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Effect of lutein extract from spinach (*Amaranthus sp*) on n-hexane fraction on bulk cooking oil peroxide numbers

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ABSTRACT
The use of cooking oil for too long can cause rancidity in cooking oil which produces cancer-triggering free radicals. This rancidity can be overcome by adding antioxidants, namely compounds that can ward off free radicals. Lutein is an antioxidant compound that contains a hydroxyl group. In this study, green spinach leaves were used as a source of lutein. Green spinach leaves have a high lutein content that is not inferior to Kenikir and Broccoli flowers. The purpose of this study was to analyze the effect of adding lutein as an antioxidant compound in bulk cooking oil. The test begins with the maceration extraction stage using n-hexane solvent, determining the Rf value of lutein with TLC using an eluent mixture of n-hexane: acetone: chloroform (6: 2: 2) and analyzed by iodometric titration method. The results show that lutein has an Rf value of 0.58, which is almost the same as the standard Rf of lutein, which is 0.59, while the lutein content in green spinach leaves is quite high at 7.86 ppm. Lutein can inhibit the rancidity process caused by an increase in the number of peroxides. At a concentration of 3% lutein has a peroxide value smaller than 1% tocopherol which in this study was used as a comparison, namely synthetic antioxidant compounds.

KEYWORDS
Antioxidants; Peroxide number; Spinach leaves; Rancidity; Lutein

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Introduction

Oil oxidation is the main reaction of damage to several types of food that contain oil. This process can result in a decrease in quality by causing changes such as smell, taste, and colour. Oil rancidity is also caused by lipid oxidation reactions through a series of reaction mechanisms. The first stage is the initial formation of free radicals (initiation), then the propagation or formation of new radicals (propagation), and the last stage (termination), which is the destruction or conversion to stable and unreactive free radicals (Zeb et al, 2017).

In protecting the body from free radical attack, antioxidant substances function to stabilize free radicals by complementing the lack of electrons from free radicals so that they inhibit chain reactions. Antioxidants are compounds that can be used to protect foodstuffs by slowing down the damage, rancidity, or discolouration caused by oxidation. Antioxidants can act as donors of hydrogen atoms or electrons so that they can stabilize free radicals (I. Gumula et al, 2014).

Rancidity in cooking oil can be inhibited by providing antioxidants. There are 2 known antioxidants, namely natural antioxidants and synthetic antioxidants. The use of synthetic antioxidants such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) can have adverse effects on human health, namely impaired liver function, lungs, intestinal mucosa, and poisoning (Reynold and Lin, 1998). Concern about the side effects of synthetic antioxidants prompted chemists to seek safer natural antioxidants. Antioxidants that are currently widely used are taken from natural ingredients such as fruits and vegetables (Aisyah et al., 2010)

The use of natural antioxidants to prevent rancidity in cooking oil has been done before, including the addition of the enzymatically synthesized antioxidant Ascorbyl palmitate (Vitamin C) in coconut oil to improve the oxidation stability of coconut oil, the addition of phenolic extracts from mangosteen rind to counteract free radicals, the addition of Carrot flour can inhibit the increase in the peroxide number, and the addition of green betel leaf extract can reduce the peroxide number (Mahardika, 2018).

There are still many natural ingredients that can be used as antioxidants, one of which is lutein. There has been a lot of research on lutein as an antioxidant compound, including the ability of lutein compounds in spinach to neutralize T-BHP oxidants in blood cells, in addition to testing the antioxidant activity of local kenikir flowers, broccoli flowers, and Chlorella pyrenoids microalgae, as well as the ability of lutein compounds in banana peels to prevent cataracts (Nasir et al, 2014).

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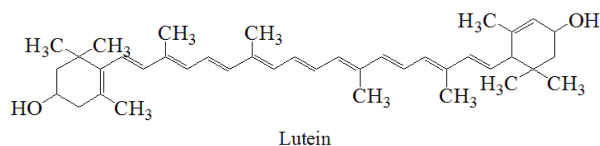


Figure 1. Lutein Compound Structure

Methods

This study was conducted in seven stages including sample preparation, extraction of lutein from spinach, determination of Rf by thin-layer chromatography, determination of lutein levels with a UV-Vis spectrophotometer, the addition of antioxidants to bulk cooking oil, standardization of 0.01 N $Na_2S_2O_3$ solution, determination of peroxide numbers by iodometric titration.

Sample preparation

Spinach leaves are weighed as much as 1000 g and then dried in the sun. Pound the dry spinach leaves to obtain spinach powder. Spinach powder was made 2 times.

Extraction of lutein from spinach

100 g of spinach powder is macerated with 400 mL n-hexane, then heated at 40^{0}C while stirring for 15 minutes. The filtrate is separated from solids. The solid was re-extracted with 200 mL n-hexane, then heated at 40 ^{0}C while stirring for 15 minutes. This extraction was repeated 3 times. The filtrate was collected and concentrated by an evaporator at 40 ^{0}C to obtain oleoresin. Oleoresin was added with 50 mL of KOH solution in 10% ethanol and heated at 65 ^{0}C for 2 hours. The extract was added with 100 mL n-hexane and then extracted with a separating funnel (repeated 3 times). The n-hexane solvent was evaporated, and the whole extract was weighed. The lutein extraction from spinach is carried out twice (Nasir et al, 2014).

Determination of Rf by Thin Layer Chromatography

The lutein extract was dotted on a silica gel plate and then eluted with a mixture of eluent n-hexane: chloroform: acetone (6:2:2) (Aizat et al, 2019). After the eluent reaches the upper limit, the Rf value is calculated and compared with the standard.

Determination of Lutein Levels with a UV-Vis Spectrophotometer

Standard Lutein Curve Creation

100 mg of pure lutein were weighed carefully, squeeze to the limit mark in a 10 mL n-hexane solution in a 100 ml flask until a concentration of 1000 ppm is obtained, then dilution is carried out for 5 variations in the concentration of 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, each concentration. The absorbance was measured at a wavelength of 445 nm (Wisatya et al, 2010).

Standard Lutein Curve Creation

The sample of the n-hexane fraction containing lutein was then measured at a maximum wavelength of 445 nm with n-hexane solvent (Muluken et al, 2019).

Addition of Antioxidants to Bulk Cooking Oil

Put 70 g of bulk cooking oil in Erlenmeyer, then add 1%, 3%, and 5% lutein in the n-hexane fraction, respectively (Hesti et al, 2019). Then heated at a temperature of 100 ^{0}C for 10 minutes. The mixture of bulk cooking oil and lutein is stored with variations in time of 1, 2, 3, and 8 days (Fri et al, 2019). As a comparison, 70 g of cooking oil without the addition of antioxidants and 70 g of cooking oil were also made with the addition of 1% Tocopherol as a control. Then the samples were stored at room temperature.

Standardization of 0.01 N $Na_2S_2O_3$ Solution

10 mL of 0.01 N KIO_3 solution pipette into the Erlenmeyer flask. then added 5 mL of 5% KI and 5 mL of H_2SO_4 2N. The mixture was titrated with 0.01 N $Na_2S_2O_3$ until the solution turned light yellow colour. Then add 1 mL of the starch indicator until it turns blue. Then it was re-titrated with 0.01 N Na2S2O3 until the blue colour disappeared. Furthermore, the concentration of $Na_2S_2O_3$ was determined (Hery et al, 2010).

Determination of Peroxide Numbers by Iodometric Titration

The sample was weighed as much as 5 grams in 100 mL Erlenmeyer, then added 30 mL of a solvent mixture of 60% glacial acetic acid and 40% chloroform. After the oil dissolves, the mixture is added with 0.5 mL of 6 M KI solution, then shaken and let stand for 2 minutes and 30 mL of water is added. After that, it was titrated with 0.01 N $Na_2S_2O_3$ until the yellow colour disappeared and a starch indicator was added to clarify the endpoint of the titration. Then it was re-titrated with 0.01 N $Na_2S_2O_3$ until the blue colour disappeared. Blanks were also made with the same treatment without samples. Titration is carried out 3 times (Hendrawati, 2011).

Results and Discussion

Extraction of Lutein from Spinach

Spinach leaf powder is macerated with n-hexane by heating at 40 ^{0}C, where the heating will release the active substance, namely lutein from spinach. When macerated, the spinach samples will experience the breakdown of cell walls and cell membranes due to the difference in pressure between inside and outside the cell so that the secondary metabolites in the cytoplasm will dissolve in the n-hexane solvent (Maimun et al, 2017). After extraction, then evaporation to obtain oleosin in the form of a paste. Oleoresin contains resins, waxes, lutein esters, and other carotenoid compounds. Lutein in nature in plants is in the form of lutein esters. The addition of KOH in ethanol is intended to degrade plant cell walls so that the lutein present in the ester-bound form becomes free, while the resin and wax will dissolve in ethanol. Furthermore, this mixture is extracted liquid-liquid using a separating funnel using n-hexane solvent. At this stage, 2 phases will be formed, and then the n-hexane phase is evaporated to obtain lutein extract.

Identification of Lutein by Thin Layer Chromatography

The TLC test results obtained 2 spots in the silica which showed that there were 2 compounds in the lutein extract obtained. The yellow spot 1 has the Rf value of the lutein compound is 0.58. This value is almost close to the standard Rf value of lutein, which is 0.59, while the orange spot 2 has an Rf value of 0.98 which is thought to be a beta-carotene compound. Both include carotenoid compounds. This shows that in the spinach extract in the n-hexane fraction there is a lutein compound.

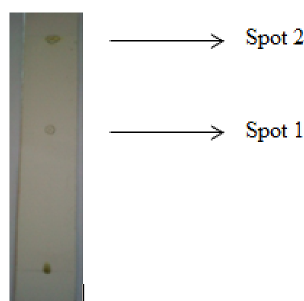


Figure 2. Test for the presence of Lutein by TLC

Lutein levels with a UV-Vis Spectrophotometer

The determination of lutein levels was carried out using a UV-Vis spectrophotometer at a wavelength of 445 nm because lutein has a chromophore group. Lutein compounds have conjugated double bonds, where this conjugated double bond is a light-absorbing chromophore that gives a yellow colour and produces a visible spectrum of light indicating the presence of lutein.

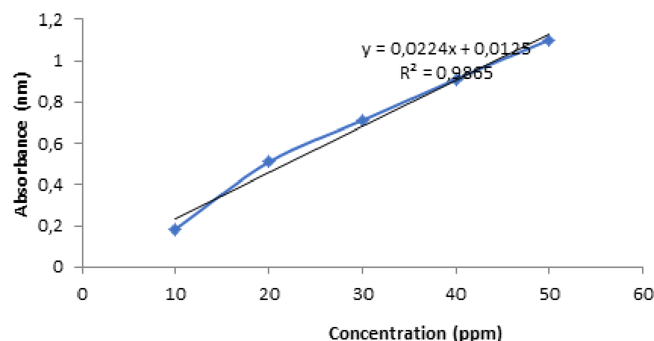


Figure 3. Lutein Standard Curve

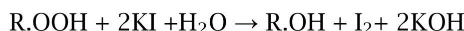
To calculate lutein levels determined on the spectrum from the UV-Vis Spectrophotometer. The linear regression equation y = bx + a state the relationship between concentration and absorbance in measurements using a UV-Vis spectrophotometer.

From the standard concentration curve to absorbance, y is the absorbance of the sample and x is the value of lutein content which will be calculated from the linear regression equation y = 0.022x + 0.012 with a correlation coefficient (R2) of 0.986. From this equation, the lutein content in spinach is 7.86 ppm.

Determination of Peroxide Numbers by Iodometric Titration

With the iodometric method, the peroxide value has been obtained which shows the amount of fat or oil that has been oxidized. Iodometric titration is carried out by dissolving an amount of oil in a mixture of glacial acetic acid: chloroform (60% : 40%), then adding the KI solution. The addition of glacial acetic acid is intended to provide an acidic atmosphere and the addition of chloroform so that the oil and acid mix. The addition of this KI solution will release a certain amount of iodine due to the reaction between fat and KI.

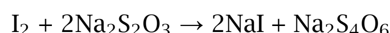
The reaction that occurs:



$$R.OOH + 2KI + H_2O \rightarrow R.OH + I_2 + 2KOH$$

The iodine formed is proportional to the number of peroxides found in bulk cooking oil. The iodine formed is then titrated with $Na_2S_2O_3$.

The reaction that occurs:



$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

The reaction mixture is then added with the starch indicator to give a blue colour. The resulting blue colour indicates that there is still iodine in the solution. The solution continued to be titrated with Na_2SO_3 until the blue colour disappeared.

Inhibition of Increase in Peroxide Numbers with Antioxidants

From all stages, antioxidant radicals will be generated, but these antioxidant radicals are relatively stable and do not have enough energy to be able to react with other fatty acid molecules to form new fatty acid radicals.

Table 1. Peroxide Numbers in Bulk Cooking Oil

Peroxide Number	Day 1 (mek/Kg)	Day 2 (mek/Kg)	Day 3 (mek/Kg)	Day 4 (mek/Kg)
Oil	10,3029	10,9848	11,7382	15,6824
+1% Tocopherol	10,0375	10,4924	10,9124	12,8617
+1% Lutein	10,1038	10,5092	11,0936	12,9725
+3% Lutein	9,8939	10,2477	10,5890	11,9789
+5% Lutein	9,7588	9,9876	10,1034	11,4890

In the table above, the peroxide number of cooking oil without the addition of antioxidants has a very large increase in peroxide number every day, whereas with the addition of lutein and tocopherol the increase in peroxide number is not too large. This is because the function of lutein and tocopherol is as an antioxidant, which causes inhibition of the oxidation process in bulk cooking oil which causes a decrease in the value of the peroxide number (Rebaya et al, 2014).

The decrease in the increase in the value of the peroxide number in bulk cooking oil with added lutein from spinach can occur because lutein can act as an antioxidant that can inhibit the oxidation process that occurs in cooking oil (Elin et al, 2018). Lutein has phenol groups as well as synthetic antioxidants that are often used which also have phenol groups such as BHA, BHT, PG, and tocopherols. The H atom from the hydroxy group of the phenolic compound from lutein will bind to the fatty acid radicals so that free radicals will not form which causes rancidity in cooking oil. Because it has a large electronegativity difference, the H from the hydroxy (OH) group is easy to escape (Rahmawati et al, 2018).

Effect of Storage Time on Peroxide Numbers in Bulk Cooking Oil

When viewed from the bar graph below, the addition of lutein to bulk cooking oil is very influential in increasing the peroxide number. Where the greater the lutein concentration added to the bulk cooking oil, the slower the oxidation process is, this can be seen from a large number of peroxide obtained (Batari, 2012). The peroxide value of the oil added with 1% lutein still exceeds the peroxide value of the oil added to the tocopherol, only at a concentration of 3% the value of the peroxide value is below the oil added with tocopherol. Therefore lutein can be used as a natural antioxidant compound which is safer than synthetic antioxidant compounds such as tocopherols (Yoki and Hitoshi, 2003).

In the bar graph, the relationship between storage time and the peroxide number below also shows that every day the peroxide number has increased. On the first day of all oil samples, the peroxide value was ± 10 mek / Kg. Only on the 8th day showed that all oil samples obtained a very high value of peroxide value, especially for oil that was not added with antioxidants, either lutein or tocopherol, reaching ± 15 mek / Kg. It can be concluded that the longer the oil storage time, the greater the value of the peroxide number.

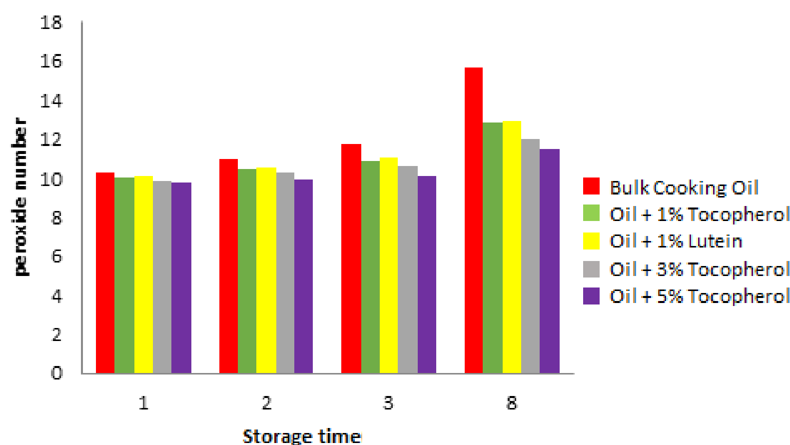


Figure 5. Chart of the relationship between storage time and peroxide number

Conclusion

Based on the results of identification by TLC, it can be concluded that in the n-hexane fraction there is lutein, which has an Rf value of 0.58 and is yellow. Testing its levels with a UV-Vis Spectrophotometer, it was found that the lutein content was 7.86 ppm. At 3% lutein content is better than 1% tocopherol in inhibiting the increase in peroxide number, and the longer the storage time, the peroxide number will increase/increase.

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References

Aisyah, S., Yulianti, E., dan Fasya, A.G. (2010). Penurunan Bilangan Peroksida dan Asam Lemak Bebas pada Proses Bleaching Minyak Goreng Bekas Oleh Karbon Aktif Polong Buah Kelor dengan Aktivasi NaCl. *AlChemy*, *1*(2), pp. 53-103. https://doi.org/10.18860/al.v0i0.1669

Aizat, W.M., Jamil, I.N., Ahmad-Hashim, F.H., Noor, N.M. (2019). Recent updates on metabolite composition and medicinal benefits of mangosteen plant. *PeerJ*, *7*, e6224. https://doi.org/io.77i7/peerj.6324

Batari, R. (2012). *Identifikasi senyawa flavonoid pada sayuran indigenous Jawa Barat*. Bogor: Fakutas Teknologi Pertanian, Institut Pertanian Bogor.

Gumula, I., Alao, J.P., Ndiege, I.O., Sunnerhagen, P., Yenesew, A., Edelyi, M. (2014). Flemingins G-O, cytotoxic and antioxidant constituents of the leaves of *Flemingia grahamiana*. *Journal of Natural Products*, *77*, 2060 – 2067.

Hendrawati, T.Y. (2011). *Studi Pengaruh Penggunaan Antioksidan Golongan Phenolik dan Aminik terhadap Ketahanan Oksidasi Minyak Sawit pada Berbagai Tingkat Kemurnian*. Bogor: Program Pascasarjana, Institut Pertanian Bogor.

Hilma, R., Herliani, and Almurdani, M. (2018). Determination of Total Phenolic, Flavonoid Content Andfree Radical Scavenging Activity of Etanol Extract Sawo Stem Bark (Manilkara zapota (L.)). *CELSciTech*, 3.

Kebede, M. and Admassu, S. (2019). Application of Antioxidants in Food Processing Industry: Options to Improve the Extraction Yields and Market Value of Natural Products. *Advancesin Food Technology and Nutritional Sciences*, *5*(2), 38-49. http://doi.org/10/17140/AFTNSOJ-5-155

Mahardika, R.G. (2018). Peningkatan Kualitas Minyak Jelantah Menggunakan Karbon Aktif dan Ekstrak Pucuk Idat. *Indonesian Journal of Pure and Applied Chemistry*, *1*, 17-23.

Maimun, T., Arahman, N., Arifah, F., Hasibuan, and Rahayu, P. (2017). Penghambatan Peningkatan Radar Asam Lemak Bebas (Free Fatty Acid) pada Buah Kelapa Sawit dengan Menggunakan Asap Cair. *Jurnal Teknologi dan Industri Pertanian Indonesia*, *9*(2), pp. 44-49. https://doi.org/10.17969/jtipi.v9i2.8469

Nasir, N.S.W., Nurhaeni, dan Musafira (2014). Pemanfaatan Arang Aktif Kulit Pisang Kepok Sebagai Adsorben untuk Menurunkan Asam Lemak Bebas dan Bilangan Peroksida Minyak Jelantah. *Natural Science: Journal of Science and Technology*, *3*(1), pp. 18-30.

Rahmawati, F., Kurniaty, L. and Bintang, M. (2019). Skrining Golongan Senyawa Aktif dan Analisis Toksisitas Ektraks Biji Kabau (*Archidendron bubalinum*). *Jurnal Widya Kesehatan dan Lingkungan*, 1(2), 153-158.

Rebaya, A., Belghith, S.I., Baghdikian, B., Leddet, V.M., Mabrouki, F., Olivier, E., Cherif, J.K. and Ayadi, M.T. (2015). Total phenolic, total flavonoid , tannin content, and antioxidant capacity of *Halimium halimifolium* (Cistaceae). *Journal of Applied Pharmaceutical Science*, *5*(1), 52-57. https://doi.org/10.7324/JAPS.2015.50110

Reynold, E. and Lin, S. (1998). The Recovery of used frying oils with various adsorbents. *Journal of food Lipids*, *V*, 1-16.

Riasari, H., Fitriansyah, S.N., Hartati, R., Anggadiredja, K. and Sukrasno. (2019). Comparison of Extraction Methods, Antioxidant Activities, Total Phenol in Seeds and Seed Shells of Kabau (*Archidendron bubalinum (Jack) IC Nielsen*) From Lampung and South Sumatra. *Pharmacognosy Journal*, 11(6), 1278-1284. http://dx.doi.org/10.5530/pj.2019.11.198

Sembiring, E.N., Elya, B. and Sauriasari, R. (2018). Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc (L.)* Roxb. *Pharmacognosy Journal*, *10*(1), 123-127. https://dx.d0i.0rg/10.5530/pj.2018.1.22

Winarsi, H. (2010). *Antioksidan alami dan radikal bebas: Potensi dan aplikasi dalam kesehatan*. Yogyakarta: Penerbit Kanisius.

Wisatya, D.K., Sarjono, P.R., Mulyani, N.S. (2010). Pengaruh Pemanasan pada Proses Pembuatan Ekstrak Kulit Buah Manggis (Garcinia mangostana Linn) terhadap Aktivitas Antimikroba. *Jurnal Kimia Sains dan Aplikasi*, *13*(2), 46-50. http://doi.org/10/14710/jksa.13.2.46-50

Yoki, Y. and Hitoshi, W. (2003). In Situ Measurement of Aggregate Formation Kinetics of Nickel(II)-Pyridylazoaminophenol Complex at the Heptane-Water Interface by Centrifugal Liquid Membrane Spectrophotometry. *Bulletin of the Chemical Society of Japan*, *76*(7), pp. 1379-1386. https://doi.org/10.1246/bcsj.76.1379

Zeb, A. and Nisar, P. (2017). Effects of High Temperature Frying of Spinach Leaves in Sunflower Oil on Carotemoids, Chlorophylls, and Tocopherol Composition. *Frontiers in Chemistry*, *5*(19). https://doi.org/10.3389/fchem.2017.00019