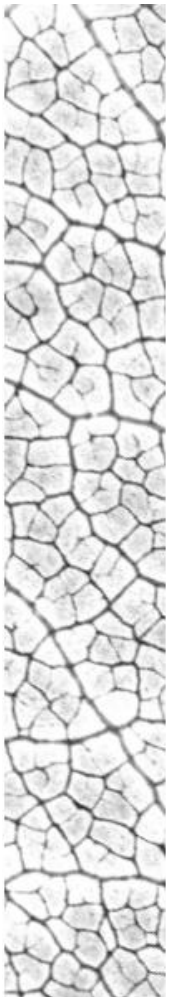

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Antioxidant activity of nutmeg fruit flesh-derived essential oil obtained through multiple drying methods

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Abstract
The purpose of this research is to study the influence of drying methods on the antioxidant activity and peroxide value of nutmeg fruit flesh-derived essential oil. This research employs a Completely Randomized Design (RAL) with 3 repetitions and nutmeg fruit flesh drying treatment (fresh/ without drying, shade-drying, sun-drying). The parameters observed include antioxidant activity test and peroxide value of nutmeg fruit flesh-derived essential oil. The research results show that nutmeg fruit flesh-derived oil contains 32 components, with major components consisting of α-pinene (18.0%), myristicin (14.1%), α-terpineol (9.4%), β-pinene (8.9%), limonene (8.5%), terpinene-4-ol (8.4%), δ-terpinene (5.9%), α-terpinolene (5.2%), and α-terpinene (4.3%). The highest antioxidant activity of nutmeg fruit flesh-derived oil is found in the shade-drying treatment, which may serve as a reducing agent in oxidation process and has good activity as an anti-free radical of DPPH. The peroxide value is lower than that of α-tocopherol, showing that nutmeg fruit flesh-derived oil has the capability to inhibit oxidation process better than α-tocopherol.

Keywords: nutmeg fruit flesh-derived oil, antioxidant activity, peroxide value

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INTRODUCTION

The Reactive Oxygen Species-ROS, like superoxide anion, hydroxyl radical, hydrogen peroxide, peroxyl radical, singlet oxygen and peroxynitrite, are known as the cause of oxidative stress triggering the development of chronic diseases like cancer, liver disease, cerebrovascular disease (Huang et al. 2005). In prevention, body has an antioxidant mechanism which is capable of removing such free (Lee et al. 2008), but in case of excessive amount of free radicals inside body, either natural or synthetic antioxidant from outside body is needed.

Antioxidant is a compound which may prevent oxidative stress caused by ROS/RNS (Oke et al. 2009). Antioxidant may neutralize any active products of metabolism like free radicals which my harm body (Safaei-Ghomi et al. 2009). Oxidation process, occurring with the free radical reaction, causes rancidity of food rich in unsaturated fatty acids (Cao et al. 2009); (Sarikurkcu et al. 2009). Synthetic antioxidant utilization is restricted since it is toxic (Gazzani et al. 1998), thus the attention is paid to natural antioxidants derived from plants for application in food industry to prevent deterioration of food quality (Singh et al. 2008), and to prevent lipid oxidation (Du and Li 2008).

A large number of spices and aromatic herbs contain chemical compounds with antioxidant property (Lindberg and Bertelsen 1995). This property is associated with various active phytochemicals including vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans, simple phenols and phenolic acids, etc. (Liu and Ng 2000). These herbs' antimicrobial and antioxidant properties make them used as preservative. One of the herbs of which potentials are commonly known and revealed is nutmeg (*Myristica fragrans* Houtt).

Maluku has abundant potential of nutmeg as its prime commodity, but only its seed and aril are traded, while its flesh is disposed of or left stacking under the trees. The flesh, on the other hand, contains active phytochemical with the main components of monoterpene hydrocarbons (61-88%), monoterpene acids (5-15%) and aromatic ethers (2-18%) (Nurdjannah 2007). One effort to utilize nutmeg fruit flesh, so that it will not be wasted, is to produce essential oil from nutmeg fruit flesh tissue through a distillation process.

The quality of essential oil is determined with some processing technologies, such as preliminary treatment (material drying). Some researches show that drying method significantly influences the yield and quality of the essential oil of aromatic plants. For example, the essential oil yield of Roman chamomile which is shade-dried is higher (1.9% w/w) than that which is sun-dried

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Fresh Shade-drying Sun-drying

Fig. 1. Nutmeg Fruit Flesh Slices with Some Drying Methods

(0.4% w/w) and oven-dried at 40˚C (0.9% w/w) (Omidbaigi et al. 2004). The carvacrol yield and content in1 *Satureja hortensis* oil which is oven-dried is higher (respectively 1.06 g and 48.1%), than that which is shade-dried (respectively 0.93 g and 46.0%) and sun-dried (respectively 0.87 g and 46.8%) (Sefidkon et al. 2006) The pulegone and menthone contents in *Mentha longifolia* oil which is sun-dried are respectively 20.2% and 38.3%; shade-dried are 18.8% and 47.6%; and oven-dried at 40˚C are quite low to none (Asekun et al. 2006).

The purpose of this research is to study the influence of drying methods on the antioxidant activity and peroxide value of nutmeg fruit flesh-derived essential oil.

RESEARCH METHOD

Research Materials and Instruments

The materials used in this research are sliced fresh nutmeg fruit flesh, clean water, aquadest, DL-α-tocopherol, ethanol 90%, diethyl ether, pure anhydrous sucrose solution, chloroform, silver nitrate solution 0.1 N, sodium chloride solution, sulfuric acid solution, alcohol, phenolphthalein, sodium thiosulfate solution, potassium iodate solution, hydrochloric acid solution, methanol and DPPH solution.

The instruments employed in this research are nutmeg fruit flesh chopping instrument, basket, boiler, thermometer, condenser, gas stove, distillate containing bottle, aerator for pumping air into cooling pipe, GCMS–QP 2010S Shimadzu, oven 105°C, analytical balance, water bath, measuring cup, pipette, Erlenmeyer flask.

Research Implementation

The fresh nutmeg fruit flesh is sliced and dried using the three methods of *fresh*, *shade-drying* and *sun-drying*. The shade-drying and sun-drying methods are performed in the drying basket. The nutmeg fruit flesh slices are dried for one day and flipped over every 2 hours. The dried nutmeg fruit flesh slices are put into distillation boiler and set in such a way so that they will be spread evenly and not too dense. The water-steam distillation is performed at 95˚C for 6 hours. The nutmeg fruit flesh-derived oil produced is collected in clean containing bottles. Afterwards, oil is separated from the water using separatory funnel. A test is then conducted to determine the components in nutmeg fruit flesh-derived oil using GCMS, antioxidant activity (DPPH Method), and peroxide value (Iodometry).

Research Design

This research employs Completely Randomized Design (RAL) with 3 repetitions and the following nutmeg fruit flesh drying methods:

A_1 = *fresh*

A_2 = *shade-drying* for 1 day

A_3 = *sun-drying* for 1 day

In case of significant or quite significant difference, the test continues to BNJ test at level of 5 percent.

RESULTS AND DISCUSSION

Nutmeg Fruit Flesh-Derived Oil

Nutmeg fruit flesh-derived oil is essential oil obtained from nutmeg fruit flesh distillation. Before distillation, the nutmeg fruit flesh is sliced and dried. The purpose of slicing is to open the glands as many as possible, while the purpose of drying is to evaporate some water out of the material. The nutmeg fruit flesh slices which are dried with some drying methods are presented in **Fig. 1**.

The observation of nutmeg fruit flesh slices dried with different drying methods shows that there is a change in physical appearance. The nutmeg fruit flesh slices dried under the sun is drier and more wrinkled. The nutmeg fruit flesh slices are then distilled to produce essential oil (**Fig. 2**). The nutmeg fruit flesh-derived oils from all of the treatments have typical smell of nutmeg oil, thus it generally meets the requirements for oil quality based on SNI 06-2388-2006. Visual observation of the oils shows that all of the nutmeg fruit flesh-derived oils from all treatments are colorless to pale yellow.

The essential oil produced in the distillation is incomplete from the fresh material, thus the oil produced is low, since the steam penetration into the material is obstructed and used more to evaporate the water. On the contrary, the drying treatments make stream penetration into the material easier, thus the hydrodiffusion process gets faster and the oil comes out more easily. The previous researches explain that a distillation will not be complete when the material

Fig. 2. Essential Oil Distillation Process from Nutmeg Fruit Flesh

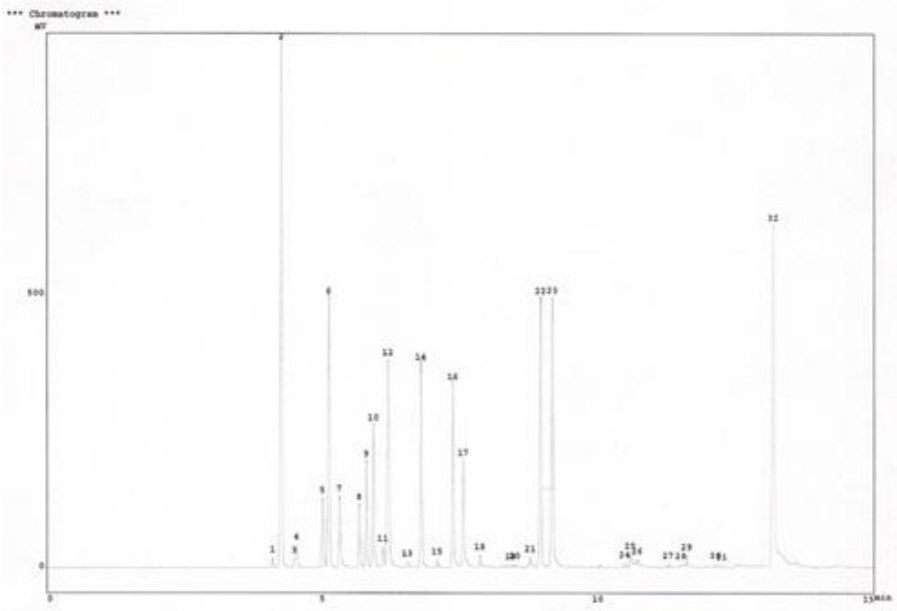

Fig. 3. GS-MS of Nutmeg Fruit Flesh-Derived Oil

processed contains much water, since the oil cannot be extracted completely, or it can only be extracted with longer distillation duration. On the contrary, dried slices will produce higher oil content (Guenther (1987) in Sipahelut and Telussa, (2011).

The chemical components of nutmeg fruit flesh-derived essential oil are identified using the Gas Chromatography-Mass Spectrometry (GC-MS) method. The principle of GC-MS is to separate components in the mixture with gas chromatography and mass spectrum may be made from each component with higher level of precision. The outcome of separation with gas chromatography is produced by chromatogram, while the outcome of Mass Spectrometry examination of each compound is referred to as spectrum (Nurhaen Dessy et al. 2016).

The identification of active compound content performed using the GC-MS method also shows that nutmeg fruit flesh-derived oil contains 32 components (**Fig. 3**), with the major components are: α-pinene (18.0%), myristicin (14.1%), α-terpineol (9.4%), β-pinene (8.9%), limonene (8.5%), terpinene-4-ol (8.4%), δ-terpinene (5.9%), α-terpinolene (5.2%) and α-terpinene (4.3%).

Table 1. Antioxidant Activity of Nutmeg Fruit Flesh-Derived Oil Obtained with Some Drying Methods

Treatment	Antioxidant activity of nutmeg fruit flesh-derived oil (%)
Fresh	24.18 a
Shade-drying	72.82 c
Sun-drying	45.66 b

The advantage of nutmeg fruit flesh-derived oil resulted in the research is that this essential oil contains oxygenated compounds more (particularly α-terpineol, terpinene-4-ol) than nutmeg seed and aril-derived oil. Oxygenated compounds are the main cause of fragrance in essential oil, and on the contrary, terpene and sesquiterpene insignificantly influence the oil's fragrance and flavor value. These compounds enhance the solubility of oil in diluted alcohol, more resistant, more stable against oxidation and resinification processes, and have stronger smell.

Antioxidant Activity of Nutmeg Fruit Flesh-Derived Oil

An antioxidant may be defined as various types of substance which are able to compete with oxidized substrate at relatively low concentration level and to inhibit or prevent substrate oxidation process (Warsito et al. 2017) The antioxidant activity test on nutmeg fruit flesh-derived essential oil is conducted with DPPH method, which is a quantitative test method to examine to what extent the activity of nutmeg fruit flesh-derived essential oil as antioxidant is. DPPH test method is a conventional method which has long been used to determine the activity of antioxidant compounds (Utomo et al. 2008) in Bahrul et al. (2014)

The data of antioxidant activity measurement results with free radical scavenging method by DPPH in **Table 1** shows that nutmeg fruit flesh-derived oil contains antioxidant activity.

Table 1 shows that the highest antioxidant activity is produced by nutmeg fruit flesh slices with shade-drying treatment (72.82%), while the lowest antioxidant activity is produced with fresh nutmeg fruit flesh slices treatment (24.18%). The inhibition activity of free radical of DPPH nutmeg fruit flesh-derived oil is determined by various antioxidant compounds contained in the nutmeg fruit flesh. Nutmeg fruit flesh slices shade-drying produces high antioxidant activity of nutmeg fruit flesh-derived oil since the water content in the material is not too high (77.56%) and there is no heat which may evaporate a large number of essential oil and oxidation, polymerization and resinification processes do not occur, thus the chemical components of essential oil is more.

With the sun-drying method, the essential oil is partially lost since the heat causes rapid diffusion process, in which the escaping water steam also brings essential oil onto the material surface and evaporates with the water steam, and the chemical components are eventually declining. In addition, sun-drying makes the membrane of nutmeg fruit flesh slices hard. According to Guenther (1987) in Sipahelut and Telussa (2011), if the amount of water in material declines or runs out, the material becomes dry and hydrodiffusion process cannot occur.

Table 2. Peroxide Value of Nutmeg Fruit Flesh-Derived Oil Obtained from Some Drying Methods

Treatment	Peroxide Value of nutmeg fruit flesh-derived oil (peroxide/1 kg)
Fresh	8.48
Shade-drying	8.58
Sun-drying	8.83

The results of this research also show that the fresh nutmeg fruit flesh slices have low antioxidant activity, since this fresh material has high water content (88%), which leads to incomplete release of essential oil during distillation. The heat derived from steam is used more to evaporate the water instead of increasing body temperature, thus the material's temperature is relatively low, causing only low release of oil.

The nutmeg fruit flesh-derived oil produced from shade-drying has higher antioxidant activity than other natural antioxidant like β-carotene (43.25%), but this value is nearly equal to the antioxidant activity of vitamin C (75.62%) and α-tocopherol (76.41%).

Peroxide Value of Nutmeg Fruit Flesh-Derived Oil

The peroxide value of a plant extract shows the extract's capability to inhibit the rate of fat oxidation. An extract which is able to inhibit oxidation rate indicated with the peroxide value of an extract may be utilized as an antioxidant. The peroxide value of nutmeg fruit flesh-derived oil produced in the research is presented in **Table 2**.

The data in **Table 2** show that the highest peroxide value is produced with sun-dried nutmeg fruit flesh slices treatment (8.83 peroxide/1 kg), and the lowest peroxide value is produced with fresh nutmeg fruit flesh slices treatment (8.48 peroxide/1 kg). Meanwhile, shade-dried nutmeg fruit flesh slices have the capability to inhibit fat oxidation rate better than fresh and sun-dried nutmeg fruit flesh slices, since with the shade-drying method, the evaporation, oxidation, polymerization and resinification processes do not occur or occur only slightly compared with sun-drying process. This result conforms to the research conducted by Sipahelut and Telussa (2011), which explains that fresh nutmeg fruit flesh slices produce less chemical components since the distillation process evaporates water more than chemical components.

Furthermore, α-tocopherol is used as a comparator in determining peroxide value, considering that α-tocopherol has been utilized to inhibit fat oxidation rate in food, and is a natural antioxidant. The peroxide value (POV) of nutmeg fruit flesh-derived oil produced is lower than the POV of α-tocopherol (111.31%).

CONCLUSION

Based on the research, we may conclude that:

1. Nutmeg fruit flesh-derived oil contains 32 components, with major components consisting of α-pinene (18.0%), myristicin (14.1%), α-terpineol (9.4%), β-pinene (8.9%), limonene (8.5%), terpinene-4-ol (8.4%), δ-terpinene (5.9%), α-terpinolene (5.2%), α-terpinene (4.3%).

2. The highest antioxidant activity of nutmeg fruit flesh-derived oil is found in the shade-drying treatment, which may serve as a reducing agent in oxidation process and has good activity as an anti-free radical of DPPH.

3. The peroxide value is lower than that of α-tocopherol, showing that nutmeg fruit flesh-derived oil has the capability to inhibit oxidation process better than α-tocopherol.

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