

The Effect of Surfactant Formulation and Salam Leaf Extract (*Syzygium Polyanthum*) on The Durability of Soybean Oil

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Abstract. The antioxidants in salam leaf extract have polar properties that affect their effectiveness in oil, thus requiring a surfactant to enhance dispersion. This study aims to investigate the impact of surfactant addition on the dispersion of salam leaf extract antioxidants in soybean oil based on iodine value and free fatty acids. The research involved extracting salam leaves and determining the best formulation with a combination of salam leaf extract and sorbitan monooleate surfactant based on antioxidant activity. The best formulation was then blended into soybean oil, which was subsequently heated discontinuously for 12 hours. The results indicated that the treatment observed based on its antioxidant activity is 0.8% salam leaf extract and 1% Sorbitan monooleate surfactant with IC₅₀ antioxidant activity of 105.4712 ppm and which belongs to the medium category. The analysis were then performed for this treatment in heating I to heating IV iodine number (102.95gI₂/100g, 102.38gI₂/100g, 102.14gI₂/100g, and 101.23gI₂/100g respectively) and the free fatty acid (0.0633%, 0.0652%, 0.1163%, and 0.1743%, respectively). The effectiveness of salam leaf extract based on iodine number, peroxide number, and free fatty acid parameters showed that the treatment with the addition of salam leaf extract combined with sorbitan monooleate surfactant gave better results than without the addition of sorbitan monooleate surfactant.

1 Introduction

Soybean oil is a vegetable oil derived from extracted soybean seeds (*Glycine max*). Soybean oil contains 85% unsaturated fatty acids which include oleic acid (23%), linoleic acid (54%), and linolenic acid (8%), and 15% saturated fatty acids which include, stearic acid (4%), and palmitic acid (11%) [1,2]. The high content of unsaturated fatty acids in soybean oil makes it susceptible to oxidative damage due to the presence of double bonds that are more prone to react with oxygen, forming peroxides.

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Lipid oxidation occurs because of the reaction between oxygen and unsaturated fatty acids (fatty acids with double bonds). Oxidation reactions lead to the deterioration of fats and oils, with rancidity as the primary consequence. These reactions can take place during storage and in high-temperature oil processing. The most prominent changes associated with oxidation include the development of an unpleasant taste and odor, alterations in color, viscosity, density, and the solubility of fats and oils [3]. These changes occur because of the presence of oxidized lipids through a series of complex reactions that produce volatile and non-volatile compounds responsible for the undesirable taste and odor in food [4].

Antioxidants are correlated with the inhibition of oxidation and can serve as a straightforward solution to enhance the stability of oils. Antioxidants are compounds capable of neutralizing free radicals by donating electrons to achieve a stable form [5]. This reaction can occur through autooxidation (oxidation by $^3\text{O}_2$), photooxidation (oxidation by $^1\text{O}_2$), and enzymatic oxidation. The formation of hydroperoxides as primary products and their decomposition into secondary products is a consequence of autooxidation and photooxidation. Secondary oxidation products include ketones, aldehydes, alcohols, lactones, hydrocarbons, esters, or others [4,6]. Natural sources of antioxidants can be found in plants, such as vegetables, fruits, spices, and herbs, which are rich in vitamins, phenolic compounds, carotenoids, and other microelements [7].

Salam leaf (*Syzygium polyanthum*), commonly used as a herbal and spice, can also be utilized as a source of natural antioxidants. Active compounds present in salam leaves include tannins, flavonoids, essential oils, citral, eugenol, triterpenoids, steroids, phenols, and saponins [8–11]. Salam leaves can function as antioxidants due to the presence of phenolic and flavonoid compounds.

Research on salam leaves has thus far been limited to determining antioxidant activities and their use in inhibiting oil degradation, but their combined utilization with surfactants have not been applied. The use of surfactants here is expected to improve the solubility of salam leaf extract, which has polar properties [5], in soybean oil. This study aims to determine the iodine value and free fatty acids in soybean oil with the addition of salam leaf (*Syzygium polyanthum*) extract and sorbitan monooleate surfactant."

2 Methods

2.1 Salam Leaves Extraction

Salam leaves are chopped to reduce their size. Subsequently, they are extracted using ethanol 96% at a ratio of 1:10 for 24 hours at room temperature. The extraction yield is filtered through filter paper, and the solvent is evaporated using a vacuum rotary evaporator at 50 °C with a speed of 80 rpm [12].

2.2 Determination of Total Phenolics

The calibration curve for Gallic Acid was prepared according to the method using the Folin-Ciocalteu phenol reagent.

Gallic Acid (50 mg) was dissolved in 1 mL of 96% ethanol, and distilled water was added to a final volume of 50 mL (concentration 1 mg/mL). Successive aliquots of the stock solution (1 mL, 1.25 mL, 1.5 mL, 1.75 mL, and 2 mL) were diluted to a final volume of 10 mL to obtain concentrations of 100 µg/mL, 125 µg/mL, 150 µg/mL, 175 µg/mL, and 200 µg/mL, respectively.

Each Gallic Acid concentration (0.2 mL) was mixed with 15.8 mL of distilled water and 1 mL of Folin-Ciocalteu reagent (diluted to a 1:1 ratio). The solution was homogenised and

allowed to stand for 8 minutes. Next, 3 mL of 10 % Na₂CO₃ was added to the Gallic Acid solution, and the mixture was shaken until homogeneous before standing at room temperature for 2 hours. The absorbance was measured at a maximum wavelength of 765 nm. The absorbance value obtained was used to construct a calibration curve for the concentration of Gallic Acid (µg/mL) [13,14].

The determination of total phenolic content using the Folin-Ciocalteu method

Salam leaf extract (100 mg) was dissolved in distilled water to a final volume of 10 mL (concentration 10 mg/mL). Then, 1 mL of the extract was diluted again to a final volume of 3 mL (1 mg/mL concentration). Next, 0.2 mL of the 1 mg/mL concentration was diluted with 15.8 mL of distilled water, and 1 mL of Folin-Ciocalteu reagent (diluted in a 1:1 ratio) was added. The extract solution was homogenised and allowed to stand for 8 min. Next, 3 mL of 10% Na₂CO₃ was added and the mixture was homogenised again before standing at room temperature for 2 hours. The absorbance was measured at a maximum wavelength of 765 nm. Total phenolic content was expressed as mg Gallic Acid Equivalent per gram (mg GAE/g) [13,14].

2.3 Determination of Antioxidant Activity

The antioxidant activity of the samples was carried out by mixing 2.0 mL of 100 µg/mL DPPH and 2.0 mL of test samples with concentrations of 10, 20, 30, 40, 50 µg/mL and adding ethanol up to 9.0 mL, left at room temperature (37 °C) for 30 minutes in a dark place. The absorbance was determined after 30 minutes and read at λ 517 nm. Three replicates were carried out for antioxidant determination. The absorbance of each solution was measured at the optimal wavelength, and the absorbance value obtained was used to calculate the percentage of antioxidant inhibition against DPPH radicals [15,16].

$$\%Inhibition = \frac{Abs\ Blank - Abs\ Sample}{Abs\ Blank} \times 100\% \quad (1)$$

Based on the percentage of inhibition data, the IC₅₀ value (50% inhibitor concentration) can be obtained, which represents the effective concentration of the antioxidant required to reduce the DPPH concentration by 50%. The IC₅₀ value is calculated using a linear regression equation that expresses the relationship between antioxidant activity and sample concentration. The linear regression equation used is $y = ax + b$, where the value of y is set to 50, and the value of x represents the IC₅₀ [15].

2.4 Application of Salam Leaf Extract into Soybean Oil

Salam leaf extract (0.8%w/v of soybean oil) and Sorbitan monooleate surfactant (1%w/v; 3%w/v; 5%w/v of soybean oil) were mixed and tested for antioxidant activity to determine the best formulation. The best formulation was then applied into soybean oil. The soybean oil is heated to 180 °C for a total of 12 hours in a discontinuous process. The heating is performed for 6 hours on the first day and another 6 hours on the second day (samples are analyzed every 3 hours).

2.5 Analysis of Iodine Value with The Wijs Method

Soybean oil (0.1 - 0.5 grams) was put into an Erlenmeyer flask and 15 mL of chloroform was added. Next, 25 mL of Wijs reagent was added and the Erlenmeyer was sealed before being kept in a dark room for 30 minutes (shaking occasionally). After this incubation period, 10 mL of 15% potassium iodide (KI) solution and 100 mL of boiling distilled water were added. The mixture was titrated with 0.1 N sodium thiosulfate (Na₂S₂O₃) while maintaining constant

stirring until the blue colour disappeared. A 0.5 mL solution of 0.5% starch was used as an indicator. The blank solution was prepared in the same way as the sample determination, but the oil sample was replaced with chloroform/CCI. Iodine value ($\text{gI}_2/100\text{g}$) is expressed as the amount of iodine in grams that can be absorbed by 100 grams of oil [17].

$$BI = \frac{V(\text{blanko} - \text{sample}) \text{Na}_2\text{S}_2\text{O}_3 \times N \text{Na}_2\text{S}_2\text{O}_3 \times 12,69}{\text{sample weight}} \quad (2)$$

2.6 Analysis Free Fatty Acid

Soybean oil (28 g) is placed into an Erlenmeyer flask, to which 50 mL of hot neutral ethanol and 2 mL of Phenolphthalein solution as an indicator are added. It is then titrated using 0.1 N NaOH until a stable pink solution is formed and remains unchanged for 30 seconds. Stirring is carried out during the titration. The volume of NaOH obtained is used to calculate the percentage of fatty acids [18].

$$\% \text{ FFA} = \frac{V \text{ NaOH} \times N \text{ NaOH} \times \text{mol.wt. linoleic acid}}{\text{sample weight} \times 1000} \times 100\% \quad (3)$$

3 Result

3.1 Total Phenolic Content

Phenolic compounds constitute a large group of secondary metabolites in plants and are widely distributed in various organs of higher plants. They play essential roles in various physiological processes of plants [19]. Phenolic compounds are phytochemicals found in nearly all plant tissues and feature aromatic rings with one or more hydroxyl groups. They can act as antioxidants [20,21]. The regression equation obtained from the measurement of a standard gallic acid solution is $y = 0.0007x + 0.0194$ with an R-value of 0.9765. From these results, the total phenolic content in ethanol extract of salam leaves is determined to be $188,1905 \pm 3,30$ mg GAE/g, meaning that the total phenolic content in salam leaf ethanol extract is equivalent to 188,1905 mg of gallic acid. Previous research revealed that phenolic compounds found in salam leaf extract include phenols, polyphenols, flavonoids, tannins, eugenol, α -tocopherol, β -tocopherol, and pyrogallol [8–11,22].

Total phenolic content has a strong correlation with antioxidant activity, i.e. the higher the total phenolic content, the stronger the antioxidant activity. Antioxidant activity IC_{50} (inhibitor concentration 50%) is declared strong if the IC_{50} value is smaller [23,24]. The ability of phenolic compounds to act as antioxidants is related to their chemical structure. The molecular structure of phenolic compounds, particularly the number and position of hydroxyl groups on the aromatic ring and the substitution pattern of the aromatic ring, provides the ability to neutralize free radicals [25,26].

3.2 Antioxidant Activity

Antioxidants are chemical compounds that donate electrons to unpaired free radicals, thereby inhibiting, delaying, or slowing down the rate of lipid oxidation reactions [27,28]. Salam leaf extract contains polar compounds, namely phenolic compounds, so surfactants are used to enhance the dispersi and performance of salam leaf extract as an antioxidant. Surfactants have both polar and nonpolar functional groups within a single molecule.

D (Salam Leaf Extract); DS1 (0,8% Salam Leaf Extract + 1% Surfactant Sorbitan Monooleic); DS2 (0,8% Salam Leaf Extract + 3% Surfactant Sorbitan Monooleic); DS3 (0,8% Salam Leaf Extract + 5% Surfactant Sorbitan Monooleic).

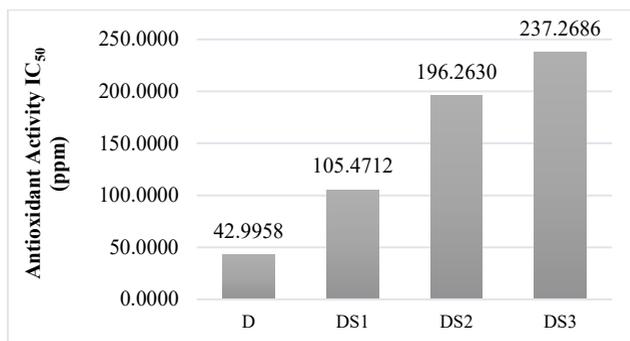


Fig 1. Antioxidant Activity IC₅₀ of Salam Leaf Extract with the Addition of Surfactant Sorbitan Monooleic.

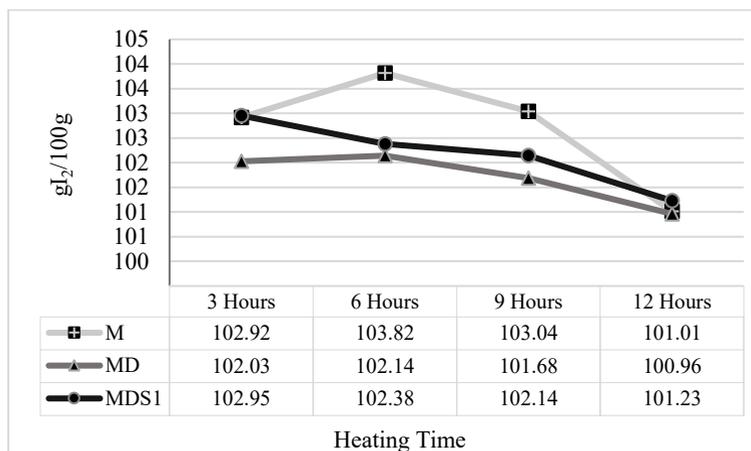
The parameter used to interpret the results of the DPPH method is IC₅₀ which is defined as the effective concentration of antioxidant required to reduce or eliminate the initial DPPH concentration by 50%. An antioxidant compound is said to be very strong if the IC₅₀ is less than 50 ppm, strong if the IC₅₀ value is between 50-100 ppm, moderate if the IC₅₀ value is between 100-150 ppm, and weak if the IC₅₀ value is between 150-200 ppm [15]. Figure 1. shows the antioxidant activity IC₅₀ of salam leaf extract (D), which is 42.9958 ppm (very strong category). The treatment of salam leaf extract with 1% sorbitan monooleate surfactant (DS1) has the highest IC₅₀ antioxidant activity, which is 105.4712 ppm (moderated category), while the treatment of salam leaf extract with 5% sorbitan monooleate surfactant (DS3) has the lowest IC₅₀ antioxidant activity, which is 237.2686 ppm (very weak category).

The results of antioxidant activity testing of salam leaf extract with the addition of sorbitan monooleate surfactant tend to show a decrease in efficiency in neutralizing free radicals as the concentration of the surfactant increases. These results suggest that antioxidant activity can be affected by the volume ratio between surfactant and antioxidant. Changes in the ratio between the two can alter the level of interaction between the surfactant and the antioxidant, and affect the access of the antioxidant to free radicals or oxygen involved in oxidative reactions (increased formation of a surfactant layer around the antioxidant molecules). There is a non-linear relationship between surfactant (lecithin) and emulsion stability where the amount of surfactant in the emulsion formation can affect the effective concentration of antioxidant (increased surfactant concentration) [29,30].

The test results indicate that the formulation of 0,8% salam leaf extract with 1% sorbitan monooleate surfactant has the strongest antioxidant activity compared to other treatments. Therefore, this formulation is used in soybean oil.

3.3 Iodine Value

The iodine number of an oil determines the quality of the unsaturated fatty acids in it; the higher the iodine number, the higher the unsaturated fatty acid content in the oil. Figure 2. shows the iodine values of soybean oil with different treatments. The treatment involving the addition of 0.8% salam leaf extract and 1% sorbitan monooleate surfactant (MDS1) has the highest iodine value compared to the treatment without the addition of salam leaf extract and sorbitan monooleate surfactant (M) and the treatment with the addition of 0.8% salam leaf extract without sorbitan monooleate surfactant (MD) during both the 3-hour and 12-hour heating periods. The average percentage of soybean oil deterioration based on iodine value in the M, MD, and MDS1 treatments was 0.63%, 0.35%, and 0.56%, respectively. Analysis of variance results for the iodine values show that the formulations for each treatment have a significant effect at a 5% confidence level. The Duncan post hoc test results showed significant differences among all treatments.



M (Control); *MD* (Soybean Oil + 0,8% Salam Leaf Extract); *MDS1* (Soybean Oil + 0,8% Salam Leaf Extract + 1% Surfactant Sorbitan Monooleic).

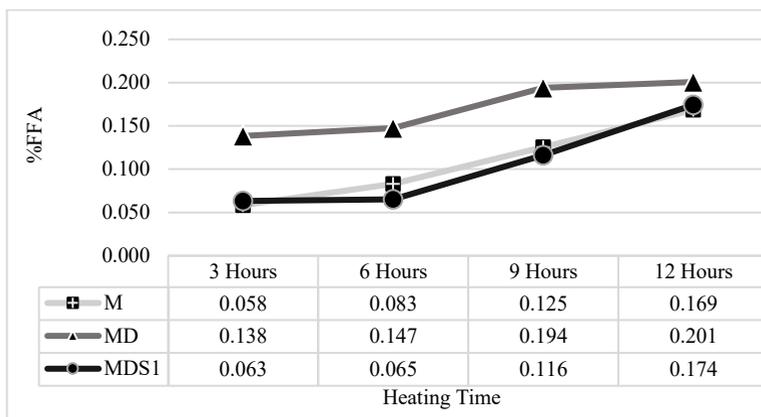
Fig 2. Graph of Average Iodine Value of Soybean Oil at Each Heating.

The result of iodine value analysis in MDS1 treatment indicates the antioxidant role of salam leaf extract and surfactant in preventing oxidation. Unsaturated fatty acids contain one or more double bonds. These bonds can coexist with weak carbon-hydrogen bonds, especially at the methylene group (CH₂) adjacent to the double bond. The weak carbon-hydrogen bonds have a low dissociation energy, making them easier to break during oxidation reactions. Therefore, the more double bonds there are, the greater the susceptibility to oxidation because there are more places for oxidation reactions. In this case, antioxidants contribute in transferring hydrogen atoms to radical species derived from lipid oxidation [31]. While surfactants play a role in improving the dispersion of extracts in soybean oil. The addition of surfactant has a positive effect in increasing the antioxidant effectiveness of salam leaf extract as an inhibitor of oxidation reaction compared to that without surfactant addition. The interaction between antioxidants and surfactant micelles may change the properties of antioxidants in terms of their solubility in emulsion systems [32].

The fluctuation in the iodine value of soybean oil from 3 hours of heating to 9 hours of heating does not significantly affect the differences in heating time. The presence of these fluctuations may be attributed to differences in the distribution of antioxidants within the oil. Dispersion and partitioning behavior in various regions of the emulsion (oil core, aqueous phase, interface) can influence the distribution of antioxidants [33].

3.4 Free Fatty Acid

Free fatty acids are hydrolysis products of the oxidation of oils and fats at high temperatures during heating [34]. An increase in free fatty acids indicates a decrease in the quality of cooking oil. Figure 3. shows the levels of free fatty acids in soybean oil for the MDS1 treatment compared to the MD treatment during heating from 3 hours to 12 hours. The results indicate that the MDS1 treatment has lower levels of free fatty acids compared to the MD treatment. This suggests that the rate of increase in free fatty acids in the MDS1 treatment is smaller than that in the MD treatment. Analysis of variance results for free fatty acids indicate that the formulations in each treatment have a significant effect at a 5% confidence level. Post-hoc Duncan tests reveal that the M and MDS1 treatments do not differ significantly from each other, while the MD treatment significantly differs from both the M and MDS1 treatments.



M (Control); *MD* (Soybean Oil + 0,8% Salam Leaf Extract); *MDS1* (Soybean Oil + 0,8% Salam Leaf Extract + 1% Surfactant Sorbitan Monooleic).

Fig 3. Graph of Average Free Fatty Acids in Soybean Oil at Each Heating.

The formation of free fatty acids in treatment M is due to the hydrolysis of triglycerides into monoglycerols, diglycerides, glicerols, and free fatty acids at high temperatures. The treatment in this study was heating for 12 hours, without frying (water from food ingredients) so that the formation of free fatty acids was relatively small. The high free fatty acids in the MD treatment may be influenced by the water content contained in the salam leaf extract which can trigger the hydrolysis reaction of triglycerides. While the MDS1 treatment, the water content cannot freely react with triglycerides due to the presence of surfactants. The presence of water can increase the possibility of oil hydrolysis reactions [35] that can contribute to an increase in the percentage of free fatty acids. Surfactants in W/O systems can play a role in regulating the interaction between the water phase and the oil phase. In the absence of surfactants, it leads to the formation of large oil droplets that are easily oxidised as only the surface part is exposed to antioxidants. The presence of surfactants is necessary to form a protective layer around the oil droplets [30,36] allowing for more even distribution and optimal antioxidant protection. This shows that the rate of increase in free fatty acids in the treatment with Sorbitan monooleate surfactant and salam leaf extract was less than the treatment with salam leaf extract without Sorbitan monooleate surfactant.

Free fatty acids can act as substrates for lipid oxidation reactions. Free fatty acids are more easily oxidised compared to other fatty acids because they are not bound in complex molecular structures such as triglycerides. The free carbon groups in free fatty acids react more easily with oxygen to form free radicals [37,38]. Free fatty acids act as pro-oxidants in vegetable oils because they have hydrophilic (carbonyl) and hydrophobic (hydrocarbon chain) groups in their structure. Carbonyl groups tend to gather on the surface of edible oils, reduce surface tension, and increase oxygen diffusion into the oil, resulting in accelerated oxidation of the oil [39].

4 Conclusion

In this study, the best formulation was based on the antioxidant activity of Salam leaf extract which was formulated with surfactant Sorbitan monooleic and the formulation of 0.8% Salam leaf extract and 1% surfactant was obtained as the best formulation with IC₅₀ antioxidant activity, namely 105.4712 ppm. The effectiveness of salam leaf extract in inhibiting oxidation reactions based on the parameters of iodine value and free fatty acids showed that the addition of Salam leaf extract with surfactant sorbitan monooleic gave better results than without the addition of surfactant sorbitan monooleate. This indicates that sorbitan monooleate surfactant

improves the dispersion of salam leaf extract in soybean oil and can play a role in reducing the interaction of soybean oil with oxygen, thereby reducing the oxidation of soybean oil.

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