Engineering safer bulk cooking oil by increasing oxidative stability with organic waste of noni (Morinda citrifolia) fruit

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Abstract. Cooking oil has brought detrimental impact on health. Many attempts have been conducted to minimize the risk of it. This study aimed at engineering safer bulk cooking oil by increasing oxidative stability with organic waste of noni fruit. Method applied was experimental with complete factorial randomized design. Factor used were solvent type (ethanol and distilled water), concentration (25% and 33%) and maceration time (2, 4, 6, 8, 16, 24 hours) of noni fruit. Parameters measured were qualitative and quantitative flavonoid, peroxide value and free fatty acid number. Statistical analysis was done by Manova followed by Games-Howell. Result showed that ethanol extract exhibit strongest flavonoid qualitatively and quantitatively within 33% concentration (48.9 Meq). The best (lowest) peroxide value can be identified on ethanol extract under 33% concentration and 24-hour maceration (2.9 ± 0.6 Meq0.2/Kg). The lowest free fatty acid (FFA) number could be achieved by 33% of ethanolic extract under 12 hours’ maceration (2.5%). Statistical analysis revealed the impact of solvent, concentration and time to peroxide value and free fatty acid value. It can be concluded that bulk cooking oil can be engineered to be safer by adding noni organic waste of noni fruit.

1. Introduction
Cooking oil is triglyceride derived from plants. Most of the physical and chemical property of cooking oil are attributed to their fatty acid composition of the triglyceride [1]. Most cooking oil used in Indonesia is palm cooking oil since it has lowest price. Cooking oil can easily change due to oxidative process which then result in rancidity of bad smell and detrimental effect on health [2,3]. Economic reason has been the major factor of reusing cooking oil in Indonesia. This condition has led to the arise of many health problems.

Many researches have been conducted on enhancing oxidative stability of cooking oil. Known synthetic antioxidants were used such as tertiary butylated hydroxy quinone (TBHQ), butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA), though they produced problems on food safety [4].

Natural antioxidant extracted from plants showed promising result on enhancing oxidative stability of palm cooking oil [5-9]. Furthermore, natural antioxidant was proven to be more potent compare to
the synthetic ones [5]. Many fruits were decades known for its antioxidant property. Unfortunately, not all kind of fruit were consumed due to some reasons. Bitter taste and fleshy look have underlying it. Noni fruit is one of the examples of the fruit mentioned. The fruit never been used as daily consumed fruit. Some has used it for remedies but most fruit will undergo their faith in the rubbish bin as waste.

Noni (Morinda citrifolia) fruit is known for decades as natural antioxidant. This fruit contain some Phyto active substances include quercetin, ursolic acid, carotene and kaempferol [10-13]. These active metabolites were proven to not only have antioxidant activities [14] but also anti-inflammatory and antidyslipidemia [15,16]. As its capacity, we try to investigate the power of noni fruit in enhancing oxidative stability of palm cooking oil. To the best of our knowledge there hasn’t been any study yet revealed the effect of noni in cooking oil’s oxidative stability. Therefore, this study to engineer safer bulk cooking oil by using noni fruit to enhance oxidative stability of the oil.

2. Methodology
This research was done in Biochemistry laboratory, faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta. Sample used was flesh fruit of noni. Complete randomized design was applied with 2x2x6 factors. Factors applied were solvent type of noni extract (ethanol and distilled water) concentration of noni extract (25% and 33%) and immersion time (2,4,6,8,16,24 hours). After being fried, noni extract was immersed in the bulk oil. Parameters used were qualitative (flavonoid assay of noni fruit) as well as quantitative (flavonoid concentration of noni fruit, peroxide number and free fatty acid number of cooking oil). Repetition was done triplicate.

2.1. Sample preparation
Morinda citrifolia fruit were collected from Jakarta. Fruit are cut into small pieces and dried for 2 days to reduce water content.

2.2. Extraction
The fruit flesh was juiced and extracted with ethanol 96% and distilled water with variations in maceration time (24 and 48 hours) and concentration series of (33% and 25%). The solvent was removed by using rotary evaporator at temperature 45℃ [17].

2.3. Qualitative flavonoid assay
30 mg of extraction was put into a test tube. Magnesium powder and a few drops of concentrated HCl was added. Positive flavonoids were yellow orange color formation.

2.4. Standard quercetin equation
A total of 2.5 mg of quercetin was weighed and dissolved in 25 ml of ethanol as standard, 100 ppm. A series of standard concentrations of 20, 30, 40, 50 and 60 ppm were made. 0.5 mL of standard liquid solution added 0.1 mL aluminium (III) chloride 10%, 0.1 mL sodium acetate 1 M and 2.8 mL distilled water. Taken one of the concentrations of a standard solution, the absorbance is measured at a wavelength of 400-800 nm.

2.5. Setting a standard quercetin curve
The standard curve is made by connecting the concentration of the standard quercetin solution with the absorbed results obtained by measurement using a UV-Vis spectrophotometer at a wavelength of 437.55 nm.

2.6. Determination of total flavonoid levels in extracts
A total of 20 mg samples was weighed and dissolved in 10 mL solvents and then centrifuged to obtain a concentration of 2000 ppm. 0.5 mL of the test sample was added with 0.1 mL aluminium (III) chloride 10%, 0.1 mL sodium acetate 1 M and 2.8 mL distilled water. After incubation for 30 minutes, the absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength of quercetin...
437.55 nm. The total flavonoids from the were calculated using a linear regression equation from the quercetin calibration curve that had been previously measured. Total flavonoid content is expressed as the number of mg quercetin equivalent to each mg of extract.

2.7. Peroxide value analysis
The sample of the mixing of the oil with an extract of 5 ml was mixed with 30 ml of glacial acetic acid and 20 ml of chloroform. Solution added 0.5 ml KI, shaken and allowed to stand for 2 minutes. The solution was added with 30 ml of distilled water and titrated with 0.01 N thiosulphate [18].

\[
\text{Peroxide value} = \frac{V \text{ thiosulphate} \times N \text{ thiosulphate} \times 1000}{\text{Sample weight}}
\]

2.8. Free fatty acid analysis
Free fatty acid counting (FFA) was firstly done in cooking oil before frying process to investigate basic FFA number of cooking oil used. 28.2 ± 0.2 g sample was weighted on Erlenmeyer. 50 ml netral alcohol was added with 2 ml phenolphthalein (PP) indicator. Titration was done with 0.1 N NaOH until pink color appear and stabile for 30 second and measurement was done with the equation below:

\[
\% \text{FFA} = \frac{m \text{N} \text{AOH} \times \text{oil weight} \times 100}{\text{sample weight} \times 100}
\]

2.9. Statistical analysis
Statistical analysis was done by Manova followed by Games Howell for post hoc analysis (sig 0.05).

3. Results and discussion

3.1. Qualitative and quantitative result of flavonoid
Qualitative measurement of flavonoid content on noni fruit waste was done in two concentrations (33% and 25%) of solvent. Solvent applied were distilled water and ethanol. Result on qualitative flavonoid can be seen in table 1.

| Solvent type  | Repetition | 33%  | 25% |
|---------------|------------|------|-----|
| Distilled water | 1          | ++   | +   |
|               | 2          | ++   | +   |
|               | 3          | ++   | +   |
| Ethanol       | 1          | +++  | +   |
|               | 2          | ++   | ++  |
|               | 3          | +++  | ++  |

Note: + sign show the color formed
More + sign indicate darker color formed

Noni fruit has been long believed to have antioxidant power. Antioxidant from plant is usually found in the form of phenolic content such as flavonoid [19]. This property of the fruit makes it very beneficial in inhibiting oxidation process in cooking oil. Oxidation processes are accelerated in the presence of water and oxygen (exposure to air), heat and light (environmental conditions), and trace metals from corrosion of containers and automotive materials [20]. The existence of antioxidant could slower the oxidation process. Hence, if applied in cooking oil will be able to enhance oxidative stability of the oil [5-9].

There are many methods to detect antioxidant such as soxlet extraction, maceration, subcritical water extraction and ultrasound-assisted extraction. However, Extraction yield and antioxidant activity are not only depending on the method but also the solvent used [21-23]. Different antioxidant compound
with varied characteristic and polarities may not be soluble in some solvent type. Previous research investigated that ethanol was the best solvent in measuring flavonoid content in plant [24].

In this research, qualitative description revealed that ethanol also being the best solvent in extracting flavonoid in noni. Though, distilled water which is also polar able to extract flavonoid in noni. The result showed lighter color compare to noni extracted with ethanol. Different extract concentration also indeed results in different color formed. It reflected that the concentration also gave impact on qualitative assay of flavonoid in noni fruit.

Confirming qualitative assay was done by measuring flavonoid quantitatively. Quantitative measurement was done by spectrophotometry with quercetin curve equation as standard. Ethanol showed higher concentration of flavonoid extraction (fig 1). Concentration of solvent also gave impact on the extraction process. The increasing extract concentration attributable to higher flavonoid content [25-28].

![flavonoid content graph](image)

**Figure 1.** Quantitative result of flavonoid content of Noni fruit (Meq).

### 3.2. Peroxide value of bulk cooking oil

Cooking oil pre-treated with noni waste under different concentration and solvent was measured for peroxide and free fatty acid value. This measurement would prove that noni application could enhance oxidative stability of the oil and quality of bulk cooking oil. Hence detrimental impact would be less likely occur.

Peroxide value is one the most important measurement of olive oil, since it is an indicator of the oxidation status of the product [29]. The peroxide value is defined as amount of peroxide oxygen per 1 kilogram of fat or oil [30].

Oxidative process which included the formation of hydroperoxide might occur due to process or storage trough photooxidation or autoxidation [31,32]. Some research has been conducted to enhance oxidative stability in cooking oil with natural antioxidant such as grape seed [33], Lamiaceae plant family [34] and even leaf extract [34] on food. Research on olive oil has also been discovered to increase the stability. Cocoa been shell and bagasse, mangos teen and lime peel were also used to lowered peroxide value of cooking oil [8,15,35-37].

Noni fruit waste used in this research also showed promising result in enhancing oxidative stability of cooking oil. Result showed that noni macerate in specific solvent used able to reduce peroxide value of bulk cooking oil (Table 1). Noni fruit in ethanol extract showed best result in minimizing the peroxide value under 33% concentration and 24 hours’ immersion of noni extract in bulk oil. Hence, this group showed the best treatment in designing oxidative stability of the oil. All peroxide value of bulk oil treated with noni revealed lower score in ethanol extraction compare to distilled water under the same concentration and immersion period. Surprisingly, 2 hours’ immersion of noni fruit extracted with distilled water slightly lowered peroxide value of bulk cooking oil. Nevertheless, it was again incline and gain its lower curve pattern. If we see detailed result of the study (Table 1), two hours’ noni immersion in cooking oil hasn’t gave impact on peroxide level of bulk cooking oil. Which was even
400% increased than national standard for peroxide allowed (10MeqO₂/Kg). Increased peroxide value of bulk cooking oil occurs due to heating process. Longer immersion of noni in cooking oil able to reduce the value quite significant. Both distilled water noni extract and ethanol extract able to give lower value within different immersion time of noni in the oil. 16 hours’ immersion of noni extract in bulk oil for 33% concentration was gained with distilled water extraction. Whilst, 4 hours’ immersion of noni ethanol extract in bulk oil able to give oxidative stability of the oil. Polyphenol found in some antioxidant plant including noni able to decrease hydroperoxide and aldehyde formation in oil [36,38,39].

### Table 2. Peroxide value of bulk cooking oil (MeqO₂/Kg).

| Solvent       | Concentration | 2 hours | 4 hours | 6 hours | 8 hours | 16 hours | 24 hours |
|---------------|---------------|---------|---------|---------|---------|----------|----------|
| Distilled water | 33%           | 46.7 ± 0.6<sup>a</sup> | 12.6 ± 0.3<sup>d</sup> | 35.2 ± 2.1<sup>e</sup> | 15.0 ± 2.1<sup>f</sup> | 8.6 ± 0.7<sup>g</sup> | 5.5 ± 0.9<sup>h</sup> |
| Distilled water | 25%           | 54.5 ± 7<sup>c</sup> | 17.0 ± 3.2<sup>e</sup> | 37.6 ± 3.2<sup>e</sup> | 23.8 ± 1.9<sup>c,g</sup> | 19.2 ± 1.0<sup>j,k</sup> | 6.7 ± 1.2<sup>i</sup> |
| Ethanol       | 33%           | 23.0 ± 19.2<sup>b</sup> | 9.9 ± 0.9<sup>e</sup> | 7.5 ± 0.4<sup>e</sup> | 9.4 ± 0.1<sup>f</sup> | 6.4 ± 0.5<sup>j</sup> | 2.9 ± 0.6<sup>l</sup> |
| Ethanol       | 25%           | 17 ± 4.9<sup>b</sup> | 15.4 ± 4.6<sup>d</sup> | 8.9 ± 0.6<sup>d</sup> | 7.7 ± 0.8<sup>d</sup> | 6.3 ± 0.3<sup>j</sup> | 3.8 ± 0.6<sup>i</sup> |

Indonesian standard for cooking oil peroxide value: 10MeqO₂/Kg [28]
Games Howell result: Different notation showed difference (sig. <0.05)

3.3. **Free fatty acid value of bulk cooking oil**

Free fatty acid parameter is fats and oil can be used to describe the extent of its deterioration due to hydrolysis of triglyceride and oxidation of unsaturated fatty acid [40,41]. The hydrolysis of triglycerides and decomposition of hydroperoxide at high temperature in the presence of moisture and air forms FFA. The maximum allowed value of FFA varies depending on the type of food being fried and number of batches during frying operation [42]. FFA value increases with increase in the number of frying cycles and heating. The increase in FFA is because of the cleavage and the oxidation of double bonds to form carbonyl compounds and low molecular fatty acids during frying. The moisture coming from fried products accelerates the hydrolysis and it is due to fact that water can promote the hydrolysis of triacylglycerol to form combination of mono and diacyl glycerol, glycerol and free fatty acids [40].

Response of FFA is shown in the table below (Table 2). Noni application on cooking oil didn’t gave impact on FFA value. FFA will formed due to hydrolysis reaction of lipid into their carboxylic acid and glycerol. Noni fruit is antioxidant which will be able to cope with oxidation process rather than hydrolysis process of cooking oil [43]. Therefore, all treatment in this study still showed high FFA value of bulk cooking oil.

Highest FFA was found in distilled water noni extraction with none of the treatment able to give low FFA. The highest of the value was found in 16 hours’ immersion of noni. Though immersion or even concentration of noni supposed to not bringing any impact on FFA. Only one experimental treatment succeeds to reach standard FFA value of 0.3%. The group was seen on 33% ethanol extract of noni fruit under 16 hours’ immersion.

### Table 3. Free fatty acid value of bulk cooking oil (%).

| Solvent       | Concentration | 2 hours | 4 hours | 6 hours | 8 hours | 16 hours | 24 hours |
|---------------|---------------|---------|---------|---------|---------|----------|----------|
| Distilled water | 33%           | 2.12 ± 0.53<sup>a</sup> | 0.68 ± 0.53<sup>a</sup> | 0.13 ± 0.14<sup>d</sup> | 0.53 ± 0.01<sup>e</sup> | 0.25 ± 0.01<sup>g</sup> | 1.63 ± 0.17<sup>k</sup> |
| Distilled water | 25%           | 2.19 ± 0.19<sup>c</sup> | 2.01 ± 0.19<sup>c</sup> | 0.88 ± 0.01<sup>d</sup> | 1.21 ± 0.01<sup>c</sup> | 0.4 ± 0.01<sup>e</sup> | 0.7 ± 0.11<sup>i</sup> |
| Ethanol       | 33%           | 0.93 ± 0.16<sup>b</sup> | 0.03 ± 0.16<sup>b</sup> | 0.04 ± 0.01<sup>d</sup> | 0.11 ± 0.01<sup>d</sup> | 0.25 ± 0.01<sup>i</sup> | 0.76 ± 0.01<sup>i</sup> |
| Ethanol       | 25%           | 0.57 ± 0.18<sup>b</sup> | 0.14 ± 0.18<sup>b</sup> | 0.02 ± 0.01<sup>d</sup> | 0.06 ± 0.01<sup>d</sup> | 0.72 ± 0.09<sup>j</sup> | 0.13 ± 0.01<sup>n</sup> |

Indonesian Standard of FFA for cooking oil: 0.3% [28]
Different notation showed difference (sig. <0.05)
4. Conclusion
Based on the result it can be concluded that ethanol extract exhibit strongest flavonoid qualitatively and quantitatively within 33% concentration (48.9 Meq). The best (lowest) peroxide value can be identified on ethanol extract under 33% concentration and 24-hour maceration (2.9 ± 0.6 Meq0/Kg). The lowest free fatty acid (FFA) number could be achieved by 33% of ethanolic extract under 12 hours’ maceration (2.5%). Statistical analysis revealed the impact of solvent, concentration and time to peroxide value and free fatty acid value. It can be concluded that bulk cooking oil can be engineered to be safer by adding noni organic waste of noni fruit.

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