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Ginger (*Zingiber officinale*) Oil as an Antimicrobial Agent for Minimally Processed Produce: A Case Study in Shredded Green Papaya

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ABSTRACT

The capacity of ginger oils, extracted by different method (hydrodistillation & solvent extraction method), to inhibit microorganisms was studied both *in vitro* and *in vivo*. Chemical compounds in oil extracts, their antimicrobial activity and Minimum Inhibition Concentration (MIC) were determined on shredded green papaya. It was found that the major constituents of ginger oil extracted by hydrodistillation method were camphene, 1,8-cineol and α-pinene, while for oil obtained by solvent extraction they were β-phellandrene and 1,8-cineol. The extracts obtained by both extraction methods inhibited *Bacillus subtilis*, *Bacillus nutto*, *Pseudomonas aerugenosa*, *Rhodoturola* sp., *Samonella newport* DMST 15675, *Samonella enteritidis* DMST 15676 and *Fusarium* sp. There was however, no effect on growth rate of *Escherichia coli*, *Campylobactor coli* NTCT 11353 and *Campylobacteor jejuni* ATCC 33291. Moreover, the MIC of both ginger oil solutions were not significantly different. In case of shredded green papaya, samples were treated with ginger oil (0, 5, 10 & 15 µL) and kept at 13ºC for 4 days. The results showed that the growth rate of microorganisms was suppressed well in applied package with 5 and 10 µL ginger oils, while with 15 µL ginger oil reduction in growth rate was observed. Major volatiles detected in headspace of treated package were α-pinene, camphene, β-phellandrene and 1, 8-cineol. Thus, Ginger oils can be used to reduce the population of microorganisms in shredded green papaya and probably also in other fresh produce and minimally processed products.

Key Words: Fresh-cut; Contamination; Antimicrobial; Essential oil; Ginger

INTRODUCTION

The purpose of minimally processing fruit and vegetables is to keep the produce fresh without losing its nutritional quality and to ensure a sufficiently long product shelf life to make distribution feasible within a region of consumption (Huxsoll & Bolin, 1989). The main problem of any product is its short shelf life, since all natural protection is removed from the produce during peeling operation while cells are damaged by peeling, trimming, slicing, grating and shredding. The metabolic changes occurring after the processing include physiological aging, microbial spoilage, and degradation of color, texture and flavor (Kabir, 1994; Varoquaux & Wiley, 1997). The deterioration of minimally processed produce is mainly caused by decompartimentalisation through natural opening and damaged tissue and preharvest infections of microorganisms. The microorganisms present grow rapidly and minimally processes fruit and vegetable quality will soon be lost (Solomon *et al.*, 2006). Abadias *et al.* (2008) also investigated the microbial quality of minimally processed products and found that microbial counts in fruit products were very low but found a higher microorganism counts in grated carrot, arugula and spinach and lower counts in cut endive and lettuce.

The use of essential oils from herbs and spices is a novel antimicrobial treatment to reduce the initial microorganism loads and those induced during processing of minimally processed fruit and vegetable. In herbs and spices, there are many antimicrobial compounds exhibiting a wide range of activities against bacteria, yeasts and fungi. Essential oils from plants have been suggested as natural preservatives not only for processed food product but also for fresh produce (Nychas & Skandamis, 2003; Burt, 2004; Fonseca, 2006). The extracts of thyme, selected spice and *Solanum torvum* have antimicrobial activities against

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bacterial and fungal (Imelouane *et al.,* 2009; Bari *et al.*, 2010; Keskin *et al*., 2010). In case of fresh produce, it can reduce the final population of normal flora in kiwi fruit, honeydrew melon, lettuce and iceburg lettuce (Wan *et al.*, 1998; Roller & Seedhar, 2002). Singh *et al.* (2002) found that the final population of *E. coli* O157:H7 in lettuce, romaine lettuce and carrot could be reduced by using thyme oil as a rinsing solution. Furthermore, the essential oils of herbs and spices could inhibit food borne pathogen (*Bacillus sp., Listeria monocytogenes, Staphylococcus aureus, Samonella sp. & Vibrio parahaemolyticus*), although *Escheriachia coli* and *Zygosaccharomyces rouxii* are not affected by some of these essential oils (Cerruti & Alzamora, 1996; Delaquis *et al.*, 2002; Valero & Salmerón, 2003; Nasar-Abbas & Halkman, 2004; Yano *et al.*, 2006; Shan *et al.*, 2007).

Ginger is an important spice in Thailand. In 2001, Thailand grew more than 30,000 million tons of ginger. It is widely used as an ingredient in the food, pharmaceutical, cosmetic and other industries. Ginger contains a unique flavor derived from both non-volatile and volatile oils. The pungent compounds are gingerol and shagaol, while zingiberene is a pre-dominant component of oils (Ravindran & Babu, 2004). Some volatile compounds having antimicrobial properties are α-pinene, borneol, camphene and linalool (Nychas & Skandamis, 2003). The medicinal properties have been mainly used for treating the symptoms of vomiting, diarrhea, light-headedness, blurred vision, dyspepsia, tremors, decrease in body temperature and high blood pressure. Furthermore, 6-gingerol and 6-shagaol can reduce viability of gastric cancer cells (Ishiguro *et al.*, 2007).

Some ginger compounds such as α-pinene, borneol, camphene and linalool are responsible for its antimicrobial activities (Nychas & Skandamis, 2003). Ginger extracts have been reported to inhibit growth of *Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus, B. subtilis, E. coli, F. moniliforme and Mycobacterium* sp. (Yamada *et al*., 1992; Hiserodt *et al.,* 1998; Thongson *et al*., 2005; Natta *et al.*, 2008; Singh *et al.*, 2008). Ginger oils showed very good inhibition of *Salinococcus roceus, H. turkmenicus* and *Halococcus morrhuae* isolated from salt cured fish (Prasad & Seenayya, 2000).

Green or unripe papaya fruit are usually used as ingredients in several Asian foods. Shredded green papaya is the main ingredient in Thai's famous papaya salad (Som-Tam). For reasons of health-concerns, shredded green papaya is used in Thai stir-fried noodle (Pad-Thai) to replace the traditional noodle, which contains a high level of carbohydrates. Both of these food items are not only being appreciated in Thailand but worldwide. This suggests that there is market potential for packaged shredded green papaya.

The aim of this research was to investigate chemical compounds and antimicrobial activities of ginger oil extracted by two difference methods namely solvent extraction and hydrodistillation. The antimicrobial activities were evaluated against microorganism cultures *in vitro* on preti dish and *in vivo* on shredded green papaya packaged in plastic box during storage at 13ºC for 4 days.

MATERIALS AND METHODS

Plant preparation: Ginger rhizome and unripe papaya were purchased from a local market in Nonthaburi province, central Thailand. Ginger was cleaned with tap water to remove soil and dust, peeled and cut into cubes of approximately 0.125 cm^3. Unripe papaya fruit was individually cleaned with tap water to remove dust and then sprayed with 70% ethanol before peeling and shredding. The shredded product was subsequently sprayed with 70% ethanol and dried for 10 min at room temperature.

Ginger oil extraction: Ginger oil was extracted by two different methods, namely hydrodistillation and solvent extraction. For hydrodistillation, 2 kg of ginger sample were mixed with 5 L distilled water. The mixture was heated in a vertical hydrodistillation unit to 100ºC and held for 24 h. The ginger oil was separated from condensed vapor through an auto oil/water separator (Natta *et al.*, 2008).

For solvent extraction, 2 kg of cut ginger were mixed with 2 L of solvent comprising diethyl ether and hexane (2:5 v/v). The mixture was left at room temperature (30±2ºC) for 24 h. The homogenate was filtered using Whatman No. 4 filter paper. The solvent was evaporated with a rotary evaporator at 40ºC, 750 mBar for 45 min (Sa-nguanpuag, 2004).

Three separated extractions, for each extraction method were carried out in a period of 4 weeks. The extracted ginger oils from the three separate extractions were mixed together and stored in amber vials at 8ºC.

Determination of headspace volatile compounds: A 250 µL of extracted ginger oil was injected into a GC/MS. The headspace volatile compounds of ginger extracts were analyzed using the splitless protocol by a Gas Chromatography/Mass Spectrometry (Agilent 6850/5973N series GC-MSD) equipped with a capillary column (HP-5 MS column: 30 m in length, 0.25 mm in diameter & 0.25 µm in film thickness). For this purpose, 100 µL of ginger extracts were put into a 25 µL glass vial, closed with an aluminum cap and silicone septum. Samples were equilibrated in a water bath at 50ºC for 30 min before headspace sample was taken through rubber septum using a gas tight syringe. The column temperature was initially set at 60ºC, held for 3 min then increased at the rate of 15ºC/min up to 180ºC, which was then held again for 5 min before increasing again at the same rate to 250ºC and held at that temperature for 20 min. The injector temperature was 250ºC and detector temperature was 280ºC. The carrier gas was helium at an average velocity at 36 cm/s. The ionization voltage was 70 eV. The identification of volatile compounds was performed by comparing the mass spectrum with

spectra in GC-MS library (National Institute of Standard & Technology, NIST98) and by comparing with data from previous studies (Chen & Ho, 1988; Onyenekwe & Hashimoto, 1999; Zancan *et al.*, 2002; Sacchetti *et al.*, 2005; Wohlmuth *et al.*, 2006; Singh *et al.*, 2008).

Activity of ginger oils against bacteria, yeasts and molds: Antimicrobial activity of ginger extracts against bacteria, yeasts and molds was evaluated both in contact and not in contact with the target microorganisms. Ten difference species of microorganisms including bacteria *(Bacillus subtilis, B. nutto, Samonella newport, S. enteritidis, Escherichia coli* O157:H7, *Pseudomonas aeruginosa, Campylobacter coli & C. jejuni*), one yeast species (*Rhodoturola glutinis*) and one fungal species (*F. oxysporum*) were selected for this study.

Preparation of microorganisms: Bacteria and yeast cultures were inoculated in Tryptic Soy Broth (TSB) and incubated in an incubator shaker at 37ºC with 200 rpm for 24 h. The cultures were diluted with saline water to obtain a suspension at concentration of 1.5×10^8 CFU/mL with a McFarland No. 0.5 standard. The spores of fungi were diluted with saline water to obtain a suspension at a concentration of 10^5 spores/mL measured by a hemocytometer. The suspension (100 µL) was spread on petri dish containing agar according to the type of microorganism. Plate Count Agar was used for bacteria, Yeast and Mold Agar was used for yeast and Potato Dextrose Agar was used for fungi. The petri dish was left at room temperature in the laminar flow for 30 min to allow the absorption of suspension.

Antimicrobial activity of ginger extracts (contact): Filter paper (Whatman No. 4) was cut into a circle of 6 mm diameter. Each cut was impregnated with 10 µL ginger extract solution (20 mg of ginger extract/mL of 10% ethanol). Four impregnated filter papers were placed on the four quadrant of the petri dish containing microorganisms. The petri dish was left for 30 min at room temperature and incubated at 37ºC for 24 h. for bacteria and yeast and 48 h for fungi. The clear zone was measured as an indication of antimicrobial activity. The experiment was carried out in triplicate for each type of microorganisms.

Antimicrobial activity of ginger extracts (non-contact): Sterile filter paper discs were impregnated with 10 µL ginger extract solution (20 mg of ginger extract/mL of 10% ethanol). The impregnated filter paper circles were placed in a covered petri dish containing microorganisms. The plate was incubated at 37ºC for 24 h. for bacteria and yeast and 48 h for fungi by reverse petri dish. The amount of microorganisms was taken as a measure of antimicrobial activity. The experiment was carried out in triplicates for each type of microorganisms.

Determination of minimum inhibition concentration (MIC): The minimum inhibition concentration of ginger oil was determined for the microorganisms, tested in the previous study. Each ginger extract was diluted into two-fold serial dilution to obtain concentration ranging between 2.5 and 20.0 mg/L. The disc diffusion assay test was performed as described above but with ginger extract solution of different concentrations. The MIC was the lowest concentration that inhibited the microorganisms, i.e. with an inhibition zone greater than 6.0 mm.

Antimicrobial activity of ginger oils on shredded green papaya: Twenty-five gram of shredded green papaya were put in 8×11×5 cm plastic boxes along with a small aluminium dish containing 6.0 mm sterile filter paper (Whatman No. 4). In each dish, 0, 5, 10 or 15 µL of ginger extract was added. The box was then closed tightly and stored at 13±2ºC for 4 days. Total microorganisms, yeast and fungi were quantitatively determined daily during 4 days of storage.

Shredded green papaya (25 g) was mixed with 225 mL of saline solution. The mixture was homogenized by a stomacher and a serial dilution was made to obtain a suitable concentration. The suspension (100 µL) was spread into a petri dish containing agar according to the type of microorganism. The results were expressed as Colony Forming Unit per milliliter (CFU/g).

Statistical analysis: The data on antimicrobial activity of ginger extracts were subjected to T–test for antimicrobial activity and Analysis of Variance (ANOVA) for microbial population growing on shredded green papaya by using SPSS version 12.0. Least Significant difference (LSD) multiple comparison test was used to determine mean differences at the 5% significance level.

RESULTS AND DISCUSSION

Volatile compounds in ginger oils: The volatile compounds in ginger oils extracted by hydrodistillation and solvent extraction were different quantitatively and qualitatively (Table I). The number and quantity of compounds detected in ginger oils extracted by the hydrodistillation method was more than that extracted by solvent method. Sesquiterpenes were the most abundant compounds in hydrodistillation ginger oils against monoterpenes in solvent extracted oils. Major volatile compounds in oil obtained by the hydrodistillation method were camphene, 1,8-cineol and α-pinene, while in solvent extracted oil the major volatiles were β-phellandrene and 1,8-cineol. The amounts of volatile compounds in oils obtained by hydrodistillation were higher than in solvent extracted oils. These results suggest that hydrodistillation can extract more volatile compounds than solvent extraction, which agree with those of Natta *et al.* (2008).

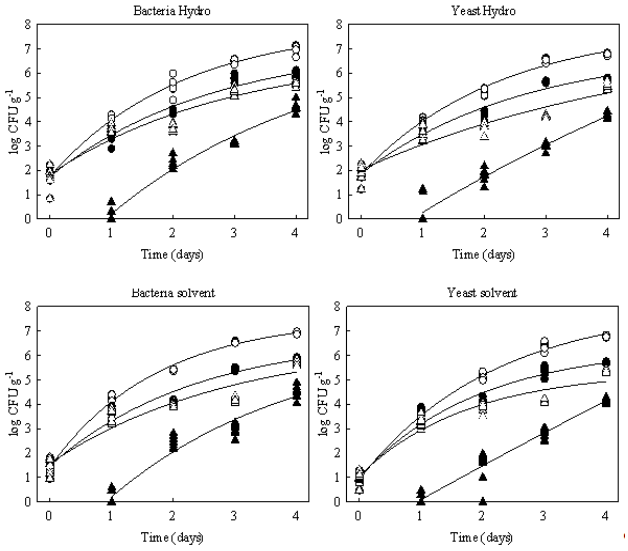
Antimicrobial activity of ginger extracts: In this study, 4 groups of microorganisms, including gram-positive bacteria, gram-negative bacteria, yeasts and molds were used to investigate the antimicrobial activity of ginger extracts. The selected microorganisms are food borne pathogen, which can contaminate produce both during preharvest and during processing (Heard, 2002).

Table I: Chemical compounds of ginger extracted by hydrodistillation and solvent extraction

Compound	Area Response (× 10^4)	
	Hydrodistillation	Solvent Extraction
Aromatic		
Toluene	-	0.84 ± 01.9
p-Cymene	3.56 ± 1.04	0.12 ± 0.07
Monoterpene		
α-pinene	138.35 ± 50.99	2.00 ± 1.13
Camphene	355.60 ± 124.55	6.36 ± 3.33
β-Pinene	20.94 ± 7.47	0.35 ± 0.19
α-Phellandrene	11.98 ± 3.60	0.36 ± 0.19
α-Terpinene	1.02 ± 0.31	-
β-Phellandrene	2.35 ± 0.88	5.92 ± 3.10
δ-Terpinene	1.12 ± 0.33	-
Terpinolene	6.00 ± 1.40	0.13 ± 0.04
3-Carene	3.15 ± 1.00	-
α-Thujene	0.73 ± 0.24	-
β-Thujene	62.36 ± 19.76	1.69 ± 0.89
Tricyclene	6.74 ± 2.53	-
Sesquiterpenes		
Ylangene	0.25 ± 0.15	-
Camphor	1.13 ± 0.30	-
α-Elemen	0.51 ± 0.22	-
α-Farnesene		0.09 ± 0.09
Germacrene D	0.26 ± 0.11	-
Zingiberene	3.41 ± 1.14	0.39 ± 0.19
β-Sesquiphellandrene	1.09 ± 0.44	-
α-Copaene	0.50 ± 0.16	-
t-Muurolene	1.74 ± 0.61	-
Allaromadendrene	1.53 ± 0.54	0.44 ± 0.40
Curcumene	2.06 ± 0.55	0.10 ± 0.10
Valencene	1.74 ± 0.61	0.44 ± 0.40
Alcohol Monoterpene		
α-Terpineol	1.72 ± 0.65	-
β-Linalool	4.54 ± 1.31	-
Borneol	2.48 ± 0.92	-
β-Citronellol	0.16 ± 0.12	0.03 ± 0.03
Aldehyde Monoterpene		
β-Citronellal	1.40 ± 0.47	-
Neral	14.39 ± 5.27	-
Geranial	14.94 ± 6.27	-
Oxide Monoterpene		
1,8-Cineole	142.98 ± 41.00	5.30 ± 3.00

Ginger oils obtained by both hydrodistillation and solvent extraction inhibited 7 out of 10 microorganisms provided the oil sample was in contact with microorganisms (Table II). The ginger oil obtained by hydrodistillation was more effective to inhibit microorganisms than oil obtained by solvent extract method, probably due to differences in number of active compounds and in their concentrations. Singh *et al.* (2008) also found that the growth rate of *Aspergillus sp.*, *Fusarium sp.* and bacteria was clearly suppressed by the ginger oil extracted by hydrodistillation when compared with solvent extraction. *B. subtilis* was the most sensitive to ginger oils from both methods with the largest inhibition zone of 23.3 and 11.7 mm diameter (hydrodistillation & solvent extraction, respectively). Previous studies indicated that, *E. coli*, *C. coli* and *C. jejuni* are not inhibited by neuther of the ginger oils, (Onyeagba *et al.*, 2004; Sofia *et al.*, 2007). The oils from both extraction methods differed non-significantly in inhibition of *B. nutto*, *S. newport* and *S. enteritidis.*

Fig. 1: Measured (dots) and simulated (lines) behaviour of bacteria (left) and yeast (right) growth, using different concentrations of ginger oils (○=0, ●=5, Δ=10, ▲=15 µL), obtained by two extraction methods



The result of non-contact assay was similar to that from disc diffusion assay (Table II). The oil obtained by hydrodistillation was more effective than that obtained by solvent extraction, showing a significant difference in antimicrobial activity. Gram negative bacteria (*E. coli, C. coli, C. jejuni*) and fingi (*Fusarium sp.*) could not be inhibited by the non-contact method, probably since the cell membranes of gram negative bacteria and fungi are more complex than those of gram positive bacteria and yeasts (Moat *et al.*, 2003). The contact assay was more effective than non-contact assay. The result of the contact assay showed that ginger oils can inhibit *Fusarium sp.*, which was not true in non-contact assay. In direct contact with microorganisms the fixed compound can interact with the microorganisms, while in the non-contact assay, only volatile compounds can do so.

Minimum inhibition concentration (MIC): The antimicrobial activity of ginger oil samples, obtained by both hydrodistillation and solvent extraction, was evaluated by measuring the diameters of the inhibition zones for all samples at the various concentrations. From these the MIC values were calculated (Table III). The MIC of ginger oils were only assessed for pre-selected microorganisms. There were no significant differences between the MIC of ginger oils extracted by hydrodistillation and by solvent extraction on *B. nutto, B. subtilis, Pseudomonas aeruginosa, Rhodoturola sp.*, and *Fusarium* sp. (Table III). The ginger oil obtained by hydrodistillation method showed stronger inhibition on *S. newport* and *S. enteritidis*.

The ginger oil samples isolated by hydrodistillation method exhibited antimicrobial activity against all microorganisms tested within a range of 2.5-10 mg/L, which was stronger than oil obtained by solvent extraction

Table II: Antimicrobial activity of ginger extract by different methods in agar disc diffusion assay and non–contact assay

Miroorganism strains	Inhibition zone (mm)		Amount of Microorganism (CFU/mL)	
	Hydro-distillation	Solvent extraction	Hydro-distillation	Solvent extraction
Bacillus nutto	12.0 ± 2.6^{a}	10.8 ± 0.9^{a}	0	0
Bacillus subtilis	23.3 ± 3.7^{d}	11.7 ± 1.0^{c}	0	0
Pseudomonas aerugenosa	17.5 ± 1.5^{f}	9.8 ± 1.3^{e}	0	0
Escherichia coli O157:H7	0	0	21.7 ± 2.0^{a}	243.7 ± 11.6^{b}
Campylobacter coli NTCT 11353	0	0	16.0 ± 4.0^{c}	160.7 ± 27.8^{d}
Campyrobacter jejuni ATCC 33291	0	0	25.3 ± 4.8^{e}	255.3 ± 27.3^{f}
Rhodoturola sp.	13.5 ± 3.1^{h}	8.8 ± 0.9^{g}	0	0
Samonella newport DMST 15675	10.2 ± 1.6^{m}	8.7 ± 0.8^{m}	0	0
Salmonella enteritidis DMST 15676	11.8 ± 1.3^{n}	11.3 ± 0.8^{n}	0	0
Fusarium sp.	14.5 ± 3.5^{y}	10.3 ± 1.5^{x}	36.7 ± 17.6^{g}	307.7 ± 33.8^{h}

Mean values with the same letter within each row are not significantly different ($p > 0.05$)

Tables III: Minimum inhibition concentration (MIC) of ginger extract by different methods in agar disc diffusion assay and non–contact assay

Microorganism strain	Minimum Inhibition Concentration (mg/L) in agar disc diffusion method		Minimum Inhibition Concentration (mg/L) in non – contact assay	
	Hydro-distillation	Solvent extraction	Hydro-distillation	Solvent Extraction
Bacillus nutto	2.5	2.5	2.5	5.0
Bacillus subtilis	2.5	2.5	2.5	5.0
Pseudomonas aerugenosa	2.5	2.5	2.5	5.0
Rhodoturola sp.	10.0	10.0	10	10.0
Samonella newport DMST 15675	2.5	5.0	5.0	10.0
Salmonella enteritidis DMST 15676	2.5	5.0	5.0	10.0
Fusarium sp.	10.0	10.0	10.0	10.0

Table IV: The estimated parameters in $y = y_{max}+(y_0-y_{max})*\exp(-k*t)$ for microbial growth in stored shredded green papaya

Conc.	Species and Oil	Variable			Standard error			R^2_{adj}	N_{obs}
		y_0	y_{max}	k	y_0	y_{max}	k		
0	Bacteria Solvent	1.43	7.53	0.58	0.06	0.13	0.03	0.99	45
5	Bacteria Solvent	1.49	6.80	0.42	0.10	0.33	0.05	0.97	45
10	Bacteria Solvent	1.57	6.60	0.34	0.15	0.74	0.09	0.90	45
15	Bacteria Solvent	-2.34	6.77	0.33	0.54	1.24	0.10	0.95	36
0	Yeast Solvent	0.92	8.03	0.46	0.07	0.21	0.03	0.99	45
5	Yeast Solvent	1.03	6.38	0.51	0.10	0.25	0.06	0.97	45
10	Yeast Solvent	1.07	5.36	0.57	0.15	0.31	0.10	0.91	45
15	Yeast Solvent	-1.34	45.80	0.03	0.33	118.88	0.08	0.96	36
0	Bacteria Hydro	1.74	7.97	0.47	0.08	0.22	0.04	0.99	45
5	Bacteria Hydro	1.74	7.22	0.38	0.10	0.42	0.06	0.96	45
10	Bacteria Hydro	1.84	6.88	0.34	0.12	0.64	0.08	0.93	45
15	Bacteria Hydro	-2.22	7.61	0.28	0.39	1.27	0.07	0.97	36
0	Yeast Hydro	1.85	7.93	0.44	0.06	0.20	0.03	0.99	45
5	Yeast Hydro	1.87	7.02	0.38	0.09	0.37	0.05	0.97	45
10	Yeast Hydro	2.03	7.72	0.20	0.12	1.67	0.09	0.91	45
15	Yeast Hydro	-1.34	14.88	0.11	0.31	8.96	0.08	0.96	36

y = Microbial population (log CFU g^{-1})
y_0 = Microbial population at initial day (log CFU g^{-1})
y_{max} = Maximum microbial population (log CFU g^{-1})
k = Rate constant (day^{-1})
t = Time (Day)

method. This seem to be due to the fact that number and concentration of major compounds of hydrodistillation ginger oil was greater than solvent extraction.

Antimicrobial activity of ginger oil on shredded green papaya: The inhibition activity of ginger oil extracts on microorganisms in packaged shredded green papaya during storage at 13ºC up to 4 day are presented in Fig. 1. The effect of ginger oil against mold growth could not be confirmed since none of the packages exhibited mold growth (data not shown). The CFU of bacteria and yeasts per gram of shredded green papaya linearly increased with time over the entire storage period, except for the shredded papaya in the box with 15 µL ginger essential oil where the linear relationship was found after day 1 ($P<0.05$). During the first day, the population declined.

Volatiles in ginger essential oil extracted by

hydrodstillation inhibited the growth of total bacteria and yeasts. The total population of bacteria and yeasts on shredded green papaya treated with ginger oil was less than that in the control (without ginger oil). The more the applied compound was volatile, the more growth of microorganisms was inhibited. Since the growth of microorganisms can be described with the exponential function, the slope of the exponential equation can indicate the growth rates of the microorganisms. The rate constant (k) of the exponential equation in Table IV is growth rate of both bacteria and yeast populations. The efficiency of inhibition of ginger oil extract by hydrodistillation method increased when the concentration was increased, as indicated in rate constant. The rate constant of ginger oil extract at 15 µL concentration was 0.28 and 0.11 day^{-1} for bacteria and yeast, respectively. Because more was the concentration of ginger oil extract in package, the greater microorganism was inhibited (Sa-nguanpuag *et al.,* 2007). Furthermore, the coefficient determination (R^2) of equation was high, indicating that this equation could be used to describe well the microbial growth under experimental conditions.

On the other hand, volatiles in ginger essential oil obtained by solvent extract inhibited the growth of total bacteria and yeast. The inhibition of ginger oil taken by solvent extract was similar to that obtained with the hydrodistillation method. The package with 15 µL of ginger oil can reduce total microorganisms and yeast on the initial day (day 1) of storage and then population of total microorganisms and yeast showed rapid growth rate until the end of storage time. The growth rates of total microrganisms in control and packages with 0, 5, 10 and 15 µL ginger extract by solvent extract were 0.58, 0.42, 0.34 and 0.33 per day, while the growth rate of yeast in control and packages with 0, 5, 10 and 15 µL ginger extract by solvent extract were 0.46, 0.51, 0.57 and 0.03 days (Table IV).

The result for non-contact assay *in vivo* was found to be similar with contact assay. The volatile ginger essential oils can inhibit microorganism both *in vitro* and *in vivo*. Volatile compounds inhibited gram-positive bacteria more strongly than gram-negative bacteria, probably because cell membranes of gram-negative bacteria are more complex than those of gram-positive bacteria. Cell membranes of gram-positive bacteria can easily interact with essential oils. The cell membrane will lose permeability and start to leakage (Burt, 2004).

CONCLUSION

The major chemical compounds in ginger extracts by hydrodistillation were camphene, 1,8-cineol and α-pinene and those by solvent extraction were β-phellandrene and 1,8-cineol. The ginger oil extracts can inhibit a wide range of microorganisms. Volatiles in ginger essential oils can reduce the population of bacteria and yeasts in shredded green papaya. This indicates that these products can be used as antimicrobial agents in packages of fresh-cut produce.

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