Effect of Hydration and Oxidation Reactions of The Chemical Composition of Kaffir lime (Cytrus hystric DC.) Oil

Warsito WARSITO1*, Edi Priyo Utomo1, and Siti Mariyah Ulfia1

1Department of Chemistry, Faculty of Mathematics & Natural Sciences, Brawijaya University Jl. Veteran 65145 Malang, Indonesia

*Corresponding author: warsito_UB@ub.ac.id

Received 14 January 2016; Revised 29 April 2016; Accepted 29 April 2016

ABSTRACT

This research aims to determine the changes of the chemical composition in the hydration and oxidation reaction of Kaffir lime (Cytrus hystric DC.) oil. The hydration reaction was carried out using amberlyst catalyst with variation time for 1.5, 3.0 and 4.5 hours. Furthermore, the oxidation process was carried out using PCC catalyst with variation time for 1.0, 1.5, and 3.0 hours. Then, the analysis of chemical composition was performed using GC-MS. The results showed that hydration reaction process for 1.5 hours had changed monoterpene, mirene and β-ocimene turned into linalool and it changes citronellal to be linalool, citronellol and dimer of ether citronellycitronellol. Moreover, the longer hydration process tends to increase the dimer of ether citronellycitronellol products. Within that process, it has turned citronellal into isopulegol and isopulegone. In addition, the longer oxidation process importantly decreases the isopulegone product.

Keywords: Kaffir lime oil, hydration, oxidation, amberlyst, PCC

INTRODUCTION

The complexity of highly volatile oil component enables a particular component or group of components have a specific activity. According to Schmidt in the Baser and Buchbauer [1], the activities of essential oils are classified into three groups. First, aromachology that provides a psychological effect when the oil is smelt. It affects for relaxation, peaceful, fresh, sensual and passionate. Second, aromatherapy which is the oils gives therapeutic effect against physical disorders such as stomach ache and psychological disorder such as depression and stress. When the oil is absorbed through the skin or inhaled, it effect is undergone. Last, aromathology (clinical aromatherapy), which is a component of the oil capable of curing illnesses. The oil is consumed orally, injections or inserted through the vagina.

A number of essential oil components that have been known as antibacterial are carvacrol, citral, eugenol, geraniol, perillaldehyde, and thymol. In addition, some essential oils also have a role as an antioxidant, repellent, insecticides, antifungal, and other activities as anti-parasitic [2]. It was also reported some monoterpenes that is the main component of essential oils contribute to providing a biological effect. For instance, limonene and β-micrene have anticonvulsant activity in rats [3]. Citronellol which is naturally present in the two form of structure as optical isomers R-(+) and S-(−) is able to reduce anxiety [4].
Kaffir lime oil has been reported to be effective against all 20 types of Salmonella and 5 other species of enterobacteria [5]. Kaffir lime oil has properties include antimicrobial, antibacterial, antifungal, antioxidant, antiviral, antiparasitic and also cytotoxic activity against leukemia cells [6].

The essential oil distilled from the twigs of the Kaffir lime plant is composed of 20 components. The main components include sabinene, micrene, limonene, linalool, and citronellal. Distillation and fractionation indicate citronellal and linalool components are distributed in each fraction with the highest citronellal levels in fractions-7. It reached 86.50% purity. Other monoterpenes hydrocarbon components isolated such as sabinene, α-pinene, micrene, β-ocimene, γ-terpinene and limonene are distributed in fractions 1-4 with sabinene levels reached to 19.83% in the fraction-1 [7].

In an effort to increase the economic value of essential oils, a wide range of approaches have been developed, such as improving the process of insulating oil with supercritical CO2 extraction technique to suppress the degradation of essential oil components. However, recent studies started to develop a method that is done by changing specific compounds through oxidation, reduction, hydrolysis/hydration and esterification to improve the aroma of essential oils that are less desirable and even raise its medicinal properties.

The deterpenation process has been developed to eliminate the limonene component which susceptible to oxidation and turn it into a more valuable component. Fang et al. tried to eliminate the monoterpenes including limonene by combining conventional vacuum distillation and supercritical carbon dioxide [8]. Similarly, Iwai et al. described supercritical CO2 extraction technique has been succeeded in effectively eliminating limonene when processing essential oils [9]. Otherwise, Nakano et al. reported that by added limonene and alcohol without a catalyst, they managed to change optically inactive compounds become optically active [10]. Pozan and Boz applied copper-magnesium oxide catalyst in attempt to provide perfume ingredient from hydrogenated of citronellol and citronellal [11].

Different approaches were carried out by converting limonene to more valuable terpenic compounds. Some oxygenated derivatives of limonene, such as limonene oxide, carvone and carveol are essential ingredients to the pharmaceutical industry. They are applied for the fragrant herb in food, perfume, cosmetic and medicine. Recently, various techniques for converting monoterpenes into terpene oxides have been developed by utilizing of metal catalysts and organic solvents [9].

The presence of monoterpenes hydrocarbons in lime oil, such as sabinene, β-micrene, β-pinene and trans-β-ocimene and some monoterpenes oxides, such as 4-terpeniol, β-citronellol, linalool and aldehydes citronellal and esters citronellyl acetate in relatively high quantity can improve their quality and application for perfumery industry. Thus, the paper is devoted to study and examine the kaffir lime oil components by enriching the oxygen content through hydrolysis or oxidation reaction. We expected that it may improve the organoleptic properties and the character of its physicochemical and hence increase the economic value of Kaffir lime oil.

**EXPERIMENT**

**Chemicals**

Kaffir lime oil applied is distillation product from leaves and twigs of Cytrus hystrix plantation by local farmer in Tulungagung, East Java (Indonesia). Other chemicals used as bought from the manufacturer (Merck or Sigma) include amberlyst, sulphuric acid, sodium sulfate anhydrate, hydrochloric acid, n-hexane, dichloromethane, nitrogen gas, and pyridinium chlorochromate.
Hydration of Kaffir lime oil

25 g lime oil was weighed and was poured into a round-bottom flask and added with 50 mL of water. The amberlyst-acid (2% v/v) is added. Then, the mixture is refluxed at 60 °C while stirred with a magnetic stirrer. The reflux product is poured into the separating funnel and is added 20 ml of n-hexane. The solution was shaken vigorously and waited to form two layers. The aqueous layer (bottom) is separated and discarded while the organic layer (top) is kept. Extraction process with n-hexane is carried out three times. Then, the combined n-hexane layers are concentrated by flowing nitrogen gas. The concentrated products are stored until analysis.

Some similar procedure above is undertaken, but each procedure is carried out with different reaction time; 1.5 h, 3.0 h and 4.5 h, respectively.

Oxidation of Kaffir lime oil

18 g of PCC was weighed and was suspended in 18 g of silica in 60 mL of dry CH₂Cl₂. Then added 15 g lime oil (in 40 mL of dry CH₂Cl₂). The solution was stirred using a magnetic stirrer for 3 hours at room temperature. The solution was filtered with silica gel and eluted using dry CH₂Cl₂ and concentrated by N₂ gas until it reaches a constant weight. Oxidation time was varied stirring for 1 h, 1.5 h, and 3.0 h.

Analysis of chemical composition of oil from hydration and oxidation products

Hydration and oxidation products were analyzed by GC-MS as follows: each sample is taken as 1 μL and then injected into the GC-MS (Shimadzu GCMS-QP 2010) with the column type RTX-wax or RTX-5ms (column length 30 m, 0.25 mm ID). Operating conditions: He carrier gas, ionizing EI (70 eV), column oven temperature of 80-305 °C (10 °C/min, the initial restrained temperature 5 minutes, the final 30 minutes), injection temperature of 300 °C, the model split injection, pressure controlled 27.4 kPa, the total flow of 90.1 mL/min, the flow in the column of 0.60 mL / min, the linear speed of 28.5 cm /sec, a cleaning column flow 3.0 mL/min. The peaks generated in TIC were scanned to create mass spectra (MS), respectively. Furthermore, mass spectra were analyzed to estimate the structure and further created chemical composition profile.

RESULT AND DISCUSSION

Changes in the chemical composition of Kaffir lime oil on hydration process with amberlyst catalyst

The use of heterogeneous Amberlyst catalysts in the hydration process of Kaffir lime oil that is composed of a complex mixture of monoterpenes hydrocarbons and oxygenated monoterpenes may experience some reaction. Two components of the hydrocarbon monoterpenes micrene and β-oicemine after hydration reaction becomes linalool on heating for 1.5 hours while refluxing the major component Citronellal encounter, at least, three reaction pathways. The first pathway experience a reduction reaction becomes citronelol, followed by some of these products have further reaction consecutively, the dehydration reaction and hydration reaction to be linalool. The second pathway produces terpinol hydrate through the carbonyl reduction reaction (cyclization) which along with the hydration of unsaturated carbon bonds, while the third pathway through a condensation reaction to form a dimer ester citronellyl citronellol. Citronellol citronellyl dimer ester product is more and more when the hydration reaction lasted longer. Otherwise, the product of linalool, citronellol and terpinol hydrate are diminished (Table 1). A schematic above reactions is presented in Figure 2.
Table 1. Changes in the composition of Kaffir lime oil component (MJP) on the hydration process with Amberlyst catalyst

| Compound           | Percentage (%) | Hydration time |
|--------------------|----------------|---------------|
|                    | 0 h | 1.5 h | 3.0 h | 4.5 h |
| Micrene            | 0.97| -     | -     | -     |
| β-Ocimene          | 0.77| -     | -     | -     |
| Linalool           | 6.10| 12.3  | 3.75  | 0.47  |
| Citronellal        | 81.5| 31.0  | 16.0  | 10.6  |
| Citronellol        | -   | 10.1  | 3.30  | 1.31  |
| Terpineol-4        | 0.50| -     | 4.80  | 1.38  |
| Terpinol hydrate   | -   | 9.84  | 32.7  | 63.2  |
| Citronellyl citronellol | -   | 30.7  | 63.2  | 63.2  |

Figure 1. (Right) Schematic transformation reaction of Kaffir lime oil components during hydration under acid-amberlyst

Chemical composition of Kaffir lime oil oxidation product

Kaffir lime oil oxidation process using PCC / SiO₂ in the methylene dichloride solvent, selectively, result in major components of Citronellal experience reduction reaction into isopulegol. Therefore, the isopulegol has a chiral carbon atom, and then the resulting isopulegol form consists of a mixture of (±) -isopulegol, (±) -neo-isopulegol, (±) -ISO-isopulegol and (±) -neoiso-isopulegol. The oxidation process which accompanied with reflux for an hour has resulted in the majority of isopulegol come through isomerization into isopulegone (Table 2). Isopulegone isomerization process will be decreased when the reflux is carried out longer and the type of reaction that occurs much more complex. As a result, the chemical composition of Kaffir lime oil oxidation products composed of components which have a very high variation (27 compounds), despite major components of citronellal which do...
not encounter reaction in the amount of 12.25%. Citronellal scheme cyclization reaction and isomerization isopulegol are presented in Figure 2.

Table 2. Changes in the composition of Kaffir lime oil (MJP) components in the oxidation process with PCC/SiO$_2$ catalyst

| Compound                        | Percentage of product at given oxidation time (%) |
|---------------------------------|--------------------------------------------------|
| Citronellal                     | 81.5 0.00 0.00 12.2                               |
| Isopulegol (mixed ±, iso, neoisopulegol) | 0.00 21.5 0.00 0.00                           |
| Isopulegone (mixed of ±, iso and neoisopulegol) | 0.00 54.5 41.8 7.58                           |

Figure 2. (Right) The transformation reactions of citronellal become isopulegol and isopulegone in the Kaffir lime oil during oxidation reaction with the PCC/SiO$_2$

CONCLUSION

The hydration reaction of Kaffir lime oil with Amberlyst catalyst resulted in minor components (micrene and β-ocimen) afford linalool. Whilst the major components, citronellal encountered hydration reaction, dehydration, reduction, and dimerization and transformed into linalool, citronellol, terpineol hydrate and citronellyl citronellol. The longer the hydration process improve citronellyl citronellol product. Moreover, oxidation of Kaffir lime oil with PCC/SiO$_2$ lead to major components of citronellal encountered cyclization and isomerization reactions, respectively, by producing isopulegol and isopulegone. Thus, the study opens the way for further research in optimization and application their bioactivity evaluation.
ACKNOWLEDGMENT

This work was supported by DPP-SPP fund from Faculty of Mathematics & Natural Sciences, Brawijaya University. We gratefully acknowledge Program Hibah Kompetisi-Program Peningkatan Kapasitas Dosen for financial support to publication in the International Conference Indonesia Chemistry Society (ICICS).

REFERENCES

[1] Başer, K.H.C. and Buchbauer, G., Essential oils (Handbook) : Science, Technology and Applications, Second edition, 2015, Taylor and Francis Group, LLC, CRC Press, London.
[2] Srisukha, V., Tribuddharat, C., Nukoolkarn, V., Bunyapraphatsara, N. Chokephaibulkit, K., Phoomniyom, S., Chuanphung, S., Sompon, S., Science Asia, 2012, 38, 212–217
[3] Viana, G.S.D, Vale, T.G., Silva, C.M.M., Matos, F.J.D., Biol. Pharm. Bull., 2000, 23, 1314–1317.
[4] Umezu, T., Ito, H., Nagano, K., Yamakoshi, M., Oouchi, H., Sakaniwa, M., & Morita, M. Life Sci., 2002, 72 (1), 91-102.
[5] Sköld, M., Karlberg, A. T., Matura, M., & Börje, A. Food Chem. Toxicol., 2006, 44(4), 538-545.
[6] Chueahongthong, F., Ampasavate, C., Okonogi, S. Timu, S. and S. Anuchapreeda, J. Med. Plant Res., 2011, 5 (14), 3097-3105.
[7] Kasuan, N., Muhammad, Z., Yusoff, Z., Rahiman, M.H.F., Taib, M.N., Haiyee, Z.A., Malaysian J. Anal. Sci., 2013, 17, 3, 359-369.
[8] Fang, T., Goto, M., Sasaki, M., & Hirose, T. J. Agric. Food Chem., 2004, 52(16), 5162-5167.
[9] Iwai, H., Matsubara, T., Kawamoto, Y., Suetsugu, T., Takamizu, A., Tanaka, M., Hoshino, M., Quitain, A. T., and Sasaki, M., J. Food Nutr. Rex., 2014, 2 (10), 718-721.
[10] Nakano, Y, Ichiyanagi,F., Naito, M.,Yang, Y. and Fujiki, M., Chem. Commun., 2012, 48, 6636-6638.
[11] Pozan, G.S. and Boz, I., Indian J. Chem. Techn., 2006, 13, 488-492.