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Preliminary Study: Effect Steam Explosion Pretreatment on the Extraction of Essential Oil from Kaffir Lime Leaves (Citrus Hystrix DC)

Yustina Wuri Wulandari
Ph.D. Student, Department of Food Science and Technology,
Gadjah Mada University, Yogyakarta, Indonesia
Chairil Anwar
Lecturer, Department of Chemistry, Gadjah Mada University, Yogyakarta, Indonesia
Supriyadi Supriyadi
Lecturer, Department of Food Science and Technology,
Gadjah Mada University, Yogyakarta, Indonesia

Abstract:
As a thermal-mechanical-chemical pretreatment, steam explosion allows a synergism process of heating that disintegrates lignocellulosic structural components, as well as shearing forces that lead to moisture expansion. Essential oil refers to a complex mixture of compounds that are volatile and have strong odor. This research aimed to identify the influence of steam explosion as pretreatment process on the micro histological features of Citrus hystrix DC leaf and the yield of essential oil using hydrodistillation extraction. The fallowing process and material variables were used steam at pressure of 1-4 bar, and kaffir leaves-water ratio of 33%. An application of steam explosion resulted in leaf tissue damaged and produced higher essential oil yield. The highest yield of essential oil 1.386% (w/w) was obtained at the saturated pressure of 2 bar. Therefore, this study concludes steam explosion as a potential process to apply in essential oil extraction.

Keywords*: Steam explosion, citrus hystrix DC, kaffir lime leaves, essential oil, yield*

1. Introduction

Citrus hytrix DC, or kaffir lime, is a citrus fruit with a very strong fragrance native to Southeast Asia. As a condiment, the leaves are used in many Malaysian and Thai dishes to impart a tangy flavor to curry and soup (Butryee et al., 2009). These leaves are also rich in vitamin E. Among the 62 edible and tropical ingredients analyzed for their α-tocopherol content, Citrus hystrix leaf (398.3 mg/kg of edible portion) ranked second after the Sauropus androgynus (426.8 mg/kg of edible portion) (Ching and Mohamed, 2001).

Thirty-eight constituents were identified in the essential oil of kaffir lime leaves representing 89% of the total oil (Waikedre et al., 2010). The oil was rich in monoterpenes (87%), which is composed of ß-pinene as a major component (10%) and low limonene content (4.7%). Its characteristics include a major content of terpinen-4-ol (13.0%), as well as α-terpineol (7.6%), 1,8-cineole (6.4%), and citronellol (6.0%). In addition, there are twenty-nine compounds identified in it (Loh et al., 2011). However, the major compound is ß-citronellal, which amounts to 66.85% of total oil. Previous studies explains that citronellal is also present in lime leaf oleoresin although the levels are lower when compared to the essential oil 25.66% (Khasanah et al., 2015).

Kaffir lime leaf oil possesses some important bioactivities such as antioxidant (Hutadilok et al., 2009), antileukemic (Ampasavate et al., 2010), antitussive, antihemorrhagic, antioxidative stress properties (Laohavechvinch et al., 2010), and antibacterial properties (Siripongvutikorn et al., 2005). Nowadays, the essential oil of kaffir lime is in demand as a fragrance in foods, perfumery, and cosmetic manufacturing (Phoungchang et al., 2008; Manosroi et al., 2005). Hydrodistillation processing has been used to isolate essential oils (Tum and Mainya, 2016). It is widely known as a conventional method in extracting essential oil from plants due to its many advantages, such as simple and cheap technology and, therefore, feasible for the extraction of essential oils in industrial level.

As a hydrolysis-based pretreatment, steam explosion releases the constitutive biomass' elements, which increases cellulose's solvent accessibility and enzyme (Glasser and Wright, 1998). Steam explosion has been widely applied in biomass conversion (Guo et al., 2011; Hooper and Li, 1996; Horn et al., 2011; Sipos et al., 2010; Viola et al., 2008). It is a thermal-mechanical-chemical pretreatment involving a synergism process of heating that allows the disintegration of lignocellulosic structural components and shearing forces that expand the moisture. Hemicelluloses tend to degrade by autohydrolysis during steaming. The delocalization and coagulation of lignin opens the structure of cell wall and widens its pore size. However, batch-wise steaming experiment has a specific problem that lies on the collection and the analysis of the by-product. Despite the potential application of steam explosion in extracting essential oils from plant materials, there are only a few articles in the literature focusing on this matter. In this research, the essential oil of Citrus hystrix leaf were obtained using hydrodistillation with steam explosion as the pretreatment process. Therefore, this research aimed to discover the influence of steam explosion, i.e., a pretreatment process, on the microhistological features of Citrus hystrix DC leaf and the yield of essential oil extraction.

2. Materials and Method

2.1. Plant Material

The sample of Citrus hystrix DC or kaffir lime leaves was collected from one local orchard in Klaten Regency, Central Java. The sortation of the mature leaves took place in the laboratory. The results were samples with the same size. These leaves were selected based on color and sheen, i.e., dark green and glossy. The whole leaves were used as experiment material after being washed under a running tap water. The leaves were collected during March-April 2017.

2.2. Steam Explosion Treatment

In this research, the steam explosion was conducted according to Wulandari et al., (2018), i.e., at a pressure of 1-4 bar. Three-hundred grams of kaffir leaves and 600 mL of water in 3 L reactor were heated with different pressures, namely 1, 2, 3, and 4 bar. When the desired pressure was reached, the explosion process was done by sudden release of the pressure on the pressure 1 bar. The treated leaves were analyzed their color, microscopic structure, ruptureness, and essential oil yield.

2.3. Hydrodistillation

A conventional hydrodistillation was performed using a Clavenger apparatus. The distillation of leaves (untreated and steam explosion-treated leaves) and water with a ratio of 1:5 produced essential oil in 2 hours. This oil was accumulated in colored vials, processed for dehydration with anhydrous sodium sulfate, and covered with a nitrogen cap. Afterward, the yield was weighed.

2.4. Colorimetric Parameter

The changes of colour leaves were identified with a Minolta CR-300 Chromameter (Japan) using the coloration scale of L, a, b parameters according to Takiwaki and Serup (1994). This measurement was for comparison purpose. These parameters were used in the Hunter-Scotfield's equation to produce the colour difference ΔE:

$$\Delta E = \pm\sqrt{(\Delta a)^2 \oplus (\Delta b)^2 \oplus (\Delta L)^2}$$

2.5. Structural Observation

The scanning electron micrographs (SEM) of kaffir leaves were captured with FEI Quanta 200. According to Wulandari et al., (2018), the leaves had to be coated with gold using the plasma sputtering method before the SEM evaluation. The micrographs were conducted in a field emission SEM (JOEL JSM-6510 LA, Japan). All of the observations on the structure of the fractured surface in this research were performed under the same magnification.

2.6. Statistical Analysis

All treatments were duplicated and analyses were done in triplicate. Data were analyzed using a one factor analysis of variance (ANOVA) and least significant differences (LSDs) were calculated at the 5% level to compare groups means using the Statistical Product and Service Solutions software package (SPSS, version 13.0).

3. Discussion

3.1. The Influence of Steam Explosion on the Morphological Features of Kaffir Lime Leaves

The colour parameters of steam explosion in treated leaves were compared to untreated leaves, as presented in Table 1. The observation included the overall changes of L value in the treated leaves. The (L*) value decreased according to increasing the pressure. This phenomenon was in line with the degradation of food color during cooking; thermal degradation of kaffir leaves' colour (Raksakantong et al., 2011a; Wanyo et al., 2011). Whereas, the a and b values of treated leaves tend to increase in line with pressure rise. Representing the total colour difference (ΔE), the L, a, b, values combination is considered as a colorimetric parameter widely applied to determine the characteristics of colour variation during steaming.

Samples	L*	a*	b*	ΔE*
Untreated	43.14±0.17e	-10.78±0.09a	13.75±0.12b	-
Treated at 1 bar	31.33±0.25d	-0.75±0.80b	15.72±0.79cd	31.49±0.51c
Treated at 2 bar	30.51±0.29c	-0.22±0.43b	17.11±0.79d	31.86±0.47c
Treated at 3 bar	28.69±0.03b	-0.26±0.07b	14.89±0.04bc	28.72±0.04b
Treated at 4 bar	25.78±0.18a	-0.31±0.13b	11.41±0.13a	24.69±0.24a

Table 1: The Colorimetric Values of Untreated (Freash) and Steamed Citrus Hystrix Leaves
[A]means±SD; N=3. Values in Same Row Marked by the Different Letters are Significantly Different P<0.05
**L= 0 for Black, +100 for White, A and B= No Specific Numerical Limits; A= (+) Is Red, (-) Is Green; B= (+) Is Yellow, (-) Is Blue; ΔE Denotes Total Color Difference*

The results presented in this work suggest that the changes in ΔE* can fairly indicate color deviation between the treated and untreated leaves. Those ΔE* decreased with increasing pressure. The colour of leaves turns to red and yellow when pressure is raised. The colour changes in kaffir lime leaves caused by a synergism process in the thermal-mechanical-chemical pretreatment of steam explosion allows may be due the non-enzymatic browing reaction, but also to the destruction of pigments present in the leaf. This actual color of the leaves was presented in Figure 1.

Figure 1: The Color of Steam Explosion in Treated and Untreated Kaffir Lime Leaves

3.2. Structural Change after Extraction by Steam Explosion

Different pressure during steam explosion produce distinguishable physical change in kaffir leaves. Figure 2a was a micrograph of the untreated leaf, which clearly can be compared with structures of the treated leaf (Figure 2b, 2c, 2d and 2e). Shape reduction and micropores expansion on the materials occur during steam explosion (Grous et al., 1986). The structures of untreated leaf showed glandular cells containing volatile oils. Figure 2b showed the typical structure after treated by steam explosion at the pressure 1 bar, glandular cells were empty but still intact. The figures showed that steam explosion ruptured and destructed the glandular trichome cell walls, inducing the formation of large cavities and intercellular space especially after applying the 3-bar pressure (Figure 2c). Furthermore, when pressure increased to 4 bar, no pores were observed in the micrograph leaves.

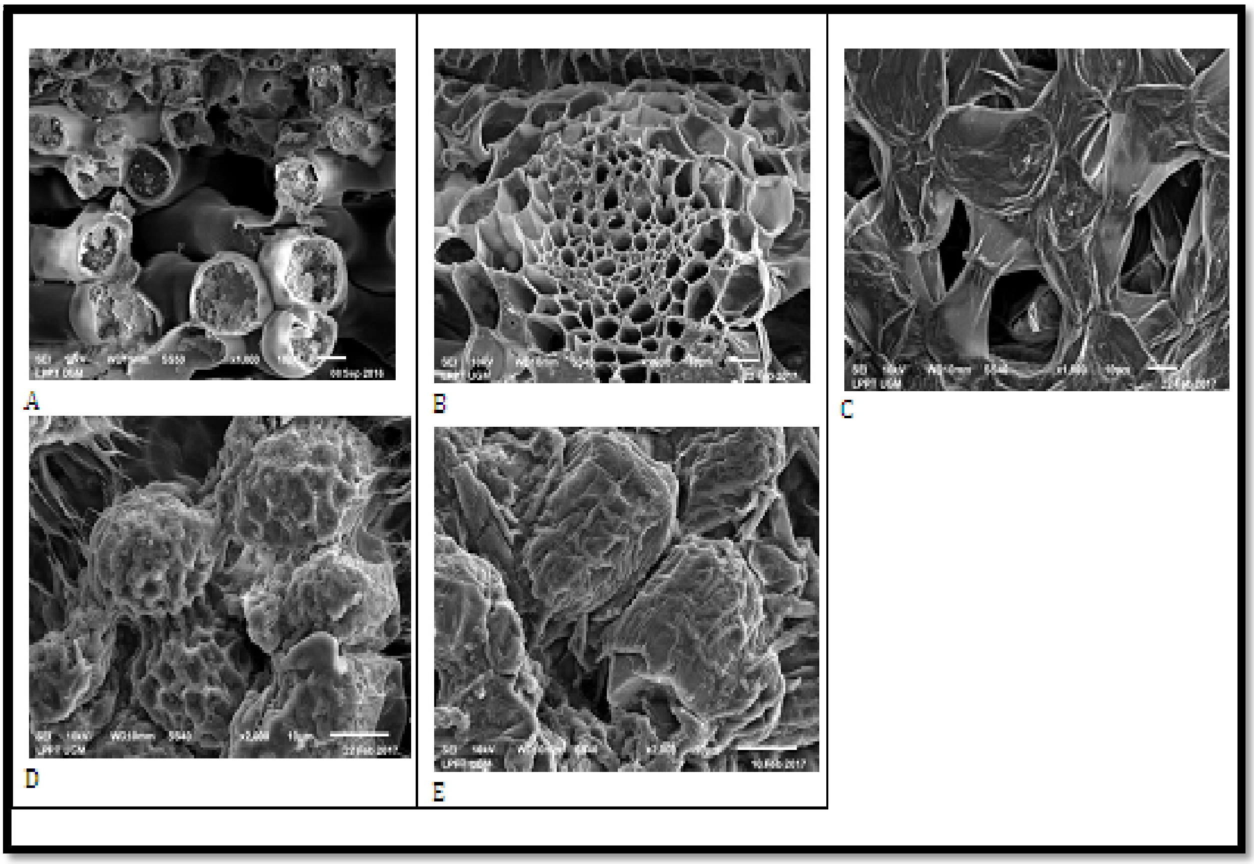

Figure 2: Scanning Electron Micrographs of Kaffir Lime Leaves: Untreated (A) and Treated With Steam Explosion at the Pressure of 1 bar (B), 2 Bar (C), 3 Bar (D), And 4 Bar (E)

3.3. Effect of Steam Explosion on the Yield of Essential Oil

The yield of kaffir lime leaves essential oils using combination process steam explosion as pretreatment and hydrodistillation process referring to Table 1. Kaffir lime leaves oil seems extracted two stages, the fist process by steam explosion, and the second was by distillation process to get an essential oil. Steam explosion aimed to disintegrate the structure of lignocellulosic matrix in leaves (Cara et al., 2006; Juan et al., 2016; Ruiza et al., 2008; Teynouri et al., 2005). The steam breaks the chemical bonds between the constitutive macromolecules of biomass. The oil in the damaged plant tissue becomes easily removed by hydrodistillation. Therefore, the oils come out of the Clevenger after 15 minutes of the starting process. As shown in Table 2, application of steam explosion as pre-treatment process prior a real extraction by hydrodistillation can increase the oil yields. However, an over pressure more than 2 bar tends to decrease the yields. Allegedly some oil has been out of the cell and carried out by the steam during explosion. This was supported by Figure 4C, where the cell treated by 2 bar pressure was more opened than cell treated by less pressure.

An appropriate steam explosion was needed to ensure the essential oil was still entrapped in the ruptured leaves but the oils comes out is still very little. This condition was very important to ensure maximum distillation yields. Steam explosion at pressure of 2 bar as pretreatment prior hydrodistillation increased the essential oil yield almost double than untreated kaffir lime leaves. The previous research is the extraction kaffir lime leaf oil using hydro distillation with aging pretreatment obtained yield 0.867±0.029% (Khasanah et al., 2015). It can also be seen that extraction of kaffir lime leaves oil using steam explosion as pretreatment can result in a higher yield 1.542 times compared to the previous research.

Pressure (bar)	Percentage yield (w/w)
Untreated	0.790±0.01a
1	1.047±0.03b
2	1.337±0.03e
3	1.210±0.02d
4	1.110±0.03e

Table 2: The Yield of Essential Oil from Citrus Hystrix Leaves
[A]means±SD; N=3. Values in Same Row Marked by the Different Letters Are Significantly Different P<0.05

4. Conclusion

Steam explosion is potentially applied in essential oil extraction. The optimum condition of steam explosion seems to cause a higher extraction efficiency than in untreated leaves due to the leaf tissue ruptured and damaged. Steam explosion at a pressure of 2 bar was recommended to keep the yield of kaffir lime leaves essential oils.

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