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Shrimp Oil-Enriched Mayonnaise Prepared Using Fish Myofibrillar Protein as a Substitute for Egg Yolk: Physical, Rheological, and Sensory Properties

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Abstract: The effect of SO (shrimp oil) at various levels (5, 10, and 15%) on the stability of mayonnaise was investigated. Droplet size (d_{32} and d_{43}), polydispersity index, and microstructure results showed an upsurge in droplet sizes with augmenting level of SO in mayonnaise (5 to 15%) ($p < 0.05$). SO imparted a bright orange color to the mayonnaise as evidenced by increased a^* and b^* values with lower L^* values ($p < 0.05$). Moreover, the impact of a fish myofibrillar protein (FMP) substitution for egg yolk (0, 25, 50, 75%) in mayonnaise containing SO (5% and 10%) was also studied. Increasing the level of FMP substitution in SO-added mayonnaise showed a dilution effect and reduced a^* and b^* values ($p < 0.05$). In addition, excessive FMP substitution up to 75% drastically increased centrifugal and thermal creaming indices, indicating lowered stability ($p < 0.05$). Nevertheless, with the augmenting FMP substitutions, the viscosity, texture, and rheological properties in mayonnaise became lower ($p < 0.05$). However, there were no differences in overall acceptability scores between 5% SO-added mayonnaise with 25% FMP substitution (SO5:FMP25) and 5% SO-added mayonnaise without FMP substitution (SO5:FMP0) ($p > 0.05$). A confocal laser scanning microscopic (CLSM) study revealed a smaller droplet and less aggregation in the SO5:FMP0 sample, compared to SO5:FMP25. The incorporation of SO and FMP substitution yielded the resulting mayonnaise, which met the requirements of a healthy food since SO is rich in PUFA and the replacement of egg yolk by FMP can contribute several health benefits. The incorporation of SO as well as FMP as substitution for egg yolk therefore has potential in the development of functional foods.

Keywords: shrimp oil; fish myofibrillar protein; mayonnaise; rheology; texture; acceptability

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1. Introduction

The n3 polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are renowned for their numerous health benefits [1]. According to European standards, adults should consume 250–500 mg of PUFA daily. However, the average intake is approximately 244 mg/day, which falls below the recommended range [2]. Thus, incorporation of n3-PUFA into regular diet can be beneficial for consumers' health. Shrimp oil (SO) is rich in n-3 PUFAs along with notable carotenoids such as β-carotene and astaxanthin, serving as major pigments [3]. SO extracted from shrimp hepatopancreas contains 10–11% lipid, which is rich in PUFAs and has several health benefits such as prevention against cardiovascular disease, diabetes, and immune response disorder. Astaxanthin is a very potent antioxidant with ten-fold higher antioxidative activity than other carotenoids [1]. It acts as an anti-inflammatory agent for the treatment of UV-induced skin aliments, thus maintaining healthy skin. With exceptional properties and nutritional benefits, SO has become the most valuable functional ingredient from shrimp processing wastes and can be used in the food and pharmaceutical industries. The health benefits of bioactive compounds in SO have been documented [4].

Mayonnaise is a widely consumed emulsion product, prepared by blending vegetable oil, egg yolk, water, vinegar, and other ingredients into a semi-solid blend of oil and water. It typically has a high fat content (65–80%). Commercial mayonnaise is often made using soybean oil (SBO), which is rich in linoleic acid, oleic acid, palmitic acid, and stearic acid [5]. Substituting SBO with SO might create a healthier mayonnaise, potentially offering more health benefits. Additionally, saturated and unsaturated fatty acids should be balanced, thus helping to achieve health-promoting goals.

On the other hand, egg yolks are crucial in making mayonnaise since they act as emulsifier and provide the structure for the finished product. However, high phosphatidylcholine in egg yolk when transformed into trimethylamine (TMA) by gut bacteria can pose health risks [6]. When TMA is absorbed and converted into trimethylamine N-oxide in the liver, the risk of atherosclerosis is increased, potentially leading to cardiovascular and cerebrovascular diseases [7]. As a result, the demand for healthier foods is challenging the food industry to find substitutes for egg yolk. Various proteins such as whey protein [8], wheat gluten [9] and soy protein [8] have been utilized as alternatives to egg yolk in oil-in-water emulsions. Despite the introduction of new food ingredients, there is little scientific information regarding the substitution of egg yolk with fish myofibrillar protein in mayonnaise.

FMP constitutes a significant amount, about 50–60%, of total fish meat protein [10]. Its unique amphiphilic nature and surface-active properties provides several functional properties, such as a gel-forming ability, water-binding capacity, and emulsifying properties [11]. FMP can adhere to fat globules by forming a strong protein layer at the interface through various configurations such as train, loop, and tail. FMP as an emulsifier shows potential in developing stable emulsions, aiding in the delivery of nutraceutical and bioactive compounds [12]. FMP, especially from underutilized species with low market value, can serve as an effective emulsifier and texture modifier in mayonnaise formulations. This utilization not only enhances the nutritional profile of the product but also adds value to the fish processing industry. Firstly, the optimal ratio of SO was optimized to obtain an emulsion with high stability. Subsequently, the formulation of mayonnaise upon substitution of egg yolk with FMP was developed. The objectives of this study were to optimize the incorporation of SO into mayonnaise via addition of SO at varying levels (5%, 10, and 15%) and to investigate the substitution of egg yolk at different levels (0, 25, 50, and 75%) with FMP.

2. Materials and Methods

2.1. Chemicals

All chemicals used were of analytical grade and sourced from Sigma (St. Louis, MO, USA). Solvents were brought from ACI Lab-scan (Bangkok, Thailand). Soy bean oil (SBO), salt, vinegar, sugar, and eggs were bought from a local supermarket in Hat Yai, Songkhla, Thailand.

2.2. Extraction of Oil from the Hepatopancreas of Shrimp

The frozen hepatopancreas from Pacific white shrimp (*Litopenaeus vannamei*) was gifted by Seafresh Industry Public Co., Ltd. (Chumphon, Thailand). SO extraction from hepatopancreas paste was performed following the procedure outlined by Gulzar and Benjakul [1]. In brief, 100 g of hepatopancreas paste and 500 mL of hexane/isopropanol mixture (1:1, v/v) were homogenized at a speed of 9500 rpm using an IKA Labortechnik homogenizer (Rawang, Selangor, Malaysia) for 2 min, followed by centrifugation (3000× g

for 15 min) at 4 °C using a centrifuge (Hitachi Koki Co., Ltd., Tokyo, Japan), and filtered using Whatman filter paper No.4. The obtained filtrate was transferred into a separating funnel and washed thrice using the same volume of distilled water. The upper hexane fraction consisting of SO was collected separately and mixed with 2–5 g of anhydrous sodium sulphate. After filtration, hexane was evaporated at 40 °C using an EYELA rotary evaporator N-1000 (Tokyo Rikakikai, Co., Ltd., Tokyo, Japan). Then, the obtained SO was placed in an amber vial, followed by nitrogen flushing, capped tightly, and kept at −40 °C.

2.3. Preparation of SO Added Mayonnaise

Mayonnaises were produced following the method of Patil and Benjakul [13]. The formulation (% on weight basis) included 8% fresh egg yolk, 4% vinegar, 1% salt, 14% sugar, 3% distilled water, and 70% oil. All the mixture except oil and vinegar were blended using a blender (Panasonic, Model MK-GB1, New Taipei City, Taiwan) at a speed of 800 rpm for 3 min. Thereafter, oil was gradually added and the mixture was blended for 5 min. Subsequently, vinegar was added and blending was performed for 3 min.

For the preparation of SO-added mayonnaise, SBO was blended with SO at SBO/SO ratios of 95:5, 90:10, and 85:15 (*v*/*v*) before adding it into the formulation. Those samples were referred to as SO5, SO10, and SO15, respectively. The sample added with only SBO served as the control (SBO). All the mayonnaise samples were placed in zip lock bag and kept at room temperature (RT) (25 ± 2 °C) for further analyses.

2.4. Analysis of SO Added Mayonnaise

2.4.1. Droplet Size and Polydispersity Index (PDI)

The droplet size and polydispersity index were assessed utilizing a Zeta Potential analyzer (ZetaPlus, Brookhaven Instruments Corporation, Holtsville, NY, USA) following the method outlined by Patil and Benjakul [13]. Before the analysis, the samples (1 g) were diluted in a 1% (*v*/*v*) sodium dodecyl sulfate (SDS) solution (20 mL) to break down the clustered droplets. To meet the manufacturer's recommendation, the refractive index and absorption used were 1.330 and 0.001, respectively.

2.4.2. Microstructure

The microstructure of mayonnaise was visualized using a light microscope EVOSTM XL Core Imaging System (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a high-resolution LCD display and a digital color camera [6]. To prepare the sample for observation, a drop of sample was directly placed on a slide without any dilution. A cover slip was then gently pressed to create a thin, even layer to ensure that no air bubbles were trapped. All observations were taken at a magnification of 40×.

2.4.3. Centrifugal and Thermal Creaming Index

Centrifugal and thermal creaming indices were measured following the methodology outlined by Wijayanti et al. [14] with slight modifications. For the creaming index (CI) analysis, approximately 15 mL ± 1 g of mayonnaise samples were placed into cylindrical plastic containers and subjected to centrifugation at 5000× *g* for 20 min at room temperature (RT). Following centrifugation, the mayonnaise was separated into two distinct phases: the top phase containing cream or oil/serum and the bottom phase consisting of the aqueous portion. The creaming index (CI) was determined using the below formula:

$$\text{CI}\ (\%) = \frac{\text{H}}{\text{H}_0} \times 100$$

where H represents the height of the oil/serum separated from the emulsion and H_0 denotes the initial emulsion height in the container.

For the thermal creaming index assessment, a similar procedure was followed, except that the samples were incubated at 80 °C in a water bath (Memmert, Schwabach, Germany) for 20 min before undergoing centrifugation.

Based on the above-mentioned analyses, SO5 and SO10 showing the smaller droplets and lower creaming indices were selected for further study.

2.5. Preparation of Fish Myofibrillar Protein (FMP)

FMP was extracted from Asian Sea bass (*Lates calcarifer*) as guided by Chanarat et al. [15]. FMP was used as an egg yolk substitute based on the preliminary research. FMP was dispersed in distilled water and dissolved by adjusting the pH to 3 using vinegar. The protein content of the FMP solution was 45 mg/mL, as determined by the Biuret assay [16]. The moisture content was found to be 95%, as determined by oven method [17]. The FMP solution was further treated with optimized ultrasound conditions (40% amplitude for 15 min) (Sonics, Model VC750, Inc., Newtown, CT, USA) with a frequency of 20 kHz ± 50 Hz and high-intensity power of 750 W to obtain the complete solubilization of FMP. The temperature was maintained below 10 °C by placing the beaker containing the sample in an ice bath. Ultrasonication was performed in a pulse mode (2 s on and 4 s off). FMP solution was used to replace the egg yolk in the basic mayonnaise formulation and the standard procedure of mayonnaise preparation was followed.

2.6. Preparation of SO-Added Mayonnaise with FMP Substitution for Egg Yolk

For FMP substitution of egg yolk, SO5 and SO10 were utilized. FMP-substituted mayonnaises were prepared by substituting egg yolk with FMP at various levels (0, 25, 50, and 75%, *w*/*w*). SO5 mayonnaise samples with FMP substitution at 0%, 25%, 50%, and 75% were named as SO5:FMP0, SO5:FMP25, SO5:FMP50, and SO5:FMP75, respectively. SO10 mayonnaise samples with FMP substitutions of 0%, 25%, 50%, and 75% were referred to as SO10:FMP0, SO10:FMP25, SO10:FMP50, and SO10:FMP75, respectively. Mayonnaise was prepared without SO and FMP substitution was used as control (SBO). Finally, mayonnaise samples were placed at RT for 8 h before analyses.

2.7. Study on Physical, Rheological, and Sensory Properties of SO-Added Mayonnaise with FMP Substitution for Egg Yolk

2.7.1. Centrifugal and Thermal Creaming Indices

Centrifugal and thermal creaming indices of SO-added mayonnaise with FMP substitution were assessed as designed by Wijayanti et al. [14].

2.7.2. Color Determination

Lightness (L^*), redness/greenness (a^*), and yellowness/blueness (b^*) of various mayonnaise samples were determined by a colorimeter (HunterLab, Model ColorFlex, VA, USA) [18]. Total difference in color (ΔE^*) and difference in chroma (ΔC^*) in comparison with control (SBO) were also calculated using the following equations:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

where ΔL^*, Δa^*, and Δb^* are the differences between the corresponding color parameter of the samples and those of control (SBO).

2.7.3. Texture Analysis

Texture properties involving firmness, consistency, and cohesiveness were assessed using the texture analyzer TA-XT2 (Stable MicroSystem, Godalming, UK) at RT following the method of Huang et al. [19].

2.7.4. Viscosity

The Viscosity analysis was conducted utilizing the Brookfield Viscometer RV Model (Brookfield Engineering laboratories, Inc., Middleboro, MA, USA). A spindle number 5 was employed for the analysis at RT, operating at a speed of 10 rpm for a duration of 30 s [20]. The viscosity result was reported in centipoise (cP).

2.7.5. Rheological Properties

The rheological properties of the mayonnaise samples were assessed by a controlled stress rheometer (RheoStress RS 1, HAAKE, Karlsruhe, Germany) equipped with parallel geometry (60 mm diameter and 1 mm gap) following the procedure of Rajasekaran et al. [21]. The samples were analyzed at RT. The linear viscoelastic range was determined using strain sweep from 0.1 to 100% at a fixed frequency of 1.0 Hz. Frequency sweep was conducted with a constant strain (0.5%) throughout the linear region and over a frequency range of 0.1 and 10 Hz. The analysis included storage modulus (G') and loss modulus (G'') as a function of frequency. Additionally, viscosity and flow curves were obtained by applying shear rate ranging from 1 to 100 s^{-1} over 2 min.

2.7.6. Sensory Properties

Sensory evaluation involved 50 untrained panelists. Each was provided with 10 g samples of mayonnaise served in white plastic cups individually coded with three different numbers. Breads were given for evaluating spreadability alongside the samples. Panelists rinsed their mouth using water between each sample assessment. The assessment covered multiple attributes including appearance, color, odor, spreadability, taste, flavor, and overall acceptability. Panelists evaluated these attributes using a 9-point hedonic scale following the guidelines outlined by Mellgard et al. [22].

Based on the above-mentioned analyses, SO5:FMP0 and SO5:FMP25 were taken for confocal laser scanning microscopy (CLSM) observations in comparison with SBO.

2.7.7. Confocal Laser Scanning Microscopy (CLSM) Images

Microstructure analysis was conducted using a confocal laser scanning microscope (CLSM) (Model FV300; Olympus, Tokyo, Japan). To the sample, a solution of Nile blue A (0.01%) was utilized and manually stirred. Subsequently, the homogeneous mixture was spread onto a microscopy slide for examination. The CLSM was operated in fluorescence mode with specific emission and excitation wavelengths (emission: 630 nm; excitation: 533 nm) for microstructure evaluation. For lipid analysis, a Helium-Neon red laser (HeNe-R) was employed during the assessment.

2.8. Statistical Analysis

The experiments were conducted in triplicate using three distinct sample lots. A completely randomized design (CRD) was employed for data analysis via analysis of variance (ANOVA). However, for sensory analysis, a randomized complete block design (RCBD) was used. Duncan's multiple range test was applied to compare mean values. The statistical package for social science software (SPSS 23 for Windows, SPSS Inc., Chicago, IL, USA) was used for data analysis.

3. Results

3.1. Effect of SO Incorporation at Different Levels on Droplet Size, Microstructure, and Stability of Mayonnaise

3.1.1. Droplet Size and Microstructure

The surface weighted mean diameter (d_{32}) and volume weighted mean diameter (d_{43}) as well as the polydispersity index (PDI) of the mayonnaise samples prepared using SO at different levels (5, 10, and 15%) are given in Table 1. The stability of an emulsion is significantly influenced by droplet size [23]. In general, d_{32} represents the average surface area of a droplet exposed to the continuous phase per unit weight of the emulsion, whereas d_{43} indicates flocculation and coalescence in the system [24]. Both d_{32} and d_{43} showed increasing trends with augmenting SO concentrations in mayonnaise from 5% to 15% ($p < 0.05$). As compared to SBO, SO5 showed larger droplets, which eventually merged with each other, known as coalescence [25]. With the upsurge in SO content, the stability of the mayonnaise was negatively affected. Among all, SO15 had larger droplet sizes, compared to SBO ($p < 0.05$). The size of the droplets produced during homogenization is often influenced by the physicochemical properties and composition of oil in the dispersed phase [26]. The nature of the oil, such as SBO or SO, plays a crucial role in the ability to create an emulsion with smaller droplets. SO was reported to have a high amount of phospholipids [27]. As a result, phospholipids, having an amphiphilic nature in SO, along with egg yolk rich in lecithin, could not be broken apart effectively during emulsification and were more likely partially dissolved in the aqueous phase. In addition, increased unsaturation in fatty acids correlated with reduced emulsifying stability [28]. As a result, SO15 containing the highest amount of SO rich in PUFAs could have a larger size and non-uniform dispersion of oil droplets. This might worsen the network or structure of the resulting mayonnaise [29].

Table 1. Droplets and polydispersity index (PDI) of mayonnaise with shrimp oil (SO) added at different levels.

Samples	d_{32} (μm)	d_{43} (μm)	PDI
SBO	1.21 ± 0.01 ^{D}	1.24 ± 0.03 ^{D}	0.11 ± 0.01 ^{D}
SO5	1.56 ± 0.01 ^{C}	1.60 ± 0.00 ^{C}	0.13 ± 0.00 ^{C}
SO10	1.83 ± 0.03 ^{B}	1.87 ± 0.01 ^{B}	0.14 ± 0.00 ^{B}
SO15	2.13 ± 0.02 ^{A}	2.16 ± 0.01 ^{A}	0.16 ± 0.00 ^{A}

Mean ± SD (n = 3). Different uppercase superscripts in the same column indicate significant differences ($p < 0.05$). SBO: mayonnaise containing soybean oil; SO5: mayonnaise containing soybean oil (95%) + shrimp oil (5%); SO10: mayonnaise containing soybean oil (90%) + shrimp oil (10%); SO15: mayonnaise containing soybean oil (85%) + shrimp oil (15%).

PDI is related to the homogeneity of oil droplet size. When the PDI is close to zero, the droplet distribution is more uniform in the mayonnaise [25]. PDI increased with the augmenting SO concentrations (5 to 15%), indicating the increased heterogenicity of the oil droplets. PDI was more likely in accordance with the distribution curve of all droplets, as shown in histograms (Figure 1). When comparing them to the SO15 sample, SO5 and SO10 showed small droplet sizes and less difference in PDI. A high level of SO incorporated into food likely provides more health benefits to consumers. The droplet sizes and distribution patterns of SBO and SO in mayonnaise were reconfirmed by visualizing the microstructure using light microscopy, as illustrated in Figure 1. Densely packed droplets were observed in the SBO sample with a much smaller droplet size as compared to that of the SO-added samples. This coincided with the narrow range of droplet size in the former, as presented in the histogram (Figure 1). This was plausibly related to the high capability of egg yolk to stabilize the SBO–water interface [30]. Among mayonnaise samples containing SO at various levels, smaller droplets with uniform dispersion were observed in SO5. Moreover, the microstructure images demonstrated that increasing the SO level from 5% to 15% resulted in an augmented droplet size. Overall, among all of the samples, the SO15 sample showed larger and more heterogenicity of oil droplets, representing a lower stability of the mayonnaise.

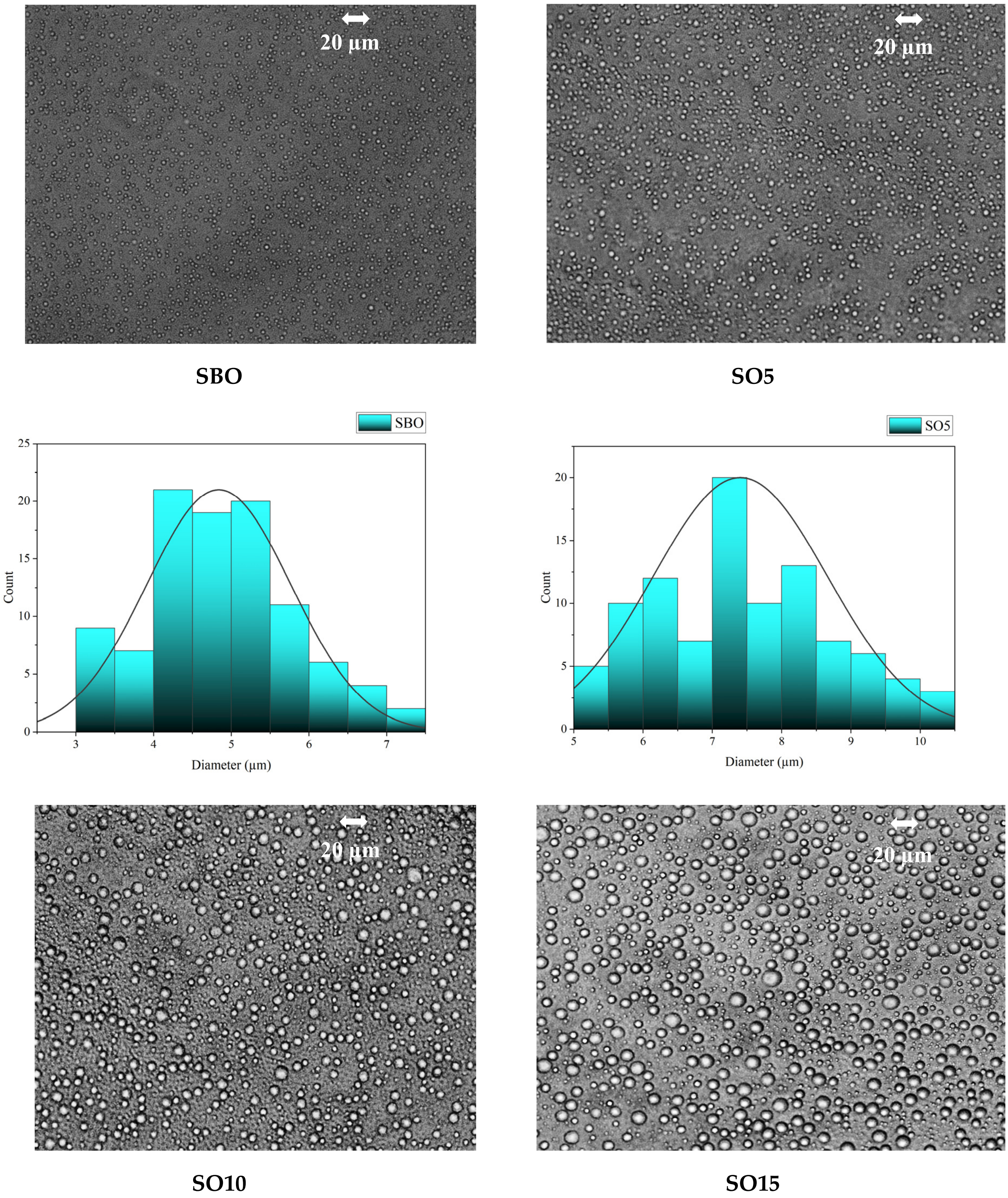

Figure 1. *Cont*.

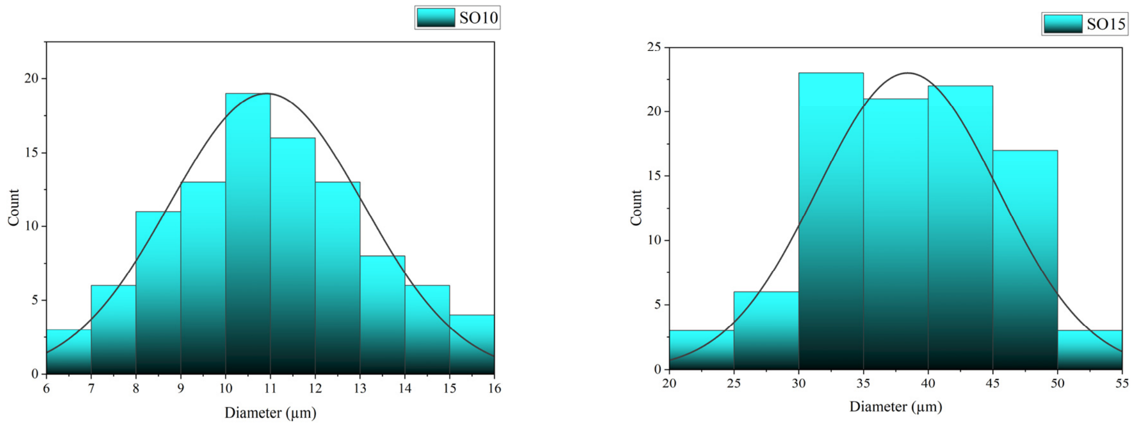

Figure 1. Microstructure and histogram (diameter) of mayonnaise prepared using shrimp oil (SO) at different levels. Observations were made at 40× magnification. SBO: mayonnaise containing soybean oil; SO5: mayonnaise containing soybean oil (95%) + shrimp oil (5%); SO10: mayonnaise containing soybean oil (90%) + shrimp oil (10%); SO15: mayonnaise containing soybean oil (85%) + shrimp oil (15%).

3.1.2. Centrifugal and Thermal Creaming Indices

Creaming serves as an indicator of emulsion instability, describing the movement of droplets towards the top of the emulsion [31]. Different centrifugal and thermal creaming indices were observed among the mayonnaise samples (Figure 2). Both centrifugal and thermal creaming indices showed a similar trend. A lower creaming index indicates greater stability [14]. Generally, the lowest centrifugal and thermal creaming indices were noticed in the SBO ($p < 0.05$). Among the SO samples, the lowest creaming index and highest stability were obtained with SO5, followed by SO10 ($p < 0.05$). The lower creaming index was more likely caused by the smaller droplet size associated with increased stability. During emulsification, protein membranes at the interface can emulsify and bind with fat particles, while a cohesive protein gel matrix can restrict the movement of fat and water [32]. However, the stability of these protein films is not solely determined by protein characteristics but is also influenced by the properties of the enclosed fat particles [33]. Variations in the amount of proteins adsorbed at the oil–water interface, protein confirmation and colloid interactions may contribute to emulsion characteristics [34]. In another study, Han et al. [35] examined the stability of emulsions as affected by the saturation degree of fatty acids. Salt-soluble pork protein was used to stabilize oleic acid, linoleic acid, and linolenic acid emulsions. The result indicated that the oleic acid emulsion exhibited the highest stability, whereas the linolenic acid emulsion displayed the lowest stability. Thus, the number of double bonds in the fatty acids significantly influenced the interfacial adsorption behavior [36]. Since SO is rich in PUFAs [27], those PUFAs negatively affected the stability of the emulsion, as witnessed by the higher centrifugal and thermal creaming indices. Thus, the addition of a higher amount of SO (15%) might increase the number of double bonds in the emulsion system. Based on the above-mentioned results, the SO5 and SO10 samples were chosen for further studies.

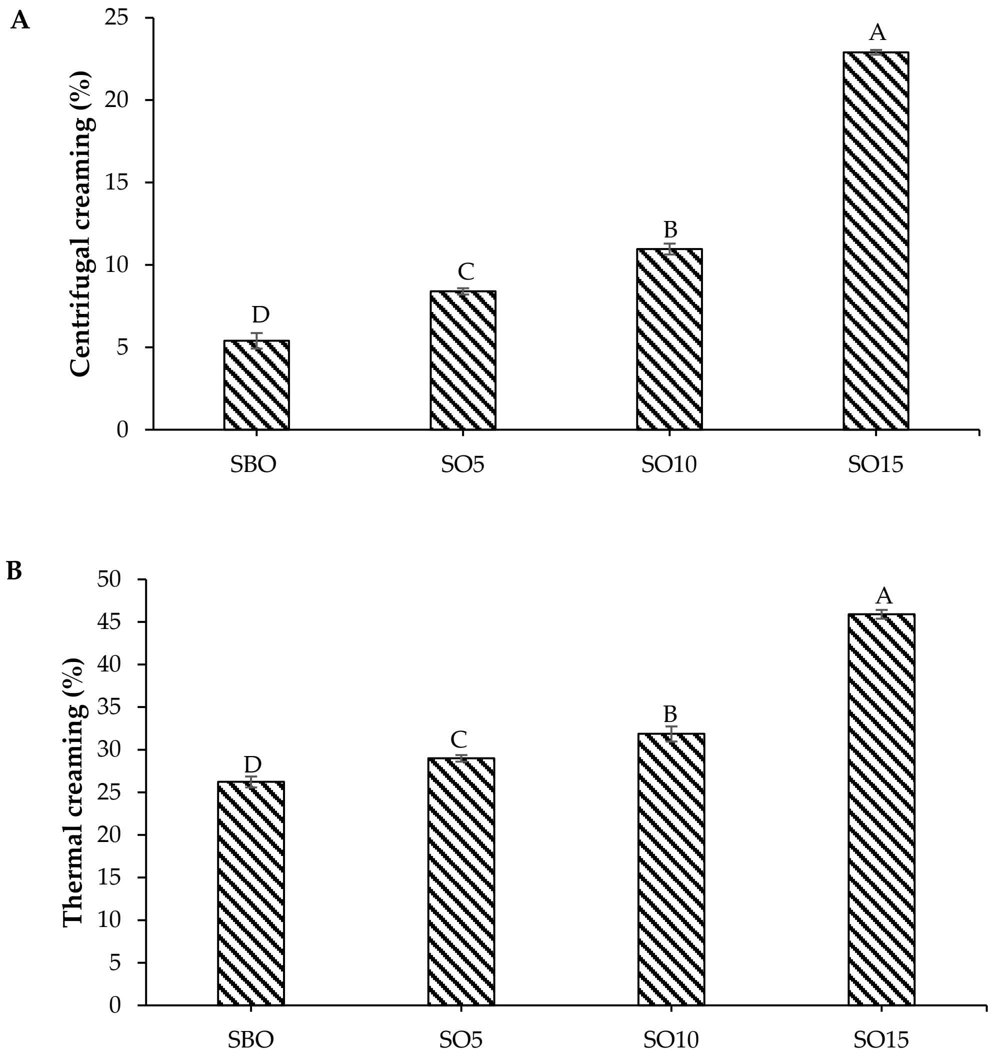

Figure 2. Centrifugal creaming index (**A**) and thermal creaming index (**B**) of mayonnaise prepared using shrimp oil (SO) at different levels. SBO: mayonnaise containing soybean oil; SO5: mayonnaise containing soybean oil (95%) + shrimp oil (5%); SO10: mayonnaise containing soybean oil (90%) + shrimp oil (10%); SO15: mayonnaise containing soybean oil (85%) + shrimp oil (15%). Bars represent the standard deviation. Different letters on the bars indicate significant differences ($p < 0.05$).

3.2. Effect of FMP Substitution for Egg Yolk on Physical, Rheological, and Sensory Properties of SO Containing Mayonnaise

3.2.1. Centrifugal and Thermal Creaming Indices

The creaming indices of SO (5% and 10%)-added mayonnaise with FMP substitution of egg yolk at different levels (0, 25, 50, and 75%) are presented in Figure 3. Both the centrifugal and thermal creaming indices showed a similar trend. Among the FMP-substituted samples (25, 50, and 75%), the lowest creaming indices were observed in the sample substituted with the lowest amount of FMP (25%). When the FMP substitution increased to 50%, there was a drastic increase in the creaming indices, representing the lowest emulsion stability and possibility of phase separation during prolonged storage. This indicates that the FMP substitute for egg yolk more likely led to a larger droplet size or less stability of the emulsion. Higher creaming is associated with a larger droplet size in the emulsion [37]. Creaming in the emulsion system primarily resulted from the emulsifier's inability to effectively reduce the interfacial tension between the dispersed and continuous phases [24]. Lecithin, found in egg yolk, acts as a small molecule emulsifier (600–900 g/mol), which might move faster to the interface and adsorb over the oil droplets to reduce its size more effectively than FMP during the emulsion formation stage as compared to FMP (20,000–200,000 Da) [38].

Therefore, FMP substitution up to 50%, of which the emulsion could withstand, was selected for further characterization.

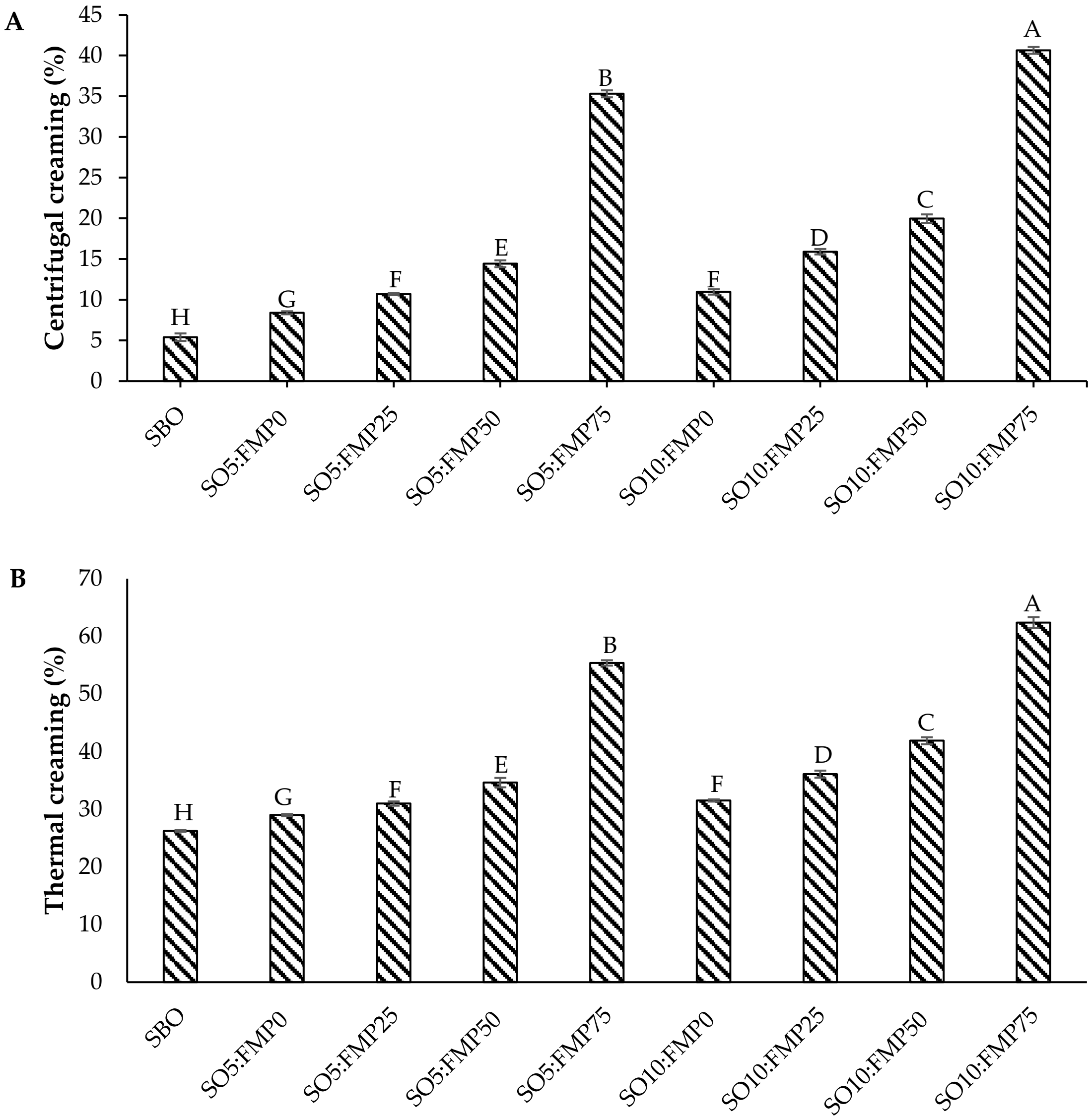

Figure 3. Centrifugal creaming index (**A**) and thermal creaming index (**B**) of mayonnaise with shrimp oil (SO) added and fish myofibrillar protein (FMP) substitution of egg yolk at different levels. SBO: mayonnaise containing soybean oil without FMP substitution; SO5 mayonnaises with FMP substitution at 0, 25, 50, and 75% were named as SO5:FMP0, SO5:FMP25, SO5:FMP50, and SO5:FMP75, respectively. SO10 mayonnaises with FMP substitution at 0, 25, 50, and 75% were referred to as SO10:FMP0, SO10:FMP25, SO10:FMP50, and SO10:FMP75, respectively. Bars represent the standard deviation. Different letters on the bars indicate significant differences ($p < 0.05$).

3.2.2. Color

The colors of the SO-added mayonnaise samples with FMP substitution at different levels (0, 25, and 50%) are presented in Table 2. Overall, the SBO mayonnaise (the control) exhibited the highest L^*, indicating a lighter color and the lowest a^* and b^*, denoting less redness and yellowness ($p < 0.05$). This was due to the absence of SO rich in astaxanthin in the SBO sample. As a result, the mayonnaise had a pale-yellow color, mainly attributed to the color of egg yolk. Among the SO-added mayonnaise samples, SO10 had a lower L^* but higher a^* and b^* values, compared to SO5 ($p < 0.05$). A higher amount of SO incorporation resulted in more redness of the mayonnaise sample. This was attributed to the presence of astaxanthin, a red carotenoid, associated with the reddish-orange color of SO [1]. In general, the augmented level of FMP substitution (0 to 50%) resulted in a higher L^*, while the a^* and b^* values were reduced coincidentally ($p < 0.05$). Generally, the difference in lightness could be attributed to varying light scattering. Light scattering and absorption depend on several factors such as size, dispersion, concentration, and the refractive index of the droplets, as well as the presence of chromophoric material [23]. Absorption is mainly responsible for the color attributes observed (redness and yellowness), while scattering influences the overall opacity or lightness of the sample [39]. Among SO-added mayonnaises with FMP substitution, SO5:FMP50 showed a higher L^* value, while the lowest value was observed in SO10:FMP0 ($p < 0.05$). For the SO5 samples, the a^* and b^* values were in the following order: SO5:FMP0 > SO5:FMP25 > SO5:FMP50 ($p < 0.05$). A similar trend in a^* and b^* values was observed among the SO10 samples. An increased L^* value with augmenting FMP substitution was related to the dilution effect of chromophoric substances like carotenoids found in egg yolk [6]. The yellowness of egg yolk is due to fat-soluble carotenoids, while FMPs do not impart color. Patil and Benjakul [13] reported that increasing the levels of fish oil in a virgin coconut oil mayonnaise increased the a^* and b^* values and reduced the L^*, owing to the presence of some indigenous pigments in fish oil. Generally, a difference in color was visually perceived when ΔE^* was above 3.7 [40]. Among all the samples, SO10:FMP0 had higher ΔE^* and ΔC^*, while SO5:FMP50 showed the lowest values when compared to the SBO sample ($p < 0.05$). The higher ΔE^* and ΔC^* were related to the colored mayonnaise, whereas lower values indicated a lighter color of the mayonnaise [41]. Thus, the color of the resulting mayonnaise was directly influenced by the incorporation of SO and FMP substitution, depending on the quantities of SO added and FMP substituted.

Table 2. Color of mayonnaise with shrimp oil (SO) added and fish myofibrillar protein (FMP) substitution of egg yolk at different levels.

Samples	L^*	a^*	b^*	ΔE^*	ΔC^*
SBO	71.07 ± 0.12 A	9.30 ± 0.12 G	40.39 ± 0.11 G	-	-
SO5:FMP0	58.53 ± 0.25 E	27.78 ± 0.18 D	47.26 ± 0.10 C	20.29 ± 0.29 D	16.09 ± 0.03 D
SO5:FMP25	61.94 ± 0.31 D	24.68 ± 0.08 E	45.26 ± 0.07 E	17.49 ± 0.15 E	11.15 ± 1.28 E
SO5:FMP50	67.35 ± 0.24 B	20.46 ± 0.15 F	42.87 ± 0.09 F	11.71 ± 0.05 F	8.40 ± 0.91 F
SO10:FMP0	53.77 ± 0.52 F	35.66 ± 0.13 A	53.21 ± 0.04 A	31.68 ± 0.91 A	25.29 ± 0.06 A
SO10:FMP25	58.26 ± 0.37 E	31.55 ± 0.34 B	49.55 ± 0.12 B	24.90 ± 0.38 B	22.17 ± 0.13 B
SO10:FMP50	63.86 ± 0.28 C	28.62 ± 0.13 C	46.49 ± 0.38 D	21.92 ± 0.31 C	18.69 ± 0.05 C

Mean ± SD ($n = 3$). Different uppercase superscripts in the same column indicate significant difference ($p < 0.05$). SBO: mayonnaise containing soybean oil without FMP substitution; SO5 mayonnaises with FMP substitution at 0, 25, and 50% were named as SO5:FMP0, SO5:FMP25, and SO5:FMP50, respectively. SO10 mayonnaises with FMP substitution at 0, 25, and 50% were referred to as SO10:FMP0, SO10:FMP25, and SO10:FMP50, respectively.

3.2.3. Texture Profile

The texture profiles of SO-added mayonnaise with FMP substitution for egg yolk at different levels (0, 25, and 50%) are presented in Table 3. The perception of mayonnaise texture by consumers involves its breakdown and chewing in the mouth before swallowing [14]. Texture analysis involves a compression technique simulating mouth behavior towards the food [42]. Textural parameters like firmness, consistency, and cohesiveness are commonly assessed for mayonnaise [43]. Firmness, related to emulsification intensity, reflects the maximum compression force [44]. Consistency, determined as the average area under the positive curve, correlates with interparticle colloidal forces and fluid viscosity balance [45]. Cohesiveness, assessed as the maximum force in the negative curve area, indicates mayonnaise stickiness. A higher negative value suggests higher stickiness [46]. The SBO sample prepared using a standard formulation was used as control and showed the highest firmness, consistency, and cohesiveness among all tested samples. SO5:FMP0 showed higher firmness, consistency, and cohesiveness, while SO10:FMP50 had the lowest values ($p < 0.05$) when compared with SBO. A higher firmness of mayonnaise indicated that oil droplets surrounded by proteins became linked together into a strong network [47]. It was noteworthy that the FMP replacement in the SO5 and SO10 samples resulted in decreased firmness, consistency, and cohesiveness, especially when higher amounts of FMP were used as a substitute to egg yolk (50%). Variation in mayonnaise texture was closely related to both emulsification conditions and droplet size. The size of droplets within the mayonnaise was thought to have an inverse correlation with textural attributes including hardness and adhesiveness [38]. Large contact surfaces between oil droplets in mayonnaise increased friction and counteracted the free flow during shearing, thus ultimately enhancing the stickiness [48]. Due to lecithin's superior emulsifying activity, compared to FMPs, the substitution of FMP likely altered the oil–water interfacial structure. As a result, the droplet size was enlarged in the mayonnaise. This increase in droplet diameter reduced the contact surface area between droplets, consequently diminishing the firmness and consistency [6]. Additionally, the reduced cohesiveness in the mayonnaise might be attributed to the dynamic viscoelastic parameter, as affected by FMP substitution in egg yolk. Therefore, incorporation of SO in mayonnaise preparation and FMP substitution for egg yolk could significantly impact the overall textural attributes of the resulting product.

Table 3. Viscosity and texture properties of mayonnaise with shrimp oil (SO) added and fish myofibrillar protein (FMP) substitution of egg yolk at different levels.

Samples	Viscosity (N.s/m^2)	Firmness (N)	Consistency (N.s)	Cohesiveness (N)
SBO	38.47 ± 0.12 ^{A}	0.64 ± 0.01 ^{A}	18.60 ± 0.61 ^{A}	−1.23 ± 0.04 ^{A}
SO5:FMP0	35.52 ± 0.11 ^{B}	0.61 ± 0.00 ^{B}	15.60 ± 0.63 ^{B}	−1.17 ± 0.03 ^{B}
SO5:FMP25	33.31 ± 0.12 ^{C}	0.56 ± 0.01 ^{C}	12.70 ± 0.65 ^{C}	−1.12 ± 0.02 ^{BC}
SO5:FMP50	27.60 ± 0.11 ^{F}	0.48 ± 0.00 ^{E}	9.24 ± 0.70 ^{D}	−0.93 ± 0.03 ^{D}
SO10:FMP0	31.72 ± 0.08 ^{D}	0.53 ± 0.01 ^{D}	13.98 ± 0.68 ^{C}	−1.12 ± 0.03 ^{C}
SO10:FMP25	29.27 ± 0.10 ^{E}	0.45 ± 0.01 ^{E}	9.11 ± 0.76 ^{D}	−0.89 ± 0.03 ^{D}
SO10:FMP50	23.63 ± 0.15 ^{G}	0.35 ± 0.01 ^{F}	7.18 ± 0.53 ^{F}	−0.78 ± 0.03 ^{E}

Mean ± SD (n = 3). Different uppercase superscripts in the same column indicate significant difference ($p < 0.05$). SBO: mayonnaise containing soybean oil without FMP substitution; SO5 mayonnaises with FMP substitution at 0, 25, and 50% were named as SO5:FMP0, SO5:FMP25, and SO5:FMP50, respectively. SO10 mayonnaises with FMP substitution at 0, 25, and 50% were referred to as SO10:FMP0, SO10:FMP25, and SO10:FMP50, respectively.

3.2.4. Viscosity

Viscosity is an inherent characteristic of liquid or semi-solid foods. It quantifies the resistance of fluid movement when subjected to shearing stress [49]. The viscosity of SO-added mayonnaise with FMP substitution at different levels (0–50%) is tabulated in Table 3. The addition of SO and FMP substitution resulted in varying viscosities of the mayonnaise. Among all samples, the highest viscosity was noticed in SO5:FMP0, while the lowest viscosity was obtained for SO10:FMP50 ($p < 0.05$). Viscosity can be directly related to the stability of the emulsion. The viscosity of the mayonnaise samples decreased with augmenting FMP substitution (0 to 50%), which might be primarily attributed to the high moisture content of FMP (95%). Typically, egg yolk has a relatively low water content around 48–50% [50]. In mayonnaise with the FMP substitution, the droplets became more scattered, leading to a decrease in intermolecular interactions. Consequently, this increased the fluidity of the mayonnaise sample [51]. Similar findings were documented by Wang et al. [6] using soybean oil body (10–50%) as a substitute for egg yolk. Increased viscosity provides a desirable mouth feel during chewing, while decreased viscosity offers advantages during high-shear operations including filling and pumping. The viscosity results aligned with the texture profiles observed in the mayonnaise (Table 3).

3.2.5. Viscoelastic Properties

The viscoelastic properties of SO-added mayonnaise with FMP substitution at different levels are shown in Figure 4. All mayonnaise samples showed linear viscoelastic response, where G′ was higher than G″, indicating the dominant elastic behavior over viscous behavior [52]. This behavior signifies a gel-like network formation within the frequency range of 0.1–100 Hz, commonly seen in concentrated emulsions (e.g., commercial mayonnaise) [7]. The increase in G′ values at higher frequencies is attributed to strong interactions between droplets, which requires more time to relax. This indicated the formation of a gel-like network formed due to entanglements of proteins at the oil–water interface between the adjacent droplets [53]. Notably, G′ values varied across all samples. Higher G′ values indicated a greater resistance to flow, possibly due to strong interactions of droplets in mayonnaise [13]. Moreover, significant differences in G′ values were observed with increasing levels of SO (5 to 10%), in which SO5:FMP0 showed higher G′ values than SO10:FMP0 ($p < 0.05$). This was more likely influenced by factors such as saturation degree and chain length of the oil. These factors affect the size and dispersion of the droplets, thereby affecting the interfacial tension and the overall network or structure of the mayonnaise [54]. On the other hand, with augmenting FMP substitution (0 to 50%), the G′ and G″ of the mayonnaise gradually decreased, which was probably due to the reduction in lecithin by FMP substitution of egg yolk. Egg yolk contains lecithin which acts as the potential emulsifier [10]. Park et al. [23] reported that the dense arrangement of oil droplets within the lipoprotein network led to an increased storage modulus (G′).

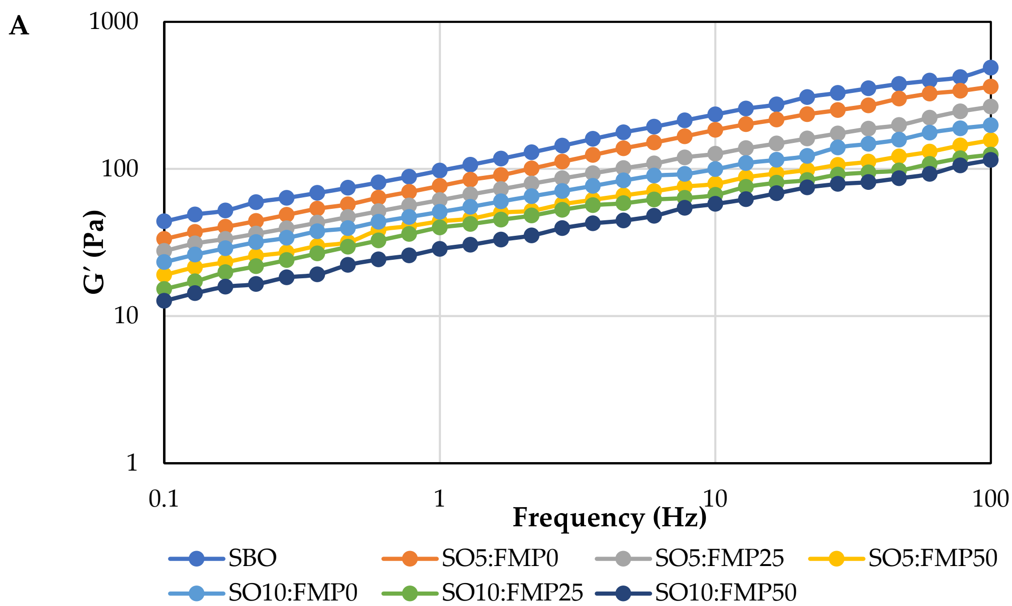

Figure 4. *Cont.*

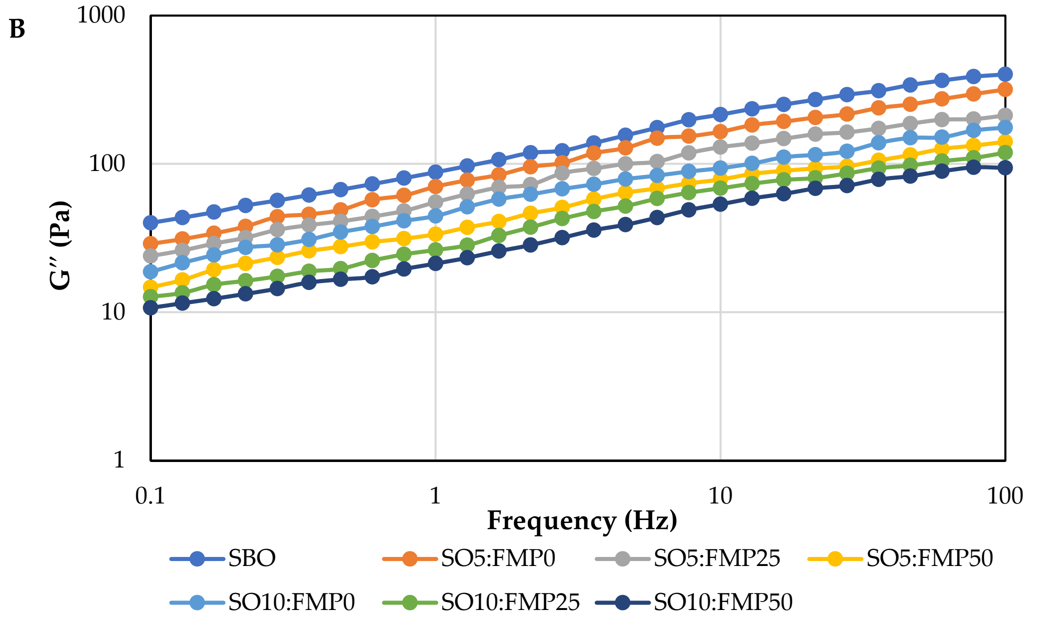

Figure 4. Storage modulus (**A**) and loss modulus (**B**) of mayonnaise with shrimp oil (SO) added and fish myofibrillar protein (FMP) substitution of egg yolk at different levels. SBO: mayonnaise containing soybean oil without FMP substitution; SO5 mayonnaises with FMP substitution at 0, 25, and 50% were named as SO5:FMP0, SO5:FMP25, and SO5:FMP50, respectively; SO10 mayonnaises with FMP substitution at 0, 25, and 50% were referred to as SO10:FMP0, SO10:FMP25, and SO10:FMP50, respectively.

3.2.6. Flow Behavior

The flow behavior of the SO-added mayonnaise with FMP substitution of egg yolk at varying levels (0, 25, and 50%) is illustrated in Figure 5. Overall, the viscosity decreased with augmenting shear rate in all mayonnaise samples, representing shear thinning behavior ($p < 0.05$). When subjecting to shear stress, the droplets in the continuous phase of the emulsion were separated from each other, causing a reorganization of the microstructure within the system [24]. Shear-thinning behavior is a common trait in different food emulsions such as mayonnaise, spreads, etc. As per Stroke's law, the viscosity tends to be inversely related to the droplet size present in the emulsion [25]. Among FMP-substituted samples, the highest apparent viscosity was observed in SO5:FMP25, whereas SO10:FMP50 showed the lowest viscosity. In general, the higher viscosity of the emulsion indicates greater stability. This is attributed to a reduction in droplet sedimentation or creaming [53]. Lower viscosity is related to a larger droplet size with a lower number of droplets per unit volume. This results in less resistance to flow or more mobility [54]. Viscosity signifies the relationship between shear stress and shear rate. Typically, as shear stress increases, viscosity decreases simultaneously. For the flow curve, as shear rate increases, shear stress also upsurges, indicating that mayonnaise displays pseudoplastic behavior characteristics of non-Newtonian fluids [51]. Generally, different oil and emulsifier types could modify the viscosity of disperse and continuous phases. This greatly influences the size of droplets during homogenization [55].

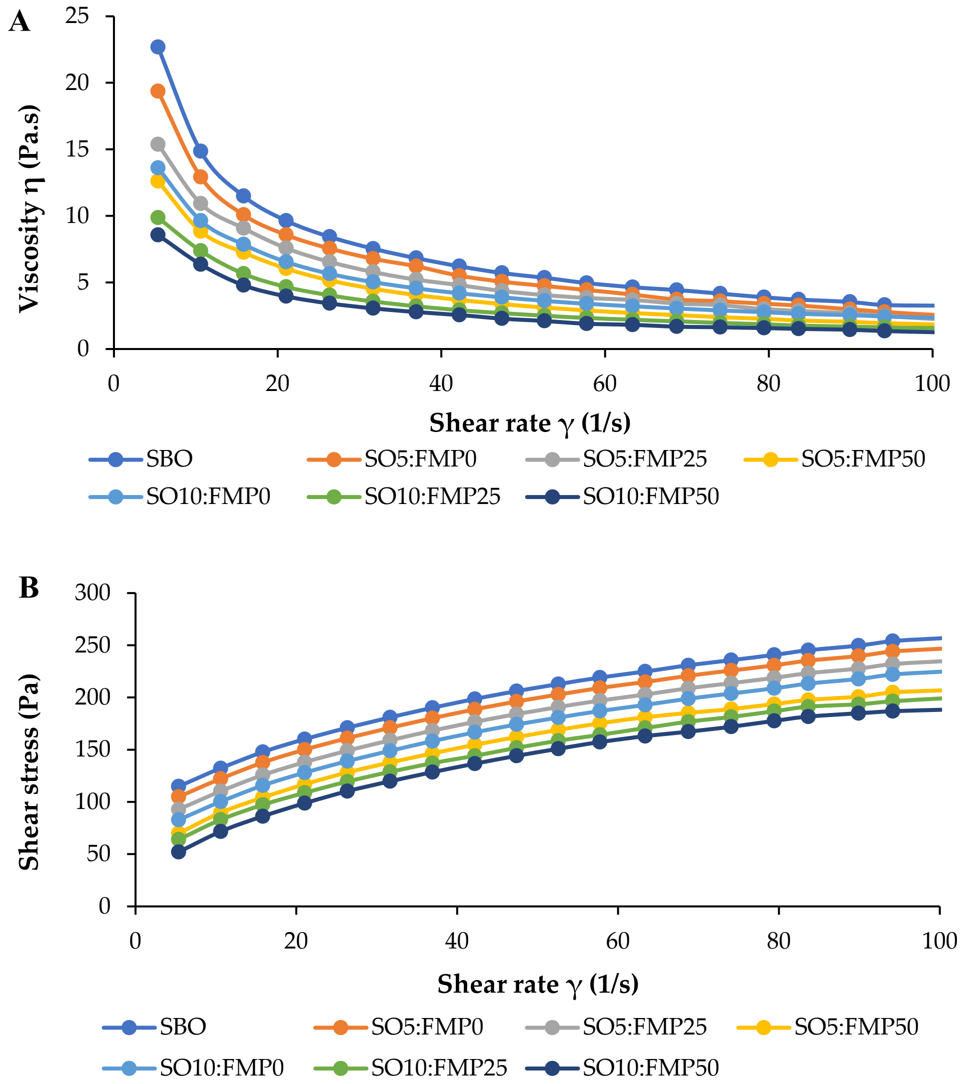

Figure 5. Viscosity (**A**) and flow curve (**B**) of mayonnaise with shrimp oil (SO) added and fish myofibrillar protein (FMP) substitution of egg yolk at different levels. SBO: mayonnaise containing soybean oil without FMP substitution; SO5 mayonnaises with FMP substitution at 0, 25, and 50% were named as SO5:FMP0, SO5:FMP25, and SO5:FMP50, respectively; SO10 mayonnaises with FMP substitution at 0, 25, and 50% were referred to as SO10:FMP0, SO10:FMP25, and SO10:FMP50, respectively.

3.2.7. Sensory Evaluation

Sensory scores of SO (5% and 10%)-enriched mayonnaise with FMP substitution at various levels (0, 25, and 50%) are shown in Table 4. SO incorporation and FMP substitution had a significant effect on the sensory attributes of mayonnaise. A higher acceptability score was observed for the SBO sample, followed by SO5:FMP0 and SO5:FMP25, respectively ($p < 0.05$). Interestingly, the appearance and color likeness scores of the SO-added mayonnaise (SO5:FMP0 and SO5:FMP25) were higher than those of SBO ($p < 0.05$). This was probably due to the attractive orange color of the mayonnaise owing to the addition of SO. For odor likeness score, at higher concentration of SO (10%), a fishy odor was detected in the resulting mayonnaise sample. Therefore, low likeness scores were obtained in the SO10 sample including SO10:FMP0, SO10:FMP25, and SO10:FMP50, compared to the SO5 samples and SBO ($p < 0.05$). Spreadability plays a vital role in mayonnaise as it directly correlates with its intended use on bread [44]. No significant difference was observed in spreadability likeness between SO5:FMP0 and SO5:FMP25 ($p > 0.05$), whereas the SBO sample showed the highest spreadability likeness score ($p < 0.05$). The difference in spreadability likeness score between the SOB sample and SO-added samples might be governed by different fatty acid compositions. SBO might be rich in saturated fatty acids, which were solidified easily [24], while SO addition could increase the amount of unsaturated fatty acids in the mayonnaise system. A high saturated fatty acids content might increase the viscosity or consistency of the SBO sample [35]. This result was consistent with the higher apparent viscosity and texture profile of the SBO sample, compared to the SO-added samples (Table 3). Furthermore, with the increase in FMP, the viscosity of the mayonnaise gradually decreased (Table 3 and Figure 4), which had a negative impact on the texture of the mayonnaise, thereby reducing its acceptability. Thus, the overall acceptability score was decreased when SO at a level of 10% was incorporated ($p < 0.05$). When FMP substitution reached 50% in SO5:FMP50 and SO10:FMP50, the mayonnaise had a fishy flavor and uneven texture, as compared to SO5:FMP0 and SO10:FMP0 (without FMP replacement). This resulted in a lower likeness score ($p < 0.05$). This result was consistent with when clover sprouts protein hydrolysate and soybean oil body were used as egg substitutes in mayonnaise. As the concentration of the substitute increased, sensory scores consistently decreased [56]. However, the comprehensive acceptability scores of SO5:FMP25 and SO5:FMP0 were not significantly different ($p > 0.05$). Additionally, no significant difference was observed between the SO5:FMP0 and SBO mayonnaise ($p > 0.05$). Therefore, mayonnaise prepared by SO incorporation at 5% and FMP substitution of 25% for egg yolk could be judged to be organoleptically acceptable.

Table 4. Sensory score of mayonnaise with shrimp oil (SO) added and fish myofibrillar protein (FMP) substitution of egg yolk at different levels.

Samples	Appearance	Color	Odor	Spreadability	Taste	Flavor	Acceptability
SBO	7.82 ± 0.23 ^{A}	7.74 ± 0.06 ^{AB}	7.94 ± 0.11 ^{A}	7.84 ± 0.02 ^{A}	7.92 ± 0.10 ^{A}	7.95 ± 0.10 ^{A}	7.92 ± 0.09 ^{A}
SO5:FMP0	8.01 ± 0.14 ^{A}	7.91 ± 0.03 ^{A}	7.80 ± 0.20 ^{B}	7.74 ± 0.14 ^{A}	7.60 ± 0.06 ^{B}	7.78 ± 0.02 ^{AB}	7.74 ± 0.15 ^{AB}
SO5:FMP25	7.78 ± 0.08 ^{A}	7.83 ± 0.09 ^{AB}	7.64 ± 0.06 ^{B}	7.62 ± 0.10 ^{AB}	7.44 ± 0.08 ^{B}	7.69 ± 0.05 ^{B}	7.62 ± 0.05 ^{BC}
SO5:FMP50	7.39 ± 0.08 ^{BC}	7.66 ± 0.14 ^{B}	7.30 ± 0.07 ^{C}	6.98 ± 0.16 ^{C}	7.10 ± 0.12 ^{C}	7.22 ± 0.09 ^{C}	7.40 ± 0.08 ^{C}
SO10:FMP0	7.50 ± 0.06 ^{B}	7.41 ± 0.06 ^{C}	7.06 ± 0.04 ^{D}	7.38 ± 0.02 ^{B}	6.70 ± 0.08 ^{D}	6.81 ± 0.04 ^{D}	6.79 ± 0.19 ^{D}
SO10:FMP25	7.27 ± 0.07 ^{BC}	7.29 ± 0.05 ^{CD}	6.91 ± 0.06 ^{D}	6.56 ± 0.15 ^{D}	6.21 ± 0.11 ^{E}	6.42 ± 0.10 ^{E}	6.42 ± 0.09 ^{E}
SO10:FMP50	7.15 ± 0.10 ^{C}	7.15 ± 0.04 ^{D}	6.41 ± 0.02 ^{E}	5.91 ± 0.10 ^{E}	5.71 ± 0.10 ^{F}	5.99 ± 0.18 ^{F}	5.83 ± 0.06 ^{F}

Mean ± SD ($n = 3$). Different uppercase superscripts in the same column indicate significant difference ($p < 0.05$). SBO: mayonnaise containing soybean oil without FMP substitution; SO5 mayonnaises with FMP substitution at 0, 25, and 50% were named as SO5:FMP0, SO5:FMP25, and SO5:FMP50, respectively; SO10 mayonnaises with FMP substitution at 0, 25, and 50% were referred to as SO10:FMP0, SO10:FMP25, and SO10:FMP50, respectively.

3.2.8. CLSM Images

CLSM is a common method employed to reveal the structure, distribution, and morphology of oil droplets within emulsions (Figure 6). CLSM images were used to observe the aggregation and droplet sizes in the mayonnaise emulsion system [21]. Regarding droplet size distribution, SBO exhibited a uniform emulsion (monodisperse), whereas the SO-added samples displayed a broad range of droplet sizes, indicating a polydisperse emulsion. The CLSM images indicated that SO5:FMP25 had slightly increased droplet sizes and more aggregation, as compared to SO5:FMP0. FMP substitution of egg yolk might alter the network structure in the continuous phase. Typically, lecithin in egg yolk has stronger emulsifying activity due to its low molecular weight and higher flexibility [23]. A denser network within the continuous phase of the emulsion could hinder droplet mobility, thus inhibiting their coalescence [6]. As expected, the oil droplets in the S05:FMP0 were smaller with less aggregation, compared to SO5:FMP25.

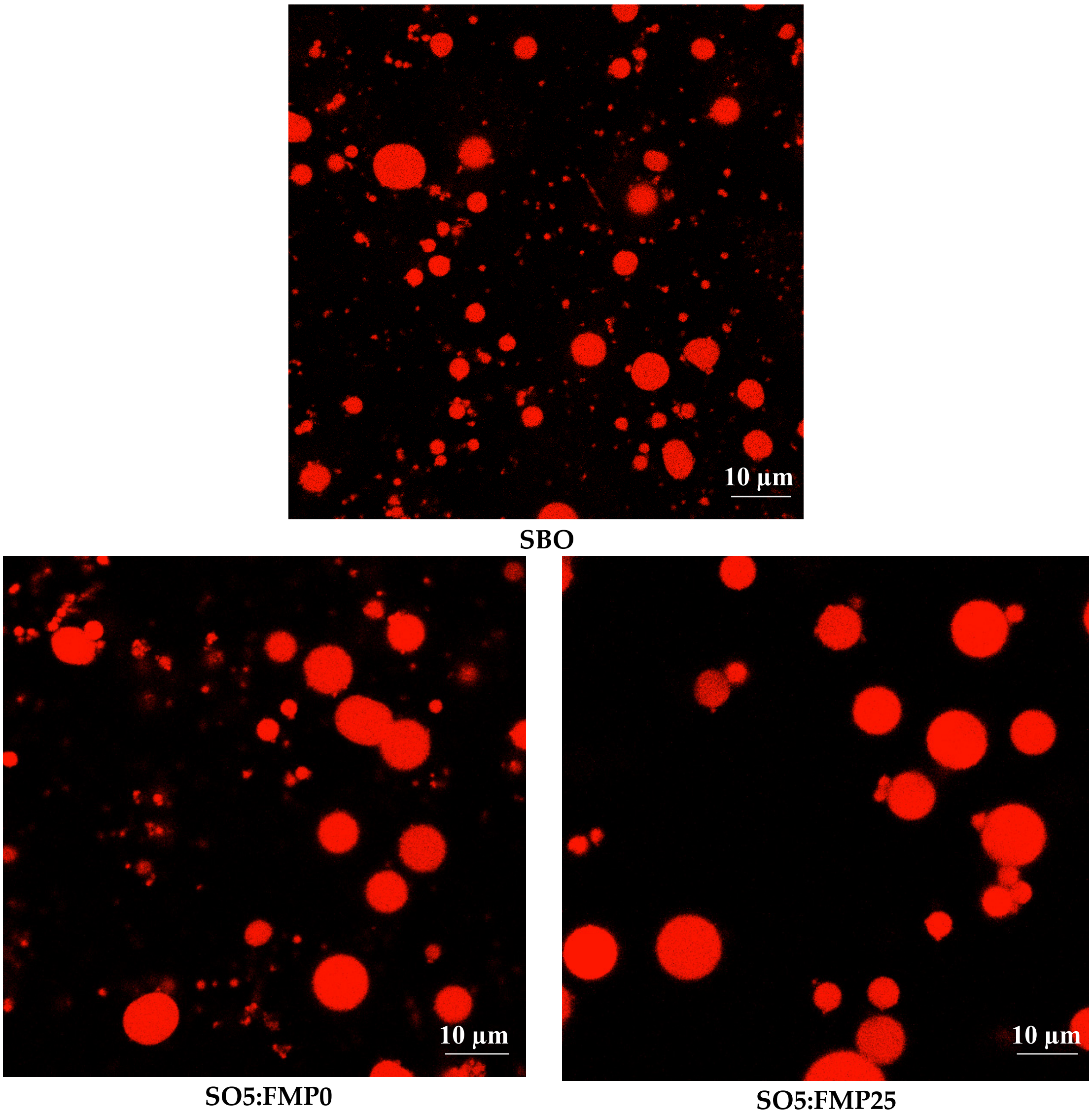

Figure 6. CLSM of SO5:FMP0 and SO5:FMP25 emulsion samples in comparison to the SBO sample. SBO: mayonnaise containing soybean oil without FMP substitution; SO5:FMP0 and SO5:FMP25: SO5 mayonnaise without and with 25% FMP substitution. Observation was made at 400× magnification.

4. Conclusions

Mayonnaise prepared using SO incorporation and FMP substitution at varying levels showed differences in color and textural properties. The color of the mayonnaise sample was affected due to the presence of astaxanthin in SO. The sample with higher amount of SO (15%) and FMP substitution (75%) was not stable. Substitution of FMP, especially in high amounts, lowered the textural and rheological properties. However, the mayonnaise prepared using 5% SO with 25% FMP substitution was acceptable based on sensory analysis when compared with the control mayonnaise (without SO and FMP substitution). Furthermore, the SO-added mayonnaise possesses health benefits due to the presence of bioactive compounds such as PUFAs and astaxanthin. Moreover, FMP acted as an alternative to egg yolk in developing a functional SO-enriched mayonnaise.

Author Contributions: B.R.: Data curation; Investigation; Methodology; Writing of original draft. A.S.: Conceptualization; Supervision; Data curation; Writing—review and editing. B.Z.: Data curation; Writing—review and editing. H.H.: Data curation; Writing—review and editing. T.P.: Data curation; Writing—review and editing. S.B.: Conceptualization; Funding acquisition; Resources;

Supervision; Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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