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Sago Starch-Lipid Complex Formation under Varying Fatty Acid Concentrations for Emulsion Stability Improvement

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Abstract

The amount of fatty acids added is critical in creating starch-lipid complexes, which can affect their formation and properties. To use the complex to stabilize oil-in-water emulsion systems, this study intends to assess the effects of fatty acid concentrations on the effectiveness of starch-lipid complex formation and the properties of the resulting complexes. Ultrasonication was used to produce starch-lipid complexes from sago starch with different fatty acid contents (10, 12.5, 15, 17.5, and 20%) for the study. The results demonstrated that adding fatty acids up to 15% concentration significantly improved the water absorption capacity (WAC). In contrast, increasing the complexing index (CI), relative crystallinity (RC), oil absorption capacity (OAC), and contact angle of sago starch-lipid complexes. When the concentration of fatty acids was raised over 15%, the properties of the starch-lipid complex did not change considerably. A concentration of 15% with CI 67.07%, RC 37.94%, WAC 1.352 mL/g, OAC 1.370 mL/g, contact angle 70.82º, emulsion capacity 36.72%, and emulsion stability 35.80% was shown to be the optimal amount of fatty acids for the production of sago starch-lipid complex. Emulsion stability and capacity can be maintained using a sago starch-lipid complex with an ideal fatty acid concentration of 15%.

Keywords: Sago, starch-lipid complex, fatty acid, concentration, emulsion

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Introduction

Starch is a polysaccharide widely found in nature and has an essential role in the food industry, such as an energy source, thickener, and texturizer (BeMiller & Whistler, 2009). Sago starch (*Metroxylon sagu*), originating from Indonesia, has great potential due to its abundance and unique functional properties. However, like most other types of starch, sago starch also has limitations in some functional properties such as heat resistance, retrogradation, and viscosity stability that can change rapidly, making it suboptimal for specific industrial applications (Tethool *et al.*, 2012). Therefore, research on starch modification is important to improve functional characteristics that are suitable for application.

The form of complexes between fatty acids and starch is one type of modification that has been extensively researched (Feng *et al.*, 2018; Wang *et al.*, 2020). Amylose chains and fatty acid molecules interact to generate starch-lipid complexes, which have an inclusion structure shaped like a double helix (Wang *et al.*, 2020). According to Feng *et al.* (2018), these complexes can modify the physicochemical characteristics of starch and enhance its thermal stability, which qualifies it for a range of particular food or industrial product uses. In forming starch-lipid complexes, pregelatinization of starch is important because it will increase the release of amylose to the medium to facilitate the formation of complexes (Chao *et al.*, 2024). Tethool *et al.* (2025) reported that the ideal sago starch pregelatinization temperature for starch-lipid complex formation is 65 ^{o}C. At higher temperatures, amylose molecules from sago starch tend to interact with each other, thus

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inhibiting the formation of starch-lipid complexes.

Fatty acid concentration is one of the important factors that influence the efficiency of starch-fat complex formation (Wang *et al.*, 2020). Several studies have shown that increasing fatty acid concentration can enhance the degree of complex formation up to a certain optimal point, but beyond that point, complex formation tends to plateau or even decrease (Garcia *et al.*, 2016; Lee *et al.*, 2020; Tang & Copeland, 2007). Studies on starch-lipid complex formation have used fatty acids at concentrations between 1-7%, with the optimal point reached within the concentration range of 3-5%, depending on the type of starch and fatty acid used. However, the complex has not yet formed optimally in forming starch-lipid complexes from sago starch and decanoic acid using fatty acid concentrations <10%. Therefore, it is necessary to investigate the effect of higher fatty acid concentrations on forming sago starch-lipid complexes.

By understanding the effect of fatty acid concentration on sago starch-lipid complex formation, information that can be useful for controlling the functional properties of starch in food and non-food industry applications is expected to be obtained. One form of utilization of starch-lipid complexes is as emulsion stabilizers (Hong *et al.*, 2018; Lu *et al.*, 2020; Tethool *et al.*, 2025). Starch-lipid complexes formed through interactions between amylose chains and fatty acids or other lipids result in helical inclusion structures that are more amphiphilic (Feng *et al.*, 2018; Wang *et al.*, 2020). This amphiphilic nature allows the complex to interact with both phases in the emulsion, increasing the emulsion system's stability. Numerous studies have demonstrated that starch-lipid complexes increase emulsions' viscosity and thermal durability while delaying the phase separation process during storage (Hong *et al.*, 2018; Lu *et al.*, 2020; Tethool *et al.*, 2025).

This study assesses the effects of changes in fatty acid concentration on the formation efficiency of sago starch-lipid complexes and the effects on the complex's physicochemical properties, which can stabilize oil-in-water emulsion systems. A better knowledge of the properties and possibilities of these complexes will enable the development and use of efficient natural emulsion stabilizers across various industries.

Methods

Material

Sago starch (*Metroxylon sago*) was acquired from a marketplace in Manokwari, West Papua Province, Indonesia. Decanoic acid was used as a fatty acid to form starch-lipid complexes (Sigma Aldrich Corporation), RBD Palm Oil to make emulsion (PT. Bina Karya Prima, Bekasi, Indonesia), commercial OSA starch (Sigma Aldrich), and other reagents of analytical grade were used in this study.

Preparation of pregelatinized sago starch-decanoic acid complex by ultrasonication

The method for creating the sago starch-lipid (SSL) complex was adapted from the study by Tethool *et al.* (2025). Initially, a suspension of sago starch with 5% starch content was heated at 65°C for 10 minutes and dehydrated at 50°C for 24 hours to obtain pregelatinized sago starch. Subsequently, 6 g of this pregelatinized starch was dispersed in 60 mL of distilled water. Decanoic acid at varying concentrations (10, 12.5, 15, 17.5, and 20% w/w of pregelatinized sago starch) was dissolved in 40 mL of absolute ethanol and added to the starch suspension. The mixture was stirred for 30 minutes and underwent ultrasonication using an ultrasonicator (TF-900N, Tefic Biotech, China), at a power density of 450 W/cm2 with pulse intervals every 4 seconds for 15 minutes. The resulting SSL complex suspension was then allowed to reach ambient temperature and then subjected to centrifugation (DM0636, DLab, China) at 1500 x g for 15 minutes. The complex was washed with 100 mL of a solution containing 50% ethanol, subjected to centrifugation for 15 minutes at 1500 x g, and then dried at 50 °C before being ground for further analysis. The resulting starch-lipid complexes were then expressed as SSL-10, SSL-12.5, SSL-15, SSL-17.5, SSL-20, respectively, the result of complex formation with fatty acid concentrations of 10, 12.5, 15. 17.5, and 20%.

Complexing Index

With some minor adjustments, the SSL complexing index (CI) was calculated using the procedure of Kang *et al.* (2020) 20 mL of deionized water was used to dissolve 2.0 g of the SSL, which was then heated to 95 °C for 30 minutes. 25 mL of deionized water was combined with 5.0 g of the resultant paste, vortexed for two minutes, then centrifuged for fifteen minutes at 1700 x g. After centrifugation, 500 µL of the supernatant was added with 15 mL of distilled water and 2 mL of an iodine solution (2.0% KI and 1.3% I2 in deionized water). At a wavelength of 690 nm, the sample's absorbance was determined using a spectrophotometer (Genesys 10S, Thermo Scientific, USA). The CI value was calculated according to equation 1, where A_{starch} represents the absorbance of native starch and $A_{complex}$ represents the absorbance of the PSS-DA complex.

$$CI\ (\%) = (A_{starch} - A_{complex}/A_{starch})\ x\ 100 \quad (1)$$

Sago starch-lipid complex characteristic

An X-Ray Diffractometer (XRD Bruker D2, Phaser, Germany) was used to measure the relative crystallinity and perform X-ray diffraction analysis on the SSL complex. Contact angle determined according Dewi *et al.* (2022), using an optical drop tool with a digital microscope. 3% SSL suspension was heated at 90 ◦C for 10 minutes, then poured into a glass dish and dried (40 ◦C) for 10 hours. A total of 2 µL of distilled water was dripped onto the SSL surface; photos were taken 45 seconds after the water was dropped. The contact angle was determined using ImageJ software.

Water absorption capacity (WAC) and oil absorption capacity (OAC) were measured according to Dewi (2023). WAC was measured by scattering 5 g of SSL in 75 mL of distilled water, agitating the mixture for an hour, then centrifuging it for 10 minutes at 3000 rpm. The starch's final weight (precipitate) was determined after the supernatant's separation. Equation 2 was used to determine WAC, where *Ws* stands for the weight of the starch and *Wsw* for the weight of the starch following water absorption.

$$WAC\ (mL/g) = [(Ws - Wsw)/Ws] \quad (2)$$

In order to determine the oil absorption capacity (OAC), 5 g (db) of SSL was dissolved in 75 mL of oil. The solution was centrifuged for 10 minutes at 3000 rpm after being shaken for an hour. After the oil layer was decanted, the weight of the remaining starch (precipitate) was noted. Equation 3, where *Ws* is the original starch weight and *Wso* is the weight of starch after absorbing oil, was used to calculate the OAC.

$$OAC\ (mL/g) = [(Ws - Wso)/Ws] \quad (3)$$

Emulsion preparation, emulsion capacity and emulsion cream stability

Oil-in-water emulsions were created following the protocol described by Tethool *et al.* (2025). The ratio of water to oil used to create the emulsion is 8:2. Palm oil and distilled water were added with SSL complexes as much as 200 mg/mL of oil. An Ultraturrax homogeniser (T25, IKA) was then used to homogenise the mixture for 3 minutes at 10,000 rpm. The resultant emulsion was put into a 50 mL centrifuge tube and spun using a DM0636 centrifuge for 10 minutes at 3000 rpm. Commercial OSA starch (c-OSA starch) from potato was used as a comparator with SSL for emulsion stabilisation ability. Equation 4 was used to compute the emulsion capacity (EC).

$$EC\ (\%) = (height\ of\ emulsion\ layer/Total\ height)\ x\ 100 \quad (4)$$

Equation 5 was used to calculate the emulsion stability (ES) during 28 days of storage at room temperature.

$$ES\ (\%) = (height\ of\ remaining\ emulsion\ layer/Total\ height)\ x\ 100 \quad (5)$$

Statistical analysis

The obtained data is expressed using the mean and standard deviation. Any significant differences between the treatments were evaluated using Duncan's post-hoc test after a one-way ANOVA with a significance level of p = 0.05. All data were statistically analyzed using SPSS (IBM Statistics Version 25, Chicago, USA.

Results and Discussion

Effect of fatty acid concentration on sago starch-lipid complexing index

The complexing index (CI) value measures the success rate of amylose-fatty acid complex formation. A higher the CI value indicates that more fatty acids are trapped and form complexes with starch (Wang *et al.*, 2024). The effect of fatty acid concentration on the CI value of the sago starch lipid complex is shown in Figure 1. Increasing the fatty acid concentration to 15% increases the CI value to 67.07%. This increase indicates that fatty acids interact effectively with amylose molecules, enhancing stability and the formation of starch-lipid complexes (Li *et al.*, 2019). This interaction is enhanced by the hydrophobic nature of the long chains of fatty acids that can fit into the amylose helix, forming stable inclusion complexes (Zhang *et al.*, 2006). Increasing the fatty acid concentration to 17.5% decreases the CI value. This decrease is due to steric hindrance at excessive fatty acid concentrations, as fatty acid molecules tend to associate with each other rather than form starch-lipid complexes (Garcia *et al.*, 2016; Karkalas *et al.*, 1995; Li *et al.*, 2019). When the fatty acid concentration is increased to 20%, the CI value tends to increase again, likely due to the formation of new types of complexes or aggregates that can stabilize the system (Li *et al.*, 2019). Changes influence this condition in the physicochemical properties of the complex, such as increased solubility or changes in bond dynamics within the complex (El-Fakharany & Redwan, 2019). Overall, adding fatty acids at 15–20% concentrations showed no significant difference in CI values ($p<0.05$). Figure 1 shows that CI values at these concentrations exhibit a plateau condition. This condition indicates that there is an optimal concentration range for fatty acid addition in the formation of starch-lipid complexes (Wang *et al.*, 2020).

Effect of fatty acid concentration on properties of sago starch-lipid complex

The success of SSL formation and the crystalline type determined by X-ray diffraction (Kang *et al.*, 2020; Wang *et al.*, 2020). Figure 2 shows the X-ray diffraction patterns of native sago starch and SSL complexes generated from different amounts of fatty acids. A C-type crystalline pattern with peaks at diffraction angles of 5, 15, 17, 23, and 27 ° is typical of native sago starch, according to several previously published studies (Dewi *et al.*, 2023; Santoso *et al.*, 2021; Tethool *et al.*, 2025). The successful creation of SSL complexes was demonstrated by the change in crystal type from C-type to V-type that occurred when fatty acid was added to sago starch. 2θ diffraction peaks at 7.44, 12.92, 19.84, and 22.46° are characteristics of the type V crystal (Kang *et al.*, 2020; Liu *et al.*, 2024; Tethool *et al.*, 2025).

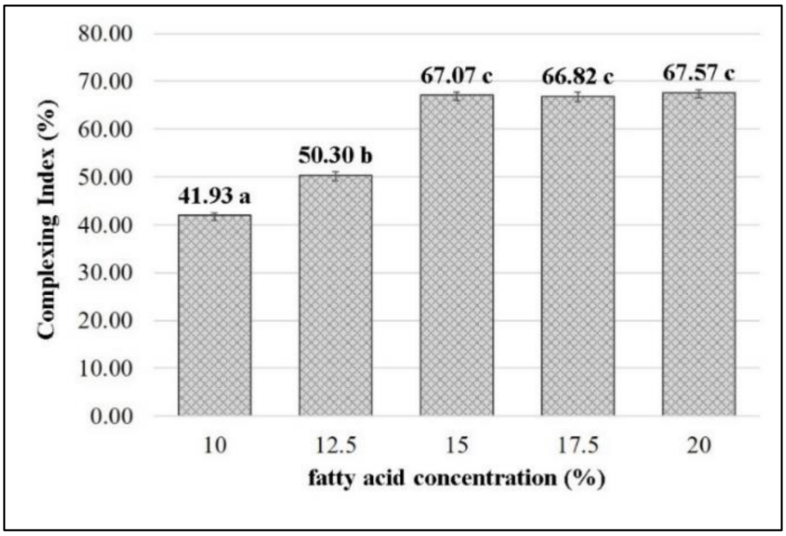

Figure 1. Effect of Fatty Acid Concentration on Sago Starch-Lipid Complexing Index

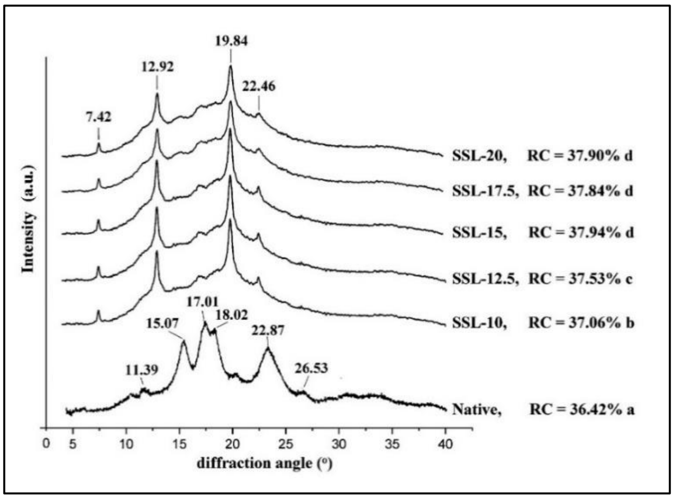

Figure 2. Effect of sago starch-lipid complex formation under varying fatty acid concentration on crystallinity

Native sago starch's relative crystallinity (RC) was 36.42%, similar to previous studies (Dewi *et al.*, 2022; Santoso *et al.*, 2021; Tethool *et al.*, 2025). SSL complex formation tends to produce higher crystallinity than native starch (Liu *et al.*, 2024). Increasing the concentration of fatty acids up to 15% significantly ($p<0.05$) increases crystallinity. This increase is related to the number of SSL complexes formed (CI value), where a higher CI value results in increased crystallinity (Tethool *et al.*, 2025). Adding fatty acid concentrations above 15% does not significantly increase the crystallinity of SSL complexes. This condition is caused by the fact that at excessive concentrations, fatty acids aggregate into micelles that do not contribute to the crystallinity of lipid starch complexes because they are not part of the SSL complex (Garcia *et al.*, 2016). Crystallinity is also related to emulsion stability. Therefore, SSL complexes with high crystallinity are expected to provide good emulsion stability.

A complex with amphiphilic properties must be used as an emulsion stabilizer (Yulianingsih & Gohtani, 2019). Therefore, the essential characteristics of modified starch for use as an emulsion stabilizer are water absorption capacity (WAC) and oil absorption capacity (OAC). The WAC and OAC properties can influence the ability of starch particles to absorb at the oil-water interface, thereby increasing emulsion stability (Farooq *et al.*, 2018; Roman *et al.*, 2022). Native sago starch has low oil-binding capacity, so the formation of SSL complexes is expected to increase OAC and decrease WAC, thereby altering the hydrophilic-hydrophobic balance. With this balance change, SSL complexes can stabilize emulsions by forming a protective layer around oil droplets, preventing coalescence and enhancing emulsion physical stability. The WAC and OAC of the resulting SSL complex are shown in Figure 3. Because native starch in intact granules tends to be insoluble in water, the SSL complex formation in Figure 3A results in a higher WAC than native sago starch. The SSL complex leaches the amylose portion from starch granules that interact with hydrophilic fatty acids. SSL complex can absorb more water as a result of this state (Feng *et al.*, 2018; Tethool *et al.*, 2025; Wang *et al.*, 2020). Meanwhile, WAC tended to drop significantly ($P<0.05$) as the concentration of fatty acids increased to 15%. According to the CI value, the more complexes that form, the more hydrophobic the characteristics become, which lowers the capacity to absorb water (Tethool *et al.*, 2025). When fatty acid concentration was increased to 15%, WAC tended to drop significantly ($P<0.05$). About the CI value, the hydrophobic characteristics of complexes will grow with their number, resulting in a decrease in their capacity to absorb water (Feng *et al.*, 2018; Tethool *et al.*, 2025; Wang *et al.*, 2020).

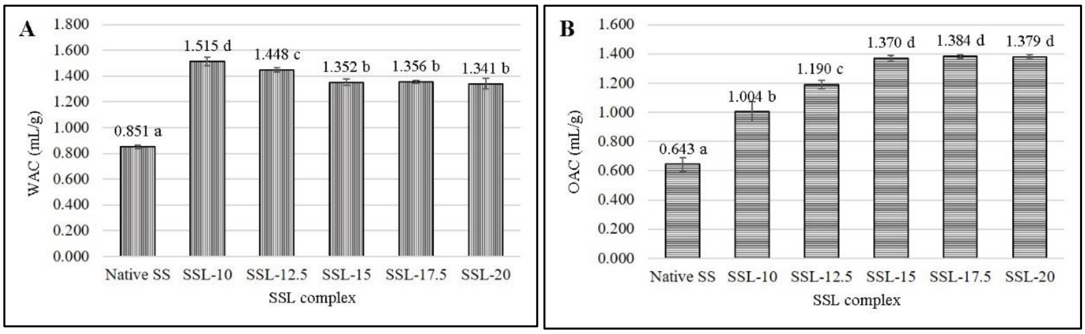

Figure 3. Effect of sago starch-lipid complex formation under varying fatty acid concentration on : A. water absorption capacity and B. oil absorption capacity

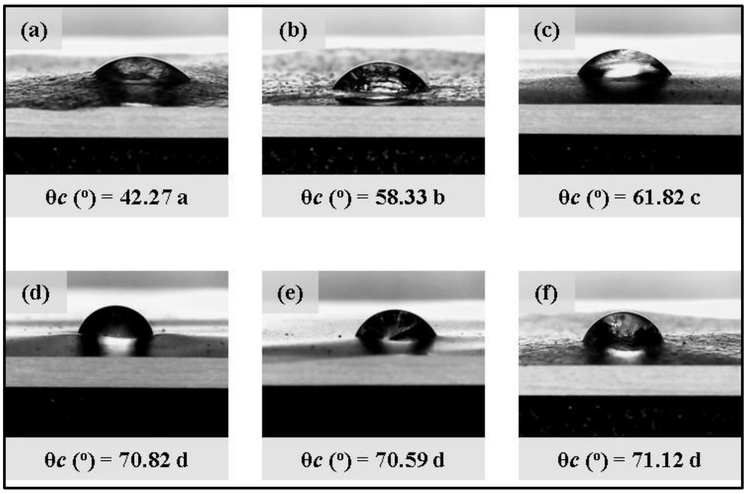

Figure 4. Effect of sago starch-lipid complex formation under varying fatty acid concentration on contact angle. (a).Native sago starch; (b) SSL-10; (c) SSL-12.5; (d) SSL-15; (e) SSL-17.5; (f) SSL-20

Figure 4 compares the contact angle of native sago starch with that of an SSL complex. Contact angle illustrates the hydrophobicity of starch (Yang *et al.*, 2017). Increasing the fatty acid concentration to 15% significantly increased the contact angle. This increase in contact angle is related to the presence of hydrophobic cavities in the complex that inhibit water absorption (Liu *et al.*, 2017; Majzoobi *et al.*, 2016). This condition is consistent with the decreased WAC and increased OAC properties (Figure 2). According to Yang *et al.* (2017), contact angles in the 60 °- 90 ° range are suitable for application to oil-in-water emulsions. The SSL product created with fatty acids at a concentration of 12.5% or more meets the Yang *et al.* (2017) criteria, so it can be used to stabilise oil-in-water emulsions.

Effect of of sago starch-lipid complex on emulsion capacity and stability

The effect of using SSL as a stabiliser on emulsion capacity (EC) and emulsion stability (ES), compared to c-OSA starch, is shown in Table 1. According to the results, the EC of the emulsion containing SSL was lower (30.25–36.60%) than that with c-OSA starch (42.99%). Increasing the amount of fatty acids in SSL formation to 15% concentration increased the EC significantly ($p<0.05$) to 36.72%. At higher concentrations (15-20%), EC values tended to be constant (not significantly different). This condition is closely related to the CI value, crystallinity, WAC, OAC, and contact angle produced. Higher CI values correlate with increased emulsion cream formation (Tethool *et al.*, 2025). The enhanced emulsion cream formation by SSL complexes is due to the interaction of the hydrophobic tails of fatty acids and amylose, resulting in hydrophobic helical cavities that facilitate the entrapment of oil within the emulsion cream (Akbari & Nour, 2018; Feng *et al.*, 2018).

Table 1. Effect sago starch-lipid complex formation under varying fatty acid concentration as a stabilizer on emulsion capacity and stability

	Emulsion Capacity (%)	**Emulsion Stability (%)**
SSL-10	30.25 ± 0.255 $^{a\ B}$	28.21 ± 0.347 $^{a\ A}$
SSL-12.5	33.83 ± 0.226 $^{b\ B}$	31.68 ± 0.509 $^{b\ A}$
SSL-15	36.72 ± 0.212 $^{c\ A}$	35.80 ± 0.212 $^{c\ A}$
SSL-17.5	36.38 ± 0.233 $^{c\ A}$	35.62 ± 0.116 $^{c\ A}$
SSL-20	36.60 ± 0.257 $^{c\ A}$	35.83 ± 0.221 $^{c\ A}$
c-OSA starch	42.99 ± 0.494 $^{d\ A}$	41.65 ± 0.208 $^{d\ A}$

* The mean ± SD is used to display the results. In the same column, lowercase superscripts indicate significant differences ($p < 0.05$) as determined by ANOVA and Duncan's test. According to the t-test, significant differences ($p < 0.05$) between EC and ES are shown by uppercase superscripts.

ES values were evaluated after storage under environmental conditions in 25 ^{o}C for 28 days. Emulsions exhibit inherent instability due to their thermodynamics, making them prone to phase separation and evolving. One of the factors affecting ES is interfacial tension, which can be managed by incorporating surfactants or other additives (Akbari & Nour, 2018). Table 1 shows that increasing the fatty acid concentration up to a concentration of 15% can increase the ES value, but the ES value is not significantly different at concentrations above that. Comparison of EC and ES values showed a significant decrease in emulsion stability at 10% and 12.5% fatty acid concentrations. However, at concentrations of 15% or more, the decrease in emulsion stability was not significant ($P<0.05$). This indicates that the use of SSL complex can maintain the stability of emulsified cream during storage. During the emulsion formation process, the SSL complex can absorb oil droplets and create an interfacial layer on the o/w emulsion that inhibits the aggregation of these oil droplets so as to maintain emulsion stability (Hong *et al.*, 2015, 2018). The SSL complex may find application as an oil-in-water emulsion stabilizer, according to the study's findings.

Conclusion

Increasing the amount of fatty acids up to 15% concentration in the formation of sago starch-lipid complexes significantly increased CI, crystallinity, OAC, and contact angle values, and decreased WAC. Increasing the fatty acid concentration above 15% resulted in starch-lipid complex characteristics that were not significantly different. The ideal condition for the amount of fatty acids to form sago starch-lipid complexes was obtained at a concentration of 15%. Using sago starch-lipid complex with an ideal fatty acid concentration can increase emulsion capacity and maintain emulsion stability. The shortcomings of the resulting starch-lipid complex are that it still has a lower emulsion capacity than OSA-modified starch, so further studies still need to be done to improve it and evaluate the stability of the natural emulsion over a more extended period.

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