J Food Sci Technol (January 2019) 56(1):443–450
https://doi.org/10.1007/s13197-018-3506-2

ORIGINAL ARTICLE

Effect of monoglyceride content on emulsion stability and rheology of mayonnaise

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Revised: 22 October 2018 / Accepted: 7 November 2018 / Published online: 27 November 2018
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Abstract The aim of the study was to determine the effects of monoglyceride content on emulsion stability and rheology of mayonnaise. For this purpose, mono (MG) and diglycerides (DG) were produced by transesterification of refined olive oil with elevating glycerol contents from 25 to 100 g and reaction times from 25 to 40 min. Maximum MG–DG yield was obtained when the reaction was performed for 40 min using 75 or 100 g of glycerol. Under these conditions, 90% of triglycerides (TG) were converted to 40% MG and 50% DG. Using a molecular distillation unit, MG was separated from the transesterification reaction mixture and purified up to 98%. Emulsifiers were then prepared by introducing distilled MG into the transesterification reaction mixture in order to increase the MG concentration from 40 to 98%, for enabling the utilization of them in mayonnaise production. The mayonnaise incorporated with 98% MG showed the highest stability and reduction in the MG concentration in the emulsifier mixture decreased the emulsion stability. Rheological measurements indicated that the control sample without emulsifier had the highest viscosity and shear stress values. The increment of the MG concentration in the emulsifier mixtures resulted in only small differences on the rheological properties of mayonnaise.

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Keywords Monoglyceride · Diglyceride · Transesterification · Molecular distillation · Emulsion stability · Rheology

Abbreviations

MG	Monoglyceride
DG	Diglyceride
TG	Triglyceride
ELSD	Evaporative light scattering detector
MD	Molecular distillation

Introduction

Mayonnaise, a semi-solid and oil-in-water emulsion, has been commonly used all over the world since it was first produced commercially in the early 1900's. Traditionally, it is a mixture of egg yolk, vinegar, oil (70–80%), salt, sugar, spices (especially mustard) and other minor ingredients (stabilizers, thickeners, etc.) (Harrison and Cunningham 1985; Depree and Savage 2001).

Due to unfavorable contact between the oil and water phases, macro emulsions are thermodynamically unstable and always breakdown over time (Dickinson 1992; Krog and Sparso 2004; McClements and Weiss 2005). To prepare kinetically stable emulsions, food industry applies surface active substances known as emulsifiers that have both hydrophilic–hydrophobic groups in their structure and stabilize the emulsion of two immiscible liquids. They reduce the interfacial tension and therefore, play a key role in producing a high stability mayonnaise (McClements and Weiss 2005).

Egg yolk is used as emulsifying agent in the mayonnaise. In addition, the use of a secondary emulsifier has some advantages for obtaining the highest emulsion

stability and the best rheological properties for processing (Franco et al. 1995). MG and DG, known as nonionic emulsifiers, are commonly used in the food, cosmetic and pharmaceutical industries. Their main applications in the food industry involve the production of mayonnaise, bread, cakes, sponge cakes, margarines, ice cream and chewing gum.

MG and DG can be produced by either enzymatically or chemically. Chemical production includes transesterification of TG with glycerol (chemical glycerolysis) by a suitable alkaline catalyst at high temperatures (200–250 °C) (Krog et al. 1996; Moonen and Bas 2004). The product might then be distilled in order to separate MG from the glyceride mixture. Short-path distillation is an alternative and successful way for this purification (Micov et al. 1997; Ferreira-Dias et al. 2001; Fregolente et al. 2010).

The microstructures of mayonnaise depend on many factors such as composition or production conditions. To produce a stable emulsion, oil droplets should be finely dispersed in the water phase (Krog et al. 1996; Moonen and Bas 2004; Maruyama et al. 2007; Nikzade et al. 2012). It was reported that type and concentration of emulsifying and stabilizing agent affect the microstructure and the rheological properties of the mayonnaise (Mun et al. 2009).

Although emulsifying properties of the partial glycerides have been studied by different authors, in our best knowledge there is an obvious lack in the literature about the influence of various MG concentrations as emulsifiers on the mayonnaise properties.

Therefore, the aim of the present study was to produce high-yielded partial glycerides with transesterification and investigate their individual effects on the stability and the rheological properties of mayonnaise.

Materials and methods

Materials

Refined olive oil was purchased from a local market in Turkey. Glycerol (anhydrous 99.0%), ethyl acetate, hexane (liquid chromatography graded), formic acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). 2-propanol and pure standards of triolein, 1-monoolein, 2-monoolein, 1-2-diolein and 1,3-diolein were purchased from Sigma Aldrich (Steinheim, Germany). All chemicals were of analytical grade.

Production of partial glycerides

Refined olive oil was subjected to transesterification reaction in order to produce MG and DG. The transesterification procedure was modified from a method previously described by Noureddini et al. (2004). A 1 L glass reactor equipped with thermometer and feed supply system was used for the transesterification reactions. 300 g of olive oil was heated up to 230 °C under vacuum, and then glycerol/catalyst (NaOH) mixture was subjected to the reaction at a flow rate of 50 mL/min. Various reaction times (from 25 to 40 min) and glycerol contents (from 25 to 100 g) were studied for the production of partial glycerides. The catalyst rate was kept constant (0.75 g) throughout the study. At the end of the reaction time, the mixture was separated into two liquid phases in a separatory funnel, and then the lower phase (glycerol) was removed while the upper phase (MG, DG, and TG) was kept at 4 °C until use.

Distillation of MG

A KDL5 short path distillation unit (UIC GmbH, Alzenau, Germany) with a surface area of 0.048 m^2 was used to distillate MG from the transesterification reaction mixture. Molecular distillation (MD) was carried out at 205 °C with a pressure of 0.01 mbar (Fischer 1998). The feeding rate, roller speed and condenser temperatures were 3 mL/min, 240 rpm and 20 °C, respectively.

Analysis of partial glycerides

Partial glycerides were analyzed using AOCS (1989) official method Cd 11d-96. Approximately 0.4 g of sample was weighed into a 10 mL flask and dissolved in hexane/2-propanol mixture (90:10, v/v), then mixed with a vortex (230v–50 Hz, Heidolph Instruments, Germany) for 1 min. The sample was then injected into a high performance liquid chromatography (HPLC, Shimadzu, Japan) equipped with an evaporative light scattering detector (ELSD, Sedex-Model 80LT, France). Separation of the glycerides (MG, DG and TG) was achieved by using a Lichrospher SI60-5 (25 cm in length, 4.6 mm internal diameter, 5 µm film thickness) fused silica capillary column (Supelco, USA) with the following working parameters: column temperature, 40 °C; ELSD drift tube temperature, 90 °C; nitrogen carrier flow rate, 1.2 L/min. Gradient elution program was applied with a mobile phase consisting of hexane (solvent A) and hexane/2-propanol/ethyl acetate/10% formic acid (80:10:10:1, v/v/v/v) (solvent B) as follows: 98% A/2% B, 0–13.4 min; 65% A/35% B, 13.4–14.2 min, 2% A/98% B, 14.2–25.2 min; 98% A/2% B, 25.2–32 min. The flow rate of mobile phase was 2 mL/min. The peaks were identified using external standards of the glycerides.

Fatty acid composition

Fatty acid compositions of the refined olive oil, the transesterification reaction mixture and the distilled monoglyceride were analyzed using a gas chromatography (Shimadzu GC-2010, Japan) equipped with a flame ionization detector (FID) and DB-23 capillary column (30 m in length, 0.25 mm internal diameter, 0.25 μm film thickness) (Agilent J&W, USA). Fatty acid methyl esters (FAME) of the samples were prepared according to the IUPAC (1987) method. The analysis was performed under the following conditions: injector, column and detector temperatures were 230 °C, 190 °C and 240 °C respectively, the injection volume was 1 μL with a split ratio of 1:80 and the carrier gas was helium at a flow rate of 1 mL/min.

Preparation of emulsifiers

Emulsifiers were prepared by introducing distilled MG into the transesterification reaction mixture (40% MG, 50% DG and 10% TG) in order to increase the MG percentage from 40 to 98%. Various emulsifier mixtures were then obtained as follows: E_1 (40% MG, 50% DG, 10% TG), E_2 (50% MG, 50% DG), E_3 (60% MG, 40% DG), E_4 (70% MG, 30% DG), E_5 (80% MG, 20% DG), E_6 (90% MG, 10% DG) and E_7 (98% MG).

Preparation of the mayonnaise

The mayonnaise samples were prepared in 100 g small batches. Each batch contained refined olive oil (80 wt%), egg yolk (10 wt%), cider vinegar (6 wt%), sugar (2 wt%), salt (1 wt%) and emulsifier (1 wt%) with elevated proportions of MG as described before (E_1–E_7). No emulsifier was used in the control sample. Mayonnaises were prepared in two steps using a standard hand mixer (220 V–50 Hz, Philips, Holland). In the first step, egg yolk, emulsifier (heated to 50 °C) and vinegar were mixed for 1 min, and then salt and sugar were added and the mixture was mixed for 1 min. Secondly, refined olive oil was introduced into the mixture very slowly and the final mixture was mixed gently for 10 min. The mayonnaise samples were stored at 4 °C prior to analyses.

Stability test

The stability of the mayonnaise was measured according to Mun et al. (2009). 15 g of the mayonnaise sample was weighted into a centrifuge tube that was tightly closed with a plastic cap and stored at 50 °C for 48 h. After storage, the sample was centrifuged at 1040 × *g* for 10 min (Hermle Labortechnik GmbH Wehingen, Germany) to separate the upper oil phase. The weight of the precipitated fraction was measured and the emulsion stability was calculated using the following equation (Eq. 1):

$$\%\ \text{Emulsion stability} = \frac{\text{Precipitated fraction weight of emulsion}}{\text{Initial weight of emulsion}} \times 100 \quad (1)$$

Rheological measurements

Rheological properties of the mayonnaise samples were investigated using a rheometer (TA.AR2000 EX, TA Instruments, New Castle, DE). Viscosities and shear stresses of the samples were measured versus time. The measurements were carried out at a constant temperature of 10 °C and parallel plate geometry with 20 mm diameter and 1 mm gap was used. Samples were allowed to rest for 5 min before performing the rheological measurements.

Statistical analysis

All experiments were conducted in triplicate. Statistical analyses were performed using the statistical software SPSS (version 20.0, SPSS Inc., Chicago, IL, USA). Tukey's test was used for the comparison of means at the 95% confidence interval. Values with $p < 0.05$ were considered as statistically significant for all cases and the data were presented as mean ± standard deviation.

Results and discussion

Mono (MG) and diglycerides (DG) produced mainly by chemical transesterification are commonly used as emulsifiers in food, cosmetic and pharmaceutical industries. Varying ratios of MG and DG are used in food industry for emulsification purpose. However, there is not sufficient knowledge about their individual efficiency on the emulsion stability and rheology of food products.

In this study, partial glycerides produced from refined olive oil by transesterification were distilled to achieve elevated MG content in the glyceride mixtures which were then used in mayonnaise in order to examine their individual effects on the emulsion stabilities and the rheological attributes of the product.

Transesterification reactions were carried out at increasing reaction times (25–40 min) and glycerol contents (25–100 g) to obtain the maximum MG and DG conversion from triglycerides (TG). Major products formed by transesterification at various times were MG and DG, but some TG still existed in the mixture (Table 1). Results demonstrated that the TG content statistically decreased

Table 1 MG and DG yields at elevated transesterification reaction time (%)

Glycerides	Reaction time (min)			
	25	30	35	40
TG	11.98 ± 0.10^{a}	11.50 ± 0.20^{a}	10.04 ± 0.40^{b}	9.71 ± 0.04^{b}
1,3-DG	37.86 ± 0.50^{a}	35.67 ± 0.15^{b}	36.68 ± 0.07^{ab}	35.77 ± 0.26^{b}
1,2-DG	13.86 ± 0.02^{b}	14.91 ± 0.41^{a}	13.75 ± 0.08^{b}	13.86 ± 0.14^{b}
1-MG	35.46 ± 0.76^{b}	36.02 ± 0.03^{b}	38.32 ± 0.64^{a}	39.54 ± 0.22^{a}
2-MG	0.65 ± 0.13^{b}	1.58 ± 0.09^{a}	1.03 ± 0.06^{b}	0.95 ± 0.07^{b}
Total DG	51.72 ± 0.53^{a}	50.58 ± 0.26^{ab}	50.43 ± 0.15^{ab}	49.63 ± 0.11^{b}
Total MG	36.11 ± 0.62^{c}	37.6 ± 0.05^{bc}	39.35 ± 0.57^{ab}	40.49 ± 0.15^{a}
Total MG and DG recovery	87.83 ± 0.09^{b}	88.18 ± 0.21^{b}	89.78 ± 0.43^{a}	90.12 ± 0.04^{a}

*Mean values in the same row with different superscript letters are significantly different ($p < 0.05$)

with the increase of the reaction time. The total TG percentage in the reaction mixture was 11.98%, 11.50%, 10.04% and 9.71% at 25, 30, 35 and 40 min, respectively. The total DG content (1,3-DG and 1,2-DG) also statistically decreased from 51.72 to 49.63%. However, the total MG content (1-MG, 2-MG) increased from 36.11 to 40.49% at 25 and 40 min, respectively. Additionally, results also indicated that the total TG conversion to MG and DG increased up to 90.12% and the maximum MG formation was obtained when the reaction time was set to 40 min. Therefore, the duration of transesterification reaction for the production of partial glycerides was selected as 40 min which was used throughout the study.

Table 2 shows the effects of elevated glycerol content on the yield of MG and TG. The maximum TG conversion and MG formation was observed when using 75 or 100 g of glycerol. There were almost no statistically significant difference in the formation of partial glycerides between 75 and 100 g of glycerol application; therefore, 75 g of glycerol was selected for further transesterification reactions. Comparison of the results in Table 2 showed that the concentration of non-reactive TG decreased dramatically with higher glycerol contents. When 25, 50, 75 and 100 g of glycerol were used in the reaction; the non-reactive TG percentages were 31.60%, 16.27%, 9.71% and 9.22%, respectively. When the glycerol content increased from 25 to 75 g, concentration of the total MG also increased from 14.33 to 40.49%. However, the total DG decreased from 53.58 to 49.63%. This increment was primarily related to the transformation of the non-reactive TG and the 1,3-DG to MG. The total MG and DG formation was greatly increased from 67.91 to 90.12%. It had previously been reported that the glycerol increment expedited higher TG conversion and increased the concentration of the DG–MG. In fact, more glycerol would change the reaction direction towards the MG formation side, which would thus affect the DG content (Noureddini et al. 2004; Zhong et al. 2014). At these conditions (40 min reaction time and 75 g glycerol), the transesterification reaction mixture contained approximately 50% of DG and 40% of MG.

MG can be distilled from TG, DG and glycerol using molecular distillation under high vacuum application. This process allows minimum product decomposition and maximum quality (Moonen and Bas 2004). MG was isolated from the transesterification reaction mixture under the distillation conditions as described in the method section. The weight of the feed and the distillate were measured as 500 g and 199 g, respectively. The distillation

Table 2 MG and DG yields at elevated glycerol content (%)

Glycerides	Glycerol content (g)			
	25	50	75	100
TG	31.60 ± 0.28^{a}	16.27 ± 0.21^{b}	9.71 ± 0.04^{c}	9.22 ± 0.01^{c}
1,3-DG	37.95 ± 0.06^{a}	37.19 ± 0.09^{b}	35.77 ± 0.26^{c}	33.95 ± 0.13^{d}
1,2-DG	15.63 ± 0.07^{a}	15.96 ± 0.03^{a}	13.86 ± 0.14^{b}	15.80 ± 0.03^{a}
1-MG	13.63 ± 0.26^{c}	29.10 ± 0.05^{b}	39.54 ± 0.22^{a}	39.12 ± 0.25^{a}
2-MG	0.70 ± 0.03^{c}	1.03 ± 0.00^{a}	0.95 ± 0.07^{b}	1.54 ± 0.09^{a}
Total DG	53.58 ± 0.02^{a}	53.15 ± 0.12^{b}	49.63 ± 0.11^{c}	49.75 ± 0.10^{c}
Total MG	14.33 ± 0.29^{c}	30.13 ± 0.05^{b}	40.49 ± 0.15^{a}	40.66 ± 0.34^{a}
Total MG and DG recovery	67.91 ± 0.27^{c}	83.28 ± 0.18^{b}	90.12 ± 0.04^{a}	90.41 ± 0.25^{a}

*Mean values in the same row with different superscript letters are significantly different ($p < 0.05$)

yield was calculated as about 40%. The reaction mixture and the purified MG composition profiles determined by HPLC are shown in Fig. 1. The transesterification reaction mixture (containing 9.71% of TG, 49.63% of DG and 40.49% of MG) was distilled and 98% of MG with small amounts of TG (0.2%) and DG (1.8%) was collected in the distillate. Similar results were reported by Krog and Sparso (2004) for the distillate composition. The purified MG consisted of 1-MG and 2-MG with varying ratios which depended on reaction temperature, fatty acid composition and presence of catalyst. It was also reported that the content of 1-MG in the distillate was generally 90–95% (Lauridsen 1976). In this study, the purified MG consisted of 95.5% of 1-MG and 2.5% of 2-MG. Additionally, it was observed that the purified MG had a solid like structure, white color and high viscosity.

Fatty acid compositions of the refined olive oil, the transesterification reaction mixture and the purified MG are shown in Table 3. Oleic (71%–73%), palmitic (12%–14%) and linoleic acids (9%–10%) were the main components of all samples. No statistically significant changes were observed in the fatty acid composition after transesterification. However, the fatty acid composition of distilled MG was statistically different from that of the refined olive oil. When compared to refined olive oil, palmitic and linoleic acids increased from 12.25 to 14.40% and 9.58 to 10.05%, respectively; while oleic acid decreased from 73.08 to 71.10% in the purified MG. These results suggest that distilled MG was mainly consisted of 1-monoolein.

Emulsion stability is defined as the ability of an emulsion to resist to the changes in its physicochemical properties over time. Food emulsions may become unstable due to the different physicochemical mechanisms such as gravitational separation (creaming/sedimentation), flocculation, coalescence, partial coalescence, Ostwald ripening and phase inversion (McClements and Weiss 2005). Therefore, emulsifying agents are used to improve both the formation and the stabilization of the food emulsions (Das

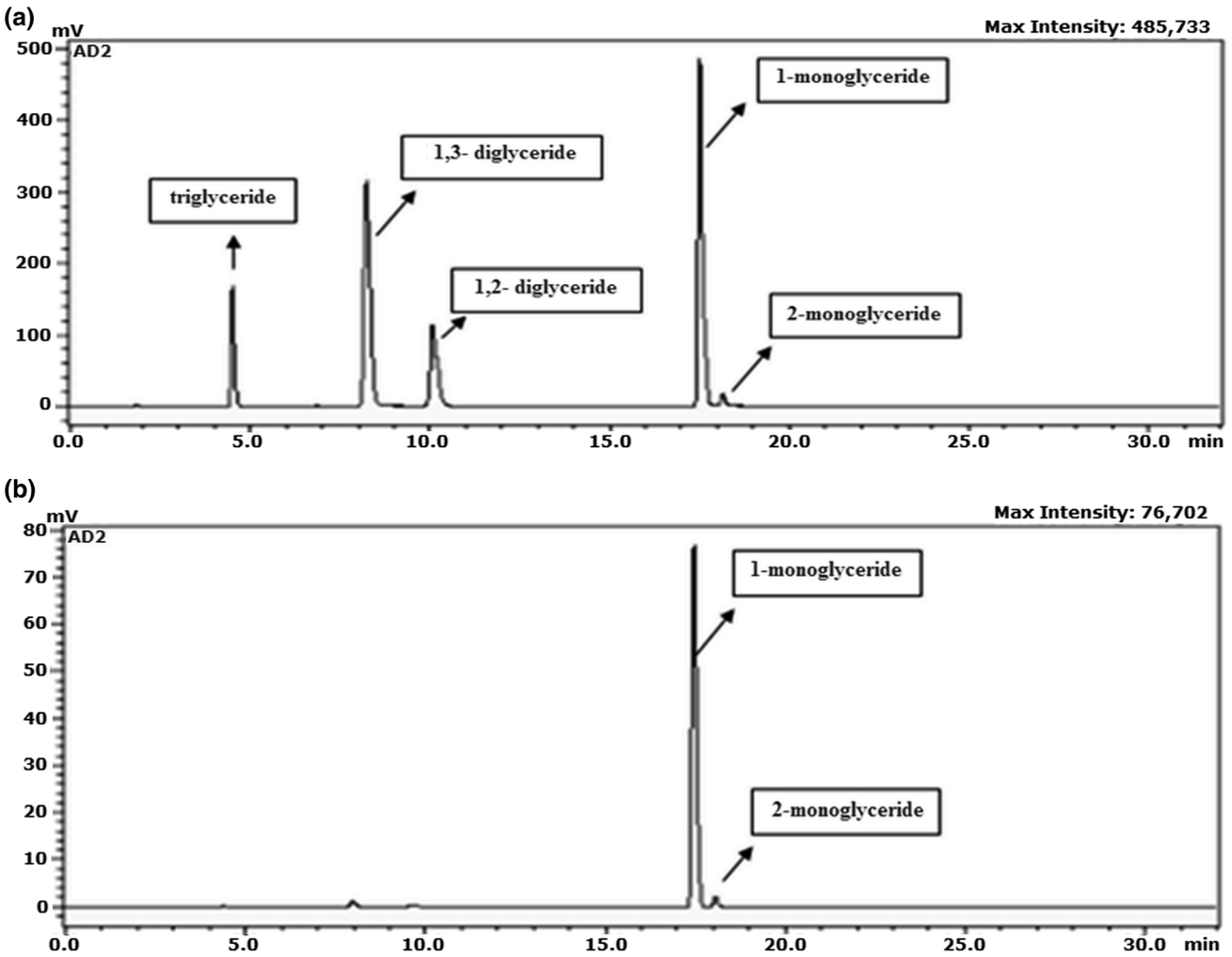


Fig. 1 HPLC profiles of transesterification reaction mixture (**a**) and purified MG (**b**)

Table 3 Fatty acid composition of the refined olive oil, transesterification reaction mixture and distilled MG samples (%)

Fatty acid	Refined olive oil	Transesterification reaction mixture	Distilled MG
C16:0	12.25 ± 0.01^{b}	12.35 ± 0.03^{b}	14.40 ± 0.06^{a}
C16:1	0.90 ± 0.00^{b}	0.87 ± 0.02^{c}	1.16 ± 0.00^{a}
C17:0	0.09 ± 0.00^{a}	0.07 ± 0.01^{a}	0.08 ± 0.01^{a}
C17:1	0.14 ± 0.00^{a}	0.12 ± 0.01^{a}	0.14 ± 0.01^{a}
C18:0	2.73 ± 0.01^{a}	2.45 ± 0.01^{b}	2.14 ± 0.18^{c}
C18:1	73.08 ± 0.12^{a}	73.22 ± 0.11^{a}	71.10 ± 0.39^{b}
C18:2	9.58 ± 0.06^{b}	9.76 ± 0.00^{ab}	10.05 ± 0.15^{a}
C18:3	0.57 ± 0.01^{a}	0.53 ± 0.01^{b}	0.56 ± 0.00^{a}
C20:0	0.41 ± 0.04^{a}	0.38 ± 0.05^{a}	0.20 ± 0.01^{b}
C20:1	0.25 ± 0.05^{a}	0.24 ± 0.05^{a}	0.14 ± 0.01^{a}

*Mean values in the same row with different superscript letters are significantly different ($p < 0.05$)

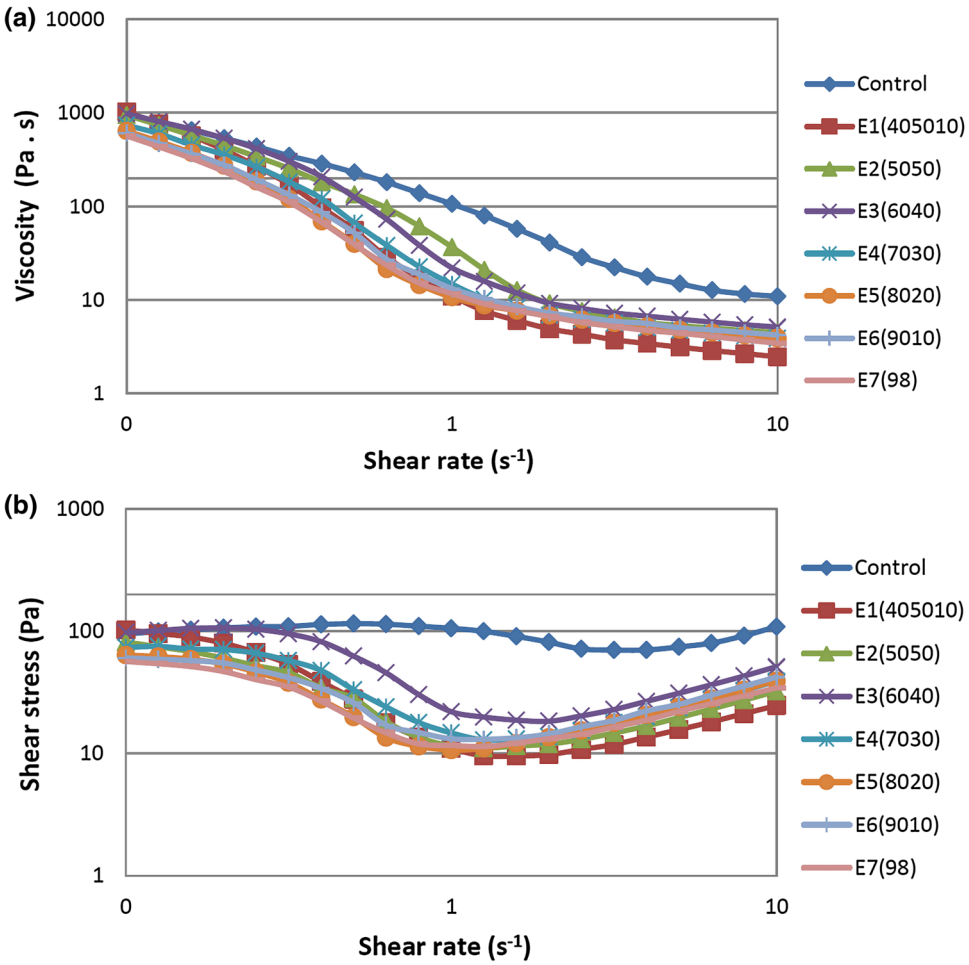


Fig. 2 Viscosity and shear stress of mayonnaise samples under different shear rate: E_1 (40% MG, 50% DG, 10% TG), E_2 (50% MG, 50% DG), E_3 (60% MG, 40% DG), E_4 (70% MG, 30% DG), E_5 (80% MG, 20% DG), E_6 (90% MG, 10% DG) and E_7 (98% MG)

and Kinsella 1990). The first two highest emulsion stability values of 73.4 ± 0.9% and 70.8 ± 1.0% were obtained from the mixtures of E_7 (98) and E_6 (90–10) samples, respectively. Moreover, the lowest stability value (51.9 ± 1.9%) was obtained from the control sample with no emulsifier. Emulsion stabilities of E_1 (40–50–10), E_2 (50–50), E_3 (60–40), E_4 (70–30) and E_5 (80–20) were measured as 59.8 ± 0.4%, 63.3 ± 0.3%, 62.4 ± 2.0%, 64.5 ± 0.3% and 65.3 ± 3.4%, respectively. These results suggest that the increasing MG content in the emulsifier mixture improves the stability of mayonnaise. Loi et al. (2019) investigated the effects of different MG and DG

compositions in a protein-stabilized model emulsion. They concluded that emulsifiers with high MG content were more effective in providing better emulsion stability.

In centrifugation method, the emulsion is subjected to a centrifugal acceleration at a specific speed and time (van Aken and van Vliet 2002). In oil-in-water emulsions, the oil droplets have a lower density than the aqueous phase. Therefore, they move towards the axis of rotation of the centrifuge, resulting in a layer of oil collected on top of the emulsion (Tcholakova et al. 2005). Figure 2 indicates the changes in the apparent viscosity and the shear stress of mayonnaise samples with different emulsifier mixtures. Initial viscosities of all samples were decreased at the end of viscosity measurement, which was due to the non-Newtonian fluid structure of mayonnaise (Nikzade et al. 2012; Li et al. 2014). The viscosity of control sample was the highest for overall experiments. The lowest viscosity value was obtained for E_7 (98) which contained the highest MG concentration when shearing rate was increased up to 1 s^{-1}. At a shearing rate of 0.79 s^{-1}, the viscosity of E_7 (98) was the lowest as 15.08 Pa. s. However, it was observed that the viscosity of E_7 (98) was the second lowest after E_1 (40–50–10) with further increment of shearing rate. In a similar manner, E_2 (50–50) had the highest viscosity at 1 s^{-1} of shearing rate, when the viscosity of control sample was ignored. It was concluded that more viscous (or solid-like) mayonnaise could be formed with the presence of not only a bulk of MG, but also a mixture of appropriate concentrations of both MG and DG which might be due to their different affinities to either water or oil droplets. According to Loi et al. (2019), MG and DG had minimal effects on the viscosity of fresh model emulsions when they were used as emulsifiers. Shear stresses were found in correlation with viscosities. The shear stress values of the mayonnaise samples decreased when shearing rate was increased up to 1 s^{-1} (except control sample). Viscosities of all samples increased when shear rate reached to 10 s^{-1}. The highest and the lowest shear stresses belonged to the control sample and E_1 (40–50–10), respectively.

Conclusion

In this study, different concentrations of partial glycerides were prepared from refined olive oil and used in mayonnaise formulations as emulsifier. According to the experimental results, increase of MG concentration in the emulsifier mixture improved the emulsion stability. The highest stability value was obtained as 73.4% for E_7 (98). According to rheological measurements, apparent viscosity and shear stress were found the highest in control sample. While mixtures of MG and DG at different concentrations resulted in better emulsion stabilities, little changes occurred in both viscosities and shear stresses of mayonnaise. However, the utilization of these mixtures caused measurable changes in both rheology and stability of mayonnaise when compared to those of control.

Acknowledgements This study was financially supported by the Scientific Research Projects Coordination Unit of Ankara University (Project No: 13L4343013).

Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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