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ULTRASONIC PREPARATION OF OLIVE OIL PICKERING EMULSION STABILIZED BY MODIFIED STARCH

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ABSTRACT

Background. In order to obtain a stable emulsion system for food products fortified by bioactive compounds, it is necessary to add an emulsifier and stabilizer. Because food has a close relationship with health, it would be better if the emulsifier and stabilizer came from natural ingredients. One natural compound which acts as a stabilizer is polysaccharides. This research involved an investigation of the stabilization of oil in water emulsion using modified polysaccharides and its complex with a surfactant.

Materials and methods. Food grade extra virgin olive oil was used as the oil phase. Pickering emulsion was prepared by adding starch particles as an emulsion stabilizer. Corn starch was used as the starch source and modified using gelatinization methods without any addition of chemicals. As for the polysaccharide-surfactant complex, the modified starch was combined with a food-grade nonionic surfactant – Tween-80. **Results.** The results show that the addition of the stabilizer did not have any specific impact on the density or viscosity of the emulsion product. However, it somehow helped to stabilise the emulsion, as proven by the

turbidity profile and the microscopic morphology investigation. **Conclusion.** Stable olive oil Pickering emulsions were successfully prepared with the assistance of an ultrasonic homogenizer and a modified corn starch-Tween 80 combination as a stabilizer.

Keywords: polysaccharides-surfactant complex, corn starch, Tween 80, emulsion stability

INTRODUCTION

The demand for natural-based supplements and active compound fortified food, enhanced with ingredients such as antioxidants, minerals, vitamins, and active compounds found in essential oils, in the market industry just keeps on growing (Guedes Silva et al., 2021). This phenomenon has encouraged the improvement of research aimed at finding an effective technology to produce supplements or food fortified by active compounds. However, it is known that fortifying products with active compounds is not an easy thing to do due to the unstable characteristics of the active compounds. Bioactive ingredients are sensitive to processing and storage conditions, presenting low stability and solubility, light and pH sensitivity, and poor bioavailability (Han et al., 2022; Yan et al., 2020).

Problems arise not only in the pharmaceutical industry but also in the food industry. The food system is a complex system containing various components

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which do not always mix well with each other. Mixtures of oils or fats and water-based ingredients are very common, and to stabilise the system, certain appropriate additives and techniques are needed, as well as methods to preserve the active compounds.

An emulsion is a dispersion or suspension of liquid matter in another liquid that does not mix under normal conditions. Oil in water (O/W) emulsions are widely known and used in food products, medicines, cosmetics, detergents, and the pharmaceutical industry. Many natural and processed foods such as milk, salad dressings, ice cream, soft drinks, and cakes are partially or exclusively made of emulsions (Luo et al., 2022). The principle of making emulsions involves mixing or homogenization without high temperatures over a relatively short time, so that the active components tend to be stable and unchanged or undamaged (Campolo et al., 2020; Xu et al., 2020). These droplets are widely used for encapsulation, preservation, and release of non-polar active ingredients such as flavours, vitamins, nutrients, antioxidants, and antimicrobials. Most emulsion is thermodynamically unstable, including oil-in-water emulsion. The first attempt to stabilise an emulsion is by reducing the chance for the droplets to aggregate with each other. The common ways are by reducing the size of the disperse phase of the droplets or by adding an emulsifier. To produce a fine, stable emulsion, highenergy methods such as high-pressure homogenization, high-speed homogenization, microfluidizationer, and ultrasound-induced emulsification are introduced (Zhou et al., 2021). The ultrasound induced process gets more attention due to it being an eco-friendly and energy-efficient process (Taha et al., 2020). The previous research has reported that ultrasonic emulsification offers several potential benefits to emulsions, such as creating a small droplet size and a narrow size distribution of emulsion, long-term stability of the emulsion, low-energy consumption, cleanness, and ease of control (Zhou et al., 2021). Considering the advantages and superior character of the ultrasonic induced emulsification process, this study was conducted by applying an ultrasound wave to produce a fine emulsion.

Nevertheless, to produce good commercial products, the addition of supporting materials that can withstand the environmental conditions is necessary, such as stabilizers, emulsifiers, thickening agents, and gelling agents (Benitez et al., 2020). Synthetic emulsifiers are commonly used in the production of emulsions, such as tweens, sodium dodecyl sulfate, and spans (McClements and Gumus, 2016). However, a high demand for more natural-based food products is common nowadays. Previous research has studied the application of natural-based emulsifiers in the emulsification process. Various food-grade polysaccharides have been utilized as stabilizers in emulsion systems, such as starch, carrageenan, pectin, and so on (Guo et al., 2021).

Starch is easy to find, abundant, biodegradable, and generally seen as a food-grade compound. Along with the application of starch as a stabilizer in the emulsification process, modification as a pretreatment is also necessary. Modification is normally conducted to obtain the hydrophobic part which exhibits interfacial activity (Gómez-Luría et al., 2019; Song et al., 2015). Some methods are conducted to modify the native nature of starch, such as octenyl succinic anhydride (OSA) modification (Agama-Acevedo and Bello-Perez, 2017), heat treatment (Cui et al., 2020), hydrolysis (Li et al., 2012), pre-gelatination, and gelatination (Gómez-Luría et al., 2019). Modified starch hydrocolloids have also been considered for imparting the desired functional properties to the emulsification application.

Polysaccharides from fucoidan were utilized to make fish oil emulsion (oil) with a water-based solvent that can be adjusted when consumed (Chang et al., 2016). Polysaccharides from fucoidan can inhibit the isoelectric aggregation of droplets and increase the lipid surface available for lipase molecules. Soy soluble polysaccharides (SSPS), gum arabic (GA), and octenyl succinate starch (OSA-S) in vegetable oil emulsions in water were used as hydrocolloid emulsifiers (Chivero et al., 2016). Soy soluble polysaccharides, GA, and OSA-S emulsified the system in the form of mayonnaise, but the emulsions cannot be stable if the amount of oil in the emulsion reaches 60% by weight. Other studies have also prepared polysaccharides from Acacia Senegal gum and Acacia gum as emulsion stabilizers on canola oil in water emulsion under acidic conditions (Hou et al., 2017). An emulsion of soybean oil and beta-carotene in a water-based solvent has also been prepared using *Ulva fasciata* polysaccharides as a stabilizer. Its stability was comparable to the gum arabic and the beet pectin stabilized emulsions (Shao et al., 2017).

On the other hand, conventional ways to produce emulsions using the addition of a surfactant have also Nafiunisa, A., Aryanti, N., Wardhani, D. H. (2022). Ultrasonic preparation of olive oil pickering emulsion stabilized by modified starch. Acta Sci. Pol. Technol. Aliment., 21(2), 183–194. http://dx.doi.org/10.17306/J.AFS.2022.1031



Fig. 1. Mechanism illustration of oil in water emulsion stabilized by: \mathbf{a} – polysaccharides, \mathbf{b} – complex of polysaccharides-surfactant

been considered. A surfactant is a substance that can significantly change the interface state of the solution system when a small amount of it is added. In the food industry, Tween 80, Tween 40, Tween 20, Span 80, polyglycerol polyricinoleate, and lecithin are the widely used surfactants (Taha et al., 2020). Physical forces such as electrostatic attraction, hydrogen bonding, and hydrophobic interactions usually hold the complexes of surfactants and polysaccharides together in stabilizing the emulsion, but they can also be held together by covalent bonds (Wang et al., 2019). Therefore, the procedure for applying the complex of polysaccharides and surfactants has a good chance of better stabilizing the emulsion. The mechanism illustration of the stabilization of an emulsion using polysaccharides and a complex of polysaccharides-surfactants is shown in Figure 1. In the process that combines a surfactant and natural polymer such as starch, the role of each moiety is still unknown. Thus, this study focused on the investigation of the physicochemical properties and the stability of the oil in water emulsion stabilized by gelatinization-modified starch and a combination of gelatinization-modified starch and surfactant. Native cornstarch was used as the source of polysaccharides and nonionic food-grade Tween-80 was used as the surfactant. The quality of the emulsion was examined based on density, viscosity, turbidity, and droplet morphology over time.

MATERIALS AND METHODS

Materials

The starch samples used in this study were non-chemically modified native corn starch of the local brand. Food grade extra virgin olive oil (100% pure, Bertolli, Enrico-Glasbest, Germany) was used as the oil phase (dispersed phase). Tween-80 purchased from the local chemical distributor was used as the surfactant. And deionized water was used as the continuous phase in all experiments.

Modification of the starch

Cornstarch was modified using gelatinization methods without any addition of chemicals (Gómez-Luría et al., 2019). Water dispersions of different starches (5.0 g/100 g) were gently stirred (250 rpm) at room temperature for 30 min for hydration and then heated in a water bath at 90°C for 20 min to fully gelatinize. Gelatinized starch dispersions (GSDs) were cooled at room temperature for 1 hour. Thereafter, GSD was centrifuged at 4000 rpm, at 25°C for 30 minutes to obtain two starch fractions: (1) precipitate (residue, rich in insoluble amylopectin) and (2) supernatant (continuous phase, rich in amylose). The precipitated fraction was discarded, while the supernatant was used to make an oil-in-water (O/W) emulsion. The solution was then thinly spread onto a flat stainless steel tray and dried at 40°C for 12 hours, the dry, solid, flat sheet was ground using a coffee grinder and sieved to obtain 100-mesh powder.

Preparation of the fine emulsion

The emulsion was prepared using a two-step procedure. The first step involved the preparation of a coarse emulsion using a homogenizer (IKA Labourtechnik), followed by the construction of the fine emulsion. The coarse emulsion was prepared by blending the stabilizer, oil phase, and water phase with and without surfactant. The emulsion stabilized using modified starch was prepared by diluting an amount of GSD powder (4%, 5%, 6% w/w) in distilled water without any addition of a surfactant. As for the emulsion stabilized using a complex of modified starch and surfactant, 5% w/w of GSD powder was diluted in distilled water, before an amount of surfactant (1%, 2%, 3% w/w) was added. After the stabilizer had fully dissolved in the water phase, olive oil was poured dropwise into the solution under slow mixing using a stirrer to reach an oil to water ratio of 2:8. Then, the solution was placed in a water bath at 25°C for 30 minutes. The mixture was homogenized using an Ultra Turrax T 25 IKA Labourtechnik (probe S 10N-10G) at 11 000 rpm for 2 min. The fine emulsion was arranged using ultrasonication on the samples at 160 W of normal power, at 20 kH for 2 min following the homogenization process by mechanical stirring. The emulsions were stored in a closed 15 ml falcon tube and placed in a tube holder to hold the tube vertically.

Emulsion characterization

Density and viscosity. Density was measured using a pycnometer. The empty pycnometer was weighed and the result was recorded (m_0) . Next, the pycnometer was filled with a liquid sample and reweighed (m_1) . The density of the liquid was obtained using the following equation 1:

Liquid density,
$$\rho = \frac{m_1 - m_0}{V_{\text{pycnometer}}}$$
 (1)

The density measurements were carried out in triplicate on each sample and the sample density was presented as the average of the triplicate experiments. All analyses were conducted at room temperature ($\pm 25^{\circ}$ C) and normal pressure (1 atm).

The viscosity of the freshly made emulsion and the emulsion after 30 days of storage was measured using a digital viscometer (RVDV-II+ Brookfield Engineering Laboratories Inc., USA). Along with the analysis, the temperature of the samples was kept at room temperature $\pm 25^{\circ}$ C. As for the equipment adjustment, LV#61 spindle was selected for all samples and the revolution rate was controlled at 60 rpm. To obtain accurate data, the torque reading limits were between 5% and 85%. The value of viscosity was recorded in C_p .

Turbidity measurement. Turbidity was calculated after determining the optical density absorbance of the

emulsion using a spectrophotometer MRC Spectro V--11D at 600 nm. The turbidity was obtained using the following equation 2 (Hien and Dao, 2021):

Turbidity, cm⁻¹ =
$$\frac{2.303 \cdot ABS}{L}$$
 (2)

where:

ABS – the absorbance measured using a UV-Vis spectrophotometer,

L(1 cm) – the scattering path length.

The value of turbidity was measured for 30 days at a certain time interval to determine the stability profile of the emulsion. Moreover, the sample was taken from the bottom layer of the emulsion in the same position for each batch of analysis, as presented in Figure 2.



Fig. 2. Simple schematic of emulsion storage for turbidity test

Microscopic visualization. Microscopic visualization of the emulsion was performed by pouring the sample onto microscope slides which were then covered with glass coverslips and observed using a biological optical microscope by SINHER model XSZ-107, connected with digital camera eyepieces (HDCE-30C) at a magnification of 10^{\times} eyepiece lens and 40^{\times} objective lens. As for the microscopic sample, the emulsion was taken from the upper part of the solution and a proper 15 times dilution was conducted before each batch of analysis.

RESULTS AND DISCUSSION

Density and viscosity characteristics of the modified starch-stabilized emulsion

The physical properties of the prepared emulsion were stabilized using modified corn starch, and the combination of modified corn starch and surfactant was conducted using the analysis of the density and viscosity of the solution. The profile of the density and viscosity of the emulsion solution is presented in Table 1. The profile of the physical properties was studied at the initial time of emulsion preparation and after a specific storage time.

Table 1 shows that the density of the generated emulsion did not show any significant difference from the initial density of the continuous phase. This is commonly found in low-disperse oil concentration O/W emulsions (Mirhosseini et al., 2008; Tadros et al., 2009). A previous study on the characteristics of orange beverage emulsion reported that the density alteration shows significant results with a high orange oil to water ratio (Mirhosseini et al., 2008). The present study was conducted on low oil concentrations at the ratio of oil to water of 2:8 weight/weight. These results only had a slight difference in density between the continuous phase and the final emulsion product. The slight increase in the final emulsion solution was predicted to be the result of the addition of gelatinized corn starch. Gelatinized corn starch was a modified starch with a high content of amylose, which possesses a high molecular density, thus giving the emulsion an increase in the density.

Table 1. Density and viscosity of the emulsion stabilized by various emulsifiers

Stabilizer	Amount	Density, g/ml		Viscosity, C_p	
		initial	final	initial	final
Gelatinized corn-starch	4	1.006	0.982	1.137	0.963
	5	1.007	0.983	1.152	0.979
	6	1.015	0.996	1.179	1.013
Gelatinized corn-starch and Tween-80	1	1.006	0.998	1.153	0.948
	2	1.008	0.998	1.179	0.973
	3	1.016	0.999	1.214	1.012

Weighting agents increase the density of the oil phase and therefore contribute to the stabilization of an emulsion through minimization of the difference in density between the different phases (Linke and Drusch, 2016). Moreover, the increase in the concentration of gelatinized-starch stabilizer also gives an increase in the density of the emulsion, even after storage, and the emulsion with a higher concentration of stabilizers was denser than the others. After storage for 30 days, the emulsion product showed a decrease in density and returned to a similar density to the continuous phase. This phenomenon is explained by the fact that along with the analysis, the emulsion part taken for sampling was on the middle layer, which is expected to be the continuous phase (water) as the oil droplet was expected to be gravitationally separated in the top layer while the separated starch settled in the bottom layer. A similar phenomenon was also found in the emulsion prepared using a complex of gelatinized starch and surfactant.

The measurement of viscosity is essential in identifying the quality of the emulsion as Stoke's law describes that the stability of an emulsion is dependent on the density of both the disperse and continuous phases, the radius of the particles (droplets), and the viscosity of the medium. The addition of gelatinized modified starch as the stabilizer in the emulsion system was one of the attempts to increase the viscosity of the solution. A previous study has discussed that an increase in viscosity was able to prevent the coalescence of the droplet particles due to the reduction in shear rates between the particles (Albano and Nicoletti, 2018). Table 1 shows that the viscosity of the emulsion increased with an increase in gelatinized-starch concentration and surfactant concentration. The addition of a stabilizer and surfactant gave the emulsion more space to be able to produce more droplets during the process. A previous study shows that the emulsion viscosity increases linearly with increasing droplet concentrations, and the viscosity increase is steeper at higher droplet concentrations (McClements, 2004). In general, the viscosity of the emulsion solution is proportional to the viscosity of the continuous phase. Accordingly, any alteration in the rheology of the continuous phase, such as the addition of a stabilizer, will influence the rheology of the whole emulsion solution (Benitez et al., 2020; McClements and Gumus, 2016).

In the present work, the apparent viscosity of the studied O/W emulsions increased as the concentration of the stabilizer increased at a fixed ratio of oil to water. This was expected due to the addition of solid content (polysaccharides in the form of gelatinized modified starch) present in the continuous phase which created a barrier between the droplet, resulting in fewer shear rates between the particles in the emulsion solution.

The viscosity of the emulsion was affected by the amount and condition of the droplets during the continuous phase. The viscosity of emulsions with a high concentration of droplets is the result of the balance between the interaction of colloids, hydrodynamic effects, and Brownian motion (McClements, 2004). Fresh emulsions tend to have more droplets of a smaller size compared to the emulsion after storage time. The flocs of droplets made the solution have low shear rates (Tadros, 2010), in which each particle had a random distribution because of its Brownian motion. In this condition, the hydrodynamic forces are not strong enough to disrupt the bonds holding the particles together, resulting in a rigid floc and relatively high viscosity (Benitez et al., 2020). As the storage time increases, the droplets start to disrupt and the shear rate increases, and the hydrodynamic forces become strong enough to disrupt and deform the flocs of droplets. Consequently, the solution becomes less resistant to fluid flow, which causes a decrease in emulsion viscosity. The viscosity reaches a constant value at high shear rates, either because all of the flocs of droplets are completely disrupted so that only individual droplets remain, or because the droplets are entirely flocculated into the oil phase again (McClements et al., 2017). After storage time, the viscosity of the emulsion decreases due to the increase in oil droplet flocculation. For the same reason, with the increase in viscosity due to the decrease in shear rates after the addition of a stabilizer, the shear rates between the particles increase as the droplets start to aggregate with each other again, resulting in a decrease in viscosity.

Turbidity characteristics of the modified starch-stabilized emulsion

Figure 3a–3c shows the impact of the composition of the polysaccharide stabilizer in the continuous phase against the turbidity in the diluted state.

Figure 4a-4c describes the addition of the surfactant



Fig. 3. Profile of turbidity of the emulsion over time for the emulsion stabilized by gelatinized-starch at concentrations of: $\mathbf{a} - 4\%$, $\mathbf{b} - 5\%$, $\mathbf{c} - 6\%$



Fig. 4. Profile of turbidity of the emulsion over time for the emulsion stabilized by a complex of gelatinized-starch and surfactant at surfactant concentrations of: $\mathbf{a} - 1\%$ weight, $\mathbf{b} - 2\%$ weight, $\mathbf{c} - 3\%$ weight

complex to the emulsion stabilized by gelatinized starch over time. For all variables, the turbidity increased as the concentration of the stabilizer increased. The degree of turbidity is an important parameter reflecting the quality of a cloudy emulsion solution (Kolniak-Ostek et al., 2013). Clouds are concentrated oil-in-water emulsions in which the turbidity results from the scattering of light by dispersed oil droplets with oil droplet sizes between 0.5 µm and 5 µm (Tan, 1998). In this context, the scattering of light is governed by the size and concentration of the dispersed phase, the ratio of refractive index (RI) between disperse and continuous phase, and the wavelength and angle of the incident light (Linke and Drusch, 2016). The physical background of the scattering of light was reviewed extensively in a previous study in which turbidity measurement was mainly used as a tool to characterize clouding agents and cloud stability.

In the present study, an increase in turbidity was found as the concentration of emulsifiers and stabilizers increased. The differences in turbidity at higher dosages can be explained by the excess emulsifier as scattering happens in every suspension because each molecule acts as a scattering centre (Kaltsa et al., 2013). Also, an excess emulsifier that forms micelles or aggregates in the continuous phase shows scattering and its impact is, therefore, more pronounced at higher concentrations. This can be derived from the chemical structure of the emulsifiers used in the present study.

Figure 3a-3c and Figure 4a-4c also show the profile of turbidity changes over time of storage, in which the results show that the turbidity of the emulsion showed a rapid decline over time for all variables of stabilizer concentration, both for the gelatinized-starch stabilizer and for the complex of gelatinized-starch and surfactant stabilizer. The turbidity measurement is a simple and inexpensive method of determining the stability of an emulsion. It represents an indirect method for the evaluation of emulsion stability by correlating the particle size distribution and the turbidity of colloidal systems (Alade et al., 2021). Previous researchers have reported the accuracy of this method compared with other techniques. Their studies found the turbidimetric method to be an efficient technique for characterizing emulsion stability. Previous research presented the theoretical and experimental turbidimetric evaluation of the stability of an acoustically prepared paraffin oil-in-water emulsion (Reddy and

Fogler, 1981), and the O/W emulsion of a heavy oil sample (Alade et al., 2021). Generally, a high turbidity of the emulsion blends indicated a high charge density and strong interaction among the polyelectrolytes, although macroscopic phase separation did not occur (Albano and Nicoletti, 2018). On the other hand, in the samples where a super-fine emulsion was observed, fewer turbidity samples indicated higher homogeneity and smaller complex sizes. Previous research also shows that a nano-emulsion of Szechuan pepper oil showed an increase in turbidity over time, and the relative size of the nanodroplets was in the range of 120-250 nm in diameter, showing very small droplet size (Shi et al., 2022). However, in this study, the droplets of the emulsion were not small enough to allow the light to pass through. The light was expected to be reflected by the droplets, resulting in a higher turbidity level at the initial investigation. In the case of the present study, where the turbidity decreased over time, the sample taken from the bottom layer of the emulsion was in the same position for all of the variables. It was expected that the bottom part of the emulsion would be more prominent by the continuous phase (water), resulting in a more transparent solution with lower turbidity. Similar results were found in a previous study where the emulsion oil droplets were in the range of 1.28–26.45 µm (Alade et al., 2021).

Microscopic visualization of the modified starch-stabilized emulsion

Figures 5 and 6 depict the microscopic morphology of the emulsion stabilized with gelatinized modified starch and a complex of gelatinized modified starch and surfactant respectively. The investigation was conducted for the freshly prepared emulsion and the emulsion after a storage time of 30 days. All of the figures were taken at the same magnification of 40 times objective lenses and 10 times ocular lenses.

The emulsion systems are packed with small-dispersed droplets in the continuous phase, which shows the characteristics of a stable emulsion. A previous study confirmed that ultrasonic wave-induced emulsification achieved a higher droplet size reduction and decreased the creaming index, resulting in an improvement in emulsion stability compared to other emulsion homogenization methods (Ferreira et al., 2016). Positive effects of ultrasonic waves on the reduction in the droplet size and the creaming index were also found in other research for producing emulsions prepared with soy protein isolate and sodium alginate (Albano and Nicoletti, 2018). The population of droplets increased along with the increase in oil content with relatively small droplets. Moreover, ultrasound-assisted emulsification generated very stable emulsions, even in low concentrations of surfactant (Kaltsa et al., 2013). Figures 5 and 6 also show the changes in the emulsion after a storage time of 30 days. It can be seen that the emulsion shows a different characteristic in which the droplets of oil in water grew in size and showed an increase in size dispersity. This phenomenon indicates that the droplet has been changed and shows signs of a growth in size. The addition of a surfactant into the polysaccharides stabilized the emulsion, allowing better results where the emulsion has smaller oil droplets. Figure 6 shows that the emulsion stabilized by a complex of surfactant and polysaccharides (modified starch) had smaller and more tightly packed droplets compared to the emulsion stabilized only by polysaccharides. Surfactants have the ability to adsorb on the surface of oil droplets and increase the repulsive interactions, thus preventing droplet coalescence and providing stable emulsions. Non-ionic surfactants such as Tween-80 promote interaction with the polysaccharide stabilizer through a steric stabilization mechanism, allowing better formation of oil droplets (Zhang et al., 2020). A previous study reported that small variations in a surfactant molecule can significantly affect the intermolecular interaction and association of the surfactant-polysaccharide complex (Grant et al., 2008).

CONCLUSION

The application of gelatinization methods to modified corn starch as a stabilizer of an emulsion was successfully conducted. The modified starch also showed good compatibility when combined with a polysaccharide-surfactant complex. The addition of modified starch from 4–6% weight and Tween-80 as a surfactant (1–3% weight) did not lead to a significant increase in density and viscosity. Both emulsion systems showed no significant effects on the physical properties of the emulsion solution, even after 30 days of storage. The density and viscosity of the emulsions only showed an average decrease of 1.68% and 16.05%, respectively, Nafiunisa, A., Aryanti, N., Wardhani, D. H. (2022). Ultrasonic preparation of olive oil pickering emulsion stabilized by modified starch. Acta Sci. Pol. Technol. Aliment., 21(2), 183–194. http://dx.doi.org/10.17306/J.AFS.2022.1031



Fig. 5. Microscopic morphology of the oil droplets of the emulsion stabilized by gelatinized-modified starch at concentrations of: $\mathbf{a} - 4\%$, $\mathbf{b} - 5\%$, $\mathbf{c} - 6\%$

for all variables. The turbidity analysis showed that the emulsion had an instability, which was proved by the decrease in the turbidity of the emulsion's bottom layer. The emulsion stabilized only by modified starch showed an average decrease in turbidity of as much as 76.5%. As for the emulsion stabilized by the complex Nafiunisa, A., Aryanti, N., Wardhani, D. H. (2022). Ultrasonic preparation of olive oil pickering emulsion stabilized by modified starch. Acta Sci. Pol. Technol. Aliment., 21(2), 183–194. http://dx.doi.org/10.17306/J.AFS.2022.1031



Fig. 6. Microscopic morphology of the oil droplets of the emulsion stabilized by a complex of gelatinized-modified starch and surfactant at surfactant concentrations of: $\mathbf{a} - 4\%$ weight, $\mathbf{b} - 5\%$ weight, $\mathbf{c} - 6\%$ weight

of modified starch and surfactant, the turbidity decreased by an average of 78%. The optical microscopic images also confirmed that the emulsion oil droplet grew in size after storage of 30 days. However, the addition of a stabilizer appeared to help prevent or reduce the instability of the emulsion.

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