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# Nutmeg's (*Myristica fragrans* Houtt) Oleoresin: Effect of Heating to Chemical Compositions and Antifungal Properties

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## Abstract

The aim of the study was to evaluate the effect of heating on the chemical compositions and antifungal properties of nutmeg's oleoresin. Nutmeg's oleoresin was obtained by two steps, distillation followed by maceration. The nutmeg's oleoresin was heated at 100°C, 120°C and 180°C. The chemical compositions were determined by GC-MS, and the antifungal properties by giant colony method. The result of the study showed that yield was 13.6%±0.2% and 24 components were identified. The nutmeg's oleoresin at 100ºC, 120ºC and 180 ºC identified 25, 21 and 20 components. Indeed, heating treatment on nutmeg's oleoresin did not decrease the antifungal properties.



Peer-review under responsibility of the organizing committee of Indonesian Food Technologist Community

Keywords: nutmeg's oleoresin, heating, chemical compositions, antifungal properties.

## INTRODUCTION

Nutmeg (Myristica fragrans Houtt) is one of the commodities which highly economic value, Indonesia is a major producer and supplier needs nutmeg and mace nutmeg. 80% of the world nutmeg from Indonesia, 20% from Grenada and the rest from Sri Lanka, Trinidad and Tobago [7].. However the reality Indonesian nutmeg commodity prices is quite low, because the low quality of nutmeg, about 55% of the seeds with the quality of BWP (Broken Wormy Punky) and mace with Broken II quality that reaches 77%. Efforts to increase the sale value of BWP quality nutmeg can be done by processing nutmeg into essential oils, nutmeg's oleoresin, and nutmeg butter.

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Oleoresin is a mixture of resins and essential oils which obtained through extraction of various spices. Oleoresin has characteristics flavor and aroma of spices which are the same as the original [1]. Oleoresin contains essential oils that make up the aroma, oleoresin also contains resins and compounds that did not volatile determine the characteristic flavor of spices. Oleoresin extraction is generally done with organic solvents, such as ethylene diklhorida, acetone, ethanol, methanol, hexane [20], ether and isopropyl alcohol [8]. The choice of solvent affects the quality and quantity of oleoresin obtained. Oleoresin were used in food processing safer extracted using ethanol solvent [9]. Extraction with polar solvents such as ethanol will be produced oleoresin with a low fat content [13]. According Rismunandar [16] nutmeg's oleoresin extraction with gradual extraction of oleoresin were will result in much more than the direct extraction. In the gradual extraction of nutmeg's essential oils is taken beforehand through steam and water distillation, then the waste is dried and followed by extraction of the percolation or maceration, Oleoresin obtained was a mixture of resins and essential oils. Extraction oleoresin was done after distillation of essential oils which will suppress the loss of essential oils contained in the oleoresin during solvent evaporation process [1].

In the food processing, nutmeg's oleoresin are often added as a flavor. Food products were usually added oleoresin or nutmeg's essential oil was products such as meat and fish, pickles, sauces, soups, biscuits and bread or cake [16, 11] These products are often damaged because of bacteria, yeast and fungi. Fungi often damage foods such as Aspergillus, Fusarium, Rhizopus, Mucor, Penicillium, Cladosporium, Eurotium, Alternaria [6, 15, 21] . One attempt to maintain the shelf life of these products is to add a component were could inhibit microbial activity. Some recent studies showed that the active substances contained in various types of spices extracts known to inhibit some pathogenic microbes or microbial food destroyer [5].

In the nutmeg's oleoresin contained essential oils, the main component of nutmeg essential oil was a hydrocarbon monoterpenes (61-88% as α-pinene, β-pinene, sabinene) monoterpenes acid (5-15%), and aromatic ether (2-18% such as myristicin, elemicin, safrole) [4]. According to Rahman, nutmeg oil has 37 components and 31.3% was terpinen-4-ol, reported that nutmeg oil has antifungal activity [14].

According previous researcher, the effect of cooking or food processing on the potential antimicrobial spices largely unknown, food processing at high temperature will damage the effect of antimicrobial (antifungal and antibacterial) of some spices [2]. Some of the reasons underlying the need for research is to determine the effect of heating on food processing temperature (100 ° C, 120 ° C and 180 ° C) on the chemical composition of nutmeg's oleoresin and a heating effect on the antimicrobial properties antifungal properties. The aim of the study was to evaluate the effect of heating on the chemical compositions and antifungal properties of nutmeg's oleoresin

## MATERIALS AND METHODS

### Materials

Sample was taken from the 5-6 month's of Nutmeg Myristica fragrans Houtt which obtained from the Ternate, North Maluku. Nutmeg is dried in the cabinet dryer until 10-15% of moisture content, then stored in a freezer until used. The organic solvent used 96% ethanol. Fungi growth medium used Dichloran Rose Bengal Chloramphenicol (DRBC) and Potato Dextrose Agar (PDA) was from Merck. Strains of Fungi Aspergillus niger FNCC 6018, 6070 FNCC Pinicillium glabrum, Mucor racemosus FNCC 6100 were obtained from the microbiology laboratory PAU-UGM, Fusarium oxysporum and Rhizopus oryzae obtained from the FTP-GMU microbiology laboratory. Other chemicals used include distilled water, Tween 80, 0.05% Tween 80 solution, caragenan, anhydrous Na2SO4, and 70% alcohol.

### Methods

#### *Preparation of nutmeg's oleoresin*

Nutmeg's oleoresin was obtained by two steps, distillation and followed by solvent extraction [16, 24]. Distillation using is water and steam distillation [13].

Nutmeg with a water content of 10-15% removed from its shell and crushed using a dry blender, 200 grams of crushed nutmeg put in distillation equipment that has been filled with water and given filter, then in distilled during ± 5 hours. Oil and water were accommodated in Clevenger removed and then separated using a separator funnel. Essential oils was obtained by separating water content by the addition of anhydrous Na2SO4, and then calculated the essential oils obtained (% db).

Distillation residue was dried using a cabinet dryer until the moisture content of 10-15%. After drying residue crushed using a blender, then screened using a 20 mesh sieve [23]. The part that passes of 20 meshsize is separated with the part which not passes, then each of them was extracted, the maceration extraction is used and fixing with 96% ethanol on ratio 1: 5 of material and solvent, extraction temperature is 40 º C with stirring for 2.5 hours, the extraction is done by using hot plate stirrer [23]. Once extracted filtered with a vacuum filter coated filter paper to separate the filtrate and the residue then filtered again using whatman paper no. 42 in order to ignore the mixture of residue and filtrate. The filtrate was cooled on 4 ° C for 1 1 hour, then was filtered to separate the nutmeg butter.

Ethanol filtrate was evaporated using rotary evaporator on 40 º C and 175 mbar. This extraction was repeated once again in the same way in order to obtain the filtrate 1 and 2 then each of them was weighed. The filtrate is a resin, the resin was mixed with essential oils obtained until a homogeneous. This mixture is called nutmeg's oleoresin.

Oleoresin chemical components were analyzed using Gas Chromatography Mass Spectroscopy (GC-MS) and nutmeg butter obtained was tested using Gas Chromatography (GC) to determine the fatty acid composition.

***Treatment of nutmeg oleoresin temperature***

Oleoresin obtained is given a heat treatment at temperatures of food processing i.e. 100ºC, 120ºC, and 180ºC, respectively. Five grams Oleoresin is placed on test tube and then screw on the cover tightly with aluminum foil and heated at each temperature treatment, the heating is done using the oven for 30 minutes. Each treatment was repeated 3 times. After heated oleoresin weighed and analyzed the chemical components using *Gas Chromatography –Mass Spectroscopy* (GC-MS)

***Analysis of Gas Chromatography Mass Spectroscopy (GC-MS)***

Nutmeg oleoresin obtained using temperature treatment were tested using GC-MS to determine the components contained in the nutmeg's oleoresin. Nutmeg oleoresin was analyzed by GC-MS under the following conditions: Shimadzu GCMS-QP2010S equipped with a RTX-5MS column (inner diameter 0:25 mm, length 30 m, and the film thickness 0.25μm) temperature of 80 ° C was increased to 250°C (4°C / min) was maintained for 20 min, injector and detector temperature 290 ° C, helium carrier gas with a flow rate of 80 mL / min, and the FID detector were used.

***Test of antifungal nutmeg's oleoresin***

In testing for the antifungal heated nutmeg's oleoresin (100ºC, 120ºC, and 180ºC). Nutmeg's oleoresin is used as antifungal materials with concentration of 10.000 ppm of the volume of media used. Oleoresin with concentrations 10.000 ppm mixed into sterile Tween 80 (2% of the total medium) until homogeneous, then mixed oleoresin into the medium after medium temperature 50-60ºC.

Test of antifungal ability against to growth rate of fungal mycelial was carried out using Giant Colony method [12]. Fungal growth parameters were measured particularly of colony diameter every day. One needle point of suspension spore concentration was inoculated of 105cfu / mL into test medium as much as 3 points each petri dish. For each treatment, it is made two replications. Incubation was carried out at a temperature of 30ºC. Each day fungal colony diameter was measured till 7 days.

***Data analysis***

Data were analyzed using a completely randomized design then followed by one-way ANOVA and Duncan's test to determine differences between treatments with a significant level ($p < 5\%$) Data analysis was performed using SPSS 15.0 for Windows.

Antifungal test results were interpreted in a graph diameter growth form vs. observation day. Percentage inhibition of fungal colonies was calculated on day 7, which is expressed with antifungal index (%) and was formulated as follows [25]:

$$\text{Antifungal index } \mathbf{(\%)} = (1\text{-}Dt / Dc) \times 100$$

Where Dt was diameter of the colony in treatment t and Dc was diameter of the colony on control.

## RESULTS AND DISCUSSION

### *Nutmeg's Oleoresin*

Nutmeg was used in this study had an average the fat and water content 33.4% and 10.6% (db), respectively. The result of the nutmeg seed extraction using extraction stages method, where the result obtained from the distillation consist of essential oils of nutmeg as much (6.47 ± 0.56)%, resin (7.14 ± 0.72)% of the weight of nutmeg and nutmeg butter as much as (16.05 ± 0.26)% of the nutmeg seed weight. Total oleoresin were obtained as mixture of essential oils and resin, than oleoresin total is further used in this study and called oleoresin. Nutmeg's oleoresin extraction result are presented in Table 1.

The essential oils were obtained yellowish (red: 0; yellow: 0.2; Blue: 0); nutmeg's butter produced yellowish-white, oleoresin was obtained blackish brown (red: 1.2, yellow: 3.8; blue: 1.2), and the total oleoresin light brown color (red: 0.7; yellow: 3.4; Blue: 0.8). Color measurements performed using a livobond Tintometer type F.

In a study conducted by Widardo [24] showed that the nutmeg seed extraction with organic solvent extraction method will gradually increase the yield of oleoresin that obtained between 11.52% - 16.51%, depending on the organic solvent used. Based on research Widardo [25] nutmeg's oleoresin which obtained from this study was similarly.

Analysis result using GC-MS showed that nutmeg's oleoresin achieve in this study contain 24 components, and the most dominant component was sabinene (41.20%). While, the component of Essential consist of 18 kinds and the most dominant was sabinene (53.16%). The result of the GC analysis of fatty acid composition of nutmeg butter constituent consists of 6 fatty acids (lauric acid, acid myristat, falmitat acid, stearic acid, oleic acid and linoleic acid) and the dominant was the myristat acid about 84.76%.

### *Heating Treatment of Nutmeg's Oleoresin*

The temperature of heating treatment of nutmeg's oleoresin was used similar with that usually used in food processing, where if the temperature increase, so the nutmeg's oleoresin weight

will reduced, due to the evaporation of essential oils greater at higher temperatures. The result of nutmeg's oleoresin heating are presented in Table 2.

The result of GC-MS analysis of nutmeg's oleoresin which no heating and heating treatment at temperature 100ºC, 120ºC and 180ºC for 30 minutes was presented in Teble 3. This Table 3 shows that the heating of nutmeg's oleoresin will appear different components and there are same components will missing due to heating. In Table 3 display the different components as effect of oleoresin heating temperatures, but the most dominant component in the no heating and heating oleoresin at temperature 100ºC, 120ºC and 180ºC was sabinene. This was probably due to sabinene which obtained from the distillation of essential oils is quite high, so the sabinene content in the total oleoresin (combination resin + essential oils) also always high. Sabinene components including terpenes groups, where these terpenes have the ability as an antioxidant and antimicrobial [22, 19].

The heating of nutmeg's oleoresin influence to chemical composition of nutmeg's oleoresin, because the effect of heating at 100ºC, 120ºC, and 180ºC will appear several new components, such as endo Bornyl acetate, α-and α-terpenil cubebene acetate and some parts are missing or undetectable, such as trans-caryophyllene, γ-cadinene, cis-sabinene hydrate, β-ocimene and α-humulene. The loss component of trans-caryophyllene, γ-cadinene, cis-sabinene hydrate, β-ocimene and α-humulene asumed due to these components have reached the boiling point, or because the existence of several components in the GC-MS analysis can not detected. While the appearance of components Bornyl acetate, α-and α-terpenil cubebene acetate still to be required in further researches.

***Antifungal Potential Test at The Heating Treatment of Nutmeg's Oleoresin***

Antifungal potency test on nutmeg oleoresin treated at 100ºC, 120ºC and 180ºC heating temperature aims to determine the ability of nutmeg's oleoresin in inhibiting the growth of fungi in food processing temperatures.

Based on the tests on various concentrations of the antifungal potential of nutmeg oleoresin was obtained that at a concentration of 10,000 ppm showed a considerable growth inhibition and clearly visible application in five types of fungi tested. In addition, the concentration of 10,000 ppm also showed inhibition of germination and sporulation. Therefore, to test potential antifungal of the heating of nutmeg's oleoresin, the 10,000 ppm concentration was applied.

The ability of inhibition was indicated by comparing the diameters observed between spore colonies where grown on medium added the heating nutmeg's oleoresin at 100ºC, 120ºC and 180ºC temperature with no heating oleoresin has the same result for the inhibition until the last day of observation (7 days). The ability of oleoresin to inhibit the growth of fungal colonies declared

with antifungal index value, which is the ratio of the size of the diameter of fungal colonies grown on media test and control media (0 ppm oleoresin).

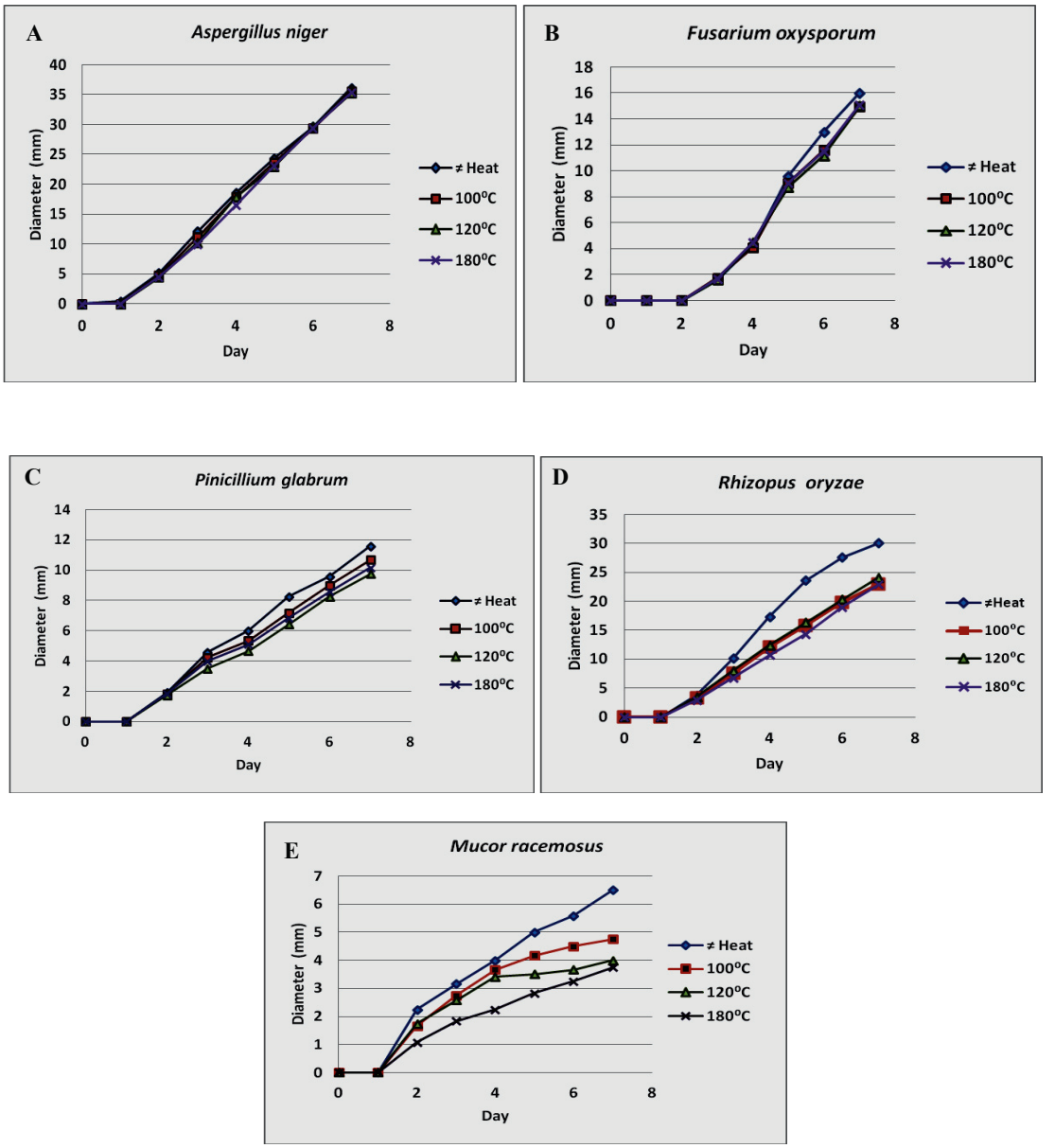


Figure 1: The Growth of fungi on various treatment temperatures nutmeg oleoresin

A) Aspergillus niger; B) Fusarium oxysporum C) Pinicillium glabrum; D) Rhizopus oryzae; and E) Mucor racemosus.

Figure 1 shows that the growth of the fungal colony diameter was higher in the fifth fungi that present in media with heating and no heating oleoresin added. It is suspected that the components of the trans-caryophyllene and α-humulene are the components that actually stimulate the growth of five fungus, as both these components are absent in three treatments on heated

oleoresin. Antifungal components on the fifth fungi tested are different that depend on fungi tested, it can be seen at the index value for the fifth largest antifungals which tested and found in media with different nutmeg's oleoresin treatment (Table 4). This proves that the changing of treatment heated oleoresin component (Table 3) causes the changing in antifungal properties.Suspected antifungal components on heating nutmeg oleoresin were α-pinene, sabinene, β-pinene, β-myrcene, α-terpinene, limonene, γ-terpinene, α-Terpinolene, and β-ocimene, these probable to play a role in inhibiting the growth of the diameter of colonies of Aspergillus niger and Pinicillium glabrum. In addition, on Fusarium oxysporum media, beside the antifungal components above, there were also three components (endo Bornyl acetate, α-and α-terpenil cubebene acetate) which suspected to play a role as well as antifungal. In Rhizopus oryzae and Mucor racemosus media, also can be found the components, such as α-pinene, sabinene, β-pinene, β-myrcene, α-terpinene, limonene, γ-terpinene, and α-Terpinolene, allegedly emergence of α-cubebene which contribute as an antifungal for Rhizopus oryzae and Mucor racemosus. However, this only assumption that require further study to determine the ability of antifungal on each component contained in the nutmeg's oleoresin for a certainty.

Based on the observations of fungal growth, it can be concluded that the nutmeg's oleoresin can inhibit fungal growth by inhibiting the mycelia growth. These result are consistent with the stated by Robinson [17] that the presence of other substances are added to the colony, such as fungicides or synthetic inhibitors of protein and cell wall can reduce the rate of extension of hyphae. Cowan [3] suggested that the cause of the inhibition of spore germination by spices and derivatives (essential oil or oleoresin) is the active components the spice that damage the membrane and cytoplasmic contents and inactivate intracellular and extracellular enzymes.

Table 1: Result of nutmeg's oleoresin extraction

| Parameters | Value |
|---|---|
| Yield (%) | 13.61 ± 0.24 |
| Specific Gravity at 25ºC | 0.885 |
| Refractive index at 25ºC | 1.489 |
| Color | Light brown (red: 0.7, yellow: 3.4, blue: 0.8) |

Table 2: The effect of heating treatment nutmeg's oleoresin

| Heating temperature (ºC) | Difference in average weight (gram) |
|---|---|
| 100 | 0 [a] |
| 120 | 0.017 [a] |
| 180 | 0.097 [b] |

Statement: The figures are followed by the same letters means are not significantly different (p <5%)

Table 3: Components of Nutmeg's oleoresin

| No | components | R.time (menit) | Peak area (%) | | | |
|---|---|---|---|---|---|---|
| | | | A | B | C | D |
| 1 | α-Thujene | 4.547 | 1.17 | 1.33 | 1.54 | 7.63 |
| 2 | α-Pinene | 4.703 | 11.36 | 11.90 | 12.34 | 12.64 |
| 3 | Camphene | 5.016 | 0.21 | 0.24 | 0.22 | 0.25 |
| 4 | Sabinene | 5.506 | 41.21 | 41.92 | 41.82 | 18.11 |
| 5 | β-Pinene, | 5.611 | 9.98 | 10.64 | 11.24 | 10.64 |
| 6 | β-Myrcene | 5.820 | 2.50 | 2.26 | 2.62 | 2.43 |
| 7 | Phellandrene | 6.243 | 0.60 | 0.45 | 0.56 | 1.09 |
| 8 | δ-Carene | 6.383 | 0.55 | 0.52 | 0.59 | 0.59 |
| 9 | α-Terpinene | 6.559 | 0.99 | 0.96 | 1.11 | 6.95 |
| 10 | Limonene | 6.904 | 4.94 | 5.23 | 5.24 | 6.73 |
| 11 | γ-Terpinene | 7.789 | 1.54 | 1.57 | 1.77 | 10.65 |
| 12 | cis-Sabinenehydrat | 8.145 | 0.56 | 0.57 | 0.58 | nd |
| 13 | α-Terpinolene | 8.749 | 0.57 | 0.58 | 0.51 | 2.64 |
| 14 | β-Ocimene | 9.198 | 0.66 | 0.64 | 0.53 | nd |
| 15 | 3-Cyclohexen-1-ol | 11.954 | 3.42 | 3.42 | 3.35 | 3.66 |
| 16 | Endo Bornyl acetate | 15.700 | nd | 0.11 | nd | nd |
| 17 | α-Cubebene | 17.858 | nd | 0.06 | nd | 0.07 |
| 18 | α-Terpenil acatat | 18.012 | nd | 0.10 | nd | nd |
| 19 | α-Copaene | 18.835 | 0.32 | 0.32 | 0.31 | 0.32 |
| 20 | trans-methyl isoeugenol | 20.189 | 2.99 | 5.93 | 5.34 | 5.37 |
| 21 | trans-caryophyllene | 20.334 | 4.17 | nd | nd | nd |
| 22 | trans-α-bergamotene | 20.816 | 0.40 | 0.29 | 0.29 | 0.26 |
| 23 | α-Humulene | 21.497 | 0.45 | nd | nd | nd |
| 24 | Germacrene | 22.419 | 0.91 | 0.18 | 0.16 | 0.11 |
| 25 | γ-Cadinene | 23.742 | 0.17 | 0.09 | nd | nd |
| 26 | Myristicin | 24.166 | 5.46 | 5.23 | 5.04 | 5.06 |
| 27 | trans-isoelimicin | 25.017 | 4.90 | 5.45 | 4.86 | 4.80 |
| | Total components | | 24 | 25 | 21 | 20 |

nd = not detected
A = Nutmeg's Oleoresin without heating
B = Nutmeg's Oleoresin heating at 100ºC
C = Nutmeg's Oleoresin heating at 120ºC
D = Nutmeg's Oleoresin heating at 180ºC

Table 4: Index antifungal at various temperatures of nutmeg's oleoresin treatment after 7 days

| Treatment Temperature (ºC) | Index of Antifungal (%) | | | | |
|---|---|---|---|---|---|
| | *Aspergillus niger* | *Fusarium 0xysporum* | *Pinicillium glabrum* | *Rhizopus oryzae* | *Mucor racemosus* |
| without heating | 45.1[a] | 67.2[a] | 52.4[a] | 59.9[a] | 91.3[a] |
| 100 | 45.8[a] | 69.5[b] | 56.2[ab] | 69.3[c] | 93.7[b] |
| 120 | 46.3[a] | 69.3[b] | 59.9[b] | 67.9[b] | 94.7[b] |
| 180 | 46.2[a] | 69.1[ab] | 58.2[b] | 69.4[c] | 95.0[b] |

Statement: The figures are followed by letters same means in one colum are not significantly different (p <5%)

Antifungal properties of nutmeg's oleoresin can be improved by adding other spices oleoresin, because of nutmeg's oleoresin the use in food usually with other spices as seasonings for example. Rasooli [18] said antimicrobial activity would be greater with the synergy between two or more active components contained in spices and derivatives such as essential oils and

oleoresins. Characteristic odor and flavor of the concentration limit use oleoresin components of essential oils and oleoresins in inhibiting microbial growth [10]. Therefore, this proves that the heating nutmeg's oleoresin in food processing temperature (temperature 100ºC, 120ºC and 180ºC) is no reducing the ability of the antifungal.

## CONCLUSION

Based on this study, the yield of nutmeg's oleoresin as much as 13.61% ± 0.24 and consist of 24 components. The heating nutmeg's oleoresin with a closed system at temperatures 100ºC not reduce the amount of oleoresin. However, the heating temperature 120ºC and 180 ºC reduce the amount of oleoresin 0.34%, and 1.94%, respectively. Furthermore, the heating temperature nutmeg's oleoresin at 100ºC, 120ºC and 180 ºC identified 25, 21 and 20 components, respectively. The most dominant component for all oleoresins either heated or no heated was sabinene. Nutmeg's oleoresin able to inhibit the fungus Aspergillus niger, Fusarium oxysporum and Pinicillium glabrum at all concentrations, and is able to inhibit the fungus Rhizopus oryzae and Mucor racemosus started at a concentration of 10,000 ppm. Nutmeg oleoresin heating at a temperature that is the temperature of the food processing 100ºC, 120ºC and 180ºC no reducing antifungal effects or growth inhibitor effects of fungal colony diameter.

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