The structuring of oil body emulsion with glycerol monostearate and its effect on physicochemical properties and lipid digestibility

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Abstract

As a promising alternative to saturated fats, monoglycerides (MAG) have been well-characterized and applied into emulsion-based food production. Oil bodies, which are stable organelles extracted aqueously from oilseeds, could be used to replace the plant oil in food production. This research aimed to structure the rapeseed oil body emulsion with glycerol monostearate (GMS) and investigate its physicochemical properties as well as the effect on lipid digestibility. GMS solidified the oil body dispersion through crystallization. The crystallization behavior in GMS-oil body emulsion (GOB) was the same as that in GMS-water gel (GW), implying that crystals formed in water phase. While different phase transitions were found in GMS-oil gel (GO). As an emulsifier, GMS also separated the aggregates of oil bodies. reducing their particle size. With the addition of GMS, the viscosity and viscoelasticity increased. GOB was more stable against the deformation under increasing shear strain, compared to other two systems. The results of lipolysis demonstrated that GMS had no effect on lipid digestibility of oil bodies and that GMS could be hydrolyzed by lipase. This project underlines the aqueous crystallization of GMS in oil body dispersion as well as the enhancement of physical properties by GMS, while no influence on lipid digestion.

Key words: oil bodies, glycerol monostearate, structured emulsion, properties, lipolysis.

Abbreviations

FFA: Free fatty acids;
GMS: Glycerol monostearate;
GOB: GMS structured oil body dispersion/emulsion/system;
GO: GMS structured oil system; GMS-oil gel;
GW: GMS-water gel
MAG: Monoglycerides;
OB: Oil bodies; oil body emulsion containing 10% lipid;
PLM: Polarized light microscopy;
SDS: sodium dodecyl sulfate;
SGF: Simulated gastric fluid;
SIF: Simulated intestinal fluid;

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1. Introduction

Saturated fats play an important role in food production of for example, cream and margarine due to their sensory and textural properties including hardness and plasticity. However, it has been proved that high intake of saturated fats might increase the risk of many health problems, for instance coronary heart diseases [1, 2]. The application of saturated fats on food products is therefore in contradiction with the increasing public demand for healthy diet. Polyunsaturated fat has been found beneficial to human health, making it a possible alternative of saturated fats in food production. Despite of its effort, direct replacement might change some mouth feel and physical properties compared to saturated fats. To deal with this technical challenge, food manufacturers and researchers have focused on finding other oil structuring strategies that can restructure the oil to play the role of saturated fats [3].

Monoglycerides (MAG) have been considered as one of the most promising alternatives to replace the saturated fats [4]. The application of MAG in food production is mainly on emulsion-based food products, and the MAG structured w/o emulsion is the currently commercially available fat-like material without saturated triglycerides [5]. It has been found that the stabilized w/o emulsion by MAG could be used as a shortening alternative in cookie production, reducing the use of saturated fat [6]. Besides, MAG could be applied to make spread and suppressed the acute triglyceride response in blood compared to commercially available butter and margarine [7].

As mentioned before, those applications are currently based on plant oil. However, a conventional oil extraction has a big impact on the environment and the use of organic solvent like hexane is believed non-natural among the public. Therefore, finding a new strategy for lipid production is of interest recently. Oil bodies (Oleosomes) are small discrete intracellular organelles which contain storage triacylglycerols surrounded by phospholipids and alkaline proteins, mainly oleosins [8]. During the current commercial process of oil extraction, oil bodies are destroyed into organic solvent for further recovery of oil. It has been found that the extracted oil bodies are stable due to the protection of oleosins and they might be used as a new source of lipid in food production [9]. The reason is that compared to the conventional oil extraction, there is no use of organic solvent and additional emulsifiers before further application, which is more sustainable and natural. To this day, there is no study using oil bodies as the source in oil structuring instead of common vegetable oil.

This current study was conducted to structure the oil bodies by using glycerol monostearate (GMS) which is categorized as long-chain saturated monoglyceride and to investigate the crystallization of GMS in the biphasic oil body-water system and the influence of GMS on physicochemical properties as well as lipid digestibility. The overview of the experimental design is displayed in Figure 1-1. Some important parameters were measured, including particle size distribution, microstructure, melting and crystallization behavior, rheological properties and the kinetics of lipolysis. This experimental design facilitated testing the hypothesis that

GMS could be absorbed into oil bodies and crystallize inside, and that the existence of GMS could influence the properties and the lipid digestion. During each measurement, the GMS-oil body emulsion system containing different amount of GMS was the experimental group while GMS-water system and GMS-oil system were comparison groups.

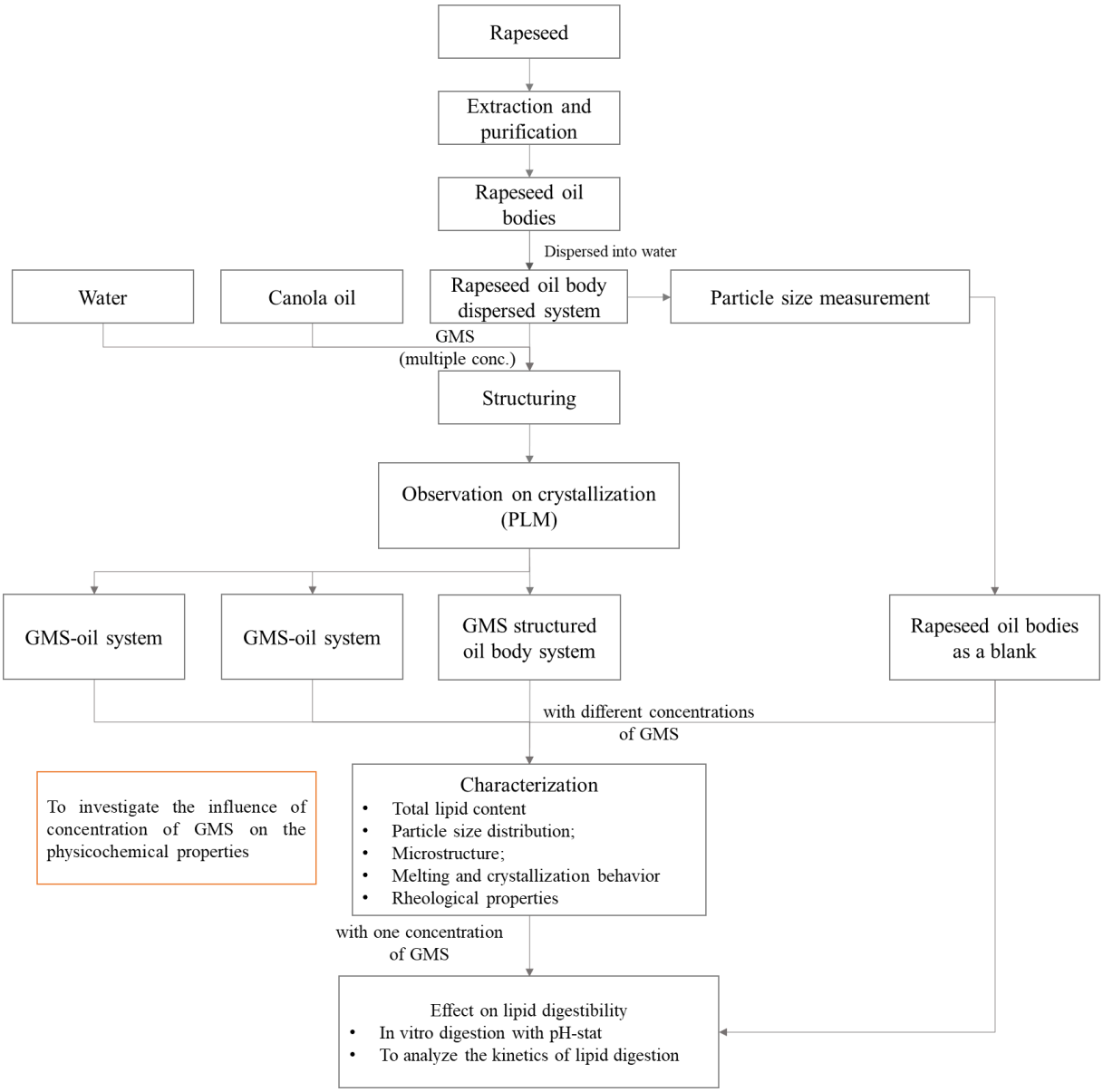

Figure 1-1. Overview of the experimental design

2. Materials and methods

2.1. Materials and regents

Canola oil was purchased from local market. Untreated Alize rapeseeds were provided by a seed producer. All chemicals used in this project were provided by FQD lab.

2.2. Sample preparation

2.2.1. Extraction and purification of rapeseed oil bodies (oleosomes)

This step was conducted according to the study of De Chirico, et al (2018) [10] with modification. To start with, seeds were soaked in 0.1M sodium bicarbonate (pH 9.5 adjusted with 1M NaOH) at ratio of 1:7 (w (seed weight) /w (weight of medium)) at room temperature for 4 hours. Head stirrer was employed here to enhance the sufficiency of soaking with a stirring speed at around 300rpm. After soaking, the mixture was ground by a laboratory blender at maximum speed for 2 minutes. The mash of seeds was then filtered through double layers of a cheesecloth. Afterwards, the filtrate was transferred into 250ml centrifuge tubes and centrifuged at 10000g for 30min at 4°C by using Beckman Highspeed centrifuge. The upper layer which was crude oil body fraction (COB) was gently collected with a spoon. The COB was then dispersed in the same medium used for soaking at ratio of 1:4 (w/w), followed by a centrifugation at 10000g for 30min. The cream layer was isolated gently using a spoon. In order to remove residue of washing solution, the isolated cream was washed again in Milli Q water (1:4 w/w) and centrifuged as before. Then, the cream layer called washed oil bodies (WOB) were collected and stored at 4°C for further study.

2.2.2. Lipid content

The total lipid content of rapeseed oil bodies (OB) was measured by Soxhlet extraction using petroleum ether 40-60°C (P.E.), following the FQD lab protocol of Soxhlet extraction (Appendix 6.1). To remove the moisture of OB, the oil body cream was pre-dried at 105°C overnight before the Soxhlet extraction. This experiment was carried out in duplicate.

2.2.3. Preparation of GMS-Oil body, GMS-Water and GMS-Oil systems

In this step, three groups of samples were prepared by oil body emulsion, water and oil with glycerol monostearate (GMS), separately. To make GMS-Oil body emulsions (GOB), WOB were firstly dispersed in water with 10% lipid content. Afterwards, OB dispersion, water and oil were heated to 60-70°C with stirring and then the GMS powder was added. The heating was stopped when the solid material had dissolved completely. The hot solution was then cooled down to the room temperature. The final concentration of GMS varied from 1 to 8% (weight of GMS/total wight of the system) and was kept the same in three systems. Three kinds of samples were considered structured when crystals could be observed under polarized light microscopy (PLM). The structured oil body emulsions (GOB) with different concentrations of GMS were used for further analysis to get insight on the influence of concentration of GMS on the physicochemical properties of the GOB. The GMS-Water system (GW) and GMS-Oil system

(GO) were used as comparison groups to verify if the properties would be similar or even better in the emulsion system. All the samples were stored at 4°C for further investigation.

2.3. Sample characterization

2.3.1. Microstructure

The microstructures of OB dispersion, GOB, GW and GO with various concentration of GMS were observed by using optical microscope Zeiss Axioskop 2 Plus (Zeiss, German) and the crystals were observed under the polarized light with suitable rotation angle between the polarizer and the analyzer. The magnification was set as 10x subjective and 50x or 100x objective. The results were recorded by the related camera. Both diluted (50 times) and undiluted samples were measured.

2.3.2. Crystalline determination

X-ray diffraction (XRD) analysis was performed upon oil body cream and oil bodies structured by 3% GMS. To concentrate, the structured oil bodies was centrifuged and the upper layer was collected for the measurement. Samples were loaded onto a glass slide and then fed to an X-ray diffractometer (X-Pert Pro; Almelo, Netherlands). The diffractometer was equipped with CuKα source ($\lambda = 1.54$ Å) operated at 35 kV and 30 mA to record wide angle X-ray diffraction patterns (WAXS) in the 2θ range of 5° to 40° at a step size of 3°/min.

2.3.3. Melting and crystallization behavior

In order to describe the melting and crystallization behaviors of GOB, GO and GW with 8% (w/w) GMS, their heat flow rates were measured via differential scanning calorimetry (DSC) according to FQD lab protocol with modification. Before measurement, 10-20mg samples were weighed and put into the hermetically sealed aluminum pans. The measurement included three steps, where the sample was firstly heated to 80℃ from 1℃ and held for 5 minutes. Afterwards, the sample was cooled to 1℃ and held for 5 minutes, followed by the second heating to 80℃ and 5-minute holding. The heating and cooling rate were 10℃ per minute. The heat flow rate (W/g (weight of sample)) was recorded and analyzed in Pyris to describe the melting and crystallization behavior. The heat flow rate of air was measured as the reference for the analysis by using an empty pan.

2.3.4. Particle size

The particle size distributions of OB dispersion, OB dispersion with SDS and GOB with 8% (w/w) GMS were measured separately using Mastersizer 3000 (Malven Instrument Ltd., UK) based on laser diffraction with refraction index of 1.455. To start with, the sample container was filled in enough Milli Q water and the rotator was turned on with the speed of around 1450rpm. Before adding samples, the measurement manual was settled followed by the system initialization and the background measurement according to the instruction of the Mastersizer 3000. The samples were added with pipette drop by drop to the device until the obscuration is above the minimum (at around 3-6%, the data quality fitted well). The measurement was done with 5 repeats at once and the average of the data was collected when the data quality and ISO

quality passed. The average particle size was reported as the surface weighted mean diameter (d3,2) when there was no flocculation or aggregation. Every time when measuring another sample, the device was cleaned with Milli Q water or even the liquid soap until the intensity at 20μm and 100μm was lower than 20% and 100%, separately. All the samples were diluted 50 times for the measurement to avoid the obscuration excessed the range. This experiment was carried out in duplicate.

2.3.5. Rheological properties

The rheological properties were measured among GOB, GW and GO in order to compare the influence of the addition of GMS at different concentrations in these three systems. The data was collected and analysed using the related software. This experiment was carried out in duplicate.

2.3.5.1. Viscosity

The viscosity of samples with the concentration of GMS ranging from 1 to 4% (w/w) was measured using Anton Paar Rheometer MCR501 (Anton Paar, Australia) with cylindrical geometries CC17 and C-CC17 (Volume of 4.7ml) at 22℃. The measurement was performed with random shear rate from 10 to 500/s and the results were showed by viscosity at a function of shear rate.

2.3.5.2. Viscoelasticity

The viscoelasticity of samples with the concentration of GMS ranging from 5 to 8% (w/w) was measured by using the same rheometer as before but with parallel-plate geometry CC25 (Volume of 0.5ml) at 22℃. Amplitude sweep test was conducted at a range of shear strain from 0.01 to 100% and under a constant frequency of 10 rads/s to describe the behavior and determine the limit of linear viscoelastic region (LVER). Parameters including shear modulus, flow and yield points were recorded for analysis.

2.4. Lipolysis in a pH-stat digestion model

A modified in-vitro digestion method with a pH-stat in the intestinal phase according to the FQD protocol was followed. Samples containing lipid (i.e. OB, GOB, oil, GO) were the experimental groups while GW and only digestive fluids were the control groups to exclude unexpected reaction during the intestinal digestion. Here the concentration of GMS for each sample was 8% (w/w). The simulated digestive fluids were freshly prepared and kept at 37℃ for at least 1 hour before the test. Each sample was dispersed into the simulated gastric fluid (SGF) in a beaker (100ml) with gently stirring (approximately 300rpm) at the beginning. Pepsin was replaced by Milli Q water and the gastric digestion was skipped to avoid unexpected enzyme reaction with lipid. After complete mixing between samples and SGF, the gastric mixture was mixed with the simulated intestinal fluid (SIF), followed by the addition of 0.3M $CaCl_2$ and bile salts (final concentration was 10mM). For the digestion, pancreatic lipase Type 2 (100-500U/mg protein) was used, whose pH was adjusted to 7 before adding to the system. A potentiometric automatic titration system 887 Titrino Plus (Metrohm, Switzerland) was

applied to monitor the release rate of free fatty acids (FFA). After adjusting the pH of the sample to 7 at 37℃, the lipase was added. Meanwhile, the titration was started. The digestion was conducted with gently stirring (300rpm) for 2 hours, during which the amount of NaOH used for titration was recorded. The amount of lipid content in all the samples was standardized as 1 gram. When the amount of GOB containing 10% lipid was 10g, the amount of GO containing 100% lipid should be 1g. The overview of the digestion process is showed in Figure 2-1. This experiment was carried out in duplicate.

The FFA release as a percentage of the initial total lipid content was calculated by applying the following formula:

$$\%FFA = 100 * \frac{V_{NaOH} * c_{NaOH} * M_{lipids}}{m_{lipids} * 2}$$

Where V_{NaOH} is the volume of NaOH (L) used for the neutralization of the released FFA, c_{NaOH} is the concentration of NaOH (M), m_{lipids} is the total mass of lipids initially presented in the substrates (1g), and M_{lipids} is the molecular weight of lipids (g/mol). Here the molecular weight of rapeseed oil is assumed as 882.92g/mol, containing 66.4% Oleic acid, 17.9% Linoleic acid, 6.6% Linolenic acid, 3.6% Palmitoleic acid and 2.1% Stearic acid. The molecular weight of rapeseed oil bodies is assumed as 974.51g/mol which is made of 11.5% Oleic acid, 11.6% Linoleic acid, 8.9% Linolenic acid, 6.5% Gadoleic acid, 3.6% Palmitic acid and 54% Erucic acid (here the impact of oleosins was ignored because 95% of oil bodies was lipid) [11].

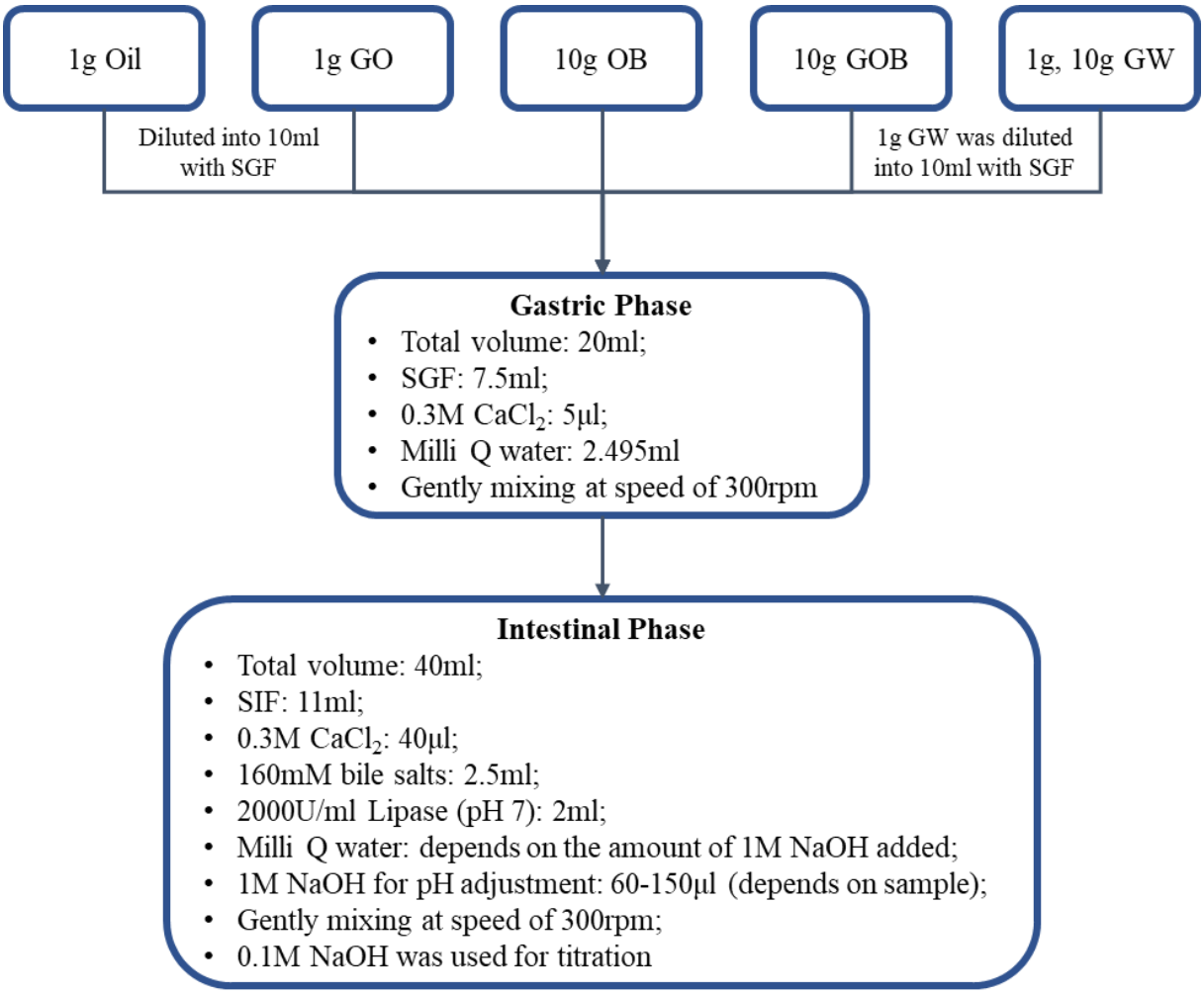

Figure 2-1. Overview of the lipolysis in the pH-stat digestion model

2.5. Statistical analysis

The data was analysed, using SPSS version 25.0. For viscosity measurement, the final viscosity was assessed by univariate analysis of variance (UNIANOVA) with samples (i.e. GMS-oil bodies, GMS-water and GMS-oil systems) and the concentration of GMS. For viscoelasticity tests, UNIANOVA was conducted to assess the yield and flow points between sample groups and GMS concentrations. Besides, the changes of storage modulus among different samples with different concentration of GMS under the increasing shear strain were assessed by the same method. UNIANOVA was also carried on to analyse the lipolysis rate among different groups (here, i.e. oil bodies, oil, GMS-oil body and GMS-oil systems).

For all the tests, the significance level was set at $p<0.05$ (2 tailed). In the Post Hoc Tests, the least significant difference (LSD) pairwise multiple comparison test and the Bonferroni test were chosen. Simple Effects Tests based on LSD were conducted to compare all pairs levels of one factor (e.g. sample groups) for each of all other factors (e.g. time, GMS concentrations).

3. Results and discussion

3.1. Lipid content and moisture content of rapeseed oil bodies

In order to prepare the oil body dispersion with 10% (w/w) oil, lipid content and moisture content of rapeseed oil body cream were determined. The lipid content of rapeseed oil bodies was averagely 95.60±0.60% in dry matter, which is in agreement with the findings by De Chirico et al. (2018) [10] whose extraction protocol was referred in this study. The moisture content of oil body cream was 34.70±2.20%, which largely depends on the practical condition. Therefore, the moisture content determination is necessary before each sample preparation.

3.2. The observation of GMS crystals in different systems

Figure 3-2 displays the microstructure observation of oil body suspension, GMS structured oil body dispersion (GOB), GMS-water gel (GW) and GMS-oil gel (GO) via polarized light microscope (PLM) under different rotation angle between the polarizer and the analyzer.

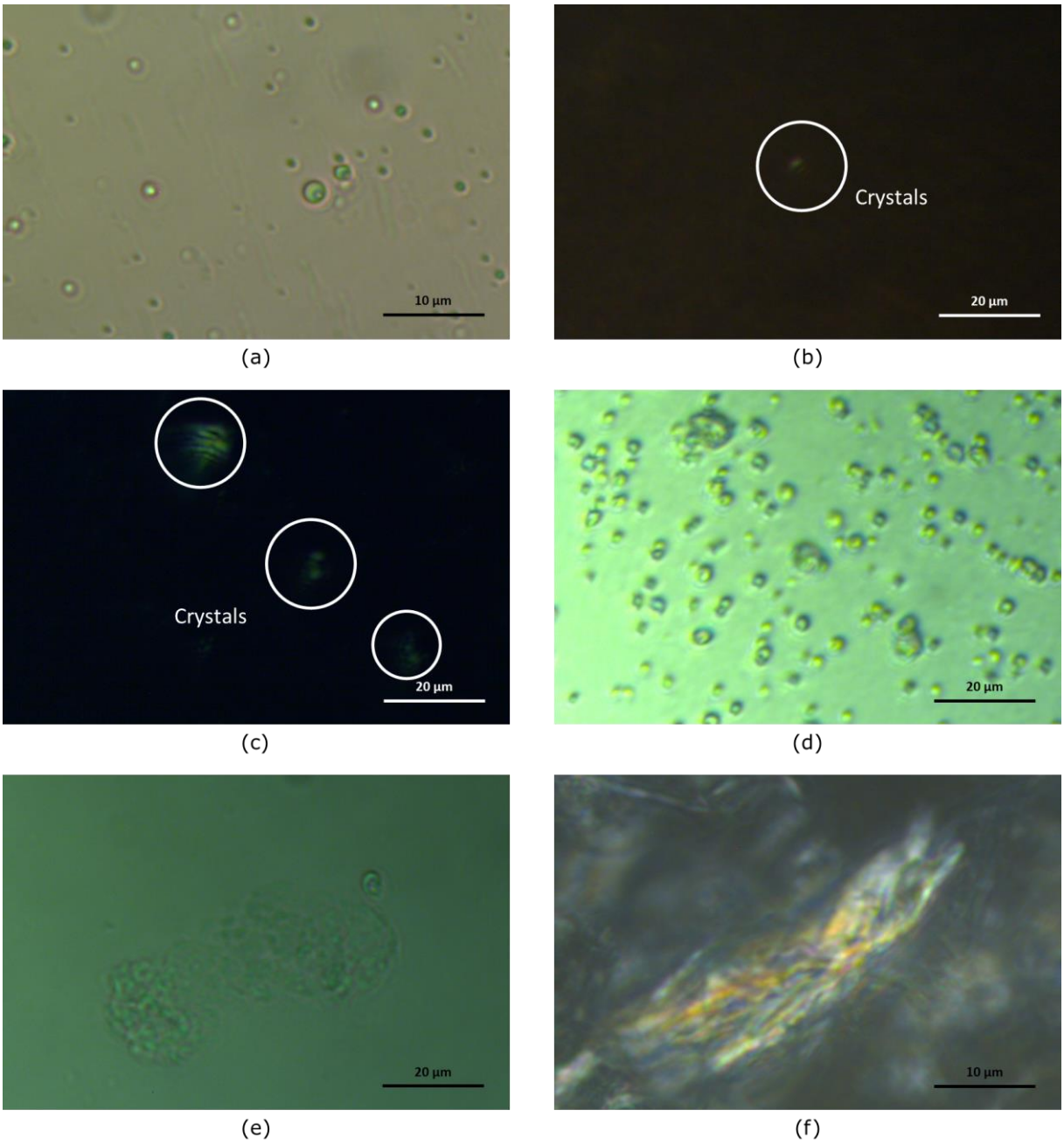

Figure 3-1. The microstructure of (a) oil body dispersion containing 10% lipid, (b) undiluted structured oil bodies with 2%GMS, (c)(d) undiluted GMS water gel with 2%GMS under different angle between the polarizer and analyser, (e) diluted GMS water gel with 2%GMS and (f) undiluted GMS oil gel. Crystals were highlighted with white circles.

As shown in Figure 3-1(b)(c)(f), crystals were only observed in the undiluted samples. Comparing the observation in Figure 3-1(c)(d)(e), the structure showed in (d) was disappeared after dilution (Figure 3-1(e)), which means that the aqueous crystals were possibly destroyed under physical motion. A similar observation was found in GOB. If the crystals were formed inside oil bodies, the dilution would not destroy them due to the protection of phospholipid layer of oil bodies against the physical change in the continuous phase. To confirm the crystallization in GOB, a crystalline determination was conducted via X-ray diffraction (XRD). There was a sharp diffraction peak produced by GOB at 2θ of 20° with intensity of around 140CPS (counts per second), while only a large amorphous scattering pattern was found in the

unstructured oil bodies (data not shown). It indicates that GMS formed crystals in GOB, corresponding to the observation under PLM. The microstructure inspected in GO is displayed in Figure 3-2(f), where some aggregates and small pieces of crystals were found. This structure is similar to that of the rosette-like MAG crystals in corn oil discovered by Kesselman and Shimoni (2007) [12] but different from the needle-like crystals in cod liver oil found by Da Pieve et al. (2010) [13]. This finding also proves that the structure of MAG crystals is influenced by the type of oil.

3.3. The melting and crystallization behaviour of GMS structured samples

As displayed in Figure 3-2, the heat flow rate (W/g) is plotted as a function of temperature to describe the melting and crystallization behaviour of oil bodies, oil and water which were structured by 8% (w/w) GMS (abbreviate: GOB, GO and GW, separately)

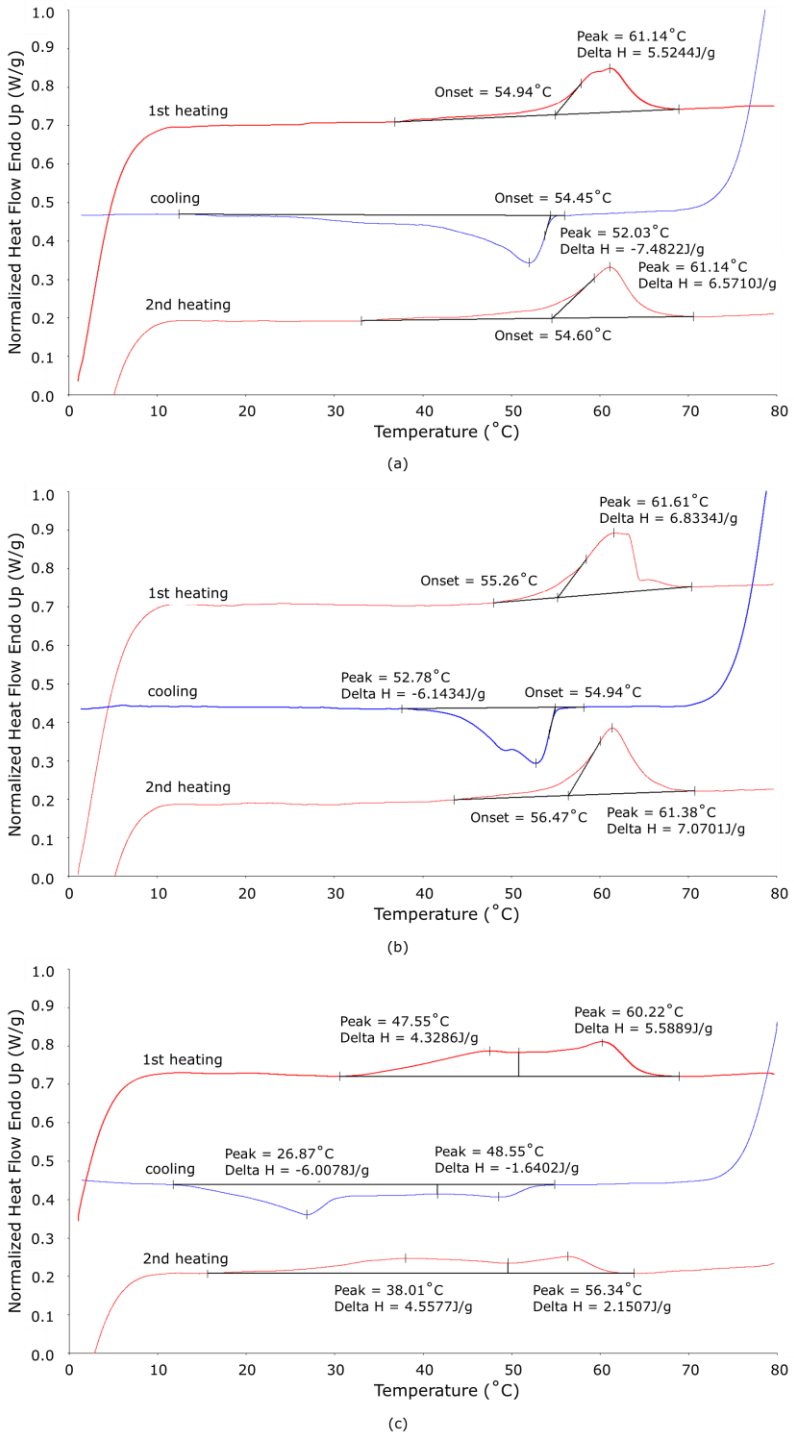

Figure 3-2. DSC melting and crystallization curves of (a) GMS structured oil body emulsion (GOB), (b) GMS-water gel (GW), and (c) GMS structured oil (GO) under heating and cooling ranging from 1 to 80°C with heating/cooling rate at 10°C/min; the concentration of GMS was 8% (w/w); Delta H = the enthalpy of transition, Peak = the temperature at the peak

In both GOB and GW (Figure 3-2(a)(b)), a similar peak at around 61°C was presented in two heating processes, while another peak was presented at around 55°C in the cooling process. It indicates that there was only one phase transition from 1 to 80°C, and the crystallizations in two systems were the same. In contrast, there were two phase transitions in GMS structured oil as shown in Figure 3-2(c). The peak at high temperature was similar with that in other two systems.

Krog (1968[14], 1997[15]) concluded the phase behavior of monoglyceride (MAG) in water system. MAG showed phase transition at different temperature and with different amount of water. When the temperature was higher than the Kraft temperature (T_k), MAG formed lamellar crystalline phase or isotropic fluid phase at various temperature and water content. With cooling to below T_k, metastable α-crystalline was formed, which readily transforms into stable anhydrous crystals called β-crystalline (coagel). Batte et al. (2007) [16] and Marangoni et al. (2007) [17] found that in an emulsion system, crystalline monostearin bilayers were formed on the surface of oil droplets and piled up together to make an encapsulated network. In addition, the crystalline transition was the same as that in only water system. On the other hand, the crystallization behavior of MAG in oil is different from the one in aqueous system. Chen et al. (2009) [18] has found that in MAG/ hazelnut oil system, there were three phases from 80 to 0°C including isotropic, inverse lamellar and sub-α crystalline. In the inverse lamellar phase, the glycerol heads exhibited a hexagonal in-plane ordering which was absent from the ordinary hydrated lamellar phase. Schematics of the crystalline structure are displayed in Appendix 6.2.

Based on the previous studies, it could be concluded that the phase transition at 61℃ in GOB and GW should be the formation of the hydrated lamellar crystalline because the Krafft temperature of GMS (approximately 50℃) is lower than this transition temperature [19]. Besides, it can be speculated that the oil bodies were encapsulated by the lamellar crystalline bilayers and water filled in between each bilayer. However, the lamellar crystals did not transform into α-crystals and even β-crystals at low temperature, as discovered in a former research. In the study of Chen et al. (2010) [20], the crystallization of GMS was sensitive to the presence of water. The Krafft temperature of GMS at which the lamellar crystalline would be transformed into α-crystalline was found to shift to lower values with the increase of water portion. According to Chen's findings, the phase transition in GOB could be explained by the existence of high-water content (90% of the system) in between the bilayer of lamellar crystalline, which inhibited the crystallization of GMS at lower temperature.

Overall, GMS performed similar melting and crystallization behavior in emulsion and water, which agree with the result from microstructure. It also provides an evident that the GMS crystallization is dominated by water phase even with the presence of oil. However, the distribution of GMS crystals in oil body system needs further verification via e.g. fluorescence or confocal laser scanning microscopy and X-ray diffraction experiments.

For GMS structured oil, the phase transition at high temperature should represent the isotropic-inverse lamellar transition. The presence of another peak at lower temperature should indicate the transformation of crystals from the inverse lamellar phase to the sub-α-crystalline phase.

3.4. The influence of GMS on the particle size distribution

In Figure 3-3, the volume fraction was plotted as a function of particle size, describing the particle size distribution among oil bodies (OB), GMS-oil body emulsion (GOB) and GMS-water gel (GW) with the concentration of GMS at 8%.

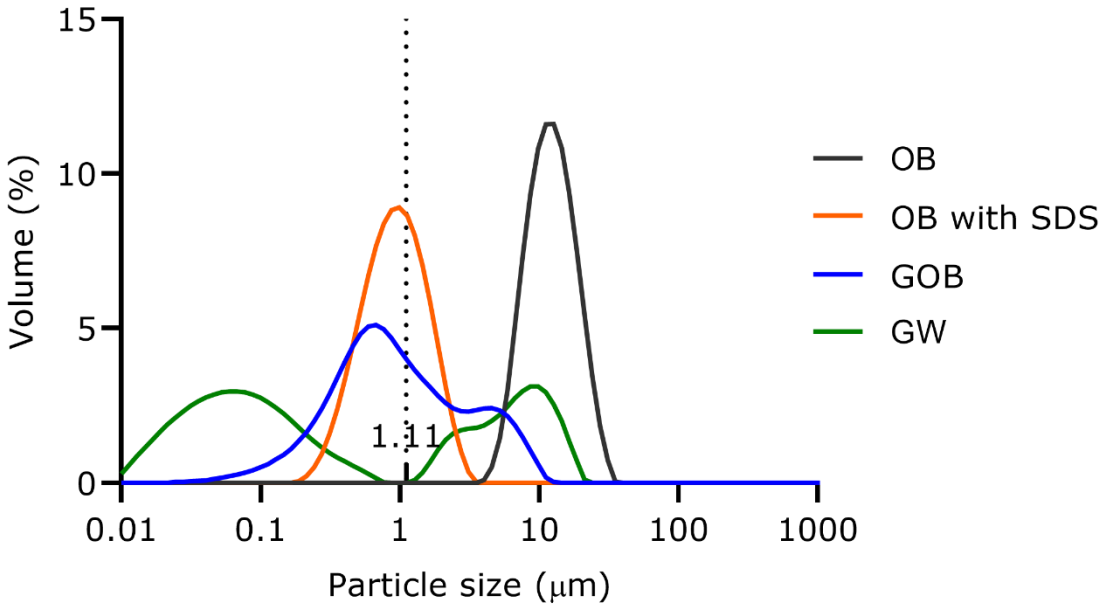

Figure 3-3. Particle size distribution of oil body dispersion (OB), SDS emulsified oil bodies (OB with SDS), oil bodies (GOB) and water gel (GW) structured by 8% (w/w) GMS

The surface weighted mean diameter (d3,2) of OB suspension was 11.9 μm, which was showed with the black line in the figure. After the addition of 0.5% sodium dodecyl sulfate (SDS), the d3,2 was 1.11μm which was comparable with the particle size determined by De Chirico et al. in 2018 [10]. The difference between OB suspension and OB with SDS implied the aggregates in the OB dispersion without homogenization, which could be separated by SDS. The left peak position of GOB was close to that of OB with SDS. It indicates that the left peak of GOB represented the particle size distribution of oil bodies, since GMS is a self-emulsifier which could be used to reduce the oil droplet size [21]. The major peaks of GW were at around 0.068, 2.75 and 9.86 μm respectively. Comparing with the particle size distribution of GOB, the presence of peaks in the range from around 3 to 10 μm in GOB and GW indicates the particle sizes of GMS crystals. Therefore, GMS not only formed crystals but also emulsified the oil bodies during the formation of GOB.

3.5. The rheological properties in oil bodies, oil and water structured by GMS at different concentrations

3.5.1. Viscosity

Figure 3-4 compares the viscosity measured among GMS structured oil bodies (GOB), GMS-water gel (GW) and GMS-oil gel (GO) at the concentration of GMS ranging from 1 to 4% with the increase of shear rate from 10 to 500s^{-1}.

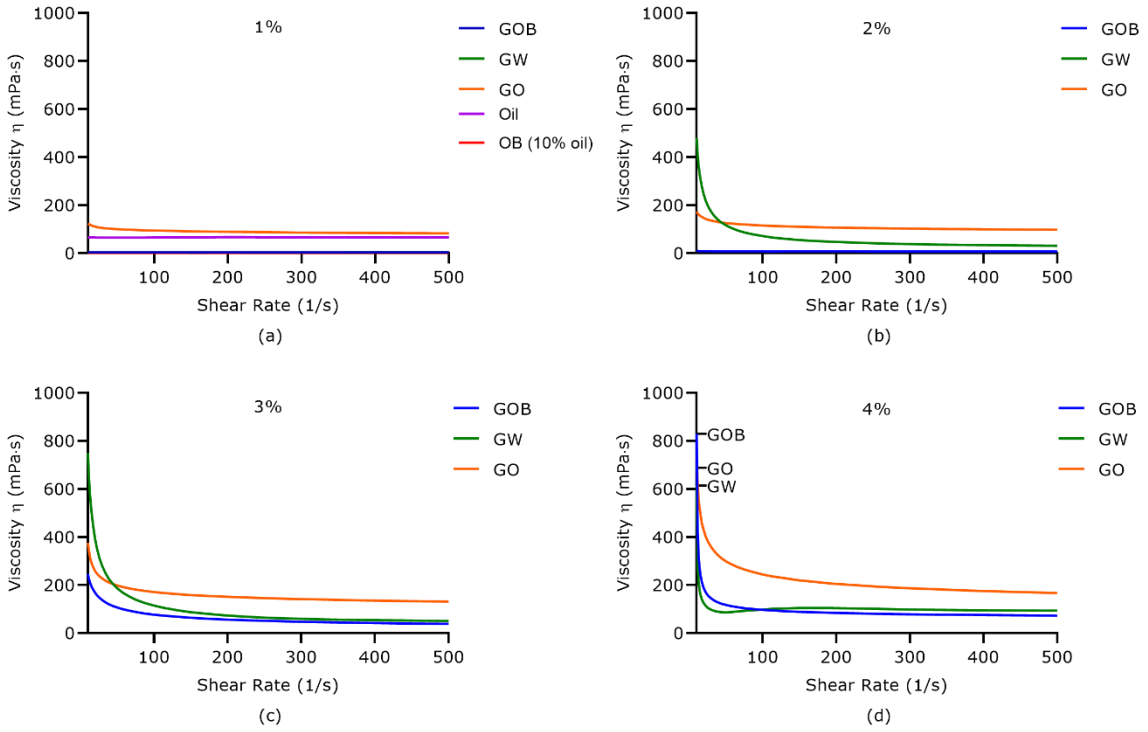

Figure 3-4. Viscosity curves of oil bodies containing 10% lipid (OB (10% oil)), oil, GMS structured oil body emulsion (GOB), GMS-water gel (GW), and GMS structured oil (GO) at the concentration of GMS ranging from 1% to 4%(w/w) under the increase of shear rate; The data showed in the figure is the mean value; the original data with standard deviation is in Appendix 6.3.1

For each group (i.e. GOB, GW and GO, respectively), the viscosity rose markedly with the increase of the concentration of GMS, since the viscosity largely depended on the volume fraction of the dispersed phase (GMS crystals) which increased with the addition of GMS in the system [22].

Comparing the results between each GMS concentration, all the samples displayed shearing thinning behavior, which became more apparent with the increase of the concentration of GMS. Shear thinning is defined as a behavior of the non-Newtonian fluids whose viscosity decreases under shear strain [23], indicating that GOB, GW and GO were non-Newtonian fluids especially at high GMS concentration. As shown in Figure 3-4(d), at high GMS concentration, GOB and GW behaved similarly that the initial viscosity sharply decreased when the shear rate increased. This is because the crystals in both oil body and water system were formed in water phase and they had similar structure. The viscosity of GOB at high shear rate was significantly lower than that of GW among all the GMS concentrations except 1%. Also, the viscosity of GOB enlarged slowly with the increase of the amount of GMS, compared to the increasing trend of GW (Figure 3-4(a)(b)(c)). This phenomenon could be influenced by the different crystalline distribution in two systems. In oil body system, GMS might firstly emulsify the oil bodies and form lamellar crystals surrounding the oil bodies, while in the water system, GMS formed crystals randomly. Therefore, at a low concentration, crystals in GOB might mainly exist on the surface of oil bodies and only a minor part dispersed in the water phase, which contributed to smaller volume

fraction than that in water system. When the concentration of GMS increased, more crystals could suspend in the water phase the thereby the volume fraction of them increased.

In contrast, GO showed a steady decreasing trend of viscosity among all the GMS concentrations and the shear thinning behavior was more apparent. Moreover, at high shear rate, the viscosity of GO was significantly higher than GOB and GW among all GMS concentrations. This could be explained by the higher initial viscosity of canola oil compared to the oil bodies. On the other hand, in the research of Chen et al. (2009) [18], a dense packing of glyceride groups was discovered in the inverse lamellar phase, which added the rigidity to make the material behaved like a gel. Moreover, the further transformation to sub-α-crystalline did not influence the existing properties. On the basis of this finding, there might be a similar packing structure in GO, which could facilitate the stability of structured oil system.

3.5.2. Viscoelasticity

An Amplitude sweep test was conducted among all the groups at different concentrations of GMS to determine the linear viscoelastic region (LVER) and describe the rheological behavior.

In Table 3-1, the comparison on the shear stress at yield and flow points between each sample and each GMS concentration is displayed. The limit of LVER is described by the shear stress at yield point, after which the structure starts to be softened with the increase of shear strain. The flow point is the crossover point at which the storage modulus is equivalent to the loss modulus, describing the transformation from elastic to viscous structure [23].

Table 3-1. Yield and flow points of GMS structured oil body emulsion (GOB), GMS-water gel (GW), and GMS structured oil (GO) at the concentration of GMS from 5% to 8%(w/w)

Concentration of GMS (%(w/w))	**GOB**		**GW**		**GO**	
	Shear Stress at yield point (Pa)	Shear Stress flow point (Pa)	Shear Stress at yield point (Pa)	Shear Stress flow point (Pa)	Shear Stress at yield point (Pa)	Shear Stress flow point (Pa)
5.00	2.74 ± 0.07^{a}	14.80 ± 0.24^{a}	1.14 ± 0.02^{e}	8.50 ± 0.16^{d}	0.10 ± 0.01^{ef}	1.60 ± 0.03^{f}
6.00	5.27 ± 0.12^{b}	33.09 ± 0.62^{b}	1.61 ± 0.10^{e}	9.14 ± 0.11^{d}	0.16 ± 0.04^{f}	3.05 ± 0.25^{f}
7.00	6.57 ± 0.68^{c}	35.04 ± 4.89^{b}	4.59 ± 0.39^{b}	30.17 ± 0.24^{e}	0.23 ± 0.02^{f}	4.60 ± 0.66^{f}
8.00	9.02 ± 0.50^{d}	43.79 ± 1.18^{c}	5.56 ± 1.78^{b}	32.92 ± 1.46^{e}	1.09 ± 0.07^{f}	8.03 ± 0.76^{g}

Note: Comparison is only conducted within same sample or same concentration or same value.

At each GMS concentration, the yield and flow point of GOB were significantly larger than of GW and GO, illustrating that the structure of GOB is stronger and more stable than other two groups under the increasing shear strain. Based on the hypothesis of crystalline structure in Chapter 3.3, in oil body system, the oil bodies could be incorporated in the crystalline network to form a more compact structure. Moreover, the oleosins on the surface of oil bodies might also interact with GMS whose functionality and mechanism needs further verification.

The yield and flow points became larger with the increasing concentration of GMS in each sample group. The significant difference was found between each two concentrations in GOB

while absent in other two groups, indicating that the GMS concentration could influence the crystalline network, especially in the water phase with the presence of oil bodies.

The storage modulus G' and loss factor tanδ are plotted in the function of shear strain in Figure 3-5. The shear modulus is used to describe the viscoelastic behavior of a material, in which the storage modulus G' represents the elastic portion while the loss modulus shows the viscous portion [24]. The ratio between the loss and storage modulus is defined as the loss factor tanδ which is a measure of the damping in a material. When the loss factor is higher than 1, the viscous component dominated and the material performed as a viscous liquid. In the contrast, the material behaves like a gel when the loss factor is lower than 1.

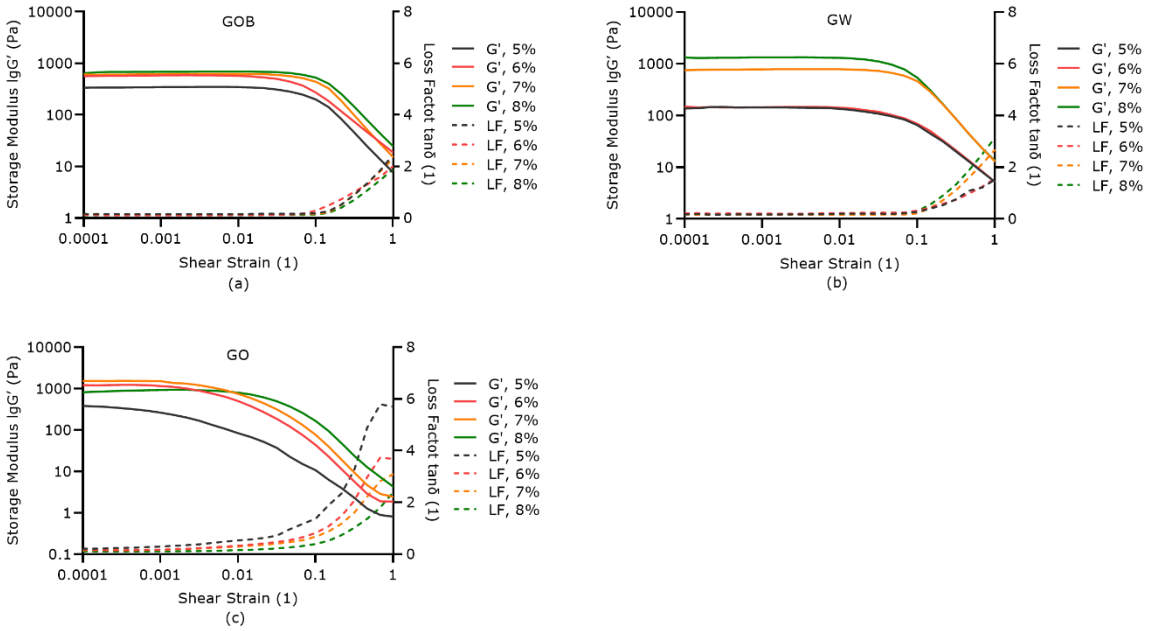

Figure 3-5. The storage modulus and loss factor of (a) GMS structured oil bodies (GOB), (b) GMS-water gel (GW), (c) GMS-oil gel (GO) at the concentration from 5% to 8% (w/w); G' = the storage modulus, LF = the loss factor; The data showed in the figure is the mean value; the original data with standard deviation is in Appendix 6.3.2

Combined with the data in Table 3-1, the loss factor was always lower than 1 in the LVER among all the groups and GMS concentrations, representing that within the region the storage modulus dominated and that all the samples performed gel-like behavior [25].

Besides, the influence of GMS concentration on the storage modulus during the increase of shear strain in each sample group was compared. For GOB (Figure 3-5(a)), at low the shear strain, there was a significant ascending of the storage modulus with the increase of concentration of GMS, except between 6% and 7%. As displayed in Figure 3-5(b), the storage modulus of GW rose when the concentration of GMS increased except between 5% and 6%. The results of GO were presented in Figure 3-6(c), where the significant difference was found between each GMS concentration under a low shear stain, except among 6, 7 and 8%.

To summarize, the storage modulus rose with the increase of the concentration of GMS in each group, except in GW with 5 and 6% GMS, the change was not obvious at high concentration (7% and above). At high shear strain, no significant difference was found between each concentration with each group. These results indicate that higher concentration of GMS could contribute to the crystallization and thereby the enhance of storage modulus.

Furthermore, the storage modulus among different samples was analysed. The storage modulus of GO is the highest among all the GMS concentration before the intersecting points, except at 8%. It illustrates that GO performed more elastic behaviour than other two groups at low shear strain, which corresponds to the result of viscosity measurement. On the other hand, the structure was also destroyed more easily by the increasing shear strain, which is in agreement with the findings in yield and flow points (Table 3-1). Additionally, there was a similar trend of the storage modulus with the increase of shear strain between GOB and GW. When the concentration of GMS was 7% and above, the storage modulus of GW was larger than GOB. Considering the existence of oil bodies in GOB, at a high concentration, the space to dissolve more GMS might not be enough anymore, inhibiting the crystallization of GMS. While the crystallinity of GMS at high concentration still need quantification via XRD and the degree of saturation of GMS needs further investigation.

3.6. The influence of GMS on the lipolysis rate in oil bodies and oil

The lipolysis profiles of GMS structured oil body emulsion (GOB) and GMS-oil gel (GO) with concentration at 8% are displayed in Figure 3-6 by plotting the released free fatty acids (FFA) and the volume of NaOH consumed in titration against the digestion time. Oil and oil body (OB) dispersion containing 10% oil were the control groups of GO and GOB, respectively. All the data were corrected by excluding the FFA release of digestive fluids.

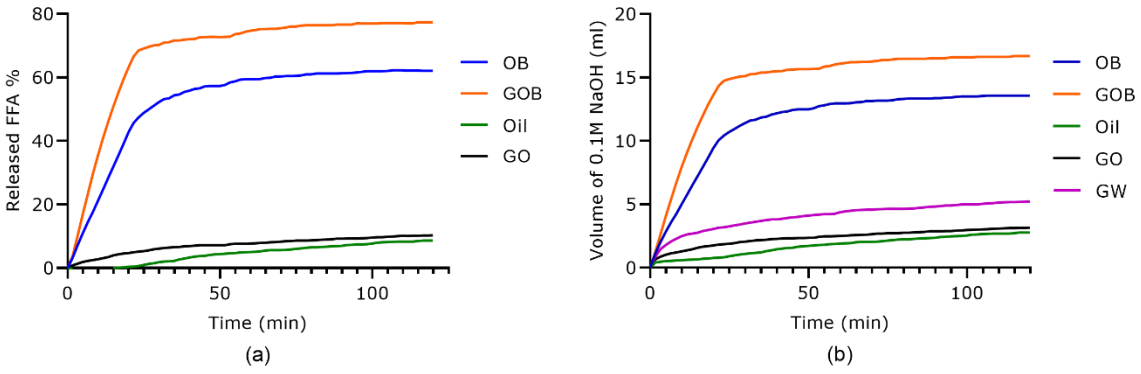

Figure 3-6. The kinetics of (a) the released FFA% of oil body emulsion containing 10% lipid (OB), GMS structured oil body emulsion (GOB), oil and GMS structured oil (GO); (b) NaOH consumption by OB, GOB, Oil, GO and GMS-water gel (GW) during lipolysis; the concentration of GMS in each sample was 8%; the influence of digestive fluids had been excluded; The data showed in the figure is the mean value; the original data with standard deviation is in Appendix 6.4

Here the concentration of GMS was 8%, at which samples were the most stable against the deformation in previous Amplitude sweep tests. In Figure 3-6(a), the FFA release levels of

GOB and GO were higher than OB and oil, respectively. A similar trend was found in the study of Ashkar et al. (2019) [26], where the GMS-based oleogel accelerated the lipid digestion compared to the unstructured oil. The authors also discovered that the increase of GMS facilitated the release of FFA by measuring samples with 10% and 15% GMS, separately. Additionally, the digestion speed of GOB in first 20 minutes was quicker, compared to OB. There are several possible factors influencing the lipid digestion. During the intestinal lipolysis, bile salts would firstly be absorbed onto the lipid surface, aiding the further absorption of lipase and colipase to hydrolyse the lipid [27]. According to the findings from the particle size distribution, the addition of GMS could contribute to the emulsification of oil bodies. As a result, the aggregates of oil bodies were separated and thereby the surface area of oil bodies increased, which might promote the accessibility to bile salts. On the other hand, GMS itself might be digested by lipase. To confirm it, another measurement on GMS-water gel (GW) containing same amount of GMS was conducted. As displayed in Figure 3-6(b), GMS did release FFA during the digestion, proving that GMS could be hydrolysed by lipase. Yet, the mechanism of how GMS functioned in oil body system during the digestion needs further verification through e.g. suitable microscopy at different time points during the lipolysis.

Moreover, there was a marked increase of FFA in the first 60 minutes among all the groups (Figure 3-6(a)), while such a significant change was not found between 60min and 120min, indicating that the digestion was almost finished within 60 minutes.

Figure 3-7 compares the difference of the undigested lipid content between each group at 60 and 120min, which has excluded the influence of GMS in GOB.

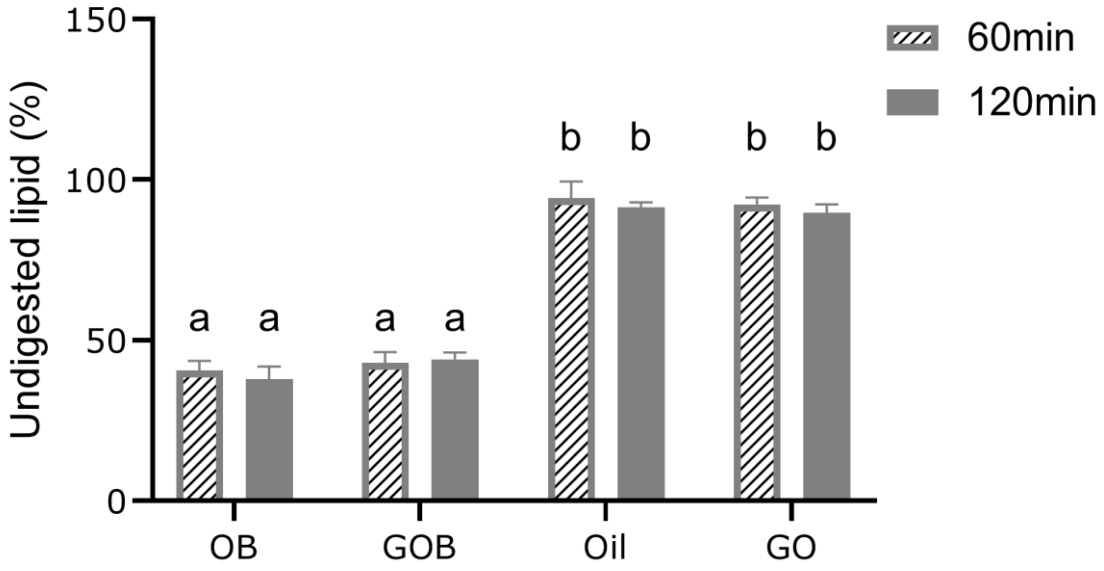

Figure 3-7. The undigested lipid of oil body emulsion containing 10% lipid (OB), GMS structured oil body emulsion (GOB), oil and GMS structured oil (GO) at 60 and 120min; the data of GOB was corrected by excluding the digestion of GMS; other data were corrected with the black digestive fluids data

After the correction which removed the digested lipid of GMS itself, the rest lipid of GOB was still similar to that of OB, indicating that GMS had no effect on the digestion of oil bodies. No significant difference was found on the undigested lipid between at 60min and at 120min, corresponding to the trend of FFA release in Figure 3-6. The GMS-water system could not be a control for GMS-oil system due to the different mediums. Thus, here the digestion of GMS-water gel was not excluded in the calculation of undigested lipid of GO. Nevertheless, there was no significant difference of the rest lipid between oil and GO, which is in line with the result of 10% GMS-canola oleogel digestion discovered by Ashkar et al. (2019) [26]. It indicates that at such a low concentration, GMS would not influence the lipolysis. To further verify the hydrolyzation of GMS in structured oil system, another in-vitro digestion of only dry GMS should be conducted.

Moreover, the undigested lipid in emulsion samples (i.e. OB and GOB) was significantly lower than the lipid in oil samples (i.e. oil and GO) although the amount of oil was equal in both systems. It has been concluded that the lipid droplet size is a key physicochemical factor in fatty acid bioavailability [28, 29]. When the lipid droplet size is smaller, the interface area between lipid and water is larger, leading to the binding of more lipase to the substrate. Here in this project, the oil samples without homogenization had larger droplet size distribution and thereby larger surface area in digestive fluids than emulsified samples. Thus, it led to an insufficient access of oil samples to bile salts and lipase.

4. Conclusions

In summary, this project investigated the crystallization of GMS and its influence on the physicochemical properties and lipid digestibility in an oil body emulsion system. The crystallization of GMS in oil body emulsion occurred in the water phase instead of the oil phase and GMS had no effect on lipid digestibility, which are opposite to what initially hypothesized. While the existence of GMS did influence the physicochemical properties as expected. In both emulsion and water system, only one phase transition occurred while in oil system, there were two phase transitions. The addition of GMS reduced the particle size of the aggregates of oil bodies, since GMS is an emulsifier. With the increase of GMS concentration, the viscosity and viscoelasticity of the system increased, indicating that more GMS crystals were formed and contributed to a stronger structure. Compared to GMS-water and GMS-oil systems, the GMS-oil body emulsion behaved more stable against the deformation by the increasing shear strain. The digestion was almost finished in first 60 minutes, where no significant difference on the extent of lipid digestion was found between GMS-structured systems and the unstructured ones. Moreover, GMS itself could be hydrolyzed by lipase. However, further verification on how GMS functioned is needed.

These findings could provide a support for the further studies that aim to investigate the application of GMS-oil body emulsions on food production, for example baking. To expand the application of structuring oil bodies, other oilseeds (e.g. soybean, peanuts and hazelnuts) and gelators (e.g. phytosterols) could be involved in future researches.

However, there are still some limitations in this project which need further verification. Since there are some proteins (oleosins) originally existing on the surface of oil bodies, the possible interaction between oleosins and GMS and the possible influence need to be analyzed and verified. Moreover, the phase behavior of GMS crystallization in oil body emulsion needs deeper identification and quantification by using related technologies like X-ray diffraction (XRD) and small-angle X-ray scattering (SAXS) during different conditions. The effect of GMS on the physicochemical properties can be further characterized in different systems with varying oil fraction. Last but not the least, emulsions are unstable systems and some properties might change overtime. The destabilization of emulsions should be taken into account in future studies.

5. References

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6. Appendix

6.1. Lipid content measurement by Soxhlet extraction

A certain amount of pre-dried cream was weighed (m(OB)) in an extraction thimble on an analytical balance and the thimble was closed with a piece of cotton wool and put into an extractor. The code on the thimble should be written by a pencil to avoid the disappearance during the extraction. The flat bottom flask with some boiling chips was weighed on an analytical balance, and the weight was recorded as m(bottle). Afterwards, at least 200ml petroleum ether 40-60℃ (P.E.) was added into the flat bottom flask. To complete the Soxhlet extraction device, the flat bottom flask was gently connected to the extractor and then they were gently installed on the device which includes a cooler and a heating block. The cold-water tap was turned on to keep the flow of cold water in the cooler before turning on the heating block at position "3". The extraction was conducted at least for 3 hours, and the time was started from the first circle of the extraction. After extraction, the heating was stopped, but the cooling was still left on until the boiling and refluxing of Petroleum ether stopped and the whole system cooled down to the room temperature. After disconnecting the flat bottom flask and the extractor from the device, all the P.E. in the extractor was rinsed and poured into the flat bottom flask and the thimble was taken out and put in a glass beaker in the fume hood overnight for the evaporation of the residue of P.E.. The P.E. in the flat bottom flask was evaporated by using Rotation Evaporators of Büchi under vacuum until there was nothing evaporated. To avoid unexpected moisture, the sample after evaporation was washed by acetone twice and then evaporated again. After evaporation, the flat bottom flask was put on the fume hood overnight to remove the residue of P.E. On next day, the flat bottom flask was weighed again on the same analytical balance as before, and the weight was recorded as m(total).

The total fat content was calculated as:

$$\text{Fat content (\%)} = \frac{\text{m(total)} - \text{m(bottle)}}{\text{m(OB)}}$$

The experiment was conducted in duplicate.

6.2. The schematics of GMS crystalline structure

6.2.1. The phase transitions from a hydrated lamellar phase to an anhydrous crystalline phase in water or emulsion system

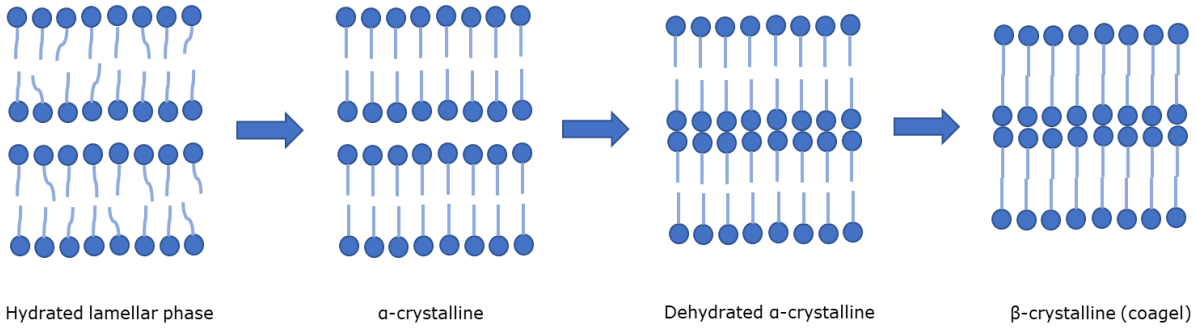

Figure 6-1. Phase transitions of monoglyceride crystals from the hydrated lamellar phase above the Krafft (i.e., chain-melting) temperature of the monoglyceride, to α-crystalline phase, to a dehydrated α-crystalline phase, and eventually to an anhydrous crystalline phase (β-gel or coagel)

6.2.2. The dense packing in the inverse lamellar phase of monoglyceride crystals in hydrophobic solutions

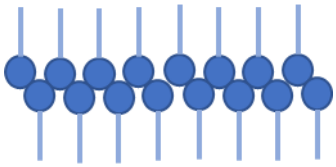

Figure 6-2. The schematic of the dense packing structure of monoglyceride crystalline in the inverse lamellar phase in hydrophobic solutions

6.2.3. The encapsulation network of monoglyceride crystals surrounding the oil droplets

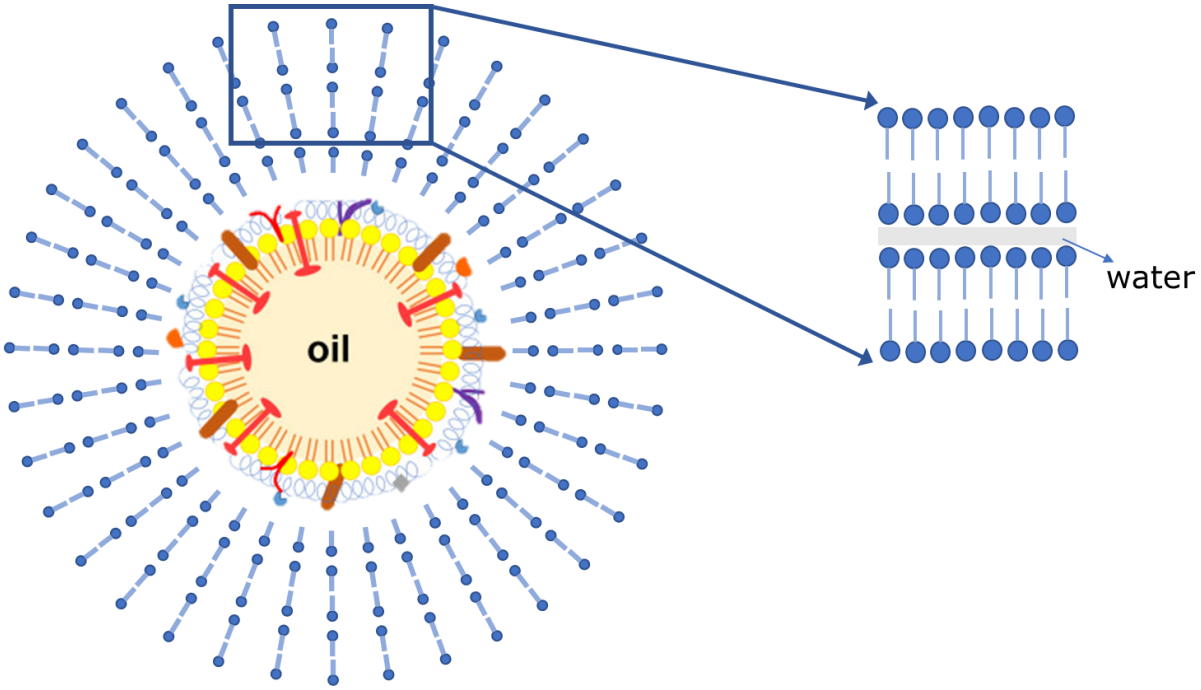

Figure 6-3. The schematic of a possible encapsulation network where the glycerol monostearate (GMS) crystalline staked together on the surface of oil bodies; Water was filled in between the bilayers of crystals

6.3. The original data of rheology measurements

6.3.1. Viscosity

6.3.1.1. Viscosity of different samples with 1% GMS

Table 6-1. Viscosity of oil bodies containing 10% lipid, oil, GMS structured oil bodies, GMS-oil gel and GMS-water gel; the concentration of GMS is 1%

Shear Rate (1/s)	GOB	GW	GO	Oil	OB (10% oil)
10	4.05±0.07	3.95±0.21	124.00±4.67	66.20±1.13	1.45±0.07
11.4	4.15±0.07	3.90±0.28	120.50±3.54	66.30±0.99	1.65±0.07
13.1	4.10±0.14	3.90±0.28	118.40±2.69	66.25±1.34	1.50±0.00
15	4.10±0.00	3.80±0.28	115.60±1.98	66.25±1.77	1.55±0.07
17.2	4.05±0.07	3.80±0.28	113.10±2.26	65.95±1.63	1.65±0.07
19.6	3.85±0.07	3.75±0.35	111.20±1.84	65.95±1.63	1.60±0.14
22.5	3.90±0.14	3.75±0.21	109.05±1.77	65.75±1.20	1.60±0.14
25.7	3.90±0.00	3.85±0.21	107.30±1.56	65.75±0.92	1.60±0.14
29.4	3.95±0.07	3.75±0.21	105.75±1.63	65.35±0.92	1.60±0.14
33.7	3.95±0.07	3.80±0.00	104.15±1.34	65.30±0.71	1.60±0.00
38.5	3.85±0.07	3.80±0.14	102.60±1.27	65.20±0.85	1.60±0.00
44.1	3.80±0.14	3.70±0.14	101.10±1.27	65.10±1.13	1.50±0.00
50.5	3.80±0.14	3.70±0.14	100.15±1.20	65.15±1.63	1.50±0.00
57.8	3.75±0.07	3.7±0.28	98.95±1.20	65.25±1.48	1.55±0.07
66.1	3.75±0.21	3.65±0.21	97.75±1.20	65.50±1.84	1.45±0.07
75.6	3.85±0.07	3.65±0.21	96.75±1.20	65.55±2.05	1.50±0.14
86.6	3.8±0.14	3.70±0.14	95.65±1.20	65.80±1.84	1.55±0.07
99.1	3.85±0.07	3.75±0.07	94.65±1.20	66.00±1.84	1.55±0.07
113	3.75±0.07	3.55±0.07	93.65±1.20	66.20±1.41	1.60±0.14
130	3.70±0.14	3.55±0.07	92.50±1.13	66.20±1.41	1.50±0.28
148	3.90±0.00	3.70±0.14	91.60±1.13	66.55±1.48	1.70±0.00
170	3.75±0.07	3.80±0.14	90.50±1.27	66.60±1.70	1.50±0.28
194	3.70±0.14	3.90±0.14	89.55±1.20	66.90±1.70	1.45±0.21
223	3.65±0.21	4.00±0.14	88.55±1.20	66.75±1.77	1.60±0.14
255	3.80±0.00	4.10±0.00	87.65±1.20	66.60±2.12	1.65±0.07
291	3.70±0.14	3.95±0.21	86.60±1.13	66.40±2.12	1.55±0.21
334	3.75±0.21	3.85±0.35	85.75±1.20	66.40±1.98	1.60±0.28
382	3.75±0.07	3.85±0.49	84.75±1.20	66.15±1.91	1.65±0.21
437	3.75±0.07	3.95±0.35	83.95±1.20	66.05±1.91	1.70±0.14
500	3.75±0.07	4.05±0.21	83.05±1.06	66.10±1.98	1.80±0.14

6.3.1.2. Viscosity of different samples with 2% GMS

Table 6-2.Viscosity of oil bodies containing 10% lipid, oil, GMS structured oil bodies, GMS-oil gel and GMS-water gel; the concentration of GMS is 2%

Shear Rate (1/s)	GOB	GW	GO
10	9.90±0.28	500.5±28.00	174.05±4.03
11.4	9.70±0.71	454.25±66.40	165.65±6.43
13.1	9.50±0.28	371.05±21.71	160.65±6.29
15	9.30±0.28	323.15±14.21	155.45±6.15
17.2	9.05±0.21	283.10±8.49	151.20±5.66

19.6	8.90±0.00	253.60±11.03	147.30±5.94
22.5	8.75±0.07	219.40±1.27	142.95±6.15
25.7	8.65±0.07	194.65±0.49	139.60±6.22
29.4	8.50±0.00	177.50±4.67	136.60±5.94
33.7	8.35±0.07	161.55±7.28	133.45±5.59
38.5	8.15±0.07	143.30±3.11	130.65±6.01
44.1	8.15±0.07	129.60±3.25	128.30±5.94
50.5	8.10±0.00	114.95±0.21	125.80±5.80
57.8	7.90±0.14	108.15±5.3	123.65±5.59
66.1	7.80±0.14	95.80±1.41	121.55±5.44
75.6	7.80±0.14	87.50±1.84	119.45±5.30
86.6	7.80±0.14	80.25±2.33	117.50±5.09
99.1	7.65±0.07	74.25±3.32	115.70±4.81
113	7.45±0.21	66.55±1.06	113.95±4.60
130	7.50±0.00	60.80±0.42	112.30±4.53
148	7.55±0.07	55.05±0.64	110.55±4.45
170	7.55±0.07	51.85±0.64	108.90±4.38
194	7.45±0.07	48.30±0.85	107.20±4.38
223	7.45±0.07	43.70±0.85	105.75±4.17
255	7.35±0.07	42.30±1.27	104.30±4.10
291	7.35±0.07	39.75±1.48	102.85±3.89
334	7.25±0.07	37.05±0.92	101.55±3.75
382	7.35±0.07	34.80±0.57	100.05±3.61
437	7.35±0.07	33.25±0.64	98.75±3.46
500	7.30±0.00	31.00±0.57	97.55±3.32

6.3.1.3. Viscosity of different samples with 3% GMS

Table 6-3.Viscosity of oil bodies containing 10% lipid, oil, GMS structured oil bodies, GMS-oil gel and GMS-water gel; the concentration of GMS is 3%

Shear Rate (1/s)	GOB	GW	GO
10	251.65±7.14	775.75±35.28	376.35±47.31
11.4	226.00±0.28	707.25±83.09	342.80±32.81
13.1	209.85±0.92	590.85±28.21	314.15±27.51
15	196.75±2.90	508.70±5.23	292.00±24.18
17.2	180.65±0.92	446.20±1.98	275.15±18.31
19.6	170.25±1.48	398.35±2.47	259.35±17.18
22.5	157.65±0.49	360.75±11.53	247.60±13.01
25.7	146.95±1.20	317.05±4.17	236.80±12.02
29.4	137.10±1.27	281.15±1.34	227.70±9.62
33.7	128.35±0.92	250.45±0.78	219.00±8.06
38.5	120.30±0.42	229.85±5.02	211.45±7.57
44.1	114.35±1.77	202.25±3.18	204.35±6.43
50.5	107.50±1.84	187.65±3.46	197.85±5.16
57.8	99.80±0.28	170.70±3.82	191.70±4.67
66.1	92.80±0.85	155.00±3.39	186.00±4.24
75.6	87.25±0.78	138.25±1.06	181.05±3.75
86.6	82.45±0.21	128.40±2.55	175.90±3.11
99.1	76.40±1.41	115.70±0.42	171.40±2.83
113	72.65±0.21	103.35±2.90	166.85±2.62
130	69.05±0.78	97.80±2.12	162.85±2.76

148	63.85±0.78	89.30±1.41	159.10±2.55
170	59.90±1.13	82.95±2.76	155.35±2.33
194	57.05±0.21	75.25±1.06	151.80±2.12
223	54.65±0.92	69.40±0.71	148.40±1.70
255	50.50±0.71	65.70±2.26	145.20±1.56
291	47.45±0.92	61.50±1.98	142.10±1.27
334	45.25±0.35	57.50±0.85	139.20±1.27
382	43.35±0.64	54.70±0.42	136.40±0.85
437	41.25±0.92	51.85±0.92	133.60±0.57
500	38.75±0.49	49.90±1.13	130.90±0.57

6.3.1.4. Viscosity of different samples with 4% GMS

Table 6-4.Viscosity of oil bodies containing 10% lipid, oil, GMS structured oil bodies, GMS-oil gel and GMS-water gel; the concentration of GMS is 4%

Shear Rate (1/s)	GOB	GW	GO
10	909.55±112.92	632.50±25.03	743.25±78.70
11.4	462.15±58.05	308.30±33.66	618.85±60.46
13.1	318.90±18.95	221.65±26.66	546.35±39.67
15	257.85±14.07	181.35±24.68	502.00±33.09
17.2	216.30±5.23	147.20±9.19	457.05±21.99
19.6	193.50±7.35	129.15±5.73	429.50±18.38
22.5	170.85±0.64	119.65±8.56	403.05±16.19
25.7	156.00±0.85	107.55±3.89	380.25±13.65
29.4	146.20±0.99	99.30±1.70	360.20±12.16
33.7	135.55±1.20	94.75±2.33	342.00±10.18
38.5	129.45±1.06	91.85±3.46	325.85±8.27
44.1	123.30±1.41	89.55±3.75	311.35±7.28
50.5	117.75±1.48	88.65±3.32	297.80±6.93
57.8	112.65±1.20	88.75±1.34	285.85±5.59
66.1	106.45±1.48	89.35±1.06	274.65±5.44
75.6	103.00±0.99	90.90±2.40	264.25±4.88
86.6	99.90±0.28	92.85±3.61	254.20±4.38
99.1	96.75±0.49	95.25±4.17	244.90±3.82
113	93.70±0.71	97.75±4.60	236.50±3.54
130	91.10±0.71	99.55±5.30	228.40±2.97
148	88.85±0.35	101.20±5.09	220.65±2.76
170	86.90±0.28	100.65±6.01	213.50±2.40
194	84.80±0.28	100.65±5.16	206.55±2.33
223	82.90±0.28	100.60±3.25	200.15±2.05
255	81.00±0.28	98.60±3.39	194.00±2.12
291	78.85±0.35	97.00±2.83	188.15±1.91
334	76.70±0.99	95.90±1.70	182.60±1.84
382	75.15±1.06	95.95±1.06	177.15±1.91
437	73.25±1.63	95.15±1.34	172.10±1.84
500	71.85±1.77	94.25±1.2	167.15±1.77

6.3.2. Viscoelasticity

6.3.2.1. The storage modulus and loss factor of GMS structured oil bodies (GOB)

Table 6-5. Storage modulus and Loss Factor of GMS structured oil bodies (GOB)at the concentration from 5% to 8% (w/w)

Shear Strain (1)	G", 5%	G", 6%	G", 7%	G", 8%	LF, 5%	LF, 6%	LF, 7%	LF, 8%
0.0001	336.82± 25.75	563.00± 3.85	609.49± 22.92	644.83± 25.49	0.17± 0.01	0.12± 0.01	0.14± 0.00	0.11± 0.04
0.000148	340.52± 9.15	566.93± 3.59	599.78± 3.70	666.00± 49.76	0.16± 0.01	0.12± 0.00	0.17± 0.01	0.12± 0.01
0.000217	339.28± 7.53	574.27± 1.00	602.97± 6.84	676.28± 38.65	0.16± 0.00	0.11± 0.00	0.15± 0.00	0.12± 0.00
0.000318	340.12± 11.65	571.57± 3.34	607.00± 7.26	679.48± 37.69	0.16± 0.00	0.11± 0.00	0.14± 0.00	0.12± 0.00
0.000466	342.23± 10.43	575.83± 3.65	609.34± 10.61	682.71± 40.23	0.16± 0.00	0.11± 0.00	0.14± 0.00	0.12± 0.00
0.000685	343.68± 10.87	575.53± 3.54	610.90± 9.50	685.04± 39.29	0.16± 0.00	0.11± 0.00	0.14± 0.00	0.12± 0.00
0.00101	344.38± 10.42	581.04± 1.31	614.62± 9.78	687.14± 38.85	0.16± 0.00	0.11± 0.00	0.14± 0.00	0.12± 0.00
0.00148	345.41± 10.38	584.17± 0.57	616.83± 10.98	688.93± 38.41	0.16± 0.00	0.11± 0.00	0.14± 0.00	0.12± 0.00
0.00217	346.33± 10.70	584.66± 1.90	619.91± 11.09	690.94± 38.80	0.16± 0.00	0.11± 0.00	0.14± 0.00	0.12± 0.00
0.00318	347.14± 10.78	584.15± 3.34	622.45± 11.67	692.63± 38.45	0.16± 0.00	0.11± 0.00	0.14± 0.00	0.12± 0.00
0.00466	347.52± 10.76	582.47± 4.12	624.46± 12.35	694.44± 37.96	0.16± 0.00	0.11± 0.00	0.14± 0.00	0.12± 0.00
0.00685	347.2± 10.84	579.63± 3.08	625.54± 13.15	695.54± 37.84	0.16± 0.00	0.12± 0.00	0.14± 0.00	0.12± 0.00
0.0101	345.37± 10.83	571.07± 3.25	624.89± 13.61	695.02± 38.64	0.16± 0.00	0.12± 0.00	0.14± 0.00	0.12± 0.00
0.0148	340.27± 10.91	557.53± 0.91	621.18± 13.44	692.15± 38.91	0.16± 0.00	0.12± 0.00	0.15± 0.00	0.12± 0.00
0.0217	329.41± 10.85	532.47± 3.04	612.16± 13.22	685.52± 38.42	0.17± 0.00	0.13± 0.00	0.15± 0.00	0.12± 0.00
0.0318	310.85± 10.47	499.27± 0.57	594.71± 12.58	671.54± 37.34	0.17± 0.00	0.12± 0.00	0.15± 0.00	0.12± 0.00
0.0466	283.41± 9.48	442.56± 6.45	564.34± 11.01	646.07± 35.24	0.17± 0.00	0.14± 0.00	0.15± 0.00	0.12± 0.00
0.0685	245.89± 8.00	369.24± 5.81	513.72± 7.99	600.67± 31.52	0.17± 0.00	0.17± 0.01	0.15± 0.00	0.12± 0.00
0.101	199.39± 5.71	271.21± 4.50	438.67± 3.30	525.45± 25.02	0.18± 0.00	0.29± 0.01	0.16± 0.01	0.13± 0.00
0.148	141.17± 1.57	184.23± 2.33	324.88± 0.37	401.21± 10.97	0.27± 0.02	0.50± 0.00	0.23± 0.00	0.20± 0.02
0.217	82.54± 0.32	115.88± 1.86	185.16± 2.96	251.49± 3.95	0.53± 0.03	0.74± 0.01	0.52± 0.02	0.41± 0.03
0.318	45.13± 0.32	73.16± 1.45	97.62± 1.63	143.07± 0.93	0.91± 0.03	1.02± 0.00	0.91± 0.02	0.71± 0.05

0.466	24.68± 0.24	46.34± 0.77	51.74± 0.55	78.9± 0.88	1.35± 0.03	1.31± 0.00	1.34± 0.01	1.07± 0.05
0.685	13.64± 0.18	29.40± 0.43	27.69± 0.22	43.4± 0.85	1.85± 0.03	1.60± 0.04	1.81± 0.01	1.47± 0.05
1.01	7.60± 0.08	18.52± 0.49	15.06± 0.18	24.38± 0.79	2.47± 0.05	2.02± 0.04	2.32± 0.01	1.90± 0.04

6.3.2.2. The storage modulus and loss factor of GMS structured oil (GO)

Table 6-6. Storage modulus and Loss Factor of GMS structured oil (GO)at the concentration from 5% to 8% (w/w)

Shear Strain (1)	G", 5%	G", 6%	G", 7%	G", 8%	LF, 5%	LF, 6%	LF, 7%	LF, 8%
0.0001	382.63±9.93	1214.20±140.71	1529.3±374.63	810.1±0.64	0.22±0.02	0.18±0.00	0.16±0.00	0.11±0.01
0.000148	370.74±13.15	1189.25±15.9	1538.00±429.92	834.84±0.38	0.22±0.01	0.16±0.02	0.16±0.01	0.10±0.00
0.000217	357.43±2.54	1220.6±153.44	1519.45±500.14	862.06±10.97	0.23±0.00	0.15±0.02	0.18±0.04	0.11±0.00
0.000318	338.01±3.92	1228.6±148.35	1550.4±448.16	880.29±18.48	0.24±0.00	0.15±0.02	0.16±0.01	0.11±0.00
0.000466	315.38±5.4	1225.65±149.55	1538.70±501.48	898.12±27.15	0.26±0.01	0.15±0.02	0.16±0.01	0.10±0.00
0.000685	290.37±2.40	1208.6±165.75	1518.95±512.3	910.01±37.63	0.28±0	0.16±0.02	0.17±0.02	0.11±0.00
0.00101	262.24±5.00	1151.7±172.96	1499.25±447.95	922.23±44.41	0.29±0.01	0.18±0.02	0.17±0.01	0.11±0.00
0.00148	230.51±0.79	1096.81±157.95	1364.45±482.74	926.3±47.53	0.33±0.00	0.19±0.02	0.19±0.02	0.11±0.00
0.00217	200.04±3.9	1000.52±149.74	1312.4±438.97	926.55±55.13	0.35±0.01	0.21±0.02	0.2±0.01	0.12±0.00
0.00318	167.77±3.63	887.29±114.47	1202.58±399.83	914.98±61.84	0.38±0.03	0.24±0.03	0.22±0.01	0.12±0.00
0.00466	133.25±1.62	763.02±99.94	1062.86±352.91	889.66±60.37	0.44±0.02	0.27±0.02	0.24±0.01	0.13±0.00
0.00685	106.66±0.91	631.75±93.10	913.07±311.74	855.77±65.49	0.49±0.02	0.3±0.02	0.27±0.01	0.14±0.00
0.0101	83.58±0.74	496.92±77.44	748.25±260.93	798.58±63.65	0.53±0.03	0.33±0.03	0.30±0.01	0.15±0.00
0.0148	67.10±4.57	374.00±57.78	587.36±203.73	716.86±57.01	0.57±0.07	0.37±0.03	0.33±0.01	0.17±0.00
0.0217	51.15±5.31	267.3±42.85	440.76±148.28	611.33±46.20	0.63±0.11	0.41±0.03	0.37±0.01	0.19±0.00
0.0318	37.18±4.13	185.86±28.72	314.81±97.72	492.69±31.89	0.71±0.15	0.47±0.04	0.41±0.01	0.22±0.00
0.0466	22.79±1.64	122.16±18.87	211.12±57.47	369.45±20.8	0.96±0.00	0.54±0.05	0.46±0.01	0.26±0.00
0.0685	15.09±1.26	75.5±11.54	131.96±30.63	257.19±15.05	1.17±0.01	0.66±0.06	0.54±0.01	0.32±0.00
0.101	10.59±0.55	43.08±6.35	76.03±14.78	164.13±11.41	1.38±0.14	0.83±0.07	0.67±0.01	0.40±0.00
0.148	6.28±0.08	22.75±2.91	39.49±6.53	93.90±8.51	1.90±0.05	1.08±0.09	0.88±0.01	0.52±0.01
0.217	3.95±0.40	11.42±1.00	19.36±2.96	47.80±5.40	2.35±0.37	1.48±0.10	1.18±0.03	0.73±0.02

0.318	2.30±0.21	5.66±0.42	9.30±1.31	23.60±2.70	3.27±0.48	2.07±0.12	1.61±0.09	1.02±0.03
0.466	1.27±0.03	2.89±0.21	4.65±0.35	12.63±1.40	4.89±0.13	2.98±0.21	2.22±0.07	1.37±0.02
0.685	0.89±0.03	1.89±0.09	2.85±0.07	7.32±0.76	5.79±0.84	3.73±0.20	2.82±0.04	1.80±0.03
1.01	0.80±0.01	1.86±0.07	2.42±0.03	4.25±0.35	5.71±1.12	3.68±0.15	3.10±0.02	2.41±0.02

6.3.2.3. The storage modulus and loss factor of GMS structured oil (GO)

Table 6-7.Storage modulus and Loss Factor of GMS structured oil (GO)at the concentration from 5% to 8% (w/w)

Shear Strain (1)	G", 5%	G", 6%	G", 7%	G", 8%	LF, 5%	LF, 6%	LF, 7%	LF, 8%
0.0001	136.45±5.81	149.00±8.77	752.05±86.34	1319.35±209.66	0.18±0.01	0.21±0.02	0.17±0.00	0.19±0.01
0.000148	138.88±4.45	143.41±10.42	763.85±85.25	1292.9±114.41	0.19±0.01	0.23±0.03	0.16±0.00	0.2±0.01
0.000217	144.59±5.28	144.68±7.97	768.61±79.71	1302.05±120.84	0.16±0.02	0.2±0.05	0.16±0.00	0.19±0.01
0.000318	146.52±5.64	143.11±8.36	773.89±79.49	1308.3±121.20	0.17±0.00	0.21±0.04	0.16±0.00	0.19±0.01
0.000466	142.79±3.46	142.83±8.71	776.07±80.21	1316.45±117.73	0.18±0.00	0.21±0.03	0.16±0.00	0.19±0.01
0.000685	142.80±3.13	143.74±8.88	778.62±79.51	1319.1±121.48	0.17±0.00	0.21±0.04	0.16±0.00	0.19±0.01
0.00101	142.91±4.21	143.98±9.31	780.71±79.51	1323.9±118.79	0.17±0.01	0.21±0.03	0.16±0.00	0.19±0.01
0.00148	142.95±2.87	145.57±8.75	782.94±80.12	1325.75±116.74	0.17±0.00	0.2±0.03	0.16±0.00	0.19±0.01
0.00217	142.64±2.95	146.25±9.40	785.03±80.39	1328.3±117.10	0.17±0.00	0.2±0.03	0.16±0.00	0.19±0.01
0.00318	142.06±2.76	146.14±8.58	786.48±80.24	1329.05±114.76	0.18±0.00	0.21±0.03	0.16±0.00	0.19±0.01
0.00466	141.14±3.43	145.99±8.42	787.16±80.00	1326.15±110.66	0.19±0.00	0.21±0.04	0.16±0.00	0.19±0.01
0.00685	139.27±4.31	145.34±8.92	786.72±79.75	1317.65±104.58	0.19±0.01	0.22±0.04	0.16±0.00	0.19±0.01
0.0101	133.96±2.55	141.6±7.92	783.55±78.45	1299.7±96.87	0.20±0.00	0.23±0.04	0.16±0.00	0.19±0.01
0.0148	127.48±1.82	136.22±8.32	775.27±76.19	1266.85±86.76	0.20±0.01	0.23±0.03	0.16±0.00	0.20±0.01
0.0217	118.02±0.59	126.72±5.64	758.2±72.02	1206.5±71.13	0.20±0.01	0.24±0.04	0.17±0.00	0.20±0.01
0.0318	108.72±0.44	117.62±6.17	725.54±64.2	1108.6±53.46	0.20±0.01	0.25±0.05	0.17±0.00	0.20±0.02
0.0466	96.71±0.97	102.69±1.35	670.77±50.32	966.18±36.63	0.20±0.01	0.24±0.04	0.17±0.00	0.20±0.02
0.0685	83.31±0.92	88.16±1.48	586.18±32.54	779.26±21.30	0.22±0.01	0.25±0.04	0.17±0.01	0.21±0.02
0.101	64.68±0.49	69.06±1.41	458.93±13.07	534.61±7.79	0.28±0.00	0.32±0.05	0.22±0.02	0.31±0.03
0.148	44.46±1.92	49.95±1.50	288.15±4.74	306.62±9.34	0.4±0.00	0.44±0.06	0.42±0.02	0.56±0.01

0.217	31.25±0.44	32.85±0.39	161.13±0.09	164.21±8.71	0.54±0.05	0.59±0.04	0.73±0.03	0.91±0.02
0.318	20.08±0.25	21.21±0.67	85.61±1.08	85.72±5.78	0.76±0.01	0.74±0.01	1.12±0.05	1.34±0.06
0.466	12.89±0.19	13.44±0.60	44.43±0.57	44.08±3.01	1.09±0.11	0.97±0.01	1.59±0.06	1.88±0.08
0.685	8.14±0.18	8.41±0.50	23.47±0.13	23.48±1.14	1.21±0.09	1.24±0.04	2.12±0.06	2.49±0.06
1.01	5.18±0.11	5.19±0.28	12.8±0.32	13.36±0.10	1.52±0.06	1.57±0.05	2.68±0.06	3.14±0.02

6.4. The original data of in-vitro digestion

6.4.1. The release of free fatty acids (FFA)

Table 6-8. The release of free fatty acids (FFA) of oil bodies (OB), oil, GMS structured oil bodies (GOB) and GMS structured oil (GO); the concentration of GMS was 8%. FFA released in only digestive fluids had been excluded.

Time (min)	OB	Oil	GOB	GO
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	3.18±0.65	-0.31±0.34	3.69±0.70	0.81±0.31
3	7.14±0.65	-0.33±0.57	10.20±0.36	1.39±0.29
5	10.89±0.76	-0.36±0.51	16.89±0.30	1.88±0.15
7	14.43±1.23	-0.38±0.66	23.27±0.81	2.25±0.02
8	17.63±2.03	-0.30±0.86	29.65±1.01	2.51±0.01
10	21.16±2.15	-0.29±0.87	35.46±1.35	2.79±0.18
12	24.73±1.95	-0.19±0.99	40.90±1.70	3.11±0.14
13	28.32±2.07	-0.16±0.92	45.99±2.09	3.45±0.31
15	31.84±2.63	-0.04±0.95	50.73±2.34	3.98±0.69
17	35.39±2.80	0.09±0.98	55.12±2.60	4.33±0.79
18	38.95±3.38	0.22±0.79	59.30±2.81	4.55±0.78
20	42.69±3.56	0.38±0.89	63.08±3.11	4.71±0.77
22	45.67±4.71	0.46±0.92	66.55±2.67	4.99±0.80
23	47.45±5.56	0.63±0.93	68.48±0.95	5.16±0.79
25	48.83±4.60	0.96±0.86	69.12±0.61	5.33±0.72
27	49.96±4.99	1.27±0.58	69.60±0.12	5.55±0.88
28	51.13±5.10	1.52±0.47	70.13±0.62	5.88±1.35
30	52.23±5.07	1.81±0.37	70.26±0.81	6.07±1.40
32	53.20±4.70	2.03±0.29	70.94±1.78	6.30±1.51
33	53.45±5.06	2.13±0.11	71.10±2.05	6.45±1.55
35	54.33±5.31	2.23±0.25	71.60±2.27	6.54±1.54
37	54.66±5.25	2.68±0.40	71.60±2.27	6.67±1.57
38	55.41±5.34	3.00±0.53	71.92±2.76	6.83±1.58
40	55.86±5.23	3.32±0.62	72.07±2.96	6.93±1.72
42	56.34±4.73	3.54±0.71	72.09±3.00	7.05±1.85
43	56.59±4.38	3.77±0.69	72.61±3.73	7.14±1.75
45	57.16±4.41	3.85±0.70	72.69±3.85	7.18±1.70
47	57.32±4.47	4.10±0.85	72.75±4.05	7.15±1.64
48	57.32±4.47	4.29±0.96	72.75±4.05	7.19±1.57
50	57.28±4.47	4.37±0.94	72.71±4.05	7.20±1.52
52	57.66±3.89	4.51±1.01	72.76±4.20	7.14±1.52

53	58.43±3.81	4.60±1.08	72.85±4.38	7.48±1.81
55	58.74±3.48	4.68±1.18	73.61±3.91	7.62±2.00
57	59.27±3.05	4.91±1.13	73.85±3.66	7.62±2.00
58	59.45±3.02	4.94±1.23	74.39±3.49	7.64±2.10
60	59.45±3.02	5.05±1.38	74.73±3.36	7.71±2.17
62	59.40±3.02	5.12±1.54	74.89±3.05	7.75±2.29
63	59.50±3.16	5.20±1.65	75.23±2.58	7.91±2.27
65	59.83±3.37	5.50±1.23	75.23±2.58	7.97±2.27
67	59.90±3.26	5.66±1.24	75.28±2.50	8.07±2.26
68	60.33±3.03	5.66±1.24	75.28±2.50	8.15±2.15
70	60.33±3.03	5.66±1.24	75.48±2.78	8.28±2.30
72	60.45±3.20	5.71±1.32	75.72±3.12	8.46±2.45
73	60.45±3.20	5.84±1.35	76.04±2.67	8.52±2.53
75	60.47±3.18	6.04±1.32	76.06±2.65	8.66±2.47
77	60.62±3.12	6.19±1.32	76.28±2.33	8.66±2.47
78	60.97±2.80	6.39±1.23	76.43±2.02	8.61±2.45
80	61.00±2.76	6.47±1.30	76.43±2.02	8.70±2.33
82	61.22±3.07	6.47±1.30	76.43±2.02	8.76±2.33
83	61.22±3.07	6.61±1.33	76.43±2.02	8.87±2.30
85	61.22±3.07	6.71±1.34	76.43±2.02	8.96±2.21
87	61.22±3.07	6.84±1.42	76.43±2.02	9.03±2.31
88	61.22±3.07	6.89±1.49	76.62±1.76	9.23±2.31
90	61.33±2.91	6.96±1.59	76.62±1.76	9.26±2.27
92	61.38±3.00	7.27±1.47	76.62±1.76	9.28±2.25
93	61.45±3.11	7.27±1.50	76.66±1.88	9.27±2.20
95	61.55±2.96	7.40±1.44	77.02±2.38	9.30±2.24
97	61.79±3.36	7.46±1.45	76.97±2.38	9.39±2.28
98	61.96±3.60	7.53±1.35	76.97±2.38	9.46±2.21
100	61.96±3.60	7.72±1.30	76.97±2.38	9.58±2.24
102	61.96±3.60	7.98±1.42	76.97±2.38	9.69±2.29
103	61.96±3.60	8.07±1.45	77.07±2.54	9.73±2.24
105	62.06±3.74	8.18±1.46	77.12±2.60	9.84±2.35
107	62.23±3.98	8.26±1.56	77.12±2.60	9.92±2.31
108	62.23±3.98	8.32±1.64	77.12±2.60	10.04±2.46
110	62.23±3.98	8.32±1.64	77.12±2.60	10.14±2.60
112	62.17±3.98	8.27±1.64	77.06±2.60	10.12±2.56
113	62.17±3.98	8.27±1.64	77.06±2.60	10.18±2.65
115	62.16±3.98	8.47±1.59	77.39±2.12	10.19±2.63
117	62.13±3.98	8.61±1.56	77.36±2.12	10.22±2.54
118	62.13±3.98	8.65±1.62	77.37±2.11	10.22±2.54
120	62.13±3.98	8.65±1.62	77.37±2.11	10.29±2.65

6.4.2. The volume of NaOH consumed in digestion

Table 6-9. NaOH consumption by oil bodies (OB), GMS structured oil bodies (GOB), Oil, GMS structured oil (GO) and GMS-water gel (GW) during lipolysis; the concentration of GMS in each sample was 8%;

Time (min)	OB	Oil	GOB	GW	GO
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

2	1.13±0.13	0.41±0.08	1.23±0.14	0.87±0.00	0.66±0.07
3	2.02±0.13	0.48±0.13	2.65±0.07	1.48±0.02	0.87±0.07
5	2.84±0.16	0.52±0.11	4.07±0.06	1.79±0.08	1.03±0.03
7	3.59±0.25	0.54±0.15	5.4±0.17	2.08±0.08	1.14±0.01
8	4.26±0.42	0.58±0.20	6.73±0.21	2.28±0.10	1.21±0.00
10	5.00±0.44	0.60±0.20	7.94±0.28	2.49±0.16	1.29±0.04
12	5.74±0.40	0.63±0.22	9.06±0.35	2.63±0.17	1.37±0.03
13	6.49±0.42	0.64±0.21	10.11±0.43	2.70±0.09	1.46±0.07
15	7.21±0.54	0.66±0.21	11.08±0.48	2.76±0.03	1.57±0.16
17	7.95±0.58	0.70±0.22	11.99±0.53	2.89±0.05	1.66±0.18
18	8.67±0.69	0.73±0.18	12.85±0.58	2.97±0.09	1.71±0.18
20	9.44±0.73	0.77±0.20	13.63±0.64	3.05±0.09	1.75±0.18
22	10.06±0.97	0.79±0.21	14.35±0.55	3.15±0.11	1.82±0.18
23	10.43±1.14	0.83±0.21	14.74±0.20	3.19±0.17	1.86±0.18
25	10.71±0.94	0.91±0.19	14.88±0.13	3.26±0.21	1.90±0.16
27	10.94±1.02	0.98±0.13	14.98±0.02	3.34±0.23	1.95±0.20
28	11.18±1.05	1.04±0.11	15.08±0.13	3.40±0.20	2.02±0.31
30	11.41±1.04	1.10±0.08	15.11±0.17	3.50±0.17	2.07±0.32
32	11.61±0.96	1.15±0.07	15.25±0.36	3.56±0.11	2.12±0.34
33	11.67±1.04	1.18±0.02	15.29±0.42	3.62±0.09	2.16±0.35
35	11.85±1.09	1.20±0.06	15.39±0.47	3.69±0.14	2.18±0.35
37	11.92±1.08	1.31±0.09	15.39±0.47	3.75±0.15	2.21±0.36
38	12.08±1.10	1.38±0.12	15.47±0.57	3.80±0.16	2.25±0.36
40	12.17±1.07	1.46±0.14	15.49±0.61	3.82±0.15	2.27±0.39
42	12.27±0.97	1.51±0.16	15.50±0.62	3.86±0.18	2.30±0.42
43	12.32±0.90	1.56±0.16	15.61±0.77	3.93±0.13	2.32±0.40
45	12.43±0.91	1.58±0.16	15.62±0.79	3.98±0.17	2.33±0.38
47	12.49±0.92	1.65±0.19	15.65±0.83	4.01±0.18	2.34±0.37
48	12.49±0.92	1.69±0.22	15.65±0.83	4.07±0.19	2.35±0.36
50	12.49±0.92	1.72±0.21	15.65±0.83	4.11±0.16	2.36±0.34
52	12.57±0.80	1.76±0.23	15.67±0.86	4.16±0.10	2.36±0.34
53	12.74±0.78	1.79±0.24	15.70±0.90	4.19±0.08	2.44±0.41
55	12.80±0.71	1.81±0.27	15.86±0.80	4.23±0.10	2.47±0.45
57	12.91±0.63	1.86±0.26	15.91±0.75	4.23±0.10	2.47±0.45
58	12.96±0.62	1.88±0.28	16.02±0.72	4.26±0.06	2.49±0.48
60	12.96±0.62	1.90±0.31	16.09±0.69	4.38±0.18	2.50±0.49
62	12.96±0.62	1.93±0.35	16.14±0.63	4.44±0.23	2.52±0.52
63	12.98±0.65	1.95±0.37	16.21±0.53	4.50±0.20	2.56±0.51
65	13.05±0.69	2.01±0.28	16.21±0.53	4.55±0.13	2.57±0.51
67	13.06±0.67	2.05±0.28	16.22±0.51	4.56±0.11	2.60±0.51
68	13.15±0.62	2.05±0.28	16.22±0.51	4.57±0.13	2.61±0.49
70	13.15±0.62	2.05±0.28	16.26±0.57	4.58±0.14	2.64±0.52
72	13.17±0.66	2.06±0.30	16.31±0.64	4.60±0.15	2.68±0.55
73	13.17±0.66	2.09±0.31	16.37±0.55	4.62±0.17	2.70±0.57
75	13.18±0.65	2.14±0.30	16.38±0.54	4.65±0.21	2.73±0.56
77	13.21±0.64	2.17±0.30	16.42±0.48	4.65±0.21	2.73±0.56

78	13.29±0.57	2.23±0.28	16.47±0.41	4.65±0.21	2.73±0.55
80	13.30±0.57	2.25±0.29	16.47±0.41	4.65±0.21	2.75±0.53
82	13.35±0.63	2.25±0.29	16.47±0.41	4.67±0.18	2.77±0.53
83	13.35±0.63	2.28±0.30	16.47±0.41	4.68±0.16	2.79±0.52
85	13.35±0.63	2.30±0.30	16.47±0.41	4.73±0.19	2.81±0.50
87	13.35±0.63	2.33±0.32	16.47±0.41	4.77±0.18	2.83±0.52
88	13.35±0.63	2.34±0.34	16.51±0.36	4.80±0.18	2.87±0.52
90	13.37±0.60	2.36±0.36	16.51±0.36	4.83±0.18	2.88±0.51
92	13.38±0.62	2.43±0.33	16.51±0.36	4.85±0.18	2.88±0.51
93	13.40±0.64	2.44±0.34	16.52±0.39	4.90±0.11	2.89±0.50
95	13.42±0.61	2.47±0.33	16.60±0.49	4.91±0.10	2.90±0.51
97	13.48±0.69	2.49±0.33	16.60±0.49	4.95±0.09	2.93±0.52
98	13.52±0.74	2.51±0.31	16.60±0.49	4.98±0.08	2.94±0.50
100	13.52±0.74	2.55±0.29	16.60±0.49	4.98±0.08	2.97±0.51
102	13.52±0.74	2.61±0.32	16.60±0.49	4.99±0.06	3.00±0.52
103	13.52±0.74	2.63±0.33	16.62±0.52	4.99±0.06	3.00±0.51
105	13.54±0.77	2.65±0.33	16.63±0.53	5.05±0.10	3.03±0.53
107	13.57±0.82	2.67±0.35	16.63±0.53	5.07±0.11	3.05±0.52
108	13.57±0.82	2.68±0.37	16.63±0.53	5.10±0.12	3.07±0.56
110	13.57±0.82	2.68±0.37	16.63±0.53	5.14±0.11	3.10±0.59
112	13.57±0.82	2.68±0.37	16.63±0.53	5.16±0.11	3.10±0.58
113	13.57±0.82	2.68±0.37	16.63±0.53	5.17±0.10	3.12±0.60
115	13.57±0.82	2.73±0.36	16.70±0.44	5.17±0.10	3.12±0.60
117	13.57±0.82	2.77±0.35	16.70±0.44	5.19±0.07	3.14±0.58
118	13.57±0.82	2.78±0.37	16.70±0.43	5.22±0.09	3.14±0.58
120	13.57±0.82	2.78±0.37	16.70±0.43	5.22±0.10	3.15±0.60