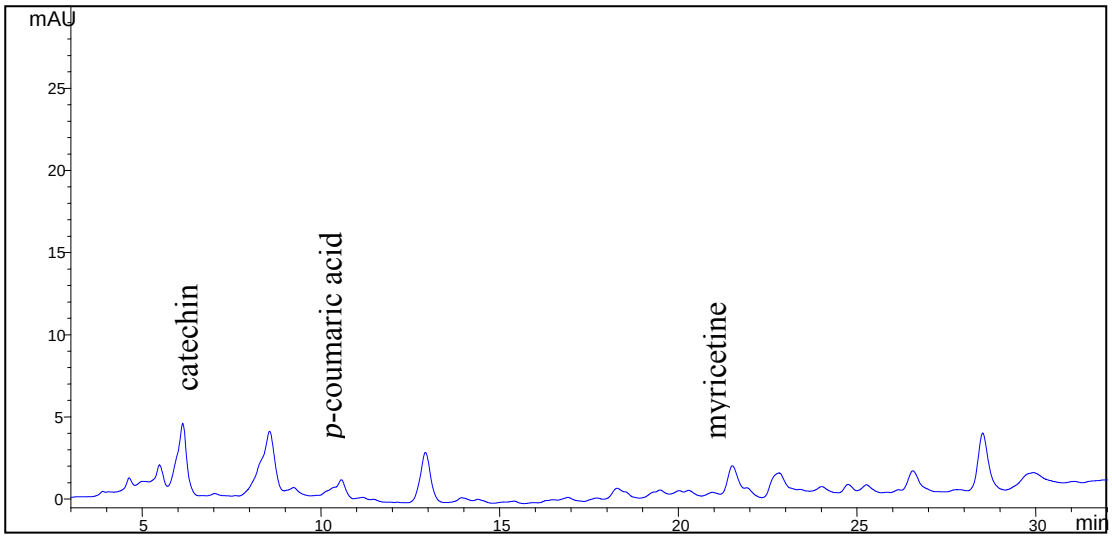
Appendix Figure B 3 Chromatograms of ethanol extract from the rhizome of galangal.



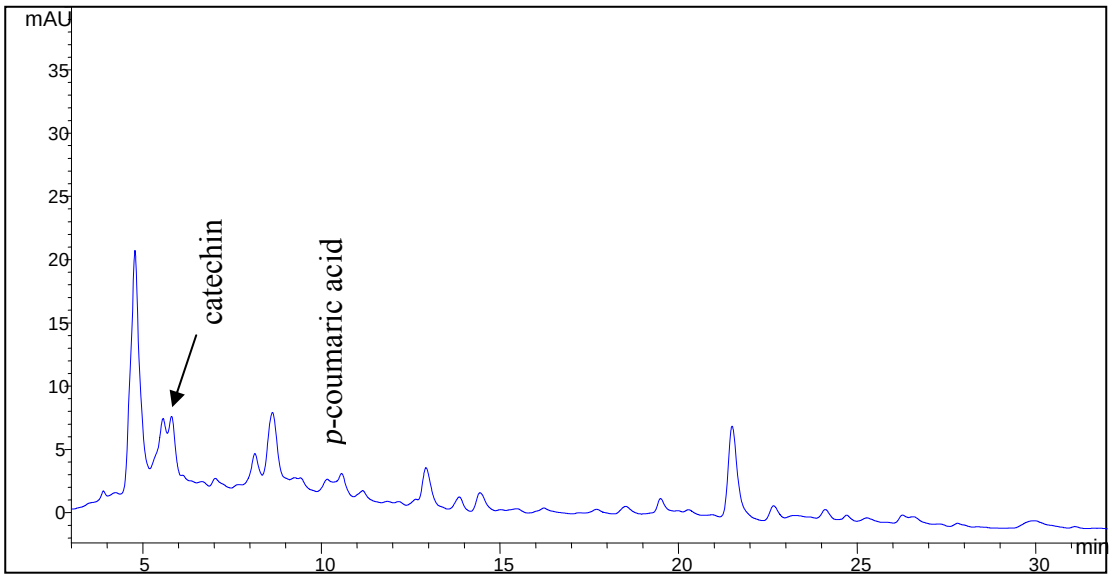
Appendix Figure B 4 Chromatograms of essential oil from the rhizome of galangal.

APPENDIX C

Chromatogram of galangal extract from HPLC



Appendix Figure C 1 HPLC chromatograms of ethanol extract from rhizome of galangal.



Appendix Figure C 2 HPLC chromatograms of crude extract from rhizome of galangal.