

Improving yield and quality characteristics of kaffir lime oil (*Citrus hystrix DC*) by solid fermentation pretreatment using tempeh yeast

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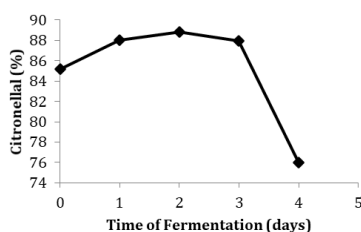
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Graphical abstract



Abstract

The industrial development of flavor and fragrance currently leads to natural ingredients. Kaffir lime is a potential ingredient developed from Indonesia, but distillation of essential oil in Indonesia generally results in low yields and quality. Fermentation as the initial treatment of distillation would improve the yield and quality of essential oil. The fermentation process is usually performed as pretreatment using cellulolytic bacteria such as *Trichoderma harzianum*, *Trichoderma viride*, or *Trichoderma reesei*, but the use of those bacteria is considered less practical for essential oil distillation process. Therefore, this research utilized tempeh yeast to increase yield and quality of essential oil. Since tempeh yeast consists of *Rhizopus* producing hyphae, it is expected that hyphae are able to penetrate the tissue, making it easier for oil to get out of the leaves. The aerobic fermentation process was carried out by continuous addition of air inside the fermenter with air compressor. Fermentation was observed within four days. The distillation was performed by using steam distillation method. The result of this study showed that leaf lime essential oil with fermentation process of day 3 produced the highest yield of 0.67%, which increased 20% compared to non-fermented leaves. The composition of leaf lime essential oil was 87.92% Citronellal, 1% β -caryophyllene, 0.3% Citronellal acetate, 0.9% Citronellol, and 1.77% linalool, which comply to local industry standard.

Keywords: essential oil, kaffir lime, fermentation

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INTRODUCTION

Consumers' preference towards natural products pave an opportunity for the essential oil industry. One of the essential oil products which possess great potential to be developed in Indonesia is kaffir lime (*Citrus hystrix DC*). The essential oil of kaffir lime leaves contains 57 types of chemical components such as 81.49% citronellal, 8.22% citronellol, 3.69% linalool, 0.31% geraniol, and other trace components (Susilo, 2016). The main component of kaffir lime is citronellal and flavor of kaffir lime leaves is derived from this component. High citronellal content in essential oil can be used as soap deodorizer, high-value perfume, liniment, toothpaste, and mouthwash (Ketaren, 1993). Its vast applications are due to citronellal's beneficial properties such as antioxidant (Lu et al., 2014) and antibacterial activities (Lopez-Romero et al., 2015).

According to a previous research, the yield of essential oil of kaffir lime leaves was around 0.47-0.88 %, depending on the method used (Sato et al., 2011; Ririn et al., 2010; Mayasari, et al. 2013). The rapid development of essential oils in the world of trade has made pretreatment necessary to improve the yield and the quality of essential oil. Through pretreatment of kaffir lime leaves such as curing, drying, slicing, and PEF, kaffir lime oil produced ranged from 0.867-0.964 % has been reported (Khasanah et al., 2015; Axnessy et al., 2014; Endarta, 2008; Imron, 2008).

Another pretreatment method of kaffir lime leaf is fermentation. According to Raharjo and Retnowati (2012), this process degrades the cell wall components tissue, and require the help of microorganisms as

a source of enzymes both natural and added microorganisms. Pretreatment using enzymes for the production of high-quality functional products from tomato processing waste has done by Zuurro et al. (2014). The enzymes have high activity in solid state fermentation (Masutti, 2012). The fermentation process is usually performed as pretreatment using cellulolytic bacteria such as *Trichoderma harzianum*, *Trichoderma viride*, or *Trichoderma reesei* but the use of those bacteria is considered less practical for essential oil distillation process. However, cellulolytic bacteria are difficult to get on the market.

To solve this, tempeh yeast was chosen as an alternative as a fermentation agent that was easily available on the market. Solid fermentation with the addition of tempeh yeast was able to increase the characteristics of physical quality and the yield of essential oil of citrus sweet peel waste (Laurita and Herawati, 2016). The result of the research showed that solid fermentation had a positive effect on the yield, with the highest yield 0.42% for a duration of 6 days of fermentation. Meanwhile, Khasanah et al. (2014) conducted preliminary treatment (fresh, solid fermentation, and liquid fermentation) with the addition of tempeh yeast to the yield and quality characteristics of cinnamon leaf essential oil. The results showed that fermentation time had a good effect on yield, with the best fermentation time on solid or liquid fermentation was on the fourth day yielding the highest yield of 0.14%. The purpose of this research is to determine the effect of fermentation with the addition of tempeh yeast to the yield and the composition of the essential oil of lime leaves (*Citrus hystrix DC*).

EXPERIMENTAL

Materials

Kaffir lime leaf was the main material in this study and it was obtained from Institute of Essential Oils, University of Brawijaya. Tempeh yeast as supporting materials was purchased from Indonesian Institute of Research (LIPI) with brand RAPRIMA.

Fermentation Process

The leaves 4 kg were fed into fermenter with the addition of 2% tempeh yeast powder as fermented agent. The fermentation process was carried out for 0, 1, 2, 3, and 4 days. Fermenter used was in the form of Styrofoam box. The aerobic fermentation process was performed under continuous addition of air inside the fermenter with air compressor. Air enters through the bottom of the fermenter with volumetric rate 0.4 L/s, then passes through the air cavity between the solid substrate and exits at the hole at the top of the fermenter. Design fermenter is shown in Fig. 1.

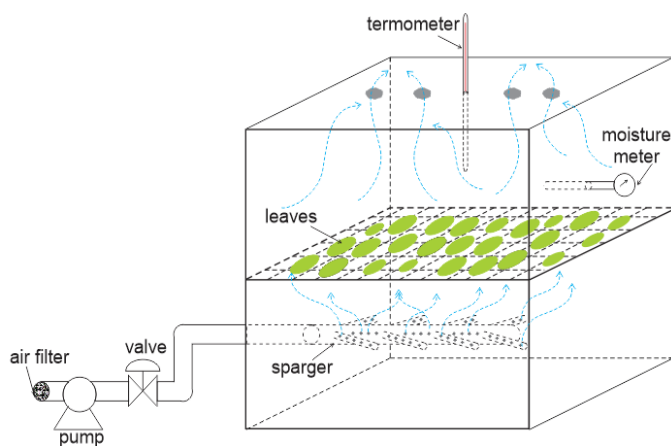


Fig. 1 Design of fermenter.

Distillation of kaffir lime leaves

The process of distillation of essential oil of kaffir lime leaves was done by using water-steam distillation method. This experiment used water-steam distillation equipment from Laboratory of Bioprocess Engineering, Brawijaya University. Both fermented and unfermented leaves were inserted into the distilled boiler. When water boils, moisture surround the leaves of kaffir lime and caused the oil components in the leaves to evaporate. Water vapor and kaffir lime leave oil were condensed until they liquified. The distillation process was carried out for 6 hours.

Separation and purification of essential oils

Distillate is a mixture of water and lime leaves oil. This distillate was moved to the separating funnel and separated during 24 hours. The separated oil was purified with anhydrous Na_2SO_4 (obtained from Laboratory of Bioprocess Engineering). The purpose was to remove all the residue-water in kaffir lime oil.

Analysis

The components of kaffir lime oil were identified by Gas Chromatography-Mass Spectrometry (GC-MS). The column type of GC-MS was RTX-5MS column. The carrier gas was helium. The column temperature was 60 °C. The temperature of GC-MS injector was 240 °C and pressure was 100 kPa. The column flow rate was 1.61 mL/min and total flow rate was 50 mL/min. The yield of process was calculated using Eq. (1).

$$\% \text{ improving yield} = \frac{\text{yield in } t_n}{\text{yield in } t_0} \times 100\% \quad (1)$$

where Yield in t_n = Yield distillation with fermentation in n days; Yield in t_0 = Yield distillation without fermentation pretreatment (0 day).

RESULTS AND DISCUSSION

Improving Yield by Fermentation of Kaffir Lime

In the aerobic fermentation process using tempeh yeast that contains *Rhizopus sp.*, lignin and hemicellulose were degraded, and the crystalline structure of the cellulose was then opened, facilitating evaporation of oil bound in vacuoles, glands internal, and external oil.

Rhizopus sp. has a vegetative cell shape in the form of yarn called hyphae. During fermentation process, hyphae penetrate the leaf tissue and release enzymes that have a function to degrade compounds contained within cell walls such as carbohydrates, fats, and proteins. In addition, *Rhizopus sp.* also produces cellulase and pectinase enzymes which are biocatalysts in cellulose and pectin degradation on plant cell walls (León and Montesano, 2013).

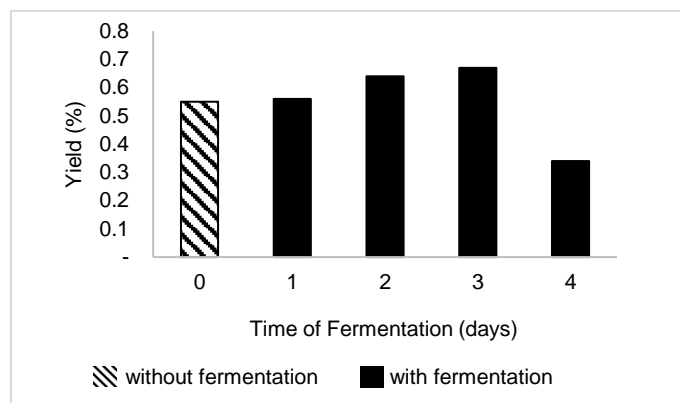


Fig. 2 Effect of fermentation time on yield of kaffir lime oil.

Fig. 2 shows the yield of kaffir lime oil for day 0 (distillation without fermentation pretreatment) and distillation with fermentation for 1, 2, 3, 4 days were 0.55%, 0.56%, 0.64%, 0.67%, and 0.34%, respectively.

Rhizopus sp. growth can be observed visually as shown in Fig. 3. The presence of white hyphae on the surface of kaffir lime leaves affected the yield of Kaffir lime oil which can be seen in Fig. 2.

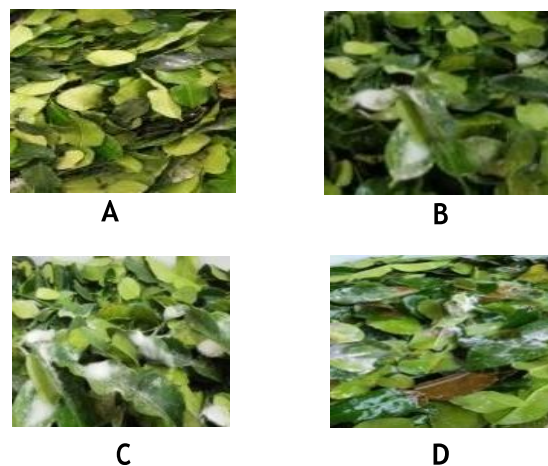


Fig. 3 Hyphae on the surface of kaffir lime leaves (a) fermentation at day 1 (b) fermentation at day 2 (c) fermentation at day 3 (d) fermentation at day 4.

The most optimum yield occurs on day 3 of fermentation. This is because degradation process of *Rhizopus sp.* occurred during fermentation process as proved by SEM images in Fig. 4. This degradation process led to the oil evaporation.

The yield product increases by 22% compared to day 0 of fermentation. On day 1 of fermentation, *Rhizopus sp.* was still adapting to its environmental conditions as well as the occurrence of enzyme synthesis. On day 2 of fermentation, it is estimated that the *Rhizopus sp.* cells wall degradation processes occurred, but on day 4 of fermentation there is a decrease in yield because of the degradation of the cell wall, causing evaporation of volatile oil bound in the internal

and external oil glands, which then was carried by air when fermentation took place before the distillation.

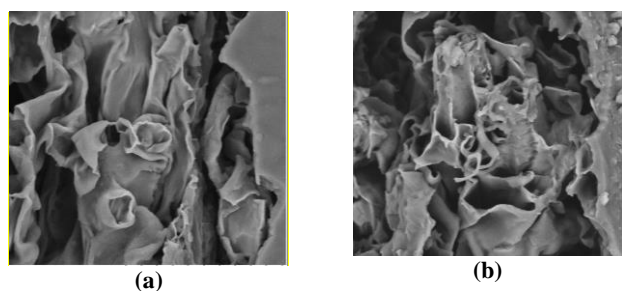


Fig. 4 SEM of Kaffir Lime Leave (a) before fermentation (b) after fermentation.

Effect of fermentation to the composition of kaffir lime essentials oils

An analysis conducted using GC-MS shows that after pretreatment fermentation 0, 1, 2, 3, and 4 days, the essential oil (OE) of the leaves of Kaffir Lime (*Citrus hystric DC*) composed of 26 components. The major constituents are shown in Table 1.

Table 1 constituents percentages identified in essential oils of kaffir lime.

Compound	RT (min)	Time of Fermentation				
		0 day (%)	1 day (%)	2 days (%)	3 days (%)	4 days (%)
Cis-Ocimene	3.15	-	-	0.07	-	-
β-Pinene	3.25	-	-	0.04	-	-
L-Linalool	3.44	1.61	-	1.55	-	-
Linalool	3.52	-	1.9	-	1.77	1.17
Citronellal	4.10	85.15	88.01	88.8	87.93	75.96
Citronellol	4.94	1.39	-	0.07	0.90	6.91
L-Citronellol	5.6	-	0.19	0.35	0.48	-
6-Octen-1-ol, 3,7-dimethyl-	5.68	-	0.47	0.78	0.19	0.76
β-Citronellol	5.79	0.13	-	-	-	-
Citronellyl Acetate	6.31	0.39	-	-	0.39	1.8
Citronellyl Propionate	6.37	0.85	-	-	-	0.81
α-Humulene	7.31	0.18	-	0.27	0.29	0.25
Isopulegol	8	-	-	0.16	-	0.15
Delta.-cadinene	8.39	0.2	-	0.26	0.18	0.23
Beta.-Bisabolene	8.55	-	0.34	-	-	0.13
Geranyl Linalool Isomer B	8.71	0.02	-	-	-	-
Caryophyllene Oxide	9.05	0.16	-	-	-	-
Trans-Caryophyllene	9.12	0.26	-	-	-	0.05
B-Caryophyllene	9.29	0.07	-	1.3	1.00	0.79
Alpha.-Trans-Sequioclogeraniol	9.49	0.28	-	-	-	-
L-B-Pinene	9.61	-	-	-	0.22	-
2-B-Pinene	9.65	-	-	-	0.41	-
Caryophylla-3,8(13)-Dien-5. Alpha.-O	10.22	-	-	-	0.20	-
Trans-Geraniol	12.14	-	-	0.02	-	0.05
Camphene	12.29	0.14	-	-	-	-
3-Octyne, 2,2,7-Trimethyl-	13.54	0.27	-	0.03	0.46	0.17
Total Identified		91.1	90.91	93.59	94.42	89.23
Others		8.9	9.09	6.41	5.58	10.77

Table 1 shows that time of fermentation can cause variation in the composition of kaffir lime oil. This is due to activity enzymes from tempeh yeast such as cellulose, pectinase, and protease which cause biotransformation process that converts one compound to another, causing isomerization and cyclization reactions (Hernandez, 2017). The major compounds among the constituents found were citronellal, citronellol, linalool, β-Caryophyllene, and Citronellyl Acetate. Based on the local flavor and fragrant industry, minimum standard for quality kaffir lime oil represented by major compound constituent, Citronellal 65 %, citronellol 1.9 %, linalool 3.5 %, β- Caryophyllene 0.2 %, and Citronellyl Acetate 1 %. The second day fermentation showed high content of citronellal but citronellol, β-Caryophyllene and citronellyl acetate were not identified. Thus, it can be concluded that day 3 of fermentation with major compound, citronellal 87 %, citronellol 0.9 %, linalool 1.77 %, β- Caryophyllene 1 %, and citronellyl acetate 0.39 % is the most optimal fermentation time because of complete major compound were clearly found in kaffir lime oil. Small amount compounds such as β-Citronellol, β-Pinene, β-Bisabolene Caryophyllene Oxide disappear because of hydrolysis, oxidation or reaction esterification (Boelens, 1997).

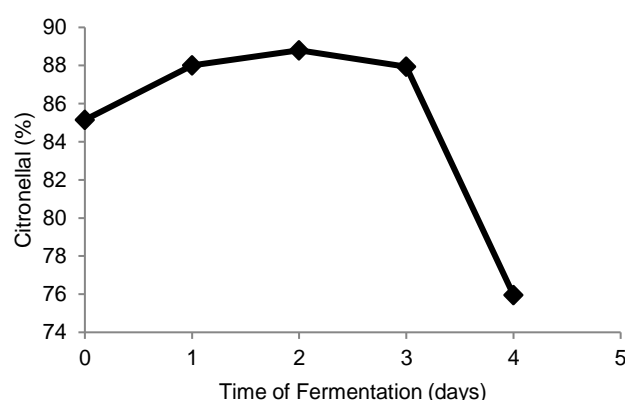


Fig. 5 Effect of fermentation on Citronellal compound in kaffir lime oil.

Citronellal is the main component of kaffir lime oil which has many uses, especially perfume and cosmetics industry (Khasanah et al., 2015) and has antioxidant and antibacterial activities (Susilo, 2016). Figure 5 shows that fermentation with tempeh yeast can increase citronellal content kaffir lime oil. This identified the existence of a biotransformation process by Rhizopus yang resulting in a cyclization reaction of monoterpenes (Citronellal) followed by secondary reactions to citronellol. The change of citronellal to citronellol is shown in the Figure 6 (Baser and Buchbauer, 2010). It also can be proven by the increasing levels of citronellol as shown in Table 1 that the amount of citronellol increased almost 6 times from day 3 to day 4.

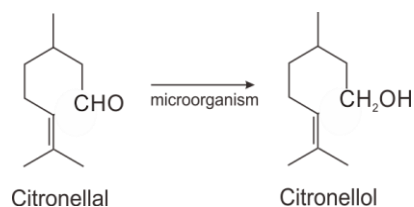


Fig. 6 Biotransformation of Citronellal to Citronellol by microorganism (Baser and Buchbauer, 2010).

CONCLUSION

Fermentation using tempeh yeast has successfully increased yield of kaffir Lime oil to 22% higher than that without fermentation. Besides, the quality of kaffir lime oil improved, which is showed by an increase in content of citronellal in the kaffir lime oil by 35% compared to local industry standard. The optimum fermentation time is on the day 3 resulting in the highest yield of 0.67%, in which the major compounds are Citronellal (87.92 %), β-Caryophyllene (1 %), Citronellol (0.90 %), Citronellyl Acetate (0.39 %).

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