UTILIZATION OF CINNAMON OIL AND CLOVE OIL AS ANTIFUNGAL AGENTS IN ACTIVE PACKAGING FOR INTERMEDIATE MOISTURE FOOD PRODUCTS

INTRODUCTION

With solid or semi-solid intermediate moisture foods (IMF) with water activities within the range 0.65-0.90, fungal growth is usually the most important microbiological spoilage problem e.g. *Eurotium amstelodami* can grow and produce toxin in durian paste (Nitimongkonchai, 1999) *Aspergillus sp., Penicillium sp., Geotrichum sp.,* and *Cladosporium sp*. can deteriorate color, taste and smell of cheese products by creating red, black or gray spots, respectively, on the cheese surface (Robinson, 1990). The development of these fungi on the IMF surface is a major obstacle for quality control in IMF industries and IMF markets. A good method of preservation should prevent these surface fungi from growing and should not inhibit growth of other useful microbes in the IMF product. Moreover it should not do any harm to human and should be accepted by customers. This would help increase the shelf life and the markets of IMF products. To find such a method of preservation is the main purpose of this project.

Essential oils are well known inhibitors of microorganisms (Burt, 2004). Cinnamon oil and clove oil are both natural preservative and flavouring substances that are not harmful when consumed in food products. There have been a number of reports of substances in each of cinnamon and clove oils that inhibit the growth of molds, yeasts and bacteria. Both cinnamon oil and clove oil added at 2% in potato dextrose agar (PDA) completely inhibited the growth of seven mycotoxigenic molds (*Aspergillus flavus*, *A. parasiticus*, *A. ochraceus, Penicillium sp. M46, P. roqueforti, P. patulum*, and P. *citrinum*) for various times up to 21 days (Azzouz and Bullerman, 1982) and could also inhibit the growth of yeasts (Canner and Beuchat, 1984). Suksrikarm (1987) similarly reported that cinnamon oil and clove oil could separately inhibit many other microbes including *Lactobacillus* sp*., Bacillus thermoacidurans, Salmonnella* sp*., Corynebacterium michiganense, Pseudomonas striafaciens,*

Clostridium botulinum, Alternaria sp.*, Aspergillus* sp.*, Canninghamella* sp.*, Fusarium* sp.*, Mucor* sp.*,* and *Penicillium* sp. Soliman and Badeaa (2000) found that ≤500 ppm of cinnamon oil can inhibit *Aspergillus flavus, A. parasiticus, A. ochraceus* and *Fusarium moniliforme* on PDA and August (1978) reported that high concentrations of cinnamon oil and clove oil could also inhibit the asexual spores of fungi. Mixtures of cinnamon and clove oils are therefore an interesting alternative to use of other chemical preservatives and appear well suited to use in active packaging systems.

Active Packaging (AP) is an innovative food packaging concept that combines advances in food technology, food safety, and packaging and material sciences in an effort to better meet consumer demands for fresh-like, safe products (Scannell *et al*., 2000). Active packaging interacts with the product or the headspace between the package and the food system to restrict growth of microorganisms and slow other quality deterioration processes (Appendini and Hotchkiss, 2002). Applications of AP include oxygen and carbon dioxide absorbers to manipulate headspace gas concentrations, devices to release flavour compounds into the product, and adding antimicrobial agents to the packaging system to control microbial growth (Vermeiren *et al*., 1999). One specific application is to incorporate essential oil(s) into the packaging to prevent the growth of microorganism (Nielsen and Rios, 2000). Modified atmosphere packaging (MAP), in which the O_2 concentration is typically lowered and the CO_2 concentration is elevated, is a well established technique for achieving related goals. This natural packaging product may open new opportunities for a new marketing in the future.

The main objective of this research was to develop an active packaging technique suitable for preserving the IMF products with a relatively long shelf life by employing the use of volatile essential oils together with the modified atmosphere and normal air environment.

OBJECTIVES

1. To Survey of types of IMF products in modern market from the retailers in Bangkok to identify products for preservation by active packaging.

2. To study effectiveness of using cinnamon and clove oil as antifungal agents in IMF products by means of an active packaging technique.

3. To develop the active packaging system for preservation of the selected IMF products with modified atmosphere condition.

4. To apply the active packaging technique developed to preserve the IMF products under normal air condition.

LITERATURE REVIEW

1. <u>IMF Product and Food Spoilage</u>.

Xerotolerant and xerophilic molds and yeasts, and halophilic bacteria, are the major spoilage organisms of intermediate moisture foods (IMF) which have water activities (a_w) of 0.65-0.90 (Pitt and Hocking, 1997; Isabel *et al*., 2000). Many microorganisms have been found on IMF including *Aspergillus flavus,* and *Penicillium roqueforti* on bread (Nielsen and Rios, 2000); *Eurotium* sp. on bakery products (Suhr and Nielsen, 2004), *Staphylococcus aureus* on honey (Mundo *et al*., 2004), *Debaryomyces hansenii,* and *Zygosaccharomyces roxii* on syrups, fruit concentrates and jams (Andrews *et al*., 1997), red halophilic cocci on salt cured fish and solar salt (Prasad and Seenayya, 2000), *Pichai membranaefaciens* on mayonnaise and cheeses; *Candida albicans* on dried meat (Pitt and Hocking, 1997), and *Mucor plumbeus* on cheeses (Taniwaki *et al*., 2001). Moulds growing on IMF may produce toxins such as citrinin, aflatoxin, and requefortine c that can be hazardous to humans (Taniwaki *et al*., 2001).

Aspergillus flavus is one of important microorganisms found on IMF products. It is easy to contaminate in material to process of food product such as bread (Pitt and Hocking, 1997), fast food (Fapohunda and Ogundero, 1990). *A. flavus* can produce alflatoxins, which is hazard to human health (Selvi *et al*., 2003). In addition, there have been many reports to show that *A. flavus* was harder to inhibit than another fungi (Matan *et al*., 2006; Magwa *et al*., 2006). For this reason, *A. flavus* has frequently been used to study the effect of essential oil, chemical or natural chemical against on growth of important microorganisms for IMF products (Nguefack *et al*., 2004; Singh *et al*., 2006).

These microorganisms are a problem for IMF products because they can produce toxins and sensory changes in IMF products. Mycotoxigens fungi have been reported in IMF products such as citrinin, cyclopiazonic acid, penitrem A, roquefortine C, sterigmatocystin and aflatoxin (Taniwaki *et al*., 2001). Toxins from

Penicillium isolates found in spanish included cyclopiazonic acids, mycophenolic acid, requefortine C, putulin or ochratoxin A (Lopez-diaz *et al*., 2001). More than 400 mycotoxins are known today, aflatoxin being the best known, and the number is increasing rapidly. Mycotoxins are secondary metabolites, which are toxic to vertebrate animals in small amounts when introduced via a natural route. The toxicity of these metabolites is very different, with chronic, termed toxicosis, being the most important to humans. However, only a few mycotoxins are well described in toxicological terms. The most important toxin effects are different kinds of cancers and immune suppression. The mycotoxins are formed during growth of moulds on foods. Some mycotoxins are only present in the mould, while most of them are excreted in the foods (Filtenborg *et al*., 1996). Table 1 shows the microorganisms of potential significance for IMF products (Conner and Beuchat, 1984; Ellis *et al*., 1993; Filtenborg *et al*., 1996).

Table 1 Microorganisms of potential significance for IMF products

Species	IMF products	Mycotoxin production
Mucor plumbeus	Apple syrup	
Pencillium comunune	Cheddar cheese	Cyclopiazonic acid
P. roqueforti	Cheddar cheese	Roquefortine
P. roqueforti	Cheddar cheese	Roquefortine
P. olsonii	Meat sausage	Cyclopiazonic acid,
P. griseofulvum		Myphenolic acid,
		Roquefortine C,
		Patulin or ochratoxin
P. nalgiovense	Sausages	Cyclopiazonic acid,
		Isofunmigaclavine A,
		Ochratoxin A,
		Isofunmigaclavine A,
		Ochratoxin A,
		Patulin, RoquefortineC.

Table 1 (Continued)

Species	IMF products	Mycotoxin production
Aspegillus flavus	Bread	Aflatoxins B_1,B_2
Eurotium repens	Rye bread	Aflatoxins, Citrinin,
P. olsonii	Meat sausage	Cyclopiazonic acid,
P. griseofulvum		
Mucor plumbeus	Apple syrup	
Pencillium comunune	Cheddar cheese	Cyclopiazonic acid
E. repens		Patulin,
Pichia membranaefaciens		Ergot alkaloids.
Saccharomyces cerevisiae		
Geotrichum candidum		
Debaryomyces hansenii		

Table Adapted from Pitt and Hocking (1997)

Microbial contamination of IMF products may result in the spoilage of the products and result in rejection by the consumer and important economic losses by the manufactures (Isabel *et al*., 2000). Therefore, many methods have been studied to prevent the growth of microorganism on IMF products such as control of water activity on IMF product, added chemical preservatives, and use of essential oils

2 Preservation of IMF foods

The methods of protection against fungal contamination in IMF foods can be physical, chemical and biological methods.

2.1 The physical methods

The moisture, gas and temperature conditions influence growth of microorganisms in IMF products. Abdullah *et al*. (2000) successfully controlled by

storing starch-based foods at a_w =0.65. Fungal did not grow in this condition until after 6 months. Another method, ozone, has been used with mixed success to inactivate contaminant microflora on meat, fruits and dry foods. The gas also is useful in detoxification and elimination of mycotoxins and pesticide residues from agricultural products (Kim *et al*., 2000).

2.2 The chemical methods

Addition of permitted food preservatives is the only feasible method of spoilage prevention in many IMF products currently under consideration. At present, choice is limited mainly to the following compounds, SO_2 and its acids, benzoic acid, parahydroxybenzoic acid and its salts, sorbic acid, and diethyl pyrocarbonate (DEPC). One of these, sorbic acid strongly prevents microorganism growth in IMF products. Sorbic acid and its water soluble salts are of major commercial significance as preservatives for food (Restaino *et al*., 1982). Sorbate has been commercially use as antimicrobial agent in foods include baked goods, cheese, confectionery products, chocolate coatings, dried fruits, butters, and salad. Concentration of sorbate of 3,000-9,000 ppm plus low temperature were important for inhibitory action against *P. cyclopium, P. viridicatum, P. crustosum* and *P. lanoso-viride* (Finol *et al*., 1982). Certain species of yeasts are more resistant and acquire a resistance to potassium sorbate. Pitt (1974) reported that growth of *Saccharomyces bailii*, a preservative tolerant yeast, was not inhibited by 0.06% of sorbic or benzoic acids in 10% glucose.

2.3 The biological methods

The biological method included herbs, spices and other fruit and plant materials (Brul and Coote, 1999). Ejechi *et al*. (1999) found phenolic acid and essential oil extracts of pepperfruit in the range of 2.5-6.5 and 1.5-3.0 mg/ml inhibited fungal spoilage tested (*Saccromyces sp*., *Candida tropicalis*, *Candida* sp., *Cryptococcus* sp., *Geotrichum* sp., *Rhizopus stolonifer*, *Aspergillus niger*, and *Fusarium* sp.) for more than 1 month. Roller and Covil (1999) studied the properties of chitosan as an antifungal in apple juice. This experiment showed the potential for

using chitosan as a natural food preservative as it inhibited growth at 25 ^{0}C. An other method of biological method is biopreservation. Biopreservation involves the use of antagonistic microorganisms and their metabolites to inhibit or destroy undesired microorganisms in food. Bacteriocins are examples of metabolites that have considerable potential in the realm of biopreservation (Scannell *et al*., 2000).

3. Essential oil

Essential oils are widely used in many of industries not only in food product for instance bakery products (Robinsfyi.com, 2004) but also in perfume (FragranceNet.com, 2004) and drugs products (Hadji-Minaglou and Bolcato, 2005). The quality of essential oil required depends on the extraction method.

3.1 Extraction method of essential oil

A range of methods is used to extract essential oil depending on the type and properties of the essential oil. Some types of essential oil are suitable to extract with cold water while some substances are dissolved in hot water. Details of extraction technique of essential oil are described in the following section.

3.1.1 Maceration

A process is comparable to making tea, whereby a linen bag containing herbs is suspended in a mixture of water and alcohol (Bokma, 2003). This method is suitable for extraction of the essential oils that are easy to disintegrate by high temperature.

3.1.2 Percolation

The round glass flash such as percolator has been used in extraction process in the industry for a long time. Small pieces of herbs with a solution are together put inside the percolator for more than 24 hours before going to squeezing

the solution from the refuse. The essential oil is sorted before using (Abdollahy *et al*., 2004).

3.1.3 Soxhlet extractor

Water above its boiling point has been used for many years as an industrial solvent. As a vapour, steam is commonly used in hydrodistillation for the isolation of volatile constituents of plant materials to provide essential oils of value in perfumery (Smith, 2002). The interest in soxhlet extraction has increased in recent years because of legal limitations of solvent residues and solvents. This makes the process more economical in the food, beverage, and pharmaceutical industries (Vági *et al*., 2005).

3.1.4 Liquid-liquid extractor

A liquid-liquid extractor is a useful method to separate compounds of a mixture. The success of this method depends upon the difference in solubility of a compound in various solvents. The difference of solubility of essential oil and solvent has formed the basis of liquid–liquid extraction for essential oil extraction (Rodrigues *et al*., 2005).

3.1.5 Extraction by Thermomicrodistillation

Thermomicro analysis and separation ovens (TAS ovens) are used to extract essential oil. Pure essential oil is identified by Thin Layer Chromatography (TLC). This method is suitable to extract a small volume of essential oil (Jolliffe, 1970).

3.2 The effectiveness of essential oils

Nowadays, essential oils are used to prevent growth of microorganisms for instant mustard and essential oil was used to inhibit growth of mould on the surface of rye bread (Nielsen and Rios, 2000). Oregano oil was showed to be a strong

inhibiter against growth of bacteria and fungi on meat (Skandamis and Nychas, 2002). Different types of essential oils are suitable to prevent growth of microbial in different foods. However, cinnamon oil and clove oil are selected for this research.

3.2.1 Cinnamon oil

Cinnamon has been popular since ancient times. Cinnamon is the dried bark of various laurel trees in the *Cinnamomun* family. One of the more common trees from which cinnamon is derived is the cassia. True cinnamon is native to Sri Lanka. The cinnamon used in North America is from the cassia tree, which is grown in Vietnam, China, Indonesia, and Central America (Ruangrungsi, 1991). Cinnamon is used in cakes, cookies, and desserts throughout the world. Cinnamon is also used in savory chicken and lamb dishes from the Middle East. In American cooking, cinnamon is often paired with apples and used in other fruit and cereal dishes. Stick cinnamon is used in pickling and for flavoring hot beverages (Leangnateethep, 1997).

3.2.2 Clove oil

Cloves are the immature unopened flower buds of a tropical tree (*Syzygium aromaticum*). When fresh, they are pink, dried, they turn to a rust-brown colour. Cloves contain 15 to 20% essential oil, which is mostly eugenol, which is a very strong antiseptic (Suksrikarm, 1987). Clove oil is often applied directly to an aching tooth, bringing immediate relief. Compounded with zinc oxide, it has been used in dentistry as a temporary tooth filling. It is a strong stimulant and carminative and used to treat nausea, indigestion and dyspepsia. Cloves are used in a number of spice mixtures including curry powders, mulling spices and pickling spices (Leangnateethep, 1997).

3.2.3 Effectiveness of cinnamon oil and clove oil

Cinnamon oil and clove oil are natural preservative substances, which are not harmful. There have been a number of reports of a distinct property of substances in cinnamon oil and clove oil, which inhibit growth of fungi. Cobley and Leslies (1963) found that 37.5 ppm of cinnamaldehyde in the cinnamon oil can inhibit *Aspergillus niger*. August (1978) reported that high concentrations of cinnamon oil and clove oil could also inhibit asexual spore of fungi. Suksrikarm (1987) has also reported that cinnamon oil and clove oil could inhibit many other microbes e.g. *Lactobacillus, Bacillus thermoacidurans, Salmonell, Corynebacterium michiganense, Pseudomonas striafaciens, Clostridium botulinum, Alternaria, Aspergillus, Canninghamella, Fusarium, Mucor, Penicillium* and *Fusarium effusm*. Nitimongkongchai (1999) successfully used 0.8% of cinnamol oil as an antifungal agent in durian paste, which increased the shelf life of durian paste up to 105 days. Cinnamon oil and clove oil at 2% level in PDA completely inhibited growth of all seven mycotoxigenic mold: *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus, Penicillium* sp. M46*, P. roqueforti, P. patulum*, and *P. citrinum* mycotoxigenic molds for various times up to 21 days (Azzouz *et al*., 1982) and could also inhibit the growth of yeasts (Conner *et al*., 1984).

3.3 The main components of cinnamon oil and clove oil

Natural active ingredient found in cinnamon oil and clove oil can be classified into 9 groups as follows:

3.3.1 Cinnamaldehyde

The cinnamon oil contains active precursor cinnamic acid, cinnamaldehyde, 4-hydroxybenzaldehyde, cinnamyl acetate, eugenol, 3-phenylpropionaldehyde, carvone, and carvacrol (Friedman, 2000). Among these, cinnamaldehyde was highly effective in inhibiting the growth of microorganisms. The antimicrobial activity order of four cinnamaldehyde congeners having both a benzyl

ring and a conjugated double bond was cinnamaldehyde, cinamic acid, cinnamyl alcohol, cinnamylacetate. It is clear that the aldehyde group has the best antibacterial activity (Chang *et al*., 2001).

3.3.2 Alcohol

Methyl alcohol and benzyl alcohol are alcohol groups found in clove oil and cinnamon oil (Chang *et al*., 2006). The alcohol group can destroy the cell membrane and also damage protein structures of microorganisms (Suksrikarm, 1987).

3.3.3 Phenolic compound

Eugenol, eugenol acetate, phenol, caryophylene, and β-caryophylene were found in clove oil and cinnamon oil. Eugenol was found to be the main active compound for clove oil (Lalko and Api, 2006). In addition, caryophylene, and β-caryophylene was hard to disintegrate at room temperature. The energy to breakdown caryophylene, and β-caryophylene was high around 17.2 and 18 kJ/mol, respectively (College, 2000). The phenolic compounds were reported to interrupt essential enzyme, reduce ATP, and destroy membrane of microorganisms (Suksrikarm, 1987).

3.3.4 Acetal and vanillin

Acetal and vanillin can interrupt the integration process of lipids that affects the osmotic barrier inside the microbial cell (Suksrikarm, 1987).

3.3.5 Linalool or 3,7-Dimethyl-1-ol, 2,6-Dimethyl-2, 7-octadien-6-ol

Vitamin E could be produced by linalool. Therefore, linalool can protect the disintegration process of essential oil. Linalool alone could not inhibit

microorganism but has to be applied with another compound to show its effectiveness (Suksrikarm, 1987).

3.3.6 Para-cymene

Para-Cymene usually is not an effective antifungal agent when used alone but when it strongly interacts with the cell membrane and when combined with carvacrol, of which it is the precursor, it does exert a positive action (Ultee *et al*., 2002). Benjilali *et al*. (1984) reported the *p*-cymene was a lightest compounds and could gave higher concentration above essential oil headspace compared with heavier compounds e.g. phenolic compounds.

3.3.7 Eucalyptol or 1,8-Epoxy-p- methane, 1,8-Cineole

Eucalyptol gives yellow colour to essential oil and produces specific flavour for essential oil (Suksrikarm, 1987).

3.3.8 Cyclohexane or 3-Cyclohexane-1-methanol

Cyclohexane produces a bright red colour in the essential oil. The cyclohexane has been used in cosmetic industry (Suksrikarm, 1987).

3.3.9 Benzaldehyde

Benzaldehyde creates flavour in essential oil. It decomposes to other substances such as benzyl alcohol, benzyl amine, dibenzyl amine, hexyl and amyl-cinnnamicaldehyde after keeping for a long time (Suksrikarm, 1987). Benzaldehyde was reported to be a strong inhibitor against mould (Smid, 1995). In addition, MoHo-Lee (2001) reported that benzaldehyde could be a safer fumigant to control stored-grain insect pests than those currently used.

3.4 Synergic action of compounds in essential oil

The synergic action between compounds has been studied for many years. The mixtures of oils (whether these provide herbaceous or spice notes) can potentially be manipulated to achieve adequate microbial control and yet may also provide a more favourable flavour profile than the use of only a single active component. Delgado *et al*. (2004) studied the bactericidal action of thymol and cymene on two *Bacillus cereus* strains (INRA-AVTZ415 and INRA-AVZ421). They reported that the synergistic effect of both natural antimicrobials showed good inhibitory on growth phase suspended. *B. cereus* cells. Normally, the synergic action observed between more than one essential oil was no chemical interaction results in a new compound, but synergistic action may also be dependent upon reactions involving numerous organic substances present in essential oils (Cassella *et al*., 2002).

Linalool and p-cymene are the precursors that are not active when used alone but they become effective when combined with other compounds such as thymene (Ultee *et al*., 2002). In this research, the synergic action between eugenol, cinnamaldehyde, p-cymene and linalool was investigated.

4. <u>Gas chromatography systems for analyzing essential oils</u>

Essential oils are complex mixtures of differently volatile substances and labial components of which the sensory perception can be changed as a result of oxidation, chemical interactions or volatilization (Baranauskienė *et al*., 2005). Gas chromatography (GC) has also been extensively used over past years in the analysis of volatile oils (Maffei *et al*., 1994) and Cheng *et al*. (2006) used GC to examine nine geographical provenances of indigenous cinnamon (*Cinnamomum osmophloeum Kaneh*.), and Ahmadi *et al*. (2005) investigated essential oil of *Oliveria decumbens* using GC.

In the case of GC, one phase is stationary and the other is mobile. The more a solute is partitioned in the mobile phase, the more it moves; in other words, the

partitioning between the stationary and mobile phases affects the time required for a solute to travel through the instrument. Gas chromatography involves a sample being vapourised and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase, which is adsorbed onto the surface of an inert solid. The instrumental components are described the following sections.

4.1 Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often depende upon the type of detector, which is used. The carrier gas system also commonly contains a molecular sieve to remove water and other impurities.

4.2 Columns

There are two general types of column, packed and capillary (also known as open tubular).

4.2.1 Packed column

A packed column contain a finely divided, inert, solid support material (commonly based on diatomaceous earth) coated with liquid stationary phase. Most packed columns are 1.5 to 10m in length and have an internal diameter of 2 - 4mm.

4.2.2 Capillary column

Capillary columns have an internal diameter of a few tenths of a millimeter. They can be one of two types; wall-coated open tubular (WCOT) or support-coated open tubular (SCOT). Wall coated columns consist of a capillary tube whose walls are coated with liquid stationary phase. In support-coated columns, the

inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

4.3 Sample injection port

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour. Slow injection of large samples causes band broadening and loss of resolution. The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash vapouriser port at the head of the column. The temperature of the sample port is usually about 50 ^{0}C higher than the boiling point of the least volatile component of the sample. For packed columns, sample sizes ranges from tenths of a microliters up to 20 microliters. Capillary columns, on the other hand, need much less sample, typically around 10^{-3} mL. For capillary GC, split/splitless injection is used.

4.4 Detector

The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample Dilution of with make-up gas will lower the detectors response. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependant detector is unaffected by make-up gas

5. Active packaging (AP)

Active packaging also encompassing so celled smart packaging, intelligent packaging, is one of the innovative food packaging concepts that has been introduced in response to the continuous changes in current consumer demand and market tends. Major active packaging techniques are concerned with substances that absorb oxygen,

ethylene, moisture, carbon dioxide, antimicrobial agents, antioxidants and flavor (Vermeiren, 1999). Active packaging (AP) performs some desired role other than providing an inert barrier between the products and external conditions, and combines advances in food technology, biotechnology, packaging and material science in an effort to comply with consumer demands for fresh like product (Scannell *et al*., 2000). Active packaging interacts with the product or the headspace between the package and the food system (Appendini and Hotchkiss, 2002)

Active packaging interacts with the product or the headspace between the package and the food system to restrict growth of microorganisms and slow other quality deterioration processes (Appendini and Hotchkiss, 2002). Applications of AP include oxygen and carbon dioxide absorbers to manipulate headspace gas concentrations, devices to release flavour compounds into the product, and adding antimicrobial agents to the packaging system to control microbial growth (Vermeiren *et al*., 1991). One specific application is to incorporate essential oil(s) into the packaging to prevent the growth of microorganism (Nielsen and Rios, 2000). Modified atmosphere packaging (MAP), in which the O_2 concentration is typically lowered and the CO_2 concentration is elevated, is a well established technique for achieving related goals.

The migration of an active substance in active packaging systems was explained by Han (2000). The active compounds were added into packaging with many methods, for example, putting the antimicrobial substance into the film by adding it in the extruder when the film or a co-extruded film is produced. However, in this research the essential oils were added into small sachet and put in into a high barrier bag. A volatile active substance developed in the headspace of the high barrier bag and could prevent growth of microorganism for a month.

Active packaging is a new method to prevent the growth of microorganism for food marketing. There have been a number of report of active packaging and these can be classified into 3 types as follows:

5.1 Modified Atmosphere Packaging (MAP)

MAP is a modern food process developed for extending the shelf life of relatively high moisture foods. MAP has been found to be effectively in preventing (Ellis *et al*., 1993) or reducing fungal growth in bakery products (Nielsen and Rios, 2000), cheese (Taniwa *et al*., 2001), beef (Dykes and Moorhead, 2001), ready-to-eat vegetables (Jacxens *et al*., 2001), prunes and raisins (Halouat *et al*., 1998). There are numerous advantages of using MAP technology, but it must be noted that there are potential problems, which also must be considered (Farber, 1991).

5.2 Antimicrobial packaging

Antimicrobial packaging can be in several forms:

- Addition of sachets/pad containing volatile antimicrobial agents into packages.

- Incorporation of volatile and non-volatile antimicrobial agent directly into polymer ie. organic acid, inorganic gas, spices.

- Coating or adsorbing antimicrobials onto polymer surfaces.

- Immobilization of antimicrobial, to polymer by ion or covalent linkages such as immobillised bacteriocins lacticin 3187 and Nisaplin®(Scannell *et al*., 2000)

- Use of polymer that are inherently antimicrobial (Appendini and Hotchkiss, 2002).

5.3 Combined packaging formats

Active packaging is an interesting alternative to the traditional use of preservative. According to Nielsen and Rios (2000) meat samples were stored in an

environment of mustard essential oil and MAP conditions. The fungal did not grow for more than two weeks in 2 µl of mustard essential oil with MAP condition. Skandamis and Nychas (2002) studied combined effect of volatile compounds of oreogano essential oil and MAP conditions. Their results showed that the essential oil had a pronounced effect on the physicochemical changes of meat samples caused by microbial of meat (Skandamis *et al*., 2002).

6. Plastic film and absorbent material use in AP systems

6.1 Types of polymer

6.1.1 Polyolefins

Low density polyethylene, linear low density polyethylene, high density polyethylene, irradiated polyethylene, polybutene, and poly (methyl pentene) are examples of polyolefins groups. LDPE film has good clarity. The basic properties of various polyethylene films are shown in Table 2. Polyethylene is non-biodegradable. Therefore, many researches have been focused in degradation of polyethylene. Arvanitoyannis *et al*., 1998 created the blends of LDPE and rice or potato starch to study mechanical properties, gas/water permeability and biodegradability before and after storage. The results suggested that the biodegradability rate of the blends was enhanced when the starch content exceeded 10% (w/w).

6.1.2 Substituted olefins

Polystyrene, poly (vinyl alcohol), poly (vinyl chloride), poly (vinylidene chloride), poly (tetrafluoroethylene) are member of this group. This group could combine with other thermoplastics to show good stability, low density, good resistance to acids and bases, moderate permeability to gases, low resistance to nonpolar solvents and to halogenous solvents (methylene chloride, for example),

excellent transparency, strong water barrier (four times higher than in the case of PET), and good resistance to polar solvents (Limam *et al*., 2005)

Table 2 Basic properties of various polyethylene films[a]

Type of polyethylene	Low density (920 kg m^{-3})	Medium density (940 kg m^{-3})	High density (960 kg m^{-3})
Moisture vapour transmission	1.4	0.6	0.3
Gas transmission			
- O_2	500	225	125
- CO_2	1350	500	350
Tensile strength (MPa)	9-15	21	28
Softening point (º C)	120-180	120-180	135-180
CH_3 groups per 1000 C´s	20-33	5-7	<1.5

[a] Table from (Robertson, 1992)

6.1.3 Copolymer of ethylene

Ethylene-vinyl acetate, ethylene-vinyl alcohol comprises this group. Ethylene-vinyl acetate (EVA) copolymers with a vinyl acetate (VA) content of 3-12% are similar in flexibility to plasticized PVC, and have good low temperature flexibility and toughness (Robertson, 1992). Ethylene-vinyl alcohol (EVOH) is hydrolyzed copolymers of vinyl acetate and ethylene. The vinyl alcohol base has exceptionally high gas-barrier properties, but is water soluble and difficult to process (Robertson, 1992). EVOH was completely undigested (Kunkel *et al*., 1995).

6.1.4 Polyesters

Polyesters are based on carbon-oxygen-carbon links, where one of the carbons is part of a carbonyl group, and are formed by the process of condensation

polymerization. Poly (oxyethylene oxyterephthaloyl) or PET is widely used in the world, and is one of the polyesters (Robertson, 1992). In 1976, PET bottles were first introduced in Japan for soy sauce. Since the first commercial debut in 1978 for the soft drink market in the U.S.A., the total amount of PET bottle usage has rapidly expanded to approximately 250 billion units in the year of 2004, corresponding to 10 million metric tons of PET resin, and a further growth by an average of over 10% annually is expected to reach beyond 300 billion units in 2006 (Shirakura *et al*., 2006).

6.1.5 Polycarbonates

Polycarbonates (PC) are polyesters of unstable carbonic acid and have carbonate linkages (Robertson, 1992). Polycarbonates are one of a potential group, of likely to be new materials used in future food packaging, but there will be new combinations of materials and new uses to which they are put.

6.1.6 Polyamides

Polyamides (PA) have carbon-carbonyl-nitrogen (amido)-carbon (Robertson, 1992). PA is useful in the industry because new materials based on polyamides can be developed for specific needs e.g. improvement of transport properties of oxygen, carbon dioxide, water, nitrogen and aromas through polymers (Del Nobile *et al*., 2002)

6.1.7 Acrylonitriles and associated copolymers

Polyacylonitrile, styrene/acrylonitrile, acrylonitrile/butadiend are conducted in this group (Robertson, 1992). This group is not suitable to use in food packaging because US FDA regulates the use of these polymers on the basis of the amount of AN that may migrate into food stimulants (Lickly *et al*., 1991). Acrylonitrile is metabolized by cytochrome P4502E1 to an epoxide, cyanoethylene oxide (CEO). CEO spontaneously decomposes to cyanide which reacts with heme

proteins such as hemoglobin to produce cyano-hemoglobin, a toxic event. CEO can be detoxified by reaction with glutathione, either enzymatically or chemically. Acrylonitrile also may exhibit acute neurotoxic effects as a result of blockage of acetylcholine pathways (Friedman and Beliles, 2002).

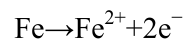
6.1.8 Cellulose acetate and regenerated cellulose

Cellulose, a polydisperse polymer with a DP of 3500 to 36,000, comprises more than one-third of the dry weight of all vegetable matter and is the world's most abundant organic compound. Regenerated cellulose is manufactured from highly purified cellulose usually derived from bleached sulfite wood pulp or cotton linters (Robertson, 1992).

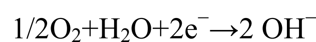
6.2 Absorbent materials

6.2.1 Oxygen and carbon dioxide absorber

Hihg CO_2-levels (10-80%) are desirable for foods such as meat and poultry (Luño *et al*., 2000) because these high levels inhibit surface microbial growth and thereby extend shelf life. The O_2 / CO_2 absorber has been developed in current market. For instance, Ageless® (Mitsubishi Gas Chemical Co., Japan) is the most common O_2-scavenging system and is based on iron oxidation. The sachets are designed to reduce O_2-levels to less than 0.01%. A rule of thumb is that 1g of iron will react with 300 cc. of O_2. The majority of currently commercially available O_2-scavengers are based on the principle of iron oxidation:

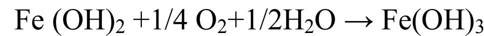


$$Fe \rightarrow Fe^{2+} + 2e^-$$



$$1/2O_2 + H_2O + 2e^- \rightarrow 2\ OH^-$$

$$Fe^{2+} + 2\ OH \rightarrow Fe\ (OH)_2$$



Some O_2-scavengers use an enzyme reactor surface that would react with some substrate to scavenge incoming O_2 (Vermeiren *et al*., 1999)

6.2.2 Antimicrobial desorbed

Antimicrobial desorbed can be formed from to sachets or pads containing volatile antimicrobial agents and adding these in to packages (Appendini and Hocking, 2002). For example, silver-ions, which inhibit a wide range of metabolic enzymes, have strong antimicrobial activity with a broad spectrum. The zeolite, which has some of its surface atoms replaced by silver, is laminated as a thin layer (3-6 µm) in the surface of the food contact polymer and appears to release silver ions as aqueous solution from the food enters the exposed cavities of the porous structure (Quintavalla and Vicini, 2002). Another desorbed, silica gel, can also adsorb water and some chemical. Silica gel is a partially dehydrated form of polymeric colloidal silicic acid. The chemical composition can be expressed as $SiO_2 \cdot nH_2O$. The water content, which is present mainly in the form of chemically bound hydroxyl groups, amounts typically to about 5 wt.% (Kopaç and Kocabaş, 2002). Calcium silicate is another of possible desorbed. The main source of calcium silicate is a mineral called wollastonite, $CaSiO_3$. Since wollastonite occurrences are much less widespread than limestone, the more common calcium oxide rich rock known as basalt could provide an alternative source of raw material (Teir *et al*., 2005). Another possible desorbed is filter paper. Nielsen *et al*., 2000 used filter paper with mustard essential oil, cinnamon oleoresin, oregano oleoresin, clove oleoresin, vanilla oleoresin, garlic essential oil to investigate inhibition of *Penicillium commune*, *P. roqueforti*, *Aspergillus flavus* and *Endomyces fibuliger*. Filter paper is made by line of filter papers. The unique features of these filters make them the optimum choice for many filtering absorption objectives (Whatman International Ltd, 2005).

7. Modeling of microbial growth response

Response surface methodology (RSM): RSM is a collection of mathematical and statistical techniques that are useful for modeling and analysing situations in which a response of interest is influenced by several variables, and the objective is to optimize this response. Therefore, the aim of RSM is the determination of the optimum operating conditions for a system under investigation or of a region of the factor space within which operating requirements are satisfied (Ahmadi, 2005). The SAS program can be used to optimize the appropriate factor. A quadratic polynomial equation was developed to predict the response as a function of independent variables and their interactions (Gunawan *et al*., 2005; Ghadge and Raheman, 2006).

$$Y = \beta_0 + \sum_{j=1}^{4} \beta_j X_j + \sum_{j=1}^{4} \beta_{jj} X_j^2 + \sum_{i<j} \beta_{ij} X_i X_j \qquad (1)$$

In this equation, Y is the predicted response, β_o is the intercept coefficient, β_j are the linear terms, β_{jj} are the squared terms, β_{ij} are the interaction terms, and X_i and X_j represent the uncoded of the independent variables. For each experimental factor the variance was partitioned into components, linear, quadratic and interaction, in order to assess the adequacy of the second order polynomial function and the relative importance or significance of the terms (Wang *et al*., 2005).

8. Product survey

Product survey is widely used in current market. Nilsson *et al*. (2004) used product survey to study the use of eco-labeling like initiatives on food products to promote quality assurance in the European Union in the recent years. The main findings of the research indicated that although small groups of consumers may be satisfied with a number of the different schemes, the majority of them fall short of providing a credible quality assurance scheme. In addition, the product survey can

help to create a new product (Kristensen *et al*., 1998). The procedures of the product survey are:

8.1 Preparation of survey instrument

The questionnaire, information of place to survey, marketing data, and local information are prepared.

8.2 Field Staff

Training session for the field staffs is held at the beginning of the data collection process to inform them of the specific procedures to be used during this data collection effort.

8.3 Supermarket Scheduling

Several supermarkets in the survey are contacted to ask permission to conduct a survey at their stores.

8.4 On-Site Data Collection

The field staff member make contact with a customer and explain the purpose of the survey. The questionnaire takes about two to three minutes to complete.

8.5 Database Preparations and Validation

Preparation of the database containing the data collected during the on-site visits involves coding, validating, editing, and processing the data.
(Agriculture and Natural Resources, 2004)

9. Texture attributes of cake

Cake is a bakery product that is spoiled by yeast and mould. The ingredients of cake include flour, wheat flour, fruit and nuts and these ingredients are often contaminated with yeasts and mould (Özilgen and Özdemir, 2001; Saxena *et al*., 2001; Freire *et al*., 2002). Normally, yeast and mould that contaminate bakery products are *Aspergillus flavus, Penicillium roqueferti* (Abellana *et al*., 2001) and these are capable of producing a harmful aflatoxin (Ellis *et al*., 1993; D'Mello and Macdonald, 1997). In addition, cake has a short shelf life of around 3 to 7 days without any preservative. Quality change of cake on storage time was studied by many scientists to understand consumer behaviour. Texture is one of the most important factors. Chuaneg and Yeh (2006) reported that the texture of cake became hard and dry on time storage. The hardness, adhesiveness, gumminess, and chewiness of cake were higher during storage. The temperature distribution in the product could also affect to the moisture movement (Baik *et al*., 2000). Moisture migration, a macroscopic migration of moisture continues for several weeks and is responsible for the loss of quality. The water activity gradient between the dough and dried fruit may become larger at higher temperature. The starchy products' moisture content is lower at higher temperature for the same water activity. This may cause an even larger activity gradient along the flour and fruit interface at high temperature, which facilitates the faster transport of water from the dough to the fruits. The diffusion and equilibrium of water in dough and fruit mixtures were explained by Karathanos (1995).

MATEIALS AND METHODS

Materials

1. Materials

1.1 Cinnamon oil (Thai China Flavours & Fragrances Industry Co., LTD. Bangkok, Thailand. and AromaSense Co., LTD. Auckland, New Zealand.)

1.2 Clove oil (Thai China Flavours & Fragrances Industry Co., LTD. Bangkok, Thailand. and AromaSense Co., LTD. Auckland, New Zealand.)

1.3 Flour

1.4 Baking soda

1.5 Wheat germ

1.6 Grated carrots

1.7 Raisins

1.8 Honey

1.9 Allspice

1.10 Salt

1.11 Nutmeg

1.12 Margarine

1.13 Chopped walnuts

1.14 Dried fruit

1.15 Water

1.16 Walnuts

1.17 Butter

1.18 Brown sugar

1.19 Eggs

1.20 Grated lemon zest

1.21 Vanilla extract

1.22 Brandy

1.23 Chopped

1.24 Mixed Berry Muffin Mix (Edmonds Muffin Mixes from Goodman Fielder Ltd., New Zealand)

1.25 Vanilla Cake Mix (Edmonds Cake Mixes from Goodman Fielder Ltd., New Zealand)

1.26 Banana Cake mix (Betty Crocker, New Zealand)

1.27 Cake mix (Wellington milling Ltd., New Zealand)

1.28 Variety fruitcake mix (Cereform Ltd., Australia)

1.29 Jamaican ginger loaf (Ernest Adams, New Zealand)

1.30 Waikato farmhouse cake (Waikato Company, New Zealand)

1.31 Rice jasmine butter cake (Kasetsart Agricultural and Agro-Industrial Product Imprevement Institute, Kasetsart University, Thailand)

2.Chemical

2.1 K-sorbate

2.2 Na-benzoate

2.3 Tween80

2.4 Eugenol standard

2.5 Cinnamaldehyde standard

2.6 Linalool standard

2.7 *p*-cymene standard

3. Media

3.1 Malt Extract Agar (MEA)

3.2 Potato Dextrose Agar (PDA)

3.3 Nutrient Agar (NA)

4. Microorganism

4.1 *Aspergillus flavus*

4.2 *Penicillium roqueforti*

4.3 *Eurotium sp.*

4.4 *Mucor plmbeus*

4.5 *Candida lipolyticus*

4.6 *Debaryomyces hansenii*

4.7 *Zygosaccharomyces rouxii*

4.8 *Pichia membranaefaciens*

5. Instruments

5.1 Questionnaire

5.2 Whatman paper NO.6

5.3 Sterile plastic plated

5.4 Vacuum chamber.

5.5 The pouch bag

5.6 Gas mixture.

5.7 Incubator

5.8 GC-MS

5.9 HPLC

5.10 Spectrophotometer

5.11 Novasina thermoconstante

5.12 Oven

5.13 Electric mixer

5.14 Tin box

5.15 Weight

5.16 Baking paper

5.17 Tray

Methods

1. Survey of types of IMF products in modern market from the retailers in Bangkok to identify products for preservation by active packaging.

1.1 Product survey and screening

1.1.1 Product survey

Scope: to survey types of IMF products in modern market from the retailer in Bangkok (department stores, supermarkets, superstores/wholesales, conveniences)

Sampling: using purposive sampling and a simple random sample without replacement.

Survey tool: observation, Internet, magazine, and handbill.

Location: the location data were obtained from Siam Future Development (2003), KSC Commercial Internet Co., Ltd. (2003), Kiwi & Kom-Kom Products Co., Ltd. (2003), Mamy Company Limited (2003), Intercosmos Media Group, Inc (2003), Department of Business Development (2003). The locations for the research survey are listed in Table 3

Time: the survey was carried out from the 3rd November to the 8th December 2003.

Table 3 Locations used in this survey.

Retailers	Branch locations
1. Department store	
- The Mall shopping complex	Bangcare, Bangkapi, Ngamwongwan, Rama 2-3, and Tapra
2. Supermarket	
- Tops supermarket	P.S. Silom, Ratchadapisek, Bangrak, Sukhumvit, Bangkae, Fashion Island, Rangsit, Jaransanitwong, Bangna, Srinakarin, Chidlom, Silom Complex, Lardprao, Ramindra, Lardyar, Wangburapa, Silom, Pinklao, Major Prapadang, Sukhumvit 41, R.C.A., Rama III, Maboonkrong, Sukapiban 3, Chok Chai 4, Prachanivet, Kaset, Rajvithi, Rama 2, Pracha-utis, Nanglynchee, Sukhumvit 24, WorldTrade Center, Thonglor, Sathorn City, and All Season Place
-Food Lion	Bangkapi, Suk Saward, Sukhumvit 83, Sukhumvit 101, Sukhumvit 69, Srinakarin, Eka Pairin, Senanikom, Pudhamonthon, Ekkamai, Sri-Yan Jaransanitwong, Songprapa, Thaparak, Samudprakarn (Pujao), Praekasa, On-Nuj, Bang-Or, Sumrong, Watcharapon, Saimai ,Bangplee, Victory Monument 1

Table 3 (Continued)

Retailers	Branch locations
- Jusco	Ratchadapisek, Lak si, Ratchtanatibeth, Wang Hin, Srinakarin, Sukapiban 1, Bangbon, Phacha-Utis, Sukhumvit 71, and Pak Kred
-Foodland	Ladprao, Ramintra, Patpong, Srinakarin, Petchaburi, Huamak, Sukhumvit, and Pattaya
-UFC Fuji Supermarket	Sukhumvit 33/1, and Sukhumvit 39.
- Plus One Supermarket	Bangplad, and Bangrampoo
- Zen Supermarket	
- Villa market	Praholyothin, Plenchit, Sukhumvit 33, Sukhumvit 49, Silom, Ratchadapisek, Sukhumvit 11, and Lang Suan
3. Superstore/ Wholesales	
- Tesco Lotus supercenter	Seacon Square Minburi Rama II Fortune Town, Ratchada, Sukapiban 1, Sukhumvit 50, Bangkhae, RamaIV, Ramindra, Chaengwattana, Laksi, Rama III, Prachachuen, Ekamai, Bandkapi, Ramindra (Express), Ladprao on Paholyothin Rd., Silom S&A, Aree, Ladprao 120, Vipawadee, Senanikom, Suthisan, Bangpakok, and Phongphet
- Big C supercenter	Chaengwattana, Ratchburana, Rajdamri, Wong Sawang, Future Park, Rangsit, Rama II, Ratthanathibeth, Hun mark,

Table 3 (Continued)

Retailers	Branch locations
	Don Muang, Fashion Island, Suksawat Bangna, Tiwanon, Ladprao, Daokanong, and Bangplee.
- Makro	Lad Prao, Cheangwattna, Srinakarin, Bangbon, Rangsit, Charansanitwong, Sathorn, and Samsen
- Carrefour	Sukapiban 3, Bang Yai, Petkasem, Ramindra, Chaengwattana, Rathanatibeth, RAMA IV, Suwintawong, Future Park, Bangkae, Sumrong, Bangbon, Ratchada, Onnuch, and Bangpakok
4. Convenience store	
- 7-Eleven	Bangrak, Sukhumvit, Bangkae, , Kaset, Rajvithi, Rama 2, Pracha-utis, Lad Prao, Cheangwattna, Srinakarin, Bangbon, Rangsit,
- FamilyMart	Kaset, Lad Prao
- Fresh Mart	Sukhumvit, Bangkae
- AM/PM	Kaset, Lad Prao
- Select	Shell gas station at Rangsit
- TigerMart	Esso gas station at Bangna
- StarMart	Caltex gas station at Rangsit
- Lemon Green	Bangchak gas station at Bangkae
- Everyday	Q-8 gas station at Rangsit
- Jiffy	Conono gas station at Bangkae

1.1.2 Product Screening

Pass/Fail screening: Four factors (Fungi, Food Law of New Zealand, Active packaging technique, and Marketing) were used in product screening. The factors were defined as follows:

1.1.2.1 Fungi

Fungi are the main spoilage organism for the selected products. This factor was decided by reference to the literature (Pitt and Hocking, 1997; Isabel *et al*., 2000; Nielsen and Rios, 2000; Suhr and Nielsen, 2004; Mundo, 2004; Andrews *et al*., 1997, Prasad and Seenayya, 2000, Taniwaki, *et al*., 2001; Lopez-diaz *et al*., 2001; Filtenborg *et al*., 1996; Conner and Beuchat, 1984; Ellis *et al*., 1993; Restaino *et al*., 1982; Filtenborg *et al*., 1996; and Isabel *et al*., 2000).

1.1.2.2 Regulations to import food product into New Zealand

The selected products had to suitable for import into New Zealand. The ingredients of the selected products must compile with the food import regulations of New Zealand. The list of prohibitive foods to be imported into New Zealand was issued in a guide to NZ (New Zealand Immigration Service, 2003).

1.1.2.3 Compatibility

The selected products could be preserved with cinnamon oil and clove oil under active packaging condition and suitable for preserving in plastic bag or plastic jar.

1.1.2.4 Marketing

The selected products were premium products having a good opportunity in the future market and having a big market size (Manager online, 2003; Krungthep Turakit online, 2003).

1.1.2.5 Water activity

The selected product had water activity (a_w) from 0.65 to 0.90.

2 Study effectiveness of using cinnamon and clove oil as antifungi in IMF product by using active packaging technique.

2.1 The inhibitory effect of the volatile gas phase of combinations of cinnamon oil and clove oil

2.1.1 Cultures

Four strains of molds and four strains of yeasts were used as test organisms as listed in Table 4. The microorganisms were obtained from the Microbiological Resources Center (MIRCEN), Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand.

2.1.2 Inoculums

The antimicrobial activity of combinations of cinnamon oil and clove oil was tested by indirect method. To prepare the test cultures for the indirect method, 15 ml of MEA in 125 ml erlenmeyer flasks was sterilized 121 ^{0}C 15 min. Then, the agar was poured into sterile plastic plated into the sterile plate (P. Intertrade Equipments Co., LTD, Thailand). Sterile plastic plates containing MEA were spread with 0.1 ml of each of the molds and yeasts

2.1.3 Preparation of Whatman paper

Whatman paper NO.6 was cut into square (5x5 cm) and immersed into cinnamon oil 2,000 µl, clove oil 2,000 µl and combination of clove and cinnamon oil 1,000 µl: 1,000 µl extract for 10 S and drained for 30 second (Skandamis and Nychas, 2002). Finally, whatman paper containing a mixture of clove and cinnamon oil was placed on to sterile plastic plates.

2.1.4 Incubation in various gaseous environments

Each sterile plastic plate was packaged individually in a pouch bags (Cryovac, Thailand) using a vacuum chamber. The pouch was 200 mm wide x 200 mm long, with permeability of 4 cc/m^2/day, MVTR 11 g/m^2/day at 25 °C. The following gas mixture was prepared using a gas mixer and used: 20% or 40% CO_2, either <0.5%, 1%, 5% or10% O_2 with the balance being N_2. The control absorbent without any volatile compound was added into the bags. The bags were than sealed hermetically. The molds and yeasts were incubated at 30 °C and the bacteria were incubated at 37 °C for 2 months.

2.1.4 Data Analysis

One-way analysis of variance (SPSS System, version 12) was used to analyze differences in antifugal effect following exposure to the different atmosphere regimes.

2.2 Minimum inhibitory volume (MIV)

MIV of cinnamon and clove oil for yeasts and molds: The molds and yeasts in Table 4 were used in this test. Sterile plastic plates containing MEA were spread with 0.1 ml of each of the molds and yeasts. Different volumes (1,000, 2,000, 3,000 or 4,000 µL) of a 1:1 mixture of cinnamon and clove oils were absorbed into

Whatman paper as in the previous case and placed inside of a pouch bag along with the inoculated plates. The bags were gas flushed with 40% CO_2, <0.05 O_2 with the balance being N_2 and sealed. The molds and yeasts were incubated at 25 °C for 2 months following Nielsen (2000).

Table 4 Microorganism used in this test and their sources

Microorganism	Culture collection no.	Source
Molds		
Penicillium roqueforti	3511	Requefort cheese
Aspergillus flavus	3566	Koji
Eurotium sp.	3243	Rice koji
Mucor plumbeus	3458	Unknown
Yeasts		
Candida lipolyticus	5655	Unknown
Debaryomyces hansenii	5265	Shrimp pastes
Zygosaccharomyces rouxii	5636	Dried preserved banana
Pichia membranaefaciens	5107	Soybean paste

2.3 Inhibition of *A. flavus* by mixtures of cinnamon and clove oils

Sterile plastic plates containing MEA were spread with 0.1 ml of *A. flavus* Combinations of cinnamon and clove oils at ratios of 1:1, 1:3, 1:5, 1:7, 1:9, and 3:1, 5:1, 7:1 and 9:1 were absorbed into Whatman paper as in the previous experiment and placed separately inside a pouch bag along with the inoculated plates. The total volume of oil mixture added was 4,000 µL in all cases. The bags were gas flushed with 40% CO_2, <0.05 O_2 with the balance being N_2, sealed and incubated at 25 °C for 45 days. The colony diameters were measured after 6, 14, 26 and 45 days without removing the plates from the bag.

2.4 Effectiveness of cinnamon oil and clove oil as an inhibitor of *Aspergillus flavus* on fruitcake

2.4.1 Selection of fruitcake recipes

Eight recipes of fruitcake were used in the experiment: 3 recipes of trial fruitcake and 5 recipes of premix fruitcakes. The selected fruitcake recipe was used to study the effectiveness of cinnamon oil and clove oil as an inhibitor growth of *Aspergillus flavus* on fruitcake as described in the this section and section 3.

2.4.1.1 The three recipes of trial fruitcake

The ingredients used in the trial fruitcakes are listed in Table 5. The processes to make a cake were: preheat oven to 180 °C, whisk the eggs, butter, sugar and vanilla together thoroughly in a large bowl, then mix flour, baking powder and mix dried fruit in a container, add eggs, butter, sugar and vanilla and pour the mix into a loaf tin and bake at 180 °C for 30 minutes.

2.4.1.2 Premix cake

2.4.1.2.1 Mixed Berry Muffin Mix (PMC_1; Edmonds Muffin Mixes from Goodman Fielder Ltd., New Zealand). The processes to make a cake was: preheat oven to 180 °C, grease a loaf tin, place muffin mix, 1 egg, 1 cup of water and 2 teaspoons butter into a mixing bowl, stir with a wooden spoon until mix and dried fruit are just combined, mixture will appear slightly lumpy, bake 30 minutes and cool in tray for 15 minutes.

Table 5 Ingredients of trial fruitcakes

Recipe I (TF_1)		Recipe II (TF_2)		Recipe III (TF_3)	
3 cup	Flour	3 cups	Flour	3 1/2Cup	Flour
2 teaspoon	Baking soda	1 tablespoon	Baking soda	1 teaspoon	Baking Soda
1 cup	Wheat germ	-	-	-	-
2 cup	Grated carrots	-	-	-	-
2 cup	Raisins	1 ½ cup	Raisins	1 ½ cup	Raisins
-	-	-	-	1 ½ cup	Golden Raisins
1 ½ cup	Honey	-	-	-	-
2 teaspoon	Cinnamon	2 tablespoons	Cinnamon	1 teaspoon	Cinnamon
2 teaspoon	Allspice	2 tablespoons	Allspice	-	-
2 teaspoon	Salt	-	-	1 teaspoon	Salt
1 teaspoon	Nutmeg	2 tablespoons	Nutmeg	-	-
½ teaspoon	Cloves	2 tablespoons	Cloves	1 teaspoon	Clove
4 tablespoons	Margarine	-	-	-	-
1 cup	Chopped walnuts	-	-	-	-
3 cup	Water	-	-	1 cup	Water
1 cup	Dried fruit	1 ½ cup	Dried fruit	2 cup	Dried fruit
½ cup	Walnuts	-	-	2 cup	Walnuts
-	-	1 ½ cup	Butter	5 tablespoons	Butter
-	-	2 cup	Brown sugar	2 cup	Sugar
-	-	4 eggs	Eggs	-	-

Table 5 (Continued)

Recipe I (TF_1)		Recipe II (TF_2)		Recipe III (TF_3)	
-	-	2 tablespoons	Grated lemon zest	-	-
-	-	2 tablespoons	Vanilla extract	-	-
-	-	½ cup	Brandy	-	-
-	-	1 ½ cup	Chopped	-	-

TF_1 = Recipe from CookingCache.com (2004)
TF_2= Recipe from Allrecipes.com (2004)
TF_3= Recipe from Robinsfyi.com (2004)

2.4.1.2.2 Vanilla Cake Mix (PMC_2; Edmonds Cake Mixes from Goodman Fielder Ltd., New Zealand). The processes to make a cake was: preheat oven to 180 ^{0}C, place paper into a box tin, place vanilla cake mix, 1 egg, ¼ cup water and 25 g soft butter into a small mixing bowl, beat on a slow speed in electric cake mixer until all ingredients are combined then on medium speed for 2 minutes (scrape down sides of bowl occasionally) and bake at 180 °C for 25 minutes.

2.4.1.2.3 Banana Cake Mix (PMC_3; Betty Crocker, New Zealand). The processes to make a cake was: preheat oven for 10 minutes at 160°C, grease a tin box with oil or cooking spray, line the base with baking paper, beat cake mix, eggs and water in a large bowl with electric mixer on low speed for 30 seconds, then on medium speed for 2 minutes. Pour into pan and gently smooth top, bake for 30 minutes or until top springs back when lightly touched in centre or skewer inserted in the centre comes out clean. Cool for 10 minutes.

2.4.1.2.4 Cake Mix (PMC_4; Wellington Milling Ltd., New Zealand). The processes to make a cake was: preheat oven for 10 minutes at 170°C, place 400 gm eggs, 200 gm water and 1,000 gm cake mix in a large bowl, mix for 1

minute on speed 1 and scrape down, mix for 5 minute on top speed, mix for a further 2 minutes on speed 2 and add fruit and bake for 30 minutes

2.4.1.2.5 Variety Fruitcake Mix (PMC_5; Cereform Ltd., Australia). The processes to make a cake was: preheat oven for 10 minutes at 170°C, add 375 gm water, 1,000 gm cake mix, and 225 gm eggs in a large bowl, using beater combine on low speed and scrape down and mix 15 gm caramel, and 100 gm syrup at this point, mix on top speed for 5 minutes, fold through mixed fruit by hand or with beater and bake for 30 minutes

2.4.1.3 Comparison of water activity of fruitcake

The trial and premix fruitcakes were baked and their compared water activity compared with 2 commercial fruitcakes (Jamaican ginger loaf (CMC_1), Ernest Adams, New Zealand and Waikato Farmhouse Cake (CMC_2), Waikato Company, New Zealand) using Aqualab Model CX-2 and moisture content by AOAC (1990)

2.4.1.4 Water activity of fruitcake during storage

Sample of the PCM_3, $PCM_4$and CMC_2 were kept inside plastic bags placed inside an incubator at 30 °C, 75%RH to measure water activity and moisture content for next 30 days. The sample was measured at 0, 7, 16, 23 and 30 days.

2.4.2 Identification of *Aspergillus flavus* from fruitcake

Sample of the PMC_5 and CMC_2 were kept at 30°C, 75%RH until the fungi grew up on the surface of fruitcake. CYA and peptone water in 500 ml Erlenmeyer flasks were sterilized 121 °C 15 min. Next, 25 g fruitcake as placed in 225 ml peptone water (10^{-1}) and dilute until 10^{-8}. One ml of each dilution was poured into sterile plastic plate, 15 ml of CYA was added to the sterile plastic plate and

mixed. The sterile plastic plates were incubated at 30°C, 75%RH for 3 days. Finally, the Pitt &Hocking (1997) was used key to determine *Aspergillus flavus* (Appendix II)

2.4.3 Effectiveness of oils in preserving fruitcake with MAP and normal air, and preliminary sensory test

The *Aspergillus flavus* from section 2.4.2 was grown in CYA (Merck Ltd, Thailand) at 25°C for 7 days. Selected fruitcake from section 2.4.1 and commercial fruitcake (CMC_2) was cut into 5x5 cm and placed inside the sterile plastic plate. Then, *A. flavus* was incubated on the surface of fruitcake and this was placed into the 200 mm wide x 200 mm long pouch bags (permeability of 4 cc/m^2/day, MVTR 11 g/m^2/day at 25 °C; Cryovac, Thailand). 0, 100, 300, 500, 700, 900, 1,100, 1,300 and 1,500 µl of cinnamon oil and clove oil at ratio 5:1 were poured into the whatman paper no.6, (∅90mm) and placed into the pouch bags. The fruitcake was packaged individually with 2 conditions as following;

2.4.3.1 Modified atmosphere condition (MAP)

The pouch bag was flushed by mixture of 40% CO_2, <0.5% O_2 with the balance being N_2.and using the gas meter for measurement concentration of CO_2 and O_2 inside the pouch bag. The bags were then sealed hermetically and incubated it at 30 °C, 75%RH for 30 days.

2.4.3.2 Normal air condition (NA)

The bags were sealed hermetically with normal air using a hand sealed and incubated at 30 °C, 75%RH for 30 days.

2.4.3.3 Preliminary Sensory test

Sensory test was done by 30 European and 30 Thai persons. One piece of fruitcake (5x5 cm) was presented in a high barrier bag for evaluation. Liking ratings on a nine-point hedonic scale was used in the test. Panellists tested three fruitcake pieces: control, 300 ml and 700 ml of cinnamon oil and clove oil and asked to order flavour and odour of the fruitcake from “dislike extremely” (scored as 1) to “like extremely” (scored as 9).

3 Developing the active packaging system for preservation of the selected IMF products with modified atmosphere conditions.

3.1 Selection of plastic film, adsorbent material and synergistic action of inhibitory volatiles

3.1.1 Selection of plastic film and adsorbent material

Jar preparation sachets were made by adding 300 µl of cinnamon oil and clove oil mixed in the volume ratio 5:1 to 0.8 g of nanostructured calcium silicate I and II (provided by Prof J. Johnston, Victoria University of Wellington, NZ), silica gel (Sigma Ltd, Australia), filter paper No 6 (Whatman International Ltd, England) and sealing these within a 5x 5 cm. polypropylene film (PP) (Packaging House, Palmerston North, NZ), Linear Low Density Polyethylene (LLDPE), Low Density Polyethylene 70 (LDPE70), Low Density Polyethylene 35 (LDPE35), Thyvex (Seal air cooperation, NZ). One sachet was placed into a 200 ml glass jar, which was established with a gas atmosphere of 40% CO_2 in N_2 at 75%RH. A 5 x 5 cm piece of variety fruitcake (Cereform Ltd., Australia) slice (~2 cm thick) was also placed inside the jar to simulate an active food packaging system. The glass jars were placed in an incubator at 30 °C, 75%RH and 1 mL gas samples were removed for analysis using a gas-tight syringe (Hamilton Gastight®, USA) at varying times via a rubber septum fitted to the lid. The flavours of essential oil in premix fruitcake were extracted using

ethyl acetate. The method to extract rice fruitcake was explained by Friedman *et al* (2000). Premix fruitcake extract (1 µl) was then injected into the gas chromatograph by using 10 µl of Hamilton syringe (Hamilton Gastight®, USA). The samples were injected into GC every day until 1 month.

GC and GC/MS analysis: GC analysis was carried out on a GC 6000 VEGA Series2 (Carlo Erba Instruments) gas chromatograph equipped with a FID and a ZB-5 (Phenomenex®NZ, Ltd, New Zealand) capillary column (30mx0.25mmx0.25 µm). The oven temperature was held at 60 °C for 0.50 min then programmed at 40 ^{0}C/min to 150 °C, and was subsequently next set at 2 °C/min to 260 °C. Other operating conditions were as follows: carrier gas, N_2 at a flow rate of 1 ml/min; injector temperature, 250 °C; detector temperature, 260 °C; split ratio, 50:1. Standard curves were separately prepared for each of *p*-cymene, linalool, cinnamaldehyde, and eugenol.

GC/MS analysis was conducted using a Shimadzu QP-5050A equipped with a DB-5 (J&W Scientific, USA) column (30mx0.25mmx0.25 µm). The carrier gas was H_2 with a flow rate 1 ml/min; other operating conditions were the same as described above.

3.1.2 Synergistic action of inhibitory volatiles

3.1.2.1 Cultures

Aspergillus flavus used in this study was isolated from fruitcake (Variety fruitcake mix, Cereform Ltd., Australia). Codes refer to strains held in the culture collection of The Institute of Food, Nutrition and Human Health (IFNHH) of the Massey University, Palmerston North, New Zealand.

3.1.2.2 Preparation of spore suspensions

Spores were obtained from mycelium grown on MEA medium at 30 °C for 7 days and were collected by flooding the surface of the plates with ~5 ml sterile saline solution (NaCl, 8.5 g/l water) containing Tween 80 (0.1% v/v). After counting the spores, the solution was standardized. Mould growth was observed after inoculation of $1x\ 10^{6}$- 10^{7}at the center of the plates (10 µl of a standardized suspension $1x\ 10^{6}$- 10^{7} spores ml^{-1})

3.1.2.3 MIC test

A completely randomized design (CRD) (Table 6) was employed to assess the effects of each major volatile of cinnamon oil and clove oil and combinations of these. The agar dilution method was used as described by Tamil Selvil *et al*. (2003). Each compound was incorporated singly in the Malt Extract Agar (MEA) at a concentration of 50, 100, 300, 500, and 700 ppm. For mixtures, equal amounts of the compounds were added to give total mass concentrations of 50, 100, 300, 500, and 700 ppm. The control was handled in the same way, but no volatile compound was added to the agar. Six replicates were prepared for each treatment. The diameter of *A flavus* colonies were examined after incubation at 30 °C for 48 h and the average value recorded. The minimum inhibitory concentration (MIC) was defined as the lowest tested concentration of test samples that resulted in a complete inhibition of visible growth of the fungal colony on the agar after 48 h incubation.

3.1.2.4 Data analysis

Colony diameters were compared by analysis of variance (ANOVA) and least significance difference (LSD) using Statistical Analysis System software (SAS v8, 2001).