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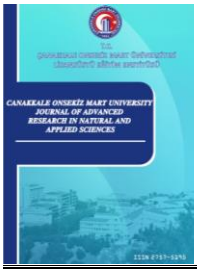

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Improving the Physical Stability of Virgin Olive Oil Mayonnaise

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Research Article

Abstract − Mayonnaise is a popular solid like sauce obtained typically from the ingredients; vegetable oil, vinegar, egg yolk, and salt. For mayonnaise production, vegetable oils with low costs are preferred. Extra virgin olive oil (EVOO), despite its high cost, is unique in that it has some very exceptional nutritional and sensorial properties and positive health promoting effects. However, EVOO mayonnaises pose some challenges in preparation and particularly in maintaining their stability for elevated periods. This study explored some options that could extend the shelf life of mayonnaise prepared from EVOO. For this purpose, two different stabilizer sodium alginate and gellan gum at two different concentrations (0.1% and 0.2%) were added to mayonnaise formulations, additionally ultrasound was applied at two different powers (40% and 70%) for 2 min. Rheological characterization revealed that all mayonnaise samples displayed a pseudoplastic behaviour which is desirable in condiments like mayonnaise. Particle size measurements revealed that oil particle diameters ranged between 2.1-25.5 μm. Real time and accelerated emulsion stability measurement were in line with each other. According to these, sodium alginate resulted in mayonnaise with the highest physical stability. Real time emulsion stability measurements revealed that all samples except control maintained their physical stability up to 20 days after preparation.

Keywords – Emulsion stabilization, *EVOO, mayonnaise, olive oil, sodium alginate*

1. Introduction

Mayonnaise is a popular solid like sauce obtained typically from the ingredients; vegetable oil, vinegar, egg yolk, and salt. The sensorial properties of this condiment is very much affected by the mouth feel of the product as well as taste. Thus, properties like appearance, structure, creaminess, and rheological properties are exceptionally important for mayonnaise. For condiments like mayonnaise, customer satisfaction is shaped by these parameters that make up the texture of the final product along with the product's physical stability (Giacintucci et al., 2016).

From a colloidal standpoint, mayonnaise is an emulsion, and like all emulsions, it is thermodynamically unstable. It is a low-pH oil-in-water emulsion characterized by high oil content, varying between 65 to 85% based on the formulation. The high oil content makes it particularly hard to maintain stability with the major component, oil being the dispersed phase an d the minor component, water being the continuous phase. The final texture and stability of the emulsion are modified by the distribution of the oil phase, the overall size of oil droplets, and the interaction between different components in the formulation. The micro-sized oil particles are stabilized by a layer of egg phospholipids present in egg yolk. These small surface-active molecules position themselves spontaneously at the interface and prevent the droplets from coalescence (Kiosseoglou & Sherman, 1983; Langton et al., 1999).

As the type of oil, mayonnaise formulations typically contain worldwide spread vegetable oils with low cost. Most used oils for this purpose are rapeseed, corn, soybean, sunflower, and canola oil. Olive oil, with its higher

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cost is not particularly preferred for mayonnaise preparation. However, extra virgin olive oil (EVOO) is unique in that it has some very exceptional nutritional and sensorial properties and positive health-promoting effects. EVOO makes up a very crucial part of dietary lipids acquired from olive tree crops especially in Mediterranean region countries such as Greece, Italy, Turkey, and Spain (Bendini et al., 2007; Boskou et al., 2006; Zampounis, 2006).

However, despite being a very nutritious oil, the use of EVOO oils as an ingredient in complex crafted foods is quite rare. This has a few different reasons. One of them is the high cost of this oil with respect to other vegetable oils, and the other reason is its unique sensorial properties. The consumers from Mediterranean regions where olive oil consumption is high are more familiar with the taste of olive oil, and mayonnaise and other sauces from olive oil could be a preference for many individuals in these regions not only because of its health benefit but also for its sensorial attributes (Bendini et al., 2007; Zampounis, 2006).

Nevertheless, replacing another vegetable oil with olive oil in complicated formulated systems like a low stability food emulsion such as mayonnaise could introduce many problems. The high dispersion degree could pose a problem in maintaining the physical stability of the product throughout its whole shelf life. The intrinsic features of an emulsion, like the main ingredients, define the colloidal attributes like the kinetic and thermodynamic stability. The existence of some components that can either negatively or positively affect emulsion stability by changing the composition of the bulk phase and especially the interface (Freer et al., 2004; Kontogiorgos, 2019).

Olive oil, particularly extra virgin olive oil, contains bioactive molecules that also have a surface affinity. These bioactive molecules are of diverse nature, but a major portion of these is made up of phenolic compounds that show powerful antioxidant activity (Bendini et al., 2007; Giacintucci et al., 2016). Recently, researchers have focused on exploring the emulsification properties of emulsions prepared with EVOO and investigating the role of surface-active compounds in it like phospholipids, free fatty acids, and phenolics. The results of these studies indicated that olive oil is unique in that the abundance of these surface-active endogenous amphiphilic molecules greatly differentiates the dynamics of emulsions prepared by it. This was related to the difference in the composition of the olive oil-water interfaces compared to other vegetable oils (C. D. Di Mattia et al., 2009; Carla D. Di Mattia et al., 2011).

To the best of our knowledge, there currently are very few studies to investigate the effect of polyphenols on the structure of real food emulsions such as mayonnaise, presumably owing to the sophistication of their formulation that could restrict the interpretation of the role of these surface-active bioactive compounds that are very low in concentration. The objective of this work was to explore the physical and structural attributes of mayonnaise prepared by extra virgin olive oils (EVOO) and go one step further than previous research on the subject; this study also aims to come up with ways to improve the physical stability of virgin olive oil mayonnaise. Mayonnaise samples were produced by using a single recipe, only differentiating in the type and amount of hydrocolloids (incorporated as a stabilizer). For this purpose, guar gum and sodium alginate was used. In addition to a standardized homogenization procedure, some samples were further homogenized via ultrasonication. Produced mayonnaise samples were then characterized by physical stability, droplet size distribution, and rheological properties.

2. Materials and Methods

2.1. Materials

Eggs (Jumbo, Large, Keskinoğlu, Turkey), apple cider vinegar (Gurme Elma Sirkesi, Kemal Kükrer, Turkey), salt (İyotlu tuz, Billur Tuz, Turkey), extra virgin olive oil (Ege Soğuk Lezzetler Naturel Sızma Zeytinyağı, Komili, Turkey) was purchased from a local grocery store in İstanbul, Turkey. Gellan Gum (CP Kelco UK Ltd, UK), Sodium Alginate (FMC Biopolymer Ltd., UK) was purchased from Sigma Aldrich.

2.2. Methods

2.2.1. Sample Preparation

Mayonnaises were prepared following a recipe studied in Giacintucci et al. (2016). Mayonnaise ingredients were used in the relative amounts of; oil (500 g), eggs (120 g), vinegar (30 g), salt (1 g). To this mixture, depending on formulation, either 0.1 and 0.2% of gellan gum or sodium alginate were added. Mayonnaises were prepared with a two-step procedure. Initially, egg yolk, vinegar, salt, and gum were mixed with a kitchen-type mixer. Then to this mixture, oil is added slowly by vigorous mixing with a lab-style high-speed homogenizer (Ultraturrax, WiseTis HG-15D,Wertheim, Germany) at 5000 rpm for 10 min. For ultrasound applied samples, the mayonnaise was later subjected to an ultrasound treatment (UW 2200, Bandelin, Sonopuls, Berlin) at either 40% or 70% max power for 2 minutes. Samples treated with ultrasound at 70% of the instrument's maximum power did not provide stable emulsions and thus were excluded from some of the measurements.

2.2.2. Experimental Design

The experimental design is given in Table 1.

Table 1
Experimental Design

Formulation	Control	G0.1	G0.2	S0.1	S0.2	US70	US40
Olive oil	+	+	+	+	+	+	+
Vinegar	+	+	+	+	+	+	+
Eggs	+	+	+	+	+	+	+
Salt	+	+	+	+	+	+	+
Ultrasound Treatment	-	-	-	-	-	+	+
Gum		0.1 % w/v Gellan Gum	0.2 % w/v Gellan Gum	0.1 % w/v Sodium Alginate	0.2 % w/v Sodium Alginate		

2.2.3. Real-time emulsion Stability

Real-time emulsion studies were conducted by monitoring of the physical stability of emulsions by taking pictures of the samples. Mayonnaises, after preparation, were placed into cylindrical sealed tubes, and these samples were put to rest at either 25ºC or 40 ºC. Photos were taken every 2-3 days over the course of 40 days.

2.2.4. Accelerated Emulsion Stability

Accelerated Emulsion Stability measurements were performed to mimic real-time emulsion stability results. Since we had limited time to observe the emulsions, it might not be possible to see the destabilization of some of the metastable mayonnaise formulations. For this, following preparation, mayonnaises were centrifuged (Sartorius, Sigma 1-14, Germany) at 6000 rpm for 20 min at 25 ºC. In case of a visible phase separation, the upper layer was identified as the oil portion, and the lower layer was identified as the serum layer. Percent creaming index (%CI) values were calculated by measuring the height of each layer and taking the ratio as shown in the following equation;

$$\%CI = \frac{H_C}{H_T} \times 100 \tag{2.1}$$

where H_C is the height of the cream layer and H_T is the total height.

2.2.5. Rheological Characterization

To characterize the flow behaviour and viscoelastic properties of mayonnaises, shear rate ramp and amplitude and frequency sweep tests were conducted. For the measurements, a cone-and-plate (40 mm diameter and 4° cone angle, 0.1425 mm gap) dynamic rheometer (Malvern, Kinexus Pro Rheometer, UK) was employed. Shear rate ramp measurements give shear stress values for changing shear rate values. For this purpose shear rate was changed between 0.1 s^{-1} to 100 $s^{-1,}$ and shear stress values were recorded for 20 sampling points for a total measurement time of 2 min. The results were found to be suitable to be defined with a power-law model;

$$\tau = K\gamma^n \tag{2.2}$$

where τ (Pa) is shear stress, K (Pa. s^n) is the consistency index, γ (s^{-1}) is shear rate, n is flow behavior index.

2.2.6. Particle Size Measurement

Light diffraction method was utilized for the measurement of the sizes of oil droplets. For this, a particle size analyzer (Mastersizer 3000, Malvern, UK) was employed. The reported droplet sizes are volume-moment mean diameter, calculated from the following equation;

$$d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \tag{2.3}$$

where n_i is the number of particles in emulsion with diameter d_i. For olive oil droplets, a refractive index of 1.46 and absorption of 0.01, and for water, a refractive index of 1.33 was used (Kirtil & Oztop, 2016). To prevent potential multi-scattering effects, emulsions were diluted approximately 10^{-4} to 10^{-7} of their initial concentration. Particle size measurements were conducted within 24 h of sample preparation.

2.2.7. Statistical Analysis

All measurements were carried out in at least three replicates. Statistical analysis software Minitab (Version 16, State College, PA, USA) was used for the analysis of variance (ANOVA) and Tukey's multiple comparison tests. Differences were considered significant for $p \leq 0.05$.

3. Results and Discussion

3.1. Real-time Emulsion Stability

Results of real-time emulsion stability can be seen in Figure 1 and Figure 2. As evident from the figures, emulsions kept at 40 °C were much quicker to destabilize that emulsions kept at 25 °C. It was possible to see the first signs of creaming 5 days after preparation for 40 °C samples, whereas 25 °C samples were relatively stable up until the 25 day mark. Temperature increases the rate of molecular movement by increasing the kinetic energy of the molecules. Viscosity is also known to be negatively correlated with temperature.

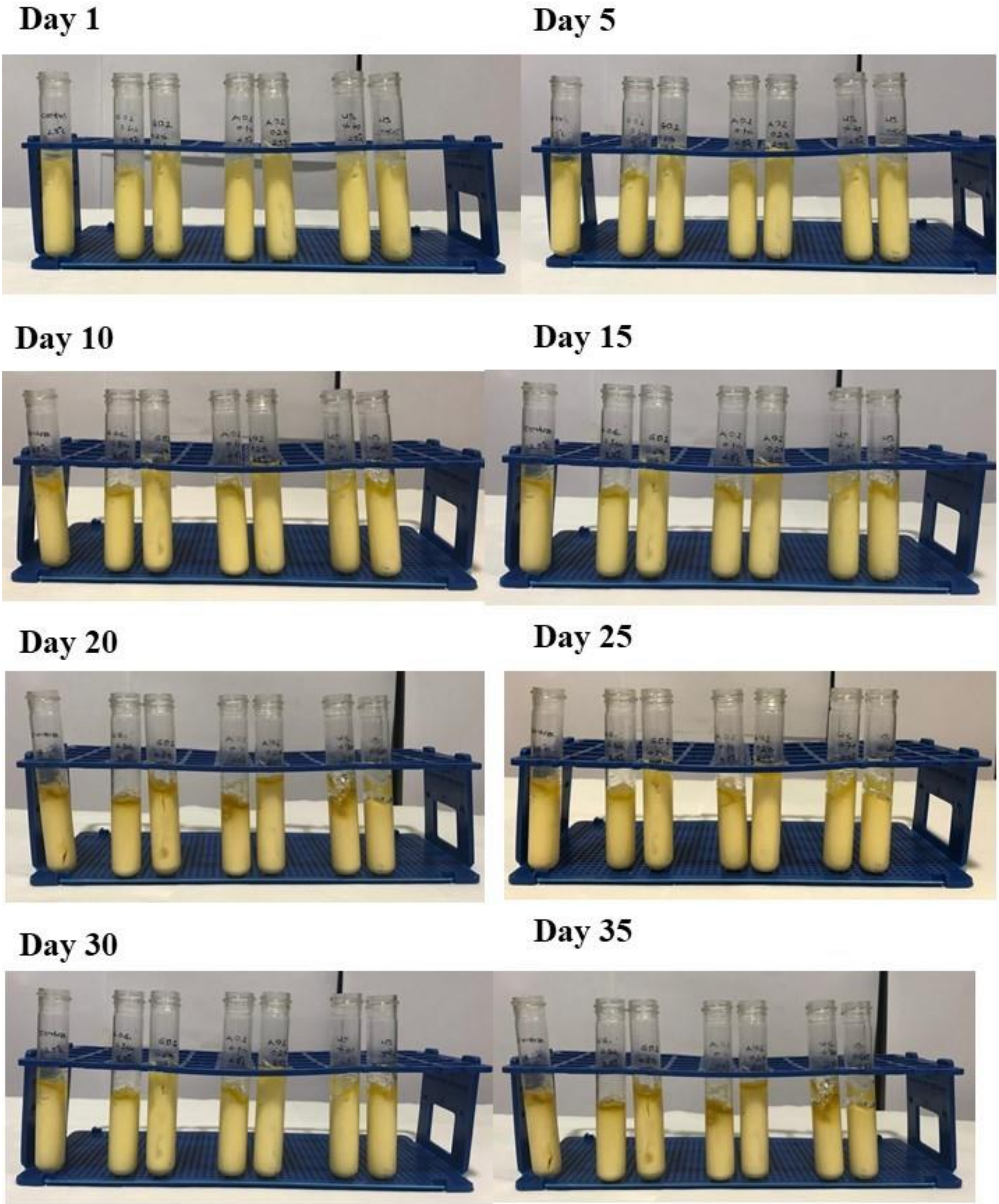

Figure 1. Real time emulsion stability measurement results at 25 ºC

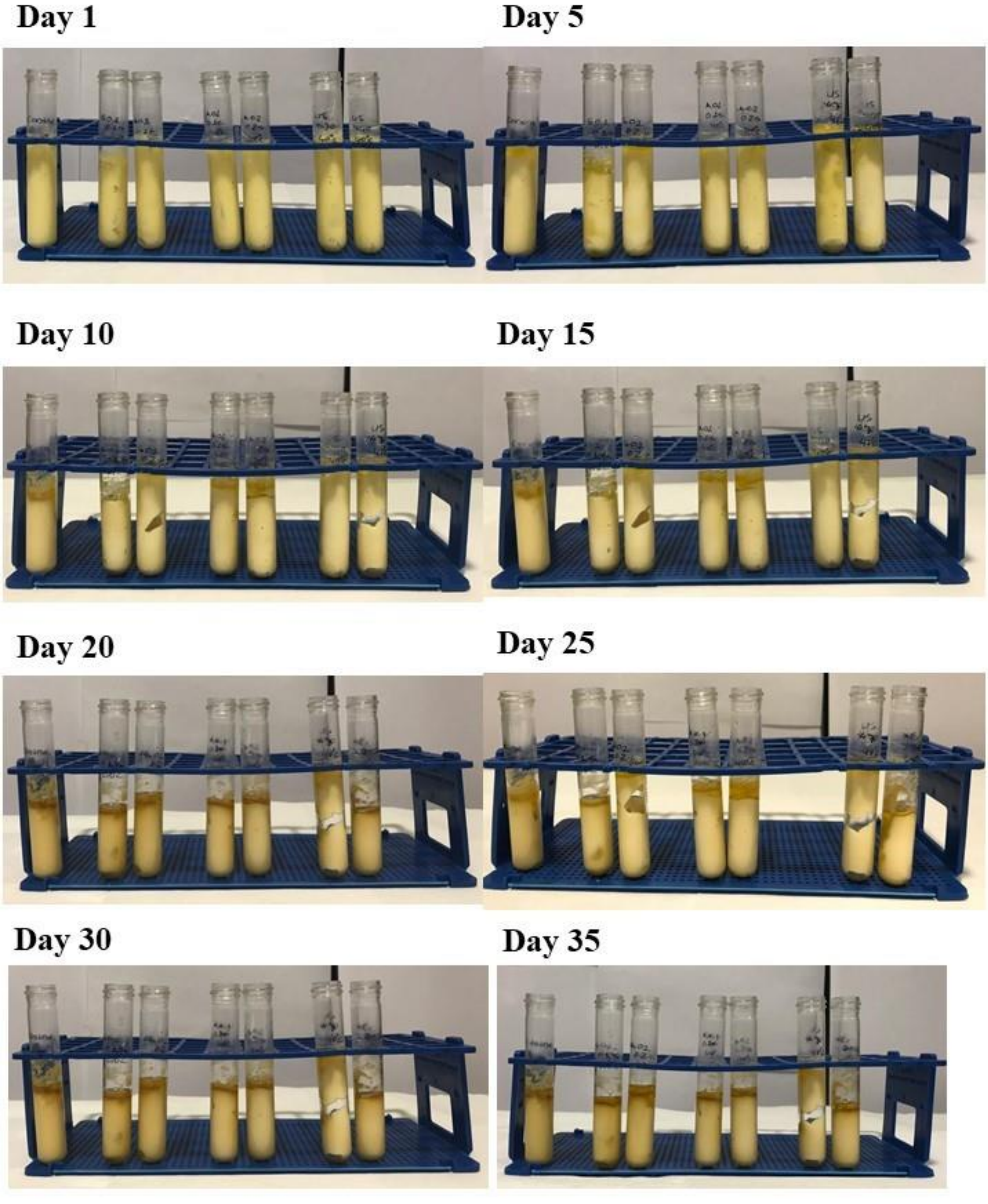

Figure 2. Real-time emulsion stability measurement results at 40 ºC

The increased molecular mobility coupled with the decreasing viscosity accelerates the rate of movement of the oil droplets. Mayonnaises are a kinetically stable system; hence they are stabilized by retardation of particle movement and collisions. However, oil particles are always under the effect of gravitational and buoyancy forces, and despite being slow they always try to move against gravity and form a cream layer at the top (Berg, 2010; Kirtil et al., 2022; Kontogiorgos, 2019). This movement is expectedly much faster at 40 ºC. At 25 ºC, mayonnaises were mostly stable for the first 20 days. After 20 days, all samples showed signs of phase separation. The control sample phase-separated before the others and had a final cream layer thicker than the other samples. This is in line with our expectation that extra virgin olive oil mayonnaises destabilize quickly and have a shorter shelf life than other plant oil-based mayonnaises. All other samples destabilized at similar rates and showed similar sizes of cream layers. At 40 ºC, the control sample was again the first to destabilize at around 5 days after preparation. This is followed by the other samples, all of which showed signs of phase separation at varying degrees at the end of 35 days.

3.2. Accelerated Emulsion Stability

Creaming index (%CI) results indicate the extent of destabilization that the mayonnaise sample went through by the vigorous centrifugal force. If there was a phase separation, it was easy to recognize it. Some of the olive oil separates itself from the rest of the emulsion and forms a clear layer on top. %CI data can be seen in Table 2. The photos of samples taken after accelerated emulsion experiments are given in Figure 3.

Table 2
Creaming index (%CI) results gathered from accelerated emulsion stability experiments

Samples	CI %
Control	7.61±0.68[d]
G0.1	5.88±0.71[d]
G0.2	17.9±1.83[c]
S0.1	0
S0.2	0
US40	38.6±1.11[b]
US70	54.3±0.98[a]

Means within the same column, followed by the different letters are significantly different ($p<0.05$).

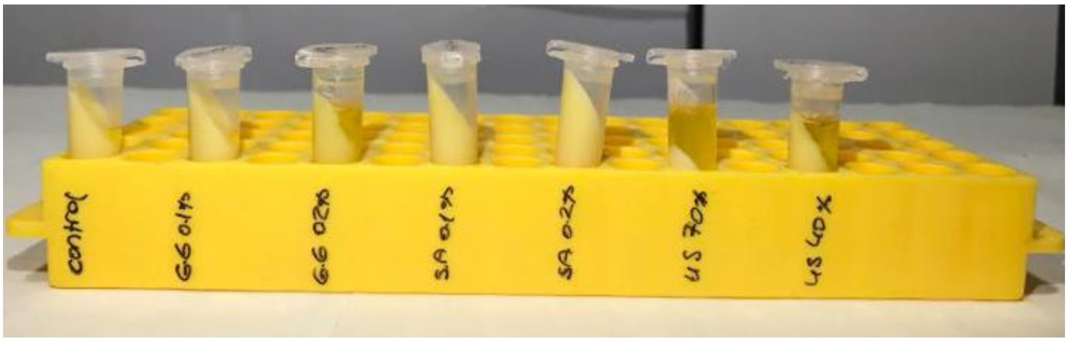

Figure 3. Results of accelerated emulsion measurements

As evident from both the photos and the %CI data, samples prepared with sodium alginate showed no phase separation. These were the only samples that didn't show any phase separation. Mayonnaises prepared with sodium alginate were found to be the most stable samples. Sodium alginate is a hydrocolloid with a high molecular weight and is commercially used as a stabilizer in many food products (Rosell et al., 2001; Vélez-Erazo et al., 2020; Yang et al., 2012). Alginate increased the viscosity of the continuous phase thereby retarded particle collisions and upward movement of particles under the effect of buoyancy force (Berg, 2010; Kontogiorgos, 2019). The accelerated emulsion studies show that alginate at both concentrations (0.1 and 0.2%) was a successful emulsion stabilizer for extra virgin olive oil mayonnaise. However, other methods were not as effective. Gellan gum was not successful in preventing phase separation. At %0.1 concentration gellan gum sample's %CI was not significantly different ($p>0.05$) than control samples. Interestingly when the amount of gellan gum increased to 0.2%, this introduced a further instability to emulsions. This could be related to the complex interactions between gellan gum and other mayonnaise ingredients and requires further analysis. Ultrasound samples showed the highest %CI values. As US power increased, %CI values also increased (up to 54.3%).

Ultrasound application was not suitable for this type of emulsion. We had observed this even right after US treatment, as there were visible signs of instability on the samples. It is a known phenomenon that if, during emulsification, samples are subjected to a homogenization method for too long after sample formation, this could break the emulsion (Berg, 2010; Dickinson, 2009). Ultrasound homogenizes emulsions by sending sound waves that can help disperse oil particles by separating large particles into smaller ones. However, if it is over-applied to an already homogenized emulsion, it can act inversely and can cause the droplets to flocculate and even merge (Dickinson, 1998, 2008). Ultrasound most likely caused the particles to merge and increase in size, which greatly accelerated the rates of creaming, and this is observed as higher %CI values. The 70% US sample was visibly unstable. Thus, it was removed from some of the analyses.

3.3. Rheological Characterization

4 shows the change of shear viscosities (Pa.s) with shear stress (s^{-1}) for all samples, including US70. As seen from the figure, shear viscosities decrease with increasing shear rate. This behavior is characteristic of pseudoplastic materials. The hydrocolloids that cause this behavior in such fluids, during flow, align themselves with the direction of flow and ease the movement of other molecules over one another. With increasing shear, this alignment is even more and more complete; thus, molecular mobility increases, which decreases viscosity (Dickinson, 2011; Vogt et al., 2015).

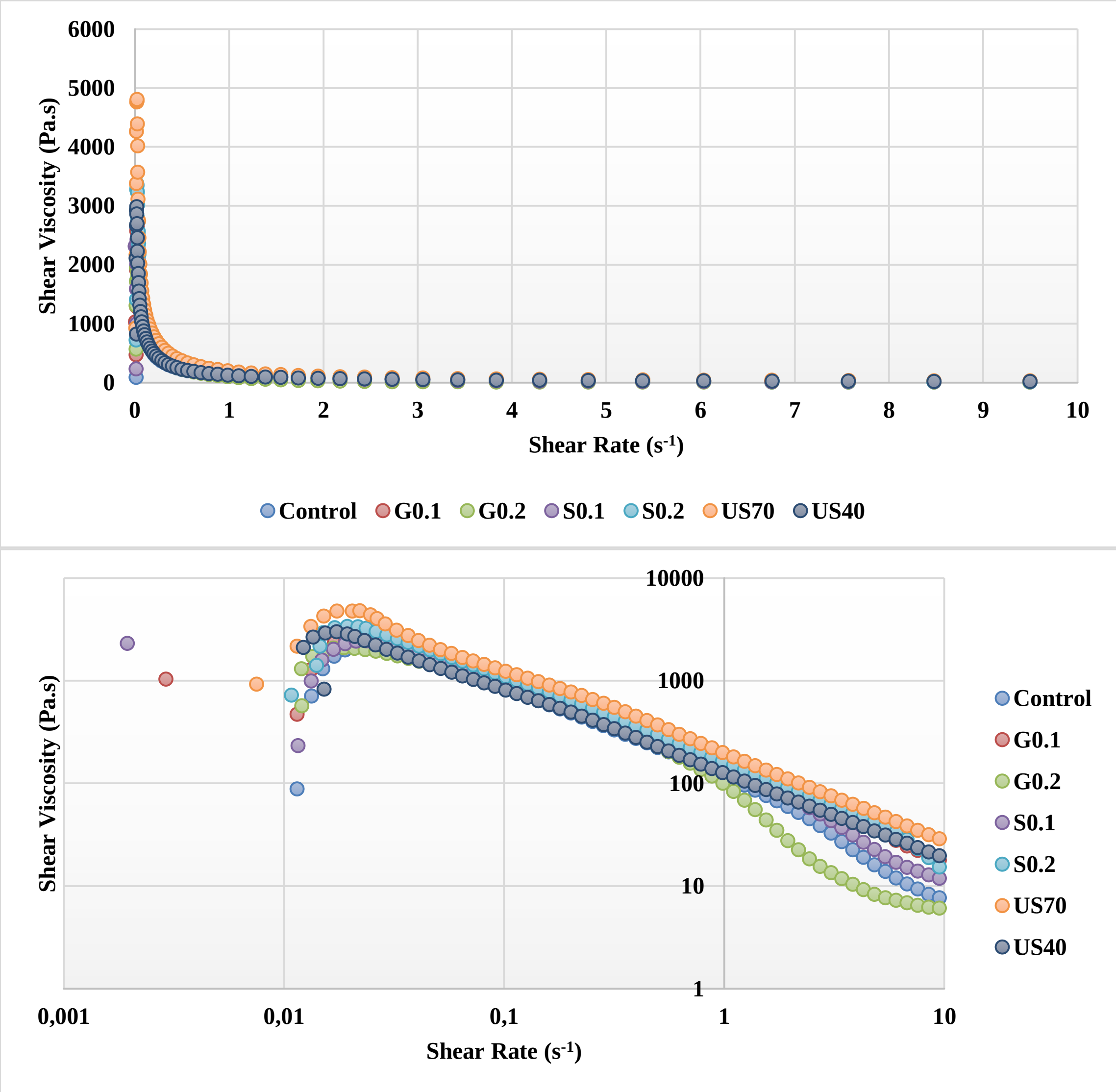

Figure 4. Change of shear viscosity with shear stress for mayonnaise samples

Shear-thinning behavior is especially essential and desirable in sauces because these condiments have to stay solid like at rest but should act more fluid-like under stress, such as during pumping or consumption (Marcotte et al., 2001). With the addition of sodium alginate and gellan gum, the shear-thinning behavior is not restricted, which is a positive result. The strong negative correlation between shear rate and viscosities was linear on a logarithmic scale. Hence, a power model fit was found to be suitable for fitting the data. This assumption was confirmed with correlation coefficients of R^2>0.90 for power-law fitting of shear rate ramp experiment results.

Table 0
Power-law fitting results of shear rate and shear stress data

	K	**n**	**R^2**
Control	91.7 ± 2.7^{e}	0.813 ± 0.07^{b}	0,90
G0.1	138.6 ± 3.3^{c}	0.709 ± 0.08^{d}	0,90
G0.2	66.7 ± 2.1^{f}	0.957 ± 0.05^{a}	0.93
S0.1	118.6 ± 4.1^{de}	0.752 ± 0.03^{c}	0.90
S0.2	154.1 ± 4.7^{b}	0.768 ± 0.07^{c}	0.95
US40	124.5 ± 5.3^{cd}	0.768 ± 0.09^{c}	0.98
US70	193.5 ± 4.8^{a}	0.754 ± 0.03^{c}	0.95

Means within the same column, followed by the different letters, are significantly different (p<0.05).

Table shows the coefficients of the power-law equation for each of the mayonnaise samples. US70 displayed the highest consistency index values. All mayonnaises displayed a shear-thinning behaviour as identified with n values lower than 1. Consistency index values are correlated with the system's resistance against the flow (Chakraborty et al., 2018; Krstonošić et al., 2020; Nikzade et al., 2012). A higher consistency index (K) indicates a more viscous structure. Considering this, US70 mayonnaise was thicker in terms of texture than others. This could be related to an abundance of oil particles of large particle sizes. It is known that in emulsions, as the particle size of the dispersed phase increases, the solution becomes more viscous (Berg, 2010; Wilde et al., 2004). The addition of sodium alginate seemed to result in higher K values than control, and K also increased with an increase in the concentration of the gum, whereas shear thinning behavior was not affected by gum concentration. One interesting finding worth noting here is that G0.2 had lower K and higher n values than G0.1. Increasing gellan gum concentration seemed to decrease mayonnaise consistency. This is in line with the accelerated emulsion stability results that showed higher %CI values in G0.2 compared to G0.1.

3.4. Particle Size Measurements

Table 4
Volume-moment mean diameter results of mayonnaise samples

Samples	**d_{43} (μm)**
Control	8.21 ± 1.1^{b}
S0.1	6.39 ± 1.2^{b}
S0.2	25.42 ± 2.1^{a}
G0.1	2.11 ± 0.4^{c}
G0.2	2.59 ± 0.2^{c}
US40	3.76 ± 0.3^{c}

Means within the same column, followed by the different letters are significantly different (p<0.05).

The size of oil particles ranged between 2.1-25.5 μm. Other studies have also reported oil particle diameters of similar sizes (Gavahian et al., 2018; Hong et al., 2018; Laca et al., 2010; Langton et al., 1999). The sample was subjected to a minimum of 1000-fold dilution procedure before measurements, and US70 sample phase-separated during this pretreatment. Hence it was not measured. Another sample that also phase-separated was S0.2. Still, we measured the particle size of this sample as, unlike US70, it was a very stable sample before dilution. However, the much larger particle sizes of this sample compared to others shows that this result could be unreliable and will be disregarded during interpretation. There was no statistical difference between the particle size results of control mayonnaise and S0.1 mayonnaise (p<0.05). This shows that alginate addition did not significantly favor emulsion formation. However, the particle sizes of G0.1, G0.2, and US40 ranged between 2.11-3.76 and were lower than the other samples. The presence of gellan gum might have provided a more efficient energy distribution in the continuous phase that resulted in better dispersion of oil particles in the aqueous phase. US40, on the other hand, despite being easily destabilized with accelerated stabilization measurements, actually displayed smaller droplet sizes than the control. This shows that US40 was mildly successful in decreasing the overall mean droplet diameter. The destabilization introduced by US could be associated with a more heterogeneous particle distribution after US application. Other studies have reported similar results where over-application of US might increase particle size heterogeneity (Dickinson et al., 1994; Kaltsa et al., 2013; Kentish & Feng, 2014; Li et al., 2019). Thus, despite an overall decrease in mean droplet sizes, the presence of large particles could have accelerated Oswalt ripening (which is one of the primary mechanisms of destabilization for mayonnaise), resulting in an overall unstable emulsion.

4. Conclusion

Mayonnaise is a popular solid-like sauce typically obtained from the ingredients; vegetable oil, vinegar, egg yolk, and salt. As the type of oil, mayonnaise formulations typically contain worldwide spread vegetable oils with low cost. Olive oil, with its higher cost, is not particularly preferred for mayonnaise preparation. However, extra virgin olive oil (EVOO) is unique in that it has some very exceptional nutritional and sensorial properties and positive health-promoting effects. EVOO is rich in surface-active compounds such as phenolics and free fatty acids. These surface-active molecules can prevent the adsorption of lecithin on oil droplets and result in emulsion destabilization. That is why it is particularly challenging to prepare mayonnaise from EVOO that has a long shelf life. This study explored some options that could extend the shelf life of mayonnaise prepared from EVOO.

Rheological characterization revealed that all mayonnaise samples displayed a pseudoplastic behaviour which is desirable in condiments like mayonnaise. None of the additives substantially changed the flow behaviour or increased the sauce thickness to levels that would be unacceptable for the consumer. Particle size measurements revealed that oil particle diameters ranged between 2,1-25.5 μm. The addition of gellan gum favored the dispersion of oil by decreasing particle sizes down to around 2 μm from 8.5 μm of control. Real-time and accelerated emulsion stability measurements were in line with each other. According to these, sodium alginate resulted in mayonnaise with the highest physical stability. These emulsions did not show any phase separation upon centrifugation at 6000 rpm for 20 min. Real-time emulsion stability measurements revealed that all samples except control maintained their physical stability up to 20 days after preparation.

In conclusion, with this study, it was possible to observe the physical stability of mayonnaise formulations prepared with different stabilizers and the incorporation of ultrasound as an additional homogenization method for mayonnaise formation. Especially with sodium alginate addition, improvements in the physical stability of EVOO mayonnaise could be achieved.

Author Contributions

Melis Coskun: Methodology, Investigation, Writing - Original Draft

Sinem Argun: Methodology, Investigation

Emrah Kirtil: Conceptualization, Data curation, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Supervision, Validation

Conflicts of Interest

The authors declare no conflict of interest.

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