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Research Article

Isolation and Medical Utilization of Nutmeg Seed Oil as Edible Coating Additive for Tatihu Fish Fillet (*Thunnus moccoyil*)

Nini Munirah Renur[1,2*], Sutrisno[3], Emmy Darmawati[3], Nurjanah[4]

[1]Fishery Product Technology Department, Tual State Fisheries of Polytechnic, Maluku, Indonesia
[2]Department of Agricultural Engineering Science, Bogor Agricultural University, Bogor, Indonesia
[3]Department of Mechanical and Biosystem Engineering, Bogor Agricultural University, Bogor, Indonesia
[4]Departmen of Fisheries Product Technology, Bogor Agricultural University, Bogor, Indonesia

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ABSTRACT

Background: Fresh fillet fish is very easy to damage so it needs proper handling to maintain its quality, one of which is the application of edible coating. Sago is a local material that is widely available in eastern Indonesia and has the potential to be used as an edible film matrix. Addition of additives such as nutmeg seed oil to the film matrix is intended to increase the protection of the film in maintaining the freshness of fish fillets. The purpose of this study was to isolate nutmeg seed oil and apply as an edible sago-based edible coating additive to the Tatihu fish fillet. The nutmeg oil isolation method uses a steam distillation system at 85°C for 5 hours. Characterization of distillation results using Gas Chromatography-Mass Spectroscopy (GC-MS) and application of edible coatings on fish fillets by dipping. The results showed that the nutmeg oil from the isolation had a rendition of 1.16%. The content of myristicin compound is 9.64%, safrol compound is 4.25% and the rest are compounds of terpenoid group. The best concentration of nutmeg oil added to the matrix made from sago was 8%, where the concentration was able to reduce the potential for microbial contamination up to 79.31% (TPC) when applied as a film on Tahitu fish fillets stored at 27 °C for 24 hours.

Keywords: Isolation, Nutmeg oil, Edible, Fish fillets

***Correspondence:** Nini Munirah Renur, Fishery Product Technology Department, Tual State Fisheries of Polytechnic, Maluku, Department of Agricultural Engineering Science, Bogor Agricultural University, Bogor, Indonesia, E-mail: nini.renur47@gmail.com

INTRODUCTION

Fish is a food group that is very susceptible to damage, unlike other meat products. The rapid deterioration of fish quality after fish die is caused by several factors including the occurrence of an enzymatic process, or the presence of spoilage microorganisms that develop on the fish's body parts (Lampila LE and McMillin KW, 2012). Fish products are very interesting because they function as a source of protein, vitamins, minerals and fats. Fish is very easily damaged so that proper handling and use of packaging can help maintain the fish quality. Various techniques to maintain the quality of fish have been used so far, ranging from simple storage, cold, pressure modification and electromagnetic field applications (Nagarajarao RC, 2016).

The main role of packaging in the food industry is to preserve and protect products from external contamination, including maintaining food safety, maintaining quality, and increasing shelf life. According to Galus S and Kadzinska J, edible film and edible coating have the same function as conventional packaging, including barrier against water vapor, gas, and flavor compounds and improve structural integrity and mechanical handling properties of food (Galus S and Kadzińska J, 2015). Edible coating is important for food products that are easily damaged such as seafood, because it is used to inhibit the growth of microbes on the surface of fresh processed products (Cagri A, *et al.*, 2004).

When the food packaging system is given antimicrobial activity, the packaging material will limit or prevent microbes from growing on the surface of the food, thereby extending the shelf life and increasing the safety of the product. The incorporation of antimicrobial agents into the food packaging system can be done through two approaches, namely: placing the antimicrobial agent into the film by adding it when the film is produced or through coating/coating on the surface of the film in direct contact with food; and release of active compounds to the food surface through sachets/pad added to the package (Quintavalla S and Vicini L, 2002). Additives used as antimicrobials must be safe and approved by certain institutions, for example nisin which is a bacteriocin produced by cationic, hydrophobic, Lactic Acid Bacteria (LAB) and amphiphilic peptides with a broad spectrum of antimicrobial activity that is approved as an additive for meat products. in several countries, namely the United States and Australia (Realini CE and Marcos B, 2014).

The use of natural extracts and essential oils that are rich in phenolic compounds can provide antimicrobial and antioxidant effects. Grape seed extract (1%) incorporated in the starch film was able to control the growth of Brochothrix thermosphacta on the surface of roast pork for 4 days. Essential oils (garlic, oregano and thyme) and their components (carvacrol and thymol) have been shown to be effective in delaying the growth of spoilage microorganisms in food products (Realini CE and Marcos B, 2014). Chitosan based film containing ginger essential oil as an active antimicrobial agent is used as the main wrapper for fish steaks. During storage the film gives a decrease in the total formation of volatile base nitrogen and lipid oxidation (Remya S, *et al.*, 2016). The nature of chitosan itself is non-toxic, antibacterial, antioxidant, film-forming, biocompatibility, and biodegradability (Renur NM, *et al.*, 2016). The matrix mixture of chitosan and gelatin made in the form of films with Oregano Essential Oil (OEA) additives shows better antimicrobial activity when compared to cinnamon oil and fennel additives (Wu J, *et al.*, 2014).

The secondary metabolite compounds found in essential oils have selectivity to certain types of bacteria. The essential oil component containing carvacrol (65.22%) and thymol (19.51%) has an influence on microbial types of mesophilic and psychrotrophic bacteria, *Pseudomonas spp., P. fluorescens, Putrefaciens shewanella*, lactic acid bacteria and family Enterobacteriaceae (Kakaei S and Shahbazi Y, 2016). Matrix films made using a mixture of chitosan-gelatin combined with grape seed extract (GSE) (1% and 2%) and *clinopodioides Ziziphora*. Essential Oil (ZEO) (1% and 2%) are able to extend the shelf life of fish fillet products (Kakaei

S and Shahbazi Y, 2016).

The addition of natural essential oils as additives to antimicrobial packaging made by previous researchers has been sufficiently developed. Indonesia is very rich in natural products, but not all natural products are used as additives for antimicrobial packaging. Maluku region is rich in spices, this is a very potential opportunity to be developed as an antimicrobial packaging additive by utilizing nutmeg oil. The use of chitosan as a basic ingredient in making a matrix can be replaced using another source of carbohydrate that is widely available in Maluku, sago. Mixing sago with gelatin is expected to produce a good matrix. The purpose of this study is to isolate clove oil from clove leaves and apply it as an additive to sago-based edible coatings.

MATERIALS AND METHODS

This study investigated the effectiveness as a treatment for dementia in the elderly of a combination of herbs that had been classified as safe for human consumption and had already been proven in clinical testing. The study used pretest-posttest control group design research during September 2020 to March 2021 based on phases of before and after herbal treatment.

Distillation of essential oils

Dried nutmeg seeds weighed 1213.07 g and then prepared a distillation kettle filled with water (Kapelle IBD, *et al.*, 2016). The chopped sample is inserted and placed on a sieve. After everything is ready, the distillery is closed and heated using a hotplate. In the next stage the evaporated water will carry oil particles and come out in the form of steam which is then flowed through a pipe to the cooler. Inside the cooler occurs condensation and water vapor mixed with oil will melt again. Then flowed to a separator to separate essential oils from water. If the results of the separation have not been separated properly, a re-separation is carried out. Distillation was carried out at 85 °C for 5 hours. The resulting oil is collected and then analyzed its constituent components using GCMS.

Analysis of chemical composition of nutmeg oil

Determination of nutmeg oil content using a Gas Chromatography-Mass Spectrometry (GC-MS Simadzu QP-5050 A series II, Class-5000 Ver 2.2) equipped with a DBMS detector with DB10 capillary column length 30 m diameter 0.25 mm, using hydrogen as a carrier gas (1.6 ml/ minute). The column temperature is 60 °C, the injector temperature is 280 °C, the detector temperature is 300 °C, the interface temperature is 320 °C, the column pressure is 100 kPa. The program lasts 39 minutes. The percentage of essential oil obtained is the percentage of oil injected (relative percentage). The molecular profile contained in essential oils was obtained by comparing the chromatograms that appeared through GC-MS digital detectors with molecular chromatograms found in library sources WILLEY 229, NIST62 LIB, and PESTICID LIB. The percentage of nutmeg oil molecule content obtained is a relative percentage.

Manufacture of antimicrobial edible coatings

A total of 10 g of sago starch that has been sieved with a size of 80 mess was dissolved in 100 ml of distilled water, stirred and filtered to obtain a starch solution, then heated at 73 °C while stirring. Gelatin was added to the mixture at a concentration of 2% and stirred, then added 4 ml glycerol and 10 g sorbitol while continuing to stir (Kakaei S and Shahbazi Y, 2016; Nafchi AM, et al., 2012). The solution was then added to nutmeg oil with various concentrations (2%, 4%, 6%, and 8%; w/w total solids) and without nutmeg oil as a control. Heating is carried out until the solution is completely gelatinized, then degassing (80 kPa, 15 minutes) to remove dissolved gas. Tatihu fish fillets are then coated by edible coating and then stored for 24 hours at room temperature (± 27 °C). After 24 hours of storage the TPC was determined.

Microbiological analysis

In the microbiological test TPC (total plate count) determination is used (Fardiaz S, 1987). The working principle of the TPC analysis is the calculation of the number of bacterial colonies in the sample (fish meat) with dilution as needed and carried out for preliminary research in triplo and in stage three is carried out in duplicate. Preparation of sample solution by mixing 5 g of sample into 45 ml of diluent solution to obtain a 10-1 dilution (1:10). Subsequently a 10-2 successive dilution is made and so on as needed. Sampling and fertilizing were carried out aseptically. The agar medium (sodium agar) is put into the 10 ml petri dish and shaken to the surface so that it is evenly distributed (the pour cup method), then allowed to stand for a while until it cools and hardens. Petri dishes that have been filled with agar and sample solution are put into an incubator in an upside-down position, which is the lid of the cup is placed at the bottom of the petri dish. The incubator temperature used was around 30°C and incubated for 2 days, then an observation was made by counting the number of colonies in the petri dish.

RESULTS AND DISCUSSION

Nutmeg oil obtained from the distillation process of dried nutmeg seed water at a distillation temperature of 85 °C for 5 hours produced a rendition of 1.16%. Nutmeg oil analyzed by GCMS showed 21 components *(Figure 1)* and the main components are presented in Table 1. The largest concentration of 14.93% for sabinene compounds and the characterization of nutmeg aroma was myristicin compound with a concentration of 9.64% and safrol compound with a concentration of 4.25%. Identification at 14.03 minutes retention time showing safrol and myristin compounds was proven by MS data *(Figure 2)*.

Table 1: The main components of nutmeg oil

No	Retention time	Name	Percentage
1	5.736	Phellandrene	4.42
2	5.918	α -pinen	13.7
3	6.86	sabinen	14.93
4	7.219	β -myrcene	7.4
5	7.735	α -terpinene	4.18
6	7.977	limonen	12.08
7	8.506	Gama-ter-pinene	5.88
8	10.317	3-cyclohexenol	5.67
9	11.674	safrol	4.25
10	14.03	miristisin	9.64

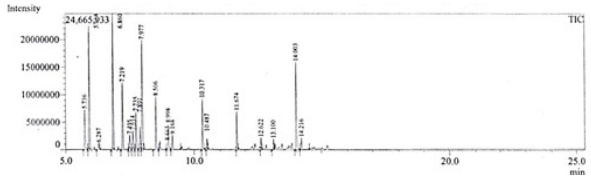

Figure 1: GC-MS spectrum of nutmeg oil

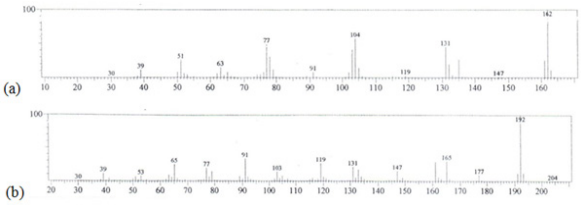

Figure 2: Spectrum of MS (a) safrol and (b) myristicin

Edible coating solution made using sago as a base material and additional nutmeg oil additives applied to tatihu fish fillets obtained different TPC data for the two variations in oil concentration. Tatihu fish fillets weighing 5 g and coated using a dip method.

The fish that were coated were left at a temperature of 27 °C for 24 hours and obtained data as shown in Table 2. The TPC test results for tatihu fish fillets are shown in *Figure 3*.

Table 2: TPC test results for coated gourd fish with additives

Additive concentration (%)	TPC	Deduction Presentation
0	29 x 108	0
2	13 x 108	55.17
4	14 x 108	51.72
6	7 x 108	75.86
8	6 x 108	79.31

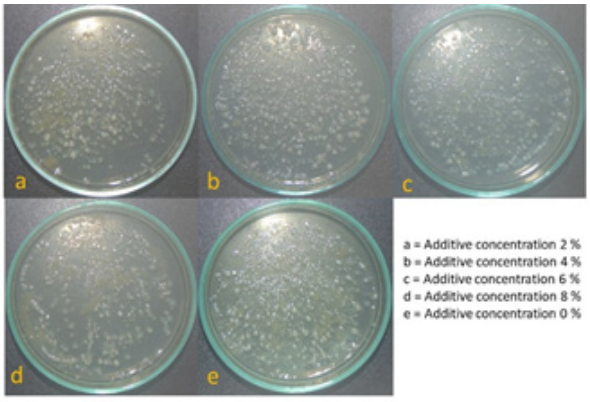

Figure 3: TPC test results for tatihu fish fillets

Based on the results of the TPC analysis, it was shown that nutmeg oil can influence the growth of spoilage bacteria in fish. Table 2 shows the effect of adding nutmeg oil can reduce the number of bacteria by more than 55%. The higher concentration of nutmeg oil can reduce the number of bacteria in fish.

CONCLUSION

Nutmeg oil isolated from dried nutmeg seeds obtained 1.16% rendamen with 9.64% myristicin content. The best concentration of nutmeg oil added to the sago matrix is 8% which can reduce the potential for microbial contamination up to 79.31% (TPC).

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