International Food Research Journal 19(4): 1733-1738 (2012)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antimicrobial activity of edible film incorporated with essential oils to preserve dried fish (*Decapterus maruadsi*)

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Article history

Received: 1 November 2011
Received in revised form: 4 July 2012
Accepted:6 June 2012

Keywords

Antimicrobial activity
edible film
essential oil
Decapterus maruadsi

Abstract

Antimicrobial activity of whey protein edible film incorporated with essential oils (cinnamon oil, clove oil, anise oil, turmeric oil, guava leaf oil, nutmeg oil and lime oil) at 2%, 4% and 6% (vol/vol) against major molds (*Aspergillus flavus*, *Penicillium* sp.) and bacteria (*Staphylococcus aureus*) found on dried fish (*Decapterus maruadsi*) were investigated using circular discs of edible film. Zones of inhibition were measured after an incubation period. The film containing anise oil was the most effective against mold than the other essential oils ($P < 0.05$). Storage tests were carried out for the dried fish packaged with edible film containing 4% and 6% (v/v) of anise oil stored at 30 ^{0}C for 28 days. This was found to extend the shelf life of the dried fish for up to 21 days. In addition, the fillets (3 cm x 2 cm) of the dried fish coated with 4% and 6% of anise oil were fried in vegetable oil for 1 - 2 min for the sensory test. Results from the sensory test showed that panelists were unable to detect the flavour and aroma differences between the control dried fish samples and those treated with 4% anise oil but a difference was detected for 6% anise oil. These findings illustrated that the shelf life of the dried fish could be extended using edible film with incorporated essential oils.

Introduction

Fish are naturally dried under the sun by spreading them on mats or trays. Considerable losses can occur during sun drying due to various causes such as rodents, birds, insects and microorganisms as well as discoloration by UV radiation (Paterson *et al*., 2003). Normally, dried fish is susceptible to spoilage caused by various microorganisms such as *Eurotium rubrum*, *Aspergillus niger*, *Aspergillus flavus* (Wheeler *et al*., 1986; Wheeler *et al*., 1988), halophilic bacteria, and yeast (Gram and Huss, 1996). Furthermore, *A. niger* and *A. flavus* are capable of producing ochratoxin A and aflatoxin, respectively, which pose a risk to human health due to their nephrotoxic, immunotoxic, mutagenic, teratogenic and carcinogenic effects (Ellis *et al*., 1993; D'Mello and Macdonald, 1997; Chulze *et al*., 2006). Chemical control has been the main means for inhibiting growth of microorganisms on fish products for many years, but consumer concerns over the use of chemicals has grown tremendously in many countries and alternative methods to preserve dried fish should be explored. For instance, edible film was reported to be used to reduce the initial microbial load and extend the shelf life of refrigerated fish (Falguera *et a*l., 2011). Moreover, the addition of an antimicrobial substance to edible film such as volatile essential oil offers better results as it lowers diffusion rates and reduces both antimicrobial concentration and the carriers which release antimicrobes onto the food surface (Avila-Sosa *et al*., 2012). And several reports have shown that the antimicrobial activity of edible film with an antimicrobial substance can preserve fish and fish products (Gómez-Estaca *et al*., 2010; Song *et al*., 2011). Therefore, the application of harmless natural preservatives extracted from herbs or plants that are commonly used in cooking, such as cinnamon and cloves, into edible film has been one of the most interesting research topics (Arora and Kaur, 1999; Nielsen and Rios, 2000; Soliman and Badeaa, 2002).

Essential oils such as cinnamon oil, anise oil and clove oil are well known inhibitors of mold, yeast, and bacteria growth. There have been a number of reports on a distinct property of substances in essential oil which inhibit the growth of mold and yeast (Arora and Kaur, 1999; Conner and Beuchat, 1984; Matan *et al*., 2006). In this study, a method to apply essential oils to an edible film based on whey protein in food products was looked at as well as the potential preservation of dried fish (*Decapterus maruadsi*).

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Materials and Methods

Essential oil

All of the essential oils were purchased from Thai China Flavours and Fragrances Industry Co., Ltd of Bangkok. The scientific names and major components of the test oils are shown in Table 1.

Preparation of inoculums

Two molds (*Aspergillus flavus* and *Penicillium* sp.) and one bacteria (*Staphylococcus aureus*) were identified from dried fish (*Decapterus maruadsi*) surfaces. Codes refer to strains held in the culture collection of the Food Technology, Center for Scientific and Technological Equipment at Walailak University.

Mold spores were obtained from mycelium grown on malt extract agar (MEA; Merck Ltd, Thailand) after incubation at 25 ^{0}C for 7 days and bacteria cell on nutrient agar (NA) after 1 day. Mold spores and bacteria cell 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). The suspensions were counted using a haemocytometer and were standardized to a concentration of 10^7 spores ml^{-1} for mold and 10^7 cell ml^{-1} for bacteria by dilution with sterile water before use. The viability of the mold strain and bacteria cell were checked using quantitative colony counts at 10^7 CFU ml^{-1}.

In vitro *assay*

The whey protein films were formed with the method described by Seydim and Sarikus (2006). Essential oils (cinnamon oil, clove oil, anise oil, turmeric oil, guava leaf oil, nutmeg oil and lime oil) were added to the film solutions in concentrations of 2%, 4%, and 6% (vol/vol) respectively. Vegetable oil was added into the film for the control treatment. The film forming solutions (27.5 ml) were cast onto 18.5 cm diameter circular Teflon® surfaces and then dried overnight at 35°C and 45 ± 5% RH. The dried weight of the film was 7.15 grams. Dried films were peeled and stored at 40 ± 5% RH. Film thickness was measured with a digital micrometer (Digimatic Micrometer, Mitutoyo, Japan) to the nearest 0.0001 mm. Measurements were taken at twelve random locations of each film sheet. Average film thickness was 0.2412 mm. Circular "discs" were cut from the edible films using a cutting well.

The antimicrobial activity of film against *A. flavus*, *Penicillium* sp., and *S. aureus* was determined by the disc diffusion assay. Sterile plates of 90 mm in diameter that contained MEA for mold and NA for bacteria were spread with 0.1 ml of each of the appropriate mold or bacteria respectively. Edible film discs (6.5 mm) in concentrations of 2%, 4%, and 6 % (vol/vol) of cinnamon oil, clove oil, anise oil, turmeric oil, guava leaf oil, nutmeg oil and lime oil were placed into each of the plates containing the spores of mold or bacteria. Edible film with vegetable oil was used as the control. The diameter of the zone of inhibition (mm) around the disc was measured after cultivation - 3 days for molds and 1 day for bacteria - at 25°C.

Table 1. List of essential oils tested

Common name	Scientific name	Major component(s)
Cinnamon oil	*Cinnamomum verum*	Cinnamaldehyde
Clove oil	*Syzygium aromaticum*	Eugenol
Anise oil	*Pimpinella anisum*	Anethole
Citronella oil	*Pelargonium citrosum*	Citronellal
Orange oil	*Citrus Aurantium*	Limonene
Tangerine oil	*Citrus reticulata*	Limonene
Turmeric oil	*Curcuma longa*	Curcumin
Guava leave oil	*Psidium guajava*	Haxanal
Nutmeg oil	*Myristica fragrans*	α-pinene, γ-terpinene
Lime oil	*Citrus x hystrix*	Limonene and α-pinene

In vivo *assay*

The round scad caught in the gulf of Thailand were obtained from the Thasala district of Nakhon Si Thammarat. The average weight of these fish was 100±10 g or 10-12 pieces/kg. Samples were frozen in a vessel and transported to the laboratory. After thawing, the fish were headed, gutted and cut into two pieces. The fish were pickle salted for 30 minutes in a clean, lid-covered plastic box previously washed with 70% ethanol. Then the fish samples (25±5 g) were sprayed individually with 10 ml of anise oil edible film solution in concentrations of 2%, 4%, and 6 % (vol/vol) respectively. Vegetable oil was added into the film for the control treatment. After that, a tray dryer (OFM Owner Food Machinery, Thailand) was used for fish drying at 80°C for 2 hours. The final water activity of the dried samples was controlled at 0.85. After drying, a 25 g piece of dried fish was placed inside a polypropylene bag and was kept at 30 ^{0}C for 28 days. At 0, 3, 7, 15, 21 and 28 days, total bacteria, yeast and mold were analyzed according to procedures 933.11 and 2002.11 of the Association of Official Analytical Chemists (AOAC, 1995), respectively.

Sensory evaluation

A fillet of the dried fish (3 cm x 2 cm) was covered with edible film containing 4% and 6% of anise oil by spraying. The edible film with vegetable oil was the control treatment. The packaging was kept at 30 °C for 24 hours.

The samples were subjected to sensory analysis

by an untrained panel (30 panelists for each test) using triangle tests. Panelists were selected from students and staff at Walailak University. The fillets of dried fish were fried in vegetable oil for 1 - 2 min. For each test, panelists were presented with three samples, consisting of a pair of Sample A (control) and a single Sample B (4% of anise oil) or a single Sample C (6% of anise oil), or the reverse. Panelists were asked to identify the different sample. The number of correct responses was used to calculate sample preference t-tests.

Statistical analysis

The experiments were performed in duplicate and repeated three times. All variables were tested for normality by applying the Kolmogorov-Smirnov test and the homogeneity of variances was assessed using Levene's test. Data transformation was done where necessary. All results were expressed as mean ± standard deviation. The data was statistically treated by one-way ANOVA and Duncan's post hoc test with $p < 0.05$ considered to be statistically significant.

Results and Discussion

Antimicrobial analysis

The inhibition of mold and bacteria by edible film with essential oil were determined by the disc method. Results are shown in Figure 1 (a-c). Anise oil (4% to 6% v/v) was the only essential oil strong enough to completely inhibit the growth of all of the microorganisms in this test. Turmeric oil at 6% v/v could inhibit the growth of *Penicillium* spp. and *S. aureus* as well but not for *A. flavus*. Cinnamon and clove oil showed some inhibition to all microorganisms. Nutmeg, guava, and lime oil were only weak inhibitors of *Penicillium* spp. and *S. aureus* but showed not good inhibition for *A. flavus*. *Aspergillus flavus* appeared to be the most resistant of the microorganisms.

Anise oil contains a number of compounds such as trans-anethole, α-trans-bergamotene, and limonene (Porta et al., 1998). Anethole is largely used as a flavour agent in the food industry, used in cakes and ice-creams and is also put in alcoholic beverages. It presents several pharmacological activities (Freire *et al*., 2005) and was found to inhibit bacteria such as *Salmonella typhimurium*, *Staphylococcus aureus* and *Vibrio parahaemolyticus* (Karapinar and Aktu , 1987), *Aspergillus parasiticus* (Karapinar, 1990), *Aspergillus niger* and *Penicillium chrysogenum* (Matan and Matan, 2008). Furthermore, a mechanism of anise oil that could prevent spore germination was found by Matan *et al*. (2011); however, further investigation is needed.

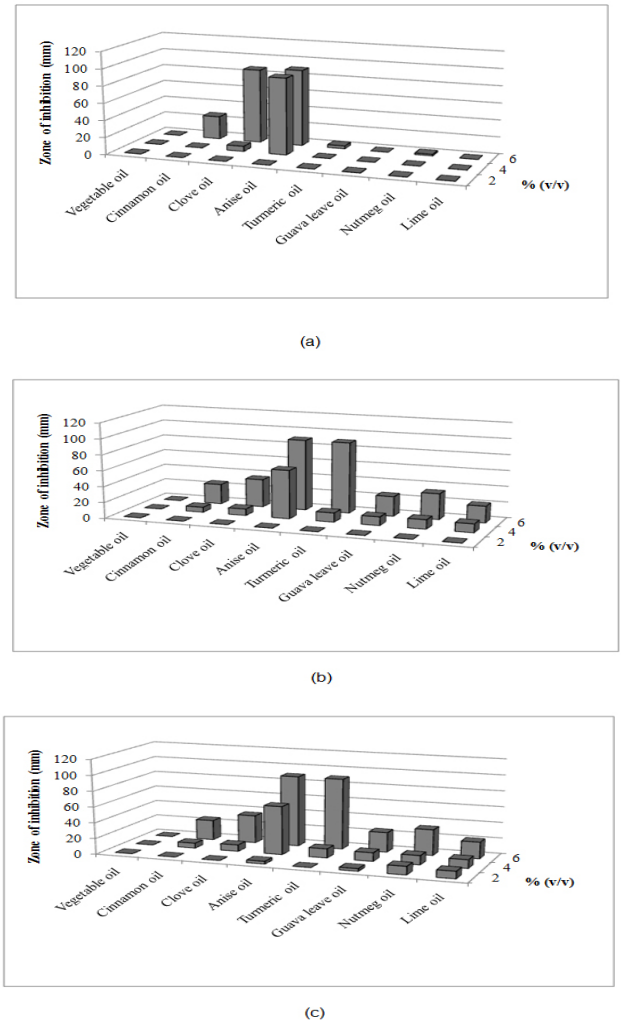

Figure 1. Zone inhibition of essential oils in edible film at a concentration of 2%, 4%, and 6 % (vol/vol) against (a) *Aspergillus flavus*, (b) *Penicillium* spp., and (c) *Staphylococcus aureus*

Cinnamon oil was reported to consist of many components such as cinnamaldehyde, eugenol, and linalool (Wang *et al*., 2005). The major component of clove oil was eugenol with a small addition of cariophyllene and humulene (Velluti *et al*., 2003; Marín *et al*., 2004). Components of cinnamon and clove oil were reported to be capable of inhibiting growth of microorganisms and Burt (2004) explained how mechanisms of cinmaldehyde and eugenol inhibit microorganisms.

In addition, nutmeg oil and turmeric oil were reported to be good inhibitors of *Penicillium chrysogenum* on the surface of rubberwood (Matan and Matan, 2007). Lime oil was also noted that it could work against *Aspergillus niger* and *Penicillium chrysogenum* (Matan and Matan, 2008). However, nutmeg oil, turmeric oil, and lime oil were not able to affect the *A. flavus* found in this test. In addition, citronella oil, orange oil and tangerine oil could not inhibit growth of molds and bacteria in this test.

Comparisons between the effectiveness of edible film incorporated with essential oils and the liquid

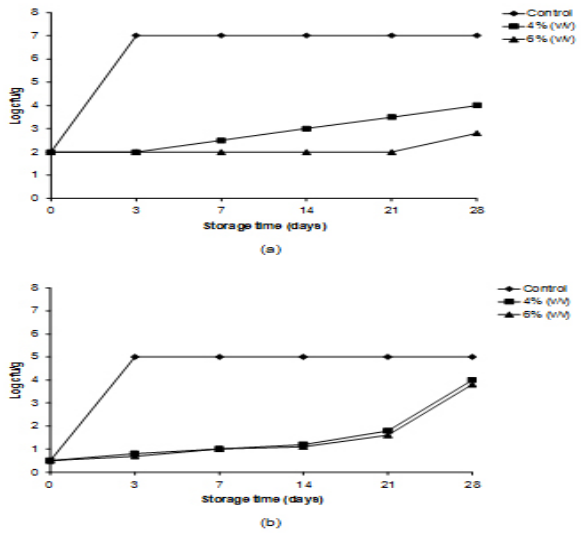

Figure 2. Total (a) bacteria, and (b) total yeast and mold found on dried fish preserved with edible film incorporated with 4 % and 6 % of anise oil

Table 2. Sensory analysis (triangle test comparison) of dried fish with (A) no essential oil, (B) preserved with 4% of anise oil, or (C), 6% of anise oil

Test	Sample	Difference
1	A-B	n.s.d
2	A-C	P<0.05
3	B-C	P<0.05

n.s.d=no significant difference (P>0.05)

phases of essential oils have shown that oil in the liquid phase is more effective in preventing spoilage than in edible film. Soliman and Badeaa (2002) found that ≤500 or 0.05 % of anise oil can inhibit *Aspergillus flavus* on an agar medium. However, the advantages of using an edible film with essential oils for food products are that it may be easy to use and it may be able to enhance food quality and extend the shelf life while reducing packaging waste. Moreover, in this regard, essential oil is a valuable component for processing biodegradable packaging which can extend shelf-life and inhibit food pathogens and spoilage.

Microbial growth in dried fish

The growth of bacteria and the growth of yeast and mold on dried fish sprayed with edible film plus anise oil (4% and 6%) is shown in Figure 2 (a-b). Bacteria (Figure 2a) plus yeast and mold (Figure 2b) grew the fastest in dried fish for the control, followed by those in edible packaging with 4% anise oil. Edible films with 6% anise oil showed slower and similar growth rates with an extended lag phase.

When the aerobic plate count reaches 10^6 cfu (colony forming units) per gram in a food product, it is assumed to be at, or near, spoilage (Chouliara *et al*., 1990). In addition, according to the Thai Industrial Standard for the surface of dried fish (UDC664.951.2), the total counts of bacteria must be $\leq 10^6$ and yeast and mold must both be $<10^2$. In this study, the limit of acceptability (10^6 cfu/g) in terms of the total viable count was 3 days for control stored in air, and >28 days for storage with 4% and 6% of anise oil edible film.

Mold growth could appear on the surface of dried fish. The control dried fish was found to spoil from yeast and mold within 3 days (10^5 cfu/g). On the other hand, edible film incorporated with anise oil at 4% and 6% could extend the shelf life of dried fish for up to 21 days ($\leq 10^2$ cfu/g).

Sensory evaluation

The comparison of the sensory evaluation between the control dried fish and the fish wrapped with edible film containing 4% or 6% of anise oil is summarized in Table 2. The result shows that the panel could not distinguish between the control (A) and the dried fish preserved with 4% of anise oil (B), while it was able to detect an overall difference between 6% of anise oil (C) with (A) or (B).

Therefore, edible film incorporated with 4% of anise oil was not only suitable for extending the shelf life of dried fish it also wasn't negative with the sensory quality of the fish.

Conclusions

The edible film incorporated with anise oil at 4% and 6 % was capable of preventing the growth of bacteria, yeasts and mold on agar plates and extended the shelf life of dried fish from 3 to 21 days. Anise oil added at 6% was detected by sensory tests on the fish, but not at 4%. Therefore 4% is considered the optimum level of anise oil based on these results. It is suggested that the technique developed in this work could be employed to extend the shelf life of dried fish products by manipulating the conditions of edible film with essential oil.

Acknowledgement

This study was supported by the Walailak University Fund.

References

AOAC. 1995. AOAC, Official Methods of Analysis (16th ed.). Association of Official Analytical Chemists, Washington, DC, USA

Arora, D.S. and Kaur, J. 1999. Antimicrobial activity of spices. International Journal of Antimicrobial Agent 12: 257-262.

Avila-Sosa, R., Palou, E., Jiménez Munguía, M.T., Nevárez-Moorillón, G.V., Navarro Cruz, A.R. and López-Malo, A. 2012. Antifungal activity by vapor contact of essential oils added to amaranth, chitosan, or starch edible films. International Journal of Food Microbiology 153(1-2): 66-72.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology 94: 223-253.

Chouliara, I., Savvaidis, I.N., Panagiotakis, N. and Kontominas, M.G. 2004. Preservation of salted, vacuum-packaged, refrigerated sea bream (*Sparus aurata*) fillets by irradiation: microbiological, chemical and sensory attributes. Food Microbiology 21(3): 351-359

Chulze, S.N., Magnoli, C.E. and Dalcero, A.M. 2006. Occurrence of ochratoxin A in wine and ochratoxigenic mycoflora in grapes and dried vine fruits in South America. International Journal of Food Microbiology 111: S5-S9.

Conner, D.E. and Beuchat, L.R. 1984. Effect of essential oils plants on growth of food spoilage yeasts. Journal of Food Science 49: 429-434.

D'Mello, J. P. F. and Macdonald, A. M. C. 1997. Mycotoxins. Animal Feed Science and Technology 69(1-3): 155-166.

Ellis, W. O., Smith, J. P., Simpson, B. K., Khanizadeh, S. and Oldham, J. H. 1993. Control of growth and aflatoxin production of *Aspergillus flavus* under modified atmosphere packaging (MAP) conditions. Food Microbiology 10(1): 9-21.

Falguera, V., Quintero, J.P., Jiménez, A., Muñoz, J.A. and Ibarz, A. Edible films and coatings: Structures, active functions and trends in their use. Trends in Food Science and Technology 22(6): 292-303.

Freire, R.S., Morais, S.M., Catunda-Junior, F.E.A. and Pinheiro, D.C.S.N. 2005. Synthesis and antioxidant, anti-inflammatory and gastroprotector activities of anethole and related compounds. Bioorganic & Medicinal Chemistry 13(13): 4353-4358.

Gómez-Estaca, J., López de Lacey, A., López-Caballero, M.E., Gómez-Guillén, M.C. and Montero, P. 2010. Biodegradable gelatine-chitosan films incorporated with essential oil as antimicrobial agents for fish preservation. Food Microbiology 27(7): 889-896.

Gram, L. and Huss, H.H. 1996. Microbiological spoilage on fish and fish products. International Journal of Food Microbiology 33(1): 121-137.

Karapinar, M. 1990. Inhibitory effects of anethole and eugenol on the growth and toxin production of *Aspergillus parasiticus*. International Journal of Food Microbiology 10(3-4):193-199.

Karapinar, M. and Aktu, S.E. 1987. Inhibition of foodborne pathogens by thymol, eugenol, menthol and anethole. International Journal of Food Microbiology 4(2): 161-166.

Marín, S., Velluti, A., Ramos, A.J. and Sanchis, V. 2004. Effect of essential oils on zearalenone and deoxynivalenol production by *Fusarium graminearum* in non-sterilized maize grain. Food Microbiology 21: 313-318.

Matan, N. and Matan, N. 2007. Biocontrol of *Penicillium chrysogenum* using nutmeg oil and turmeric oil. KMITL Science and Technology Journal 7(52): 192-196.

Matan, N. and Matan, N. 2008. Antifungal activities of anise oil, lime oil and tangerine oil against molds on rubberwood (*Hevea brasiliensis*). International Biodeterioration and Biodegradation 62(1): 75-78.

Matan, N., Rimkeeree, H. Mawson, A.J., Chompreeda, P., Haruthaithanasan, V. and Parker, M. 2006. Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. International Journal of Food Microbiology 107(2): 180-185.

Matan, N., Saengkrajang, W. and Matan, N. 2011. Antifungal activities of essential oils applied by dip-treatment on areca palm (*Areca catechu*) leaf sheath and persistence of potency upon storage. International Biodeterioration and Biodegradation 65: 212-216.

Nielsen, P.V. and Rios, R. 2000. Inhibition of fungal growth on bread by volatile components from spices and herbs, and possible application in active packaging, with special emphasis on mustard essential oil. International Journal of Food Microbiology 60: 219-229.

Paterson, B., Goodrick, B. and Frost, S. 2003. Controlling the quality of aquaculture food products. Trends in Food Science and Technology 8: 253-257.

Porta, G.D., Taddeo, R., D'Urso, E. and Reverchon, E. 1998. Isolation of clove bud and star anise essential oil by supercritical CO_2 extraction. LWT-Food Science and Technology 31(5): 454-460.

Seydim A.C. and Sarikus, G. 2006. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. Food Research International 39(5): 639-644.

Soliman, K.M. and Badeaa, R.I. 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and Chemical Toxicology 40: 1669-1675.

Song, Y., Liu, L., Shen, H., You, J. and Luo, Y. 2011. Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (*Megalobrama amblycephala*). Food Control 22(3-4): 608-615.

Thai Industrial Standards Institute. 1994. Thai industrial standard for dried fish (UDC664.951.2). Bangkok, Thailand.

Velluti, A., Sanchis, V., Ramos, A.J., Egido, J. and Marín, S. 2003. Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. International Journal of Food Microbiology 89(2-3): 145-154.

Wang, S-Y., Chen, P-F. and Chang, S-T. 2005. Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. Bioresource Technology 96(7): 813-818.

Wheeler, K.A. and Hocking, A.D. 1988. Water relations of *Paecilomyces variotii*, *Eurotium amstelodami*, *Aspergillus candidus* and *Aspergillus sydowii*, xerophilic fungi isolated from Indonesian dried fish. International Journal of Food Microbiology 7(1): 73-78.

Wheeler, K.A., Hocking, A.D., Pitt, J. I. and Anggawati, A. M. 1986. Fungi associated with Indonesian dried fish. Food Microbiology 3(4): 351-357.