Lime (*Citrus aurantifolia*) Peel Effect on Peroxide Value of Cooking Oil

S Rahayu\(^1\)*, Supriyatin\(^2\), T R Fauziah\(^1\)

\(^{1}\)Biology Department, Faculty of Mathematics and Sciences, Universitas Negeri Jakarta.

\(^{2}\)Biology Education Department, Faculty of Mathematics and Sciences, Universitas Negeri Jakarta

*Corresponding author: srirahayu@unj.ac.id

**Abstract.** Cooking oil is a staple in everyday life. Economic has been the reason of its repeated use. The quality of cooking oil can be known from taste, colour, and aroma. In terms of aroma, rancidity is caused by the presence of peroxide. Peroxide is a sign of oil breakdown or damage due to oxidation, which causes a rancid odour. Lime peel is used as an antioxidant because it contains vitamin C, flavonoids, and carotenoids. Damage to cooking oil can’t be prevented but can be lowered by giving antioxidants from lime peel. This study aims to determine the effect of lime peel extract on the peroxide number in cooking oil. Antioxidant content of lime peel was measured by spectrophotometric absorbance of vitamin C, flavonoid and carotene content. Peroxide value was assessed by titration. Oil clarity was also measured by spectrophotometric to confirm peroxide value. The method used in this study was an experiment using a complete randomized design with two factorials. The first factor is the type of solvent (distilled water, ethanol, and chloroform). The second factor is the extract concentration (70, 80, and 90) μg / ml. Peroxide number measurement was analysed using ANOVA (p <0.05) and continued with the Duncan test. Antioxidant content showed that lime peel contains vitamin C, flavonoid and carotene with highest in vitamin C. Peroxide value measurement obtained that distilled water extract concentration of 90 μg / ml had the lowest peroxide number of 0.56 M Equiv O\(_2\) / Kg while the highest value was found in distilled water extract of 80 μg / ml concentration (0.96, 56 M Equiv O\(_2\) / Kg). Clarity of the oil was found best at 90μg/ml concentration (0.38). It can be concluded that lime peel extract significantly gave effects on peroxide number of cooking oil. Both concentration and extract solvent determine the peroxide number.

1. **Introduction**

Cooking oil is a staple in everyday life. Economic has been the reason of its repeated use without wise consideration on detrimental health effect due to low quality of the oil. The quality of cooking oil can be evaluated from smell, weight, color, foreign matter, appearance, cold point, peroxide value and rancidity [1]. In terms of aroma, rancidity is caused by the presence of peroxide. Peroxide is a sign of oil breakdown or damage due to oxidation, which causes a rancid odor [2,3,4]. Repeated cooking leads to corresponding rancidity and spontaneous deterioration of cooking oil [5]. Oxidation in cooking oil might be happening due to photo-oxidation and autooxidation [6,7].

The process of oxidation in cooking oil can’t be hindered but the rate might be lowered. Some ways have been studied to minimize this rapid process such as storage and low store temperature [8], the use of synthetic antioxidant [9] as well as natural antioxidant [10,11]. Antioxidant has been useful
strategies to reduce deterioration in cooking oil. Nevertheless, the use of synthetic antioxidant has been restricted due to its possible carcinogenic effect [12]. Many researches were then focused on the use of natural antioxidant from plants like grape seed, chestnut leaves, green tea [13,14]. Recently, the use of natural residual source of antioxidant has been very interesting since it will not only reduce disposal but also add value to the by-product [15,16,17].

Lime (Citrus) is famous sour and fresh fruit around the world. It has been decades known as powerful antioxidant attributed to the present of bioactive compound such as ferulic acid, hydro cinnamic acid, cyanidin glucoside, hesperidin, vitamin C, carotenoid and naringin content [18]. Among its family, citrus aurantifolia is the species with high antioxidant capacity proved by hesperidine, total phenolic, flavonoid in it [19]. Not only the fruit flesh, the peel also has beneficial effect. Instead of the fact, lime peel is usually discarded or only used as garnish. Optimal use of the lime peel hasn't been seriously investigated. This study would like to assess the use of lime peel as strategy to not only lower the rate of oxidation of cooking oil but also reduce waste and add more value on by product.

2. Experimental Details

2.1. Research Method

The research would like to study on the effect of lime peel on peroxide value of cooking oil. Complete randomized design (CRD) applied in this study with 3x4 factorial. Factors contribute were solvent type (chloroform, distilled water, ethanol) and concentration (0, 70, 80, 90 µg/ml). Each treatment has 5 repetition. Preliminary study was done to detect antioxidant property of lime peel on vitamin C, total flavonoid and carotenoid content by spectrophotometric method. Instead of peroxide value, oil clarity was also measured to assure the quality of cooking oil after treated with antioxidant given.

2.2. Antioxidant assay on lime peel

Antioxidant detection was done by spectrophotometer to detect the content of Vitamin C, total flavonoid and carotenoid in lime peel. Vitamin C determination was done by pipetting 2,3,4, and 5 ml of ascorbic acid. 4 ml of 2,6-diklorophenol indophenol was added and 0,4% oxalic acid was added until the volume reaches 10 ml. The absorbance was the measured in 516 nm [20]. Regression equation was used to measure the quantity of vit C by adding 2,6-diklorophenol indophenol into 1ml of samples to make 10ml volume. It was then shake and the absorbance was measured in 516 nm.

Total flavonoid content was measured by aluminium chloride colorimetry assay. Standard of catechin (20,40,60,80,100 mg/l) was added with 4 ml of distilled water, 0.3 ml of NaNo2. After 5 minutes 0,3 ml of 10% was added. At the 6th minute, 2 ml 1M NaOH was add and 10 ml volume was reach with distilled water. The solution was mixed well, and absorbance was measured in 510 nm [21].

Carotenoid content was evaluated by preparing series of standard carotene (3,6,9,12,15 µg/mL) and measure the absorbance on 454 nm [22]. Equation formed was then used to measure the quantity of carotene on samples.

2.3. Peroxide value measurement

Peroxide value was measured by titration. 5 g of cooking oil was added with 30 ml of 95% acetic acid, it was then shake and 0,5 ml of KI was added. The solution was shake for 1 min and 30 ml distilled water was added. The solution was titrated with 0.01N Na2S2O3 and observed to up to yellow color disappeared. 0,5 ml og 1% starch was then added titrated again until blue color disappeared.
2.4. Cooking Oil Clarity

Clarity has been used as one indicator of destruction in cooking oil. Clarity was analyzed with spectrophotometer (448 nm). The highest the absorbance, the worse quality observed from cooking oil.

2.5. Data Analysis

Data was analyzed by two-way Anova and followed by DMRT to investigate differences among groups of treatment.

3. Result and Discussion

3.1. Antioxidant property of lime peel

Antioxidant property of plant was determined by their chemical constituent. Vitamin C has the most abundance source and famous antioxidant [23]. Lime fruit was one of the most well-known source of vitamin C. Instead of vitamin C, flavonoid has been the second popular antioxidant compound. It has been reported that flavonoid compound of plants, which contain hydroxyl, are responsible for radical scavenging property of most plants [21]. Carotene, yet not as well known as Vit C and flavonoid, is one of plant pigment with antioxidant, anti-inflammation and anti-cancer properties [24]. To evaluate antioxidant power of lime peel we measure its Vit C, flavonoid and carotenoid as seen in fig 1 below:

![Antioxidant property of lime peel](image)

**Figure 1.** Antioxidant property (Vit C, Flavonoid and carotenoid content) of lime peel

Based on the result it was known that lime peel contains bioactive antioxidant compound of vitamin C, flavonoid and carotenoid. Vitamin C was the dominant antioxidant found in lime peel followed by flavonoid and carotene. Many researches have revealed the existence of the antioxidants in lime peel [10,17,18,19] with methoxylated flavones and glycoside flavones content. Carotene

Solvent used of distilled water on lime peel showed highest vit C (24.74 mg/ml) content and the lowest on chloroform (7.48 mg/ml). Flavonoid was also seen highest in distilled water extract (1.45 mg/ml) and lowest in chloroform (0.41 mg/ml). Whilst carotenoid was observed higher in chloroform but lowest in distilled water (0.45 mg/ml). Solvent used in this research was categorized as polar (distilled water) and semi-polar solvent (ethanol and chloroform). Vitamin C as one of powerful antioxidants vitamins has higher polarity compare to vitamin A and E. It has four hydroxyl group while vitamin E only has one and vitamin A has no hydroxyl group. The hydroxyl part assures the
polarity of vitamin C on polar solvent used (distilled water) [25]. Flavonoid, as vitamin C is also known to be antioxidant of water phase. It is one of the most diverse and widespread group of natural compounds and probably act as the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties [19]. Different with the two antioxidants discussed, carotenoid is a lipid phase antioxidant. The lipophilic manner of carotenoid generate synergism on active radical scavenger kinetically controlled at interfaces [26].

3.2. Peroxide Value of Used cooking oil
The best test for autoxidation (oxidative rancidity) is determination of the peroxide value (PV). PV value is useful to assess the extent to which spoilage of oil has taken place. Addition of natural antioxidants into the frying oil is the best way of enhancing oxidative stability [27]. Lime peel addition into cooking oil before frying process proven to reduce peroxide value formed as shown in fig 2 below:

![Figure 2. Peroxide value (Mequiv O₂/Kg) of used cooking oil with lime peel application](image)

The result showed that peroxide value was seen lowest on 90µg/ml lime peel concentration with distilled water solvent (0.56 Mequiv O₂/kg). Highest value was investigated on 80 µg/ml lime peel concentration with distilled water solvent (0.96 Mequiv O₂/ kg) which is closest to control (1 Mequiv O₂/ kg). Ethanol extract of lime peel has high effect on lowering peroxide with 80 and 90 µg/ml concentration showed by lower value (0.64 Mequiv O₂/kg). On the other hand, chloroform lime peel extract exhibited the same high peroxide value on 70 and 90 µg/ml concentration (0.72 Mequiv O₂/kg) compare to 80 µg/ml (0.6 Mequiv O₂/kg)

During frying, oxidation occurs more rapidly than storing and cause production of peroxide and volatile compounds such as aldehydes, ketones, carboxyclic acid and other undesirable chemicals [5,28]. Fortifying cooking oil with natural antioxidant has been extensively studied [29,30,31]. Nevertheless, study on the use of waste biomass product such as lime peel was still low. Lime peel as given before frying process has been proven gave effect on peroxide value as other natural antioxidants did [32, 33].
3.3. Cooking Oil Clarity
Cooking oil colour, as mentioned before is used as one indicators of decomposition [2,3]. Foamy and dark colour of oil cooked was due to the broken fatty acid chain release on heating [34]. Lime peel administration on cooking oil clarity was observed in fig 3 below:

![Fig 3. Clarity of cooking oil on lime peel application](image)

Clarity was achieved under distilled water lime peel extract of 90µg/ml concentration (0.38). The result confirms the peroxide analysis of treatment given where this group was chosen as the best in lowering peroxide number. It was then assumed that this treatment lime peel extract group was able to lower detrimental effect of frying process in cooking oil. The highest absorbance was shown in chloroform lime peel extract of 70µg/ml concentration. This condition occurred since lime peel antioxidant wasn’t optimally extracted with this solvent type. Further, it was unable to lower peroxide and clarity of the cooking oil.

Thermal polymerization is a process followed by heating with or without oxygen. Heat will then break the oil chain and this broken chain will form polymer. This polymer is responsible in causing dark color of the oil [35].

4. Conclusion
This study exhibits the beneficial use of waste product from lime peel in cooking oil oxidative stability. Antioxidant assay showed that lime peel contains vitamin C, flavonoid and carotene with highest in vitamin C. Clarity of the oil was found best at 90µg/ml concentration (0.38). It can be concluded that lime peel extract significantly gave effects on peroxide number of cooking oil. Both concentration and extract solvent determine the peroxide number.

Acknowledgement
We would like to thank biology department laboratory for all materials and facility support. We would like to also express highest appreciation and thank to Hibah Ristek Dikti under the scheme of PDUPT for research funding.

References
[1] Mehmood, Thair, et al. 2012 J. Chem. Soc. Pakist. 34 3.
[2] Anwar F, Bhanger M I and Kazi T G 2003 J. Am. Oil Chem. Soc. 80 151–5.
[3] Li X, Wu X, Liu R, Jin Q and Wang X 2015 J. Food. Engineer. 166 349–55.
[4] Guillén M D, and Cabo N 2002 Food. Chem. 77 503–10.
[5] Godswill A C, Amagwula I O, Igwe V S and Gonzaga A I 2018 Sci. Technol. Engineer. 4 4.
[6] Yaakob Z, Narayanan B N, Padikkaparambil S, Unni K S and Akbar PM 2014 Renew. Sust. Energy. Rev. 35 136–53.
[7] Christensen E and McCormick R L 2014 Fuel. Proc. Technol. 128 339–48.
[8] Jinxia J, Prieto M A, Barreiro M F, Carvalho A M, Oliveira, M B P P et. al. 2016 Food. Bioprod Proc. 98 28.
[9] Barba F J, Zhu Z, Koubaa M, Sant’Ana A S and Orlien V 2016 Trends. Food Sci. Technol 49 96-109.
[10] Rahayu S S, Nabila D N 2019 Int. J. Innov. Technol. Explor. Engineer. 8 226-229.
[11] Caleja J, Barros L, Antonio A L, Oliveira B P P et. al. 2017 Food. Chem. 216 342-346.
[12] Lorenzo J M, González-Rodríguez R M, Sánchez M, Amado I R and Franco D 2013 Food. Res. Int. 54 611–620.
[13] Pateiro M, Lorenzo, J M, Amado I R and Franco D 2014 Food. Chem.. 147 386–394.
[14] Amado I R, Franco D, Sánchez M, Zapata C, and Vázquez J A 2014 Food. Chem. 165 290-299.
[15] Putnik P, Kovačević D B, Jambrik A R, Barba F J, Cravotto G, et al. 2017 Molecules. 22 680
[16] Babbar N, Oberoi H S, Uppal D S, and Patil R T 2011 Food. Res. Int. 44 391-396.
[17] Xu G, Liu D, Chen J, Ye X, Ma Y and Shi J 2008 Food. Chem. 106 545-551.
[18] Ghafar M F, Prasad K N, Weng K K, and Ismail A 2010 Afr. J. Biotecnol. 9 326-330.
[19] Widiastuti H 2015 Jurnal Fitofarmaka Indonesia 2 72-75.
[20] Atanassova M, Georgieva S and Ivancheva K 2011 J. Univ. Chem. Technol. Metallurr. 46 81-88.
[21] Octaviani T, Guntarti A and Susanti H 2014 Pharmačiana 4 101-109.
[22] Narayana K, Vergheese S and Jacob S S 2009 Exp. Toxicol. Pathol 6 553–563.
[23] Saini R K, Nile S H and Park S W 2015 Food. Res. Int. 76 735–750.
[24] Lung J K S and Destiani D P 2017 Farmaka. 15 53-62.
[25] Skibsted L H 2012 J. Agr. Food. Chem. 60 2409-2417
[26] Kaleem A, Aziz S and Iqtedar M 2015 FUUAST. J. Biol. 5 191-196.
[27] Nassehimia H and Ahrai F 2016 J. Health. Res. Comm. 1 64-69.
[28] Rahayu S and Supriyatun 2017 AIP Conf. Proc. 1868 090016.
[29] Bera D, Lahiri D and Nag A 2006 J. Food. Engineer. 74 542-545.
[30] Aladedunye F A 2014 Europ. J. Lipid Sci. Technol. 116 688–706.
[31] Alizadeh L, Nayebozadeh K and Mohammad A 2016 J. Food. Sci. Technol. 53 611- 620.
[32] Karakaya S and Şimşek Ş 2011 J. Am. Oil. Chem. Soc. 88 1361-1366.
[33] Vaisali C, Belur P D and Regupathi, I 2016 LWT-Food Sci. Technol. 69 153–160.
[34] Serjouie A, Tan C P, Mirhosseini H and Che Man Y B 2010 Am J. Food. Technol. 5 310-323.