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Physical and functional properties of plant-based pre-emulsified chewable gels for the oral delivery of nutraceuticals

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ABSTRACT

Gelatin-based pre-emulsified chewable gels have presented advantages over traditional tablets, bulk oils, hard and soft capsules for oral delivery. Ethical, ecological, and religious considerations have increased the demand for plant-based gelling agents which can be formulated into chewable emulsion gels. Plant-based polysaccharide emulsion gels prepared with agar and pectin were compared to gelatin emulsion gels regarding rheological, textural, and functional properties. The agar emulsion gel had higher gelling/melting temperatures (T_g: 40 °C, T_m: 90 °C) than the gelatin emulsion gel (T_g: 37 °C, T_m: 45 °C), whereas pectin emulsion gel had a more similar gelling/melting profile to the gelatin formulation (T_g: 38 °C, T_m: 54 °C). Texture analyses revealed that the agar emulsion gel had a harder and more brittle texture, whereas pectin emulsion gel had a softer texture than the gelatin emulsion gel. Pectin emulsion gels had the largest average droplet size (32 μm), followed by agar (13 μm) and gelatin emulsion gels (1 μm). The *in vitro* lipolysis experiments indicated that the polysaccharide emulsion gels were lipolyzed to a lower extent and had a slower initial lipolysis rate (agar: 2.8 μmol FFA/*sec*, pectin: 4.3 μmol FFA/*sec*), compared to the gelatin emulsion gel (24.9 μmol FFA/*sec*). The industrial potential and challenges of the polysaccharide emulsion gels were evaluated, and the results show that plant based pre-emulsified chewable gels can be manufactured for the oral delivery of nutraceuticals.

1. Introduction

Food supplements and nutraceuticals are consumed to complement a diet with micronutrients, aiming to enhance health and provide medical benefits (DeFelice, 1995; Santini & Novellino, 2017). Essential fatty acids, vitamins, minerals, and carotenoids are among important nutraceuticals which may be incorporated into an applicable dosage form (Chen, Remondetto & Subirade, 2006; Karuna & Prasad, 2015). Typical oral delivery forms include soft and hard gel capsules, tablets, elixirs, syrups, and chewables (Adepu & Ramakrishna, 2021; Dille, Hattrem & Draget, 2018b). There has been an increasing demand for the development of user-friendly dosage forms, in which a chewable delivery system is considered to be a practical alternative (Dille, Hattrem & Draget, 2018a). The most common chewable delivery forms are similar to gummies/confectioneries in appearance, in which sweeteners, acidulants, aromas and colorants are used to make a well-tasting product. Besides having a good palatability, which is important for compliance, the product also has the uniformity of a tablet, and its chewable texture removes the need for water in the process of ingestion. Although chewable dosage forms offer a user-friendly design, their development and production have been challenging. In the formulation, the water-soluble actives are typically in a solubilized state, while fat-soluble actives need to be mixed throughout the continuous water phase as small oil droplets (Dille et al., 2018a). This may be especially challenging if the payload of the fat soluble active ingredient is high, as mixing of the oil phase requires a more complex production process, while maintaining the stability of the oil phase throughout the manufacturing process. The chemical stability of the embedded ingredient may to a certain extent be ensured by innovative formulation and packaging technology, while to obtain a stable droplet size at elevated temperatures for a longer period of time, the choice of emulsifier, and gelling agent is of high importance.

Emulsion gels *i.e.*, gelled emulsions, are complex soft-solid colloidal materials which consist of an emulsion and a gel (Dickinson, 2012). Emulsion gels can be classified as emulsion droplet-filled gels and emulsion droplet-aggregated gels (Dickinson, 2012; Lin, Kelly & Miao, 2020b). In emulsion droplet-filled gels, a polysaccharide or protein gel constitutes the continuous gel matrix which contains embedded emulsion droplets (Gravelle & Marangoni, 2021). If the emulsion droplets strongly interact with the gel network, the droplets are generally referred to as active fillers. The term inactive filler is used for systems in which the emulsion droplets have little to no interaction with the surrounding gel network (Chen & Dickinson, 1999). In the context of

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oral delivery of nutraceuticals, emulsion gels are convenient delivery forms compared to traditional capsules, bulk oils or liquid emulsions which may present compliance issues for pediatric and geriatric populations, as well as patients suffering from dysphagia. In addition, emulsion gels provide physical stability and mechanical properties to emulsions (Lu, Mao, Hou, Miao & Gao, 2019).

Due to their unique texture and emulsifying capability, chewable gelatin-based emulsion gels have been studied, characterized, and patented (Dille et al., 2018a, 2018b; Hattrem, Dille, Seternes, Ege & Draget, 2018, 2015; Haug & Draget, 2007). Gelatin-based emulsion gels typically exhibit an active filler effect, whereas polysaccharide-based emulsion gels made with hydrocolloids with low surface activity typically exhibit an inactive filler effect (Dille et al., 2018a; Koç et al., 2019). One challenge with gelatin-based gels is the low melting temperature of gelatin which may result in the instability of the delivery system in warm climates during transportation or storage of the products (Baydin, Aarstad, Dille, Hattrem & Draget, 2022). Another disadvantage is the animal origin of gelatin since it is obtained from the connective tissues of cattle, pigs, fish or poultry (Schrieber & Gareis, 2007). Ecological, ethical, and health concerns, religious constraints as well as dietary restrictions may endorse vegetarian or vegan diets for individuals (Leitzmann, 2014). In the recent years, there has been an increase in the number of people who follow a plant-based diet (Alcorta, Porta, Tárrega, Alvarez & Vaquero, 2021). This has resulted in an increase in the demand for plant-based food alternatives in the market (Noguerol, Pagán, García-Segovia & Varela, 2021; Stannard, 2018).

Although gelatin is an animal-derived biopolymer, terms such as plant-based or “veggie gelatin” have been used to describe plant hydrocolloids with gelling properties (Alipal et al., 2021; Lestari, Octavianti, Jaswir & Hendri, 2019). Emulsion gels have been prepared with polysaccharides such as agar (Kim, Gohtani & Yamano, 1997, 1996, 1999; Yamano, Kagawa, Kim & Gohtani, 1996), pectin (Lupi et al., 2015), carrageenans (Fontes-Candia, Ström, Lopez-Sanchez, López-Rubio & Martínez-Sanz, 2020; Sala, de Wijk, van de Velde & van Aken, 2008), cellulose (Jiang et al., 2019), and other polysaccharides (Dun et al., 2020; Weiss, Scherze & Muschiolik, 2005; Yang et al., 2019). Although most plant-based hydrocolloids can act as stabilizing agents of emulsions due to structuring, thickening or gelling of the continuous phase, they usually lack the surface active properties to act as emulsifying agents (Dickinson, 2009). Therefore, an emulsifying agent is commonly included in combination with a plant-based gelling agent to provide sufficient emulsifying and stabilizing capacity in such systems (Banerjee & Bhattacharya, 2011; Shao et al., 2020). Another challenge with plant-based emulsion gels is their texture since gelatin has a characteristic elastic, chewy, melt-in-the-mouth texture which is very difficult to mimic with plant-based alternatives (Karim & Bhat, 2008; Schrieber & Gareis, 2007). Other issues with polysaccharide emulsion gels have been reported as stability, syneresis, and the complexity of the systems (Lin, Kelly, Maidannyk & Miao, 2020a; Yue et al., 2022).

In addition to being more practical and user-friendly than traditional oral delivery forms, gelatin-based emulsion gels have been shown to have comparable stability and dissolution kinetics to a standard oral tablet (Dille et al., 2018b). Furthermore, the bioavailability of omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) significantly increased when orally delivered in a chewable gelatin-based emulsion gel compared to bulk oil (Haug et al., 2011). Digestibility of food emulsion gels are commonly studied through *in vitro* lipolysis experiments to preliminarily investigate the functionality of formulations. *In vitro* lipolysis studies of gelatin emulsion gels indicated the relation between the gel mesh size and speed of lipid digestion (Sarkar et al., 2015). *In vitro* lipolysis of agar emulsion gels was studied and compared with emulsions without agar present in the formulation, suggesting limitations of digestion due to entrapment of oil droplets in the agar network (Wang, Neves, Kobayashi, Uemura & Nakajima, 2013). Similarly, the lipolysis extent of pectin emulsion gels were lower compared to liquid emulsions (Yang et al., 2022). The methodology and digestive parameters of the *in vitro* lipolysis experiment may have a large impact on the results of the digestion studies (Li, Hu & McClements, 2011; Lin & Wright, 2018; Mella, Quilaqueo, Zúñiga & Troncoso, 2021). Therefore, for comparative analyses, it is of importance to test different formulations in a standardized *in vitro* lipolysis set-up (Li et al., 2011). To the authors’ knowledge, a study comparing the extent of *in vitro* lipolysis of emulsion gels prepared with gelatin, agar, and pectin, has not been previously published.

The present study focuses on two polysaccharide-based emulsion gels: agar and pectin. Agars are moderately sulfated galactans, obtained from red seaweed, and are a mixture of agarose and agaropectin (Araki, 1956; Sousa, Rocha & Gonçalves, 2021). Pectins are a group of complex heteropolysaccharides, mainly containing galacturonic acid residues, and are obtained from terrestrial plants (Hua, Wang, Yang, Kang & Zhang, 2015). Commercial pectins may be amidated or methylesterified to varying degrees (Da Silva & Rao, 2006; Zeeb, Roth & Endreß, 2021). The scope of this study was to characterize emulsion gels made with polysaccharides, agar, and pectin, and to compare the rheological and textural properties of these gels with a traditional gelatin emulsion gel. In addition, agar and pectin gels that do not contain oil were compared to corresponding emulsion gels to study the impact of oil on the rheology and texture of the gels. Along with the comparison of the physical and textural properties of the gels, their functionalities were studied with gastrointestinal *in vitro* lipolysis experiments. The object of this investigation was to develop plant-based emulsion gels for the oral delivery of nutraceuticals and evaluate their potential as alternatives to gelatin-based emulsion gels.

2. Materials and methods

2.1. Materials

Agar (Gelagar HDR 300) was purchased from B&V, Italy. Bovine gelatin (Type B 160 Bloom, Batch# 643208) was provided by Gelita®, Germany. Xylitol was provided by Danisco, UK. Sorbitol was purchased from Food Innovation, Norway. Malic acid (MA) and trisodium citrate dihydrate (TCD) were provided by MerckMillipore, USA. Corn oil (lot# MKCH1635) and ascorbic acid were purchased from Sigma, USA. Citrem (Grinsted N 12 veg kosher, batch# 4011722438) was purchased from Danisco, Denmark. Hydrolyzed sunflower lecithin (Giralec HE-60) was provided by Lasenor, Spain. GENU® pectin (LM-104 AS-FS) was provided by CP Kelco, Denmark. Calcium citrate tetrahydrate (CCT) was provided by Gadot Biochemical Industries, Israel. Bile extract porcine (lot# SLCC9272) was purchased from Sigma Aldrich, USA. Pancreatin from porcine pancreas (8xUSP, lot# SLBZ5739) and lipase from porcine pancreas (100–500 U/mg, lot# SLBZ7254) were purchased from Sigma Aldrich, USA. Rabbit gastric lipase (RGE15, 15 U/mg lipase and 500 U/mg pepsin, lot# BCBV8659) was purchased from Lipolytech, France.

2.2. Composition and preparation of the gels

Agar gel (AG) was prepared by mixing agar and deionized water (18.2 MΩcm Stakpure OmniaPure, Germany) at 90 °C for 30 min with magnetic stirring. After agar had completely dissolved, 0.06 (w/w)% Witafrol was added, and the solution was degassed using the Diaphragm Vacuum Pump (Vacuubrand, MZ 2C) until no air bubbles were visible. The water loss was compensated after degassing. The solution was allowed to gel at ambient temperature, *i.e.*, an average of 22 °C laboratory environment. Agar emulsion gel without oil (AWO), *i.e.*, agar gel with sugar alcohols and buffer salts, was prepared by first dry mixing agar, sorbitol, and xylitol. Ambient temperature water was added to the powder mixture and the contents were mixed at 90 °C for 30 min with magnetic stirring. The temperature was reduced to 65 °C and MA was added to the mixture and mixed for 10 min. Afterwards, TCD was added to the mixture and mixed for 10 min. Agar emulsion gel (AEG) was prepared in

Table 1
Abbreviations and compositions of the gels. All ingredients are given as weight percentage (w/w).

Ingredient	Function	Agar gel (AG)	Agar emulsion gel without oil (AWO)	Agar emulsion gel (AEG)	Pectin emulsion gel without oil (PWO)	Pectin emulsion gel (PEG)	Gelatin emulsion gel (GEG)
Water	Solvent	92.85	35.51	26.10	33.88	25.26	21.00
Agar	Gelling	7.15	2.72	2.00	–	–	–
Pectin	agent	–	–	–	3.76	2.8	–
Gelatin		–	–	–	–	–	8.60
Citrem	Emulsifier	–	–	1.50	–	–	–
Xylitol	Sweeteners	–	38.64	28.40	38.10	28.4	28.40
Sorbitol		–	19.05	14.00	18.78	14	14.00
MA	Buffer	–	1.36	1.00	2.01	1.5	1.00
TCD	salts	–	2.72	2.00	2.68	2	2.00
Ascorbic acid		–	–	–	0.47	0.35	–
CCT	Calcium salt	–	–	–	0.32	0.24	–
Lecithin	Emulsifier	–	–	–	–	0.45	–
Corn oil	Oil	–	–	25.00	–	25.00	25.00

a similar way to AWO, with the exception of first adding TCD to the solution when the temperature was reduced to 65 °C. After TCD was fully dissolved, citrem was added to the mixture and mixed for 15 min with magnetic stirring. Afterwards, MA was added and mixed for 10 min. The water phase was degassed as described above. The temperature of the mixture was reduced to 50 °C and preheated corn oil at this temperature was included in the mixture. The water phase and the oil phase were homogenized at 50 °C using T18 digital ultra-turrax® (IKA®, USA) at speed 9.8k rpm for 8 min.

Pectin emulsion gel without oil (PWO), *i.e.*, pectin gel with sugar alcohols and buffer salts, was prepared by first dry mixing pectin and sorbitol, and then adding water and mixing with magnetic stirring at 75 °C for 25 min. Afterwards, xylitol, TCD, MA, ascorbic acid and CCT were dry mixed and gradually added to the mixture with a total mixing time of 20 min. The water phase was degassed as described above. Pectin emulsion gel (PEG) was prepared similarly to PWO. After degassing, the temperature was reduced to 60 °C, preheated lecithin was added, and mixed for 20 min. Preheated corn oil was added and homogenized at 60 °C with 9.8k rpm for 8 min.

Gelatin emulsion gel (GEG) was prepared by mixing gelatin with water for 30 min with magnetic stirring at 60 °C. Afterwards, sorbitol, xylitol, TCD and MA were added and mixed for 10 min after each addition. Preheated corn oil at 60 °C was added to the aqueous phase and homogenized at ambient temperature at 9.8k rpm speed for 5 min. The emulsion gel was degassed as described above.

The composition of the gels is given in Table 1. For AG, AWO and AEG, the ratio of agar to water was kept constant at 0.77. For PWO and PEG, pectin to water ratio was kept constant at 0.11.

2.3. Rheological characterization

Small amplitude oscillatory shear (SAOS) measurements on the gels were performed with a rheometer (Malvern Kinexus ultra+, Westborough, United States). The upper geometry was serrated PP40X SW1648 SS for all agar gels, whereas the lower plate was serrated PLS40X S1586 SS for AG and KNX0127, curved sandblasted 50 mm for AWO and AEG. For PWO, PEG and GEG, the lower plate was PL61 ST S2579 SS, and the upper geometry was CP4/40 40 mm diameter 4° cone angle. A solvent trap was used for AG, PEG and GEG to prevent evaporation during the measurement. The protocol given in Baydin et al. (2022) was followed. Instrument calibration (zero gap) was performed prior to analysis. After gel preparation, approximately 2 g of gel was placed on the Peltier temperature controlled lower plate, which was heated up to 60 °C. The rheometer was operated in 0.1% shear strain controlled mode and the frequency was set to 1 Hz. The chosen strain was confirmed to be within the linear viscoelastic region through a strain sweep performed on AG (one of the most brittle gels tested in this study) between 0.001 and 100% strain (Supplementary Figure 1). The viscoelastic properties of the sample were obtained by using a temperature gradient of 2 °C/min, with a start and end temperature at 60 °C and a holding time of 15 min at 20 °C for PEG and GEG. For AG, AEG and AWO, the end temperature was 90 °C and oscillation continued for 10 min at 90 °C. The results were analyzed using rSpace for Kinexus software (version 1.75) and Microsoft Excel (version 2201). The gelling temperature (T_g) and melting temperature (T_m) of the samples were estimated as the temperature at which the phase angle corresponded to 45° in the cooling and heating process, respectively. The maximum storage modulus (G'_{max}, Pa) was determined as the highest measurement point during setting at 20 °C.

2.4. Texture profile analyses

Texture properties of the gels were analyzed with TA.XT plusC Texture Analyser (Stable Micro Systems Ltd., UK). Upon preparation, the gels were cast using cylindrical molds of standard dimensions (19.6 mm height, 8 mm diameter). The gels were set at ambient temperature for 24 h prior to analysis. Single compression analysis and standard texture profile analysis (TPA) were performed at ambient temperature, using a 5 kg load cell for AG, AWO, AEG, PEG and PWO, and a 30 kg load cell for GEG and 75% strain TPA test of AG. P/35 35 mm diameter cylinder aluminum probe supplied by Stable Micro Systems Ltd. was used for both single compression and TPA analyses. For the 75% strain single compression, pre-test and post-test speeds were 2 mm/s, while the test speed was 0.1 mm/s and the trigger force was 5 g. Strain height was measured automatically during compression. Max force (g) and strain at failure (%) data was obtained from the fraction moment of the gels. Young's modulus (N/m^2) was calculated from the following equation where gradient (N/m) was calculated by the ratio of force at 2% and 3% strain:

$$Young's\ modulus\ \left(N/m^2\right) = \frac{Gradient\ (N/m) \times\ height\ of\ the\ gel\ (m)}{Area\ of\ the\ gel\ \left(m^2\right)}$$

Area of the gel was the contact area of the gel with the probe which was calculated from the surface area of the uniform cylinder molds.

The texture profile analysis (TPA) was carried out with 20%, 30% and 75% strain to mimic different components of mastication. Pre-test, test, and post-test speeds were 1 mm/s, and the trigger force was 5 g. Strain height was measured automatically during compression. Hardness, adhesiveness, resilience, cohesiveness, springiness, and chewiness parameters were calculated from the TPA data which were analyzed with the Exponent connect software. Gumminess parameter was excluded from the analysis since it is comparable to chewiness. Gumminess is valid for semi-solid materials, whereas chewiness is applicable for solids (Bourne, 2002).

Table 2
Compositions of control gels for *in vitro* lipolysis. All ingredients are given as weight percentage (w/w).

Ingredient	Agar emulsion gel control (AC)	Pectin emulsion gel control (PC)	Gelatin emulsion gel control (GC)
Water	52.60	50.71	46.00
Agar	2.00	–	–
Pectin	–	2.80	–
Gelatin	–	–	8.60
Xylitol	28.40	28.40	28.40
Sorbitol	14.00	14.00	14.00
MA	1.00	1.50	1.00
TCD	2.00	2.00	2.00
Ascorbic acid	–	0.35	–
CCT	–	0.24	–

2.5. Syneresis measurements

Syneresis measurements were based on weight loss of the gels upon freezing at −30 °C and thawing at ambient temperature (approximately 22 °C). The gel was weighed and sealed with an airtight foil, *i.e.*, aluminum blister. After freezing for 12 h and thawing for 4 h at ambient temperature, the gel was weighed again and the difference in gel weight was normalized to percentage loss.

2.6. Water activity measurements

The water activity (a_w) of the gels was measured with HygroPalm HC2-AW (Rotronic, Switzerland) at ambient temperature. The sample was placed into the measurement chamber and the water activity was recorded after 15 min.

2.7. Droplet size analyses

Droplet sizes of the emulsion gels were measured with the Mastersizer 3000 Hydro MV (Malvern, UK). After AEG and PEG were prepared, approximately 2 g of sample was dissolved in water at 50 °C. For GEG, the solvent was 0.1 M HCl. Solutions were added to the water cell drop by drop until an obscuration rate of 5–18% was obtained. The dispersant refractive index was set to 1.330 and 1.470, for water and corn oil, respectively. Particle absorption index was 0.010 for all emulsion gels. The data collected from the detectors was analyzed by the Mastersizer software. The software provided average droplet size parameters (D[4, 3] volume mean diameter and D[3, 2] surface mean diameter) of five measurements, as well as the droplet size distribution of each emulsion gel.

2.8. In vitro *lipolysis*

In vitro lipolysis was performed either as gastrointestinal (gastric lipolysis followed by intestinal lipolysis) stage or intestinal stage only. Digestion in the gastric stage was not monitored. Therefore, the gastric stage should be considered as a pretreatment before the intestinal stage. In addition to emulsion gels (GEG, PEG, and AEG) control gels of each emulsion gel (GC, PC and AC) were tested with gastrointestinal *in vitro* lipolysis setup. Control gels were prepared as described in Section 2.2 for the emulsion gels apart from not containing oil or emulsifier (Table 2). To mimic mastication, the polysaccharide gels were pushed through a metal mesh with pore size 1.7 mm (Endecott, England), whereas gelatin gels were dissected with a scalpel into similar size pieces as the polysaccharide gels. The *in vitro* lipolysis protocol previously reported by Dille and Draget, (2021) was followed with modifications.

In vitro lipolysis of the gels was performed at 37 °C. For the gastric stage, 1.5 g of gel was mixed with a NaCl solution. Rabbit gastric extract was added, and the pH was quickly and manually reduced to 3. The pH combination electrode was A 1622 M DI (SI Analytics, Germany) coupled to the titrator 7000-M1/20, TitroLine (SI Analytics, Germany). The simulated gastric fluid (SGF) was kept at constant stirring for 1 h. The final volume of the SGF was 10 ml, and the concentrations of NaCl and gastric lipase were 80 mM and 4 mg/ml, respectively. Afterwards, the contents of the SGF were transferred to a beaker containing simulated intestinal fluid (SIF).

The SIF was formed by adding 450 mg bile extract porcine (Sigma, USA, lot#SLCC9272) and water into the beaker and stirring the contents until the bile extract was fully dissolved. 12.5 ml salt mix solution (40 mM $CaCl_2$/536 mM NaCl/dH_2O) was added to the beaker and kept in magnetic stirring for 10 min. The pH was adjusted to 7 by manually adding droplets of 1 mM and 0.1 mM NaOH. Pancreatin from porcine pancreas (Sigma, USA, lot#SLBZ5739) and lipase from porcine pancreas (Sigma, USA, lot#SLBZ7254) were dissolved in water in separate Eppendorf tubes through vortex mixing. The titrator was set to maintain the pH at 7 by adding droplets of 0.1 mM NaOH. The pH-stat method was initiated after simultaneously adding 1 ml of each enzyme mixture into the beaker. The final concentrations of the SIF components were 9 mg/ml bile extract porcine, 10 mM $CaCl_2$, 134 mM NaCl, 1.2 mg/ml pancreatin and 1.2 mg/ml lipase. The final volume of the SIF was 50 ml. The SIF was kept at constant stirring for 1 h while the data from the pH-stat method were recorded through the titrator.

For tests with only the intestinal *in vitro* lipolysis, the salt mix contained 40 mM $CaCl_2$ and 600 mM NaCl to reach a final concentration of 10 mM $CaCl_2$ and 134 mM NaCl in SIF without the SGF. For the *in vitro* lipolysis curves, the total lipolysis was assumed to start when pH initially reached pH 7, *i.e.*, the data points until pH 7 were removed and the total NaOH consumption was calculated after this point. The initial rate of the *in vitro* lipolysis reaction was calculated from the slope of the total lipolysis curve between 4 and 80 s.

2.9. Microscopy

Photomicrographs of the emulsion gels were taken after 1 h of gastric lipolysis using inverted light microscope Axio Observer Z1 (Carl Zeiss Microscopy GmbH, Germany). A small amount of SGF was transferred with a Pasteur pipette on the microscope slide, covered with a cover slip and examined using the microscope. The ZEISS ZEN pro digital imaging software (Version 2.3) was used to photograph the samples.

2.10. Statistical analyses

All statistical analyses were performed using IBM® SPSS® Statistics software version 28.0.1.0 (142). One-way analysis of variance (ANOVA) with post-hoc Tukey's honest significant difference (HSD) test was performed. Statistically significant differences were reported if $p < 0.05$.

3. Results and discussion

The emulsion gels were characterized and compared using water activity (a_w), syneresis, SAOS, large scale deformation, and droplet size measurements. To study the impact of oil in the polysaccharide gels, AEG and PEG were also compared to their "without oil" (WO) versions, AWO and PWO, respectively. In addition, AWO and AEG were compared

Table 3
Water activity (a_w) and syneresis of the gels presented as% water loss. For a_w and syneresis measurements, three and 6–9 replicates per each sample were analyzed, respectively. For a_w, the standard deviation was smaller than 0.01 for each gel. The abbreviations used for the gels are given in Table 1. *This result was due to minor adhesion of the gel to the aluminum blister. Different letters in superscript indicate statistically significant differences between gels. For both a_w and syneresis $p < 0.001$ between different gels.

Gel	a_w	Average syneresis (%)
AG	0.94 ^{d}	5.6 ± 0.7 ^{d}
AWO	0.77 ^{bc}	4.7 ± 0.6 ^{c}
AEG	0.77 ^{c}	3.2 ± 0.2 ^{b}
PWO	0.74 ^{a}	1.5 ± 0.3 ^{a}
PEG	0.76 ^{b}	1.7 ± 0.4 ^{a}
GEG	0.73 ^{a}	1.0 ± 0.5 *

to a pure agar gel (AG). In the WO gels, the aqueous phase was identical to the emulsion gels; for any ingredient in the WO gel, solute/water ratio is equal to its corresponding emulsion gel. Because of this, similar a_w was observed for emulsion gels and their WO versions. The a_w of different emulsion gels were proportional to the water they contain in the formulation (Table 1, Table 3). As expected, the a_w of AG was much higher than AWO and AEG, highlighting the importance of co-solutes to decrease the a_w of food systems below 0.85 to prevent microbial growth as recommended by the U.S. Food and Drug Administration (2018).

Syneresis, *i.e.*, expulsion of water from a gel, is a common phenomenon for agar gels (Armisen & Gaiatas, 2009). As expected, AG showed the highest syneresis (Table 3). The addition of sugar alcohols to AG significantly lowered syneresis for AWO, in accordance with lower a_w. The inclusion of oil in the formulation further reduced syneresis for AEG significantly. In a previous study with alginate emulsion gels, this was suggested to be due to oil droplets acting as barriers for water transport (Lević et al., 2015; Lin et al., 2020a). It is known that increasing agar concentrations result in lower syneresis (Banerjee & Bhattacharya, 2011). The current data suggest that the inclusion of sugar alcohols and oil also contribute to reducing syneresis due to a reduced amount of water as well as a greatly reduced a_w (free water) in the system. Pectin gels are known to exhibit syneresis during storage as a result of aging (Rao, Van Buren & Cooley, 1993). Although syneresis was lower for both PEG and PWO compared to agar gels, the inclusion of oil did not reduce the syneresis of the pectin gel (Table 3).

GEG was not included in the statistical tests for syneresis (Table 3). In this system, syneresis was considered negligible since no sweating was observed. This is in accordance with literature (Mizrahi, 2010). In general, syneresis in food gels is an undesirable phenomenon since it may impact the quality and stability of the product (Banerjee & Bhattacharya, 2011; Mizrahi, 2010). However, for adhesive gels which exhibit stickiness to their packaging material, a controlled amount of syneresis may promote lubrication due to surface liquid (Saha & Bhattacharya, 2010). During the syneresis measurements no stickiness was observed with agar or pectin gels.

3.1. Small amplitude oscillatory shear measurements

Although agar/water ratio was kept constant for the agar gels (AG, AWO, and AEG), it should be noted that AG had a much higher overall concentration of agar in the total formulation. In addition, water-soluble polyols increase the total volume of the water phase, resulting in a lower agar/water phase ratio for AWO and AEG. Consequently, AG had the significantly highest G'_{max} among agar gels, followed by AWO and AEG, although without significant differences (Table 4). In accordance with the literature, inactive fillers decrease gel strength compared to an oil-free gel (Dickinson & Chen, 1999; Dille, Haug & Draget, 2021b). Similarly, PWO had a higher G'_{max} than PEG, although the difference was not statistically significant. Among emulsion gels, AEG had the highest and PEG had the lowest G'_{max}, respectively.

Table 4
G'_{max}, T_m, and T_g of emulsion gels, their "without oil" versions and a pure agar gel. The results are presented as the average of three independent replicates ± standard deviation. The abbreviations used for the gels are given in Table 1. N/A indicates that data could not be obtained since the phase angle has not reached 45° with the current temperature gradient. The results were obtained with a temperature gradient of 2 °C/min and are expected to vary between different temperature gradients. Different letters in superscript indicate statistically significant differences between gels. For all three parameters $p < 0.001$ between different gels.

	G'_{max} (kPa)	T_m °C	T_g °C
AG	145.9 ± 33.0 ^{d}	N/A	50.7 ± 6.8 ^{b}
AWO	73.9 ± 1.7 ^{c}	87.4 ± 1.1 ^{c}	39.1 ± 1.1 ^{a}
AEG	44.1 ± 7.8 ^{bc}	90.1 ± 0.2 ^{c}	40.2 ± 1.3 ^{a}
PWO	6.1 ± 0.2 ^{ab}	50.5 ± 2.2 ^{b}	36.3 ± 1.0 ^{a}
PEG	3.2 ± 0.3 ^{a}	54.2 ± 2.7 ^{b}	38.3 ± 1.8 ^{a}
GEG	16.8 ± 1.7 ^{ab}	45.3 ± 1.3 ^{a}	37.0 ± 0.5 ^{a}

Although the T_m of AG could not be obtained, it is expected to be above 90 °C, due to high agar concentration (Lahrech, Safouane & Peyrellasse, 2005). The T_m and T_g of AWO and AEG were similar with a slightly higher T_m for AEG. The T_m of AEG (90 °C) was significantly higher than PEG (54 °C) and GEG (45 °C) which were also significantly different (Table 4). A high T_m may be advantageous for the storage and textural stability of polysaccharide-based gel products in warm climates. The stability of gelatin gels is known to be compromised at high storage temperatures due to the acid hydrolysis of gelatin (Baydin et al., 2022; Van den Bosch & Gielens, 2003). However, the high T_m of AEG may present challenges during commercial production since it would require critical temperature control to ensure the quality of the final product. Although agar gels have a reversible gelation process without significant compromising the gelling properties (Imeson, 2009), AEG had a lower G'_{max} after re-melting the gel (data not shown). For industrial production, PEG may be a more manufacturable formulation alternative to GEG since it has relatively similar T_m and $T_g$values to GEG and it did not exhibit large decreases in G'_{max} after re-melting (data not shown). It should be noted that PEG may pose challenges for industrial applications due to the complexity and sensitivity of its formulation to, *e.g.*, pH and calcium concentration (Burey, Bhandari, Rutgers, Halley & Torley, 2009; Zeeb et al., 2021).

3.2. Texture characteristics of the gels

Consumer acceptance of emulsion gels for oral delivery highly depend on their mechanical properties which describe their response to deformation, such as mastication, *i.e.*, chewing (Aguayo-Mendoza et al., 2020). Texture characteristics of the gels were analyzed with a single compression and TPA tests with three maximum strains. The average curves of single compression with 75% strain are shown in Fig. 1A. The parameters of this test demonstrated that AG had the significantly highest Young's modulus among all gels, indicating high stiffness/rigidity. Although the inclusion of sugar alcohols (AWO) significantly decreased the Young's modulus, it significantly increased the maximum force and strain at failure of the agar gel (Table 5). The lower Young's modulus of AWO is due to a lower overall agar concentration in the formulation compared to AG. Strain at failure coincides with the sensory deformability of the material (Koç et al., 2019). As discussed in Section 3.1, AG had a much higher overall concentration of agar in the total formulation compared to AWO and AEG. The stiffness order of agar gels (AG > AWO > AEG) obtained with single compression were in accordance with the G'_{max} values obtained by SAOS measurements (Table 4).

Both agar and pectin WO gels had significantly higher Young's modulus, maximum force and strain at failure, compared to their corresponding emulsion gels, although the difference was not significant for the Young's modulus of PWO and PEG (Table 5). As expected, the inclusion

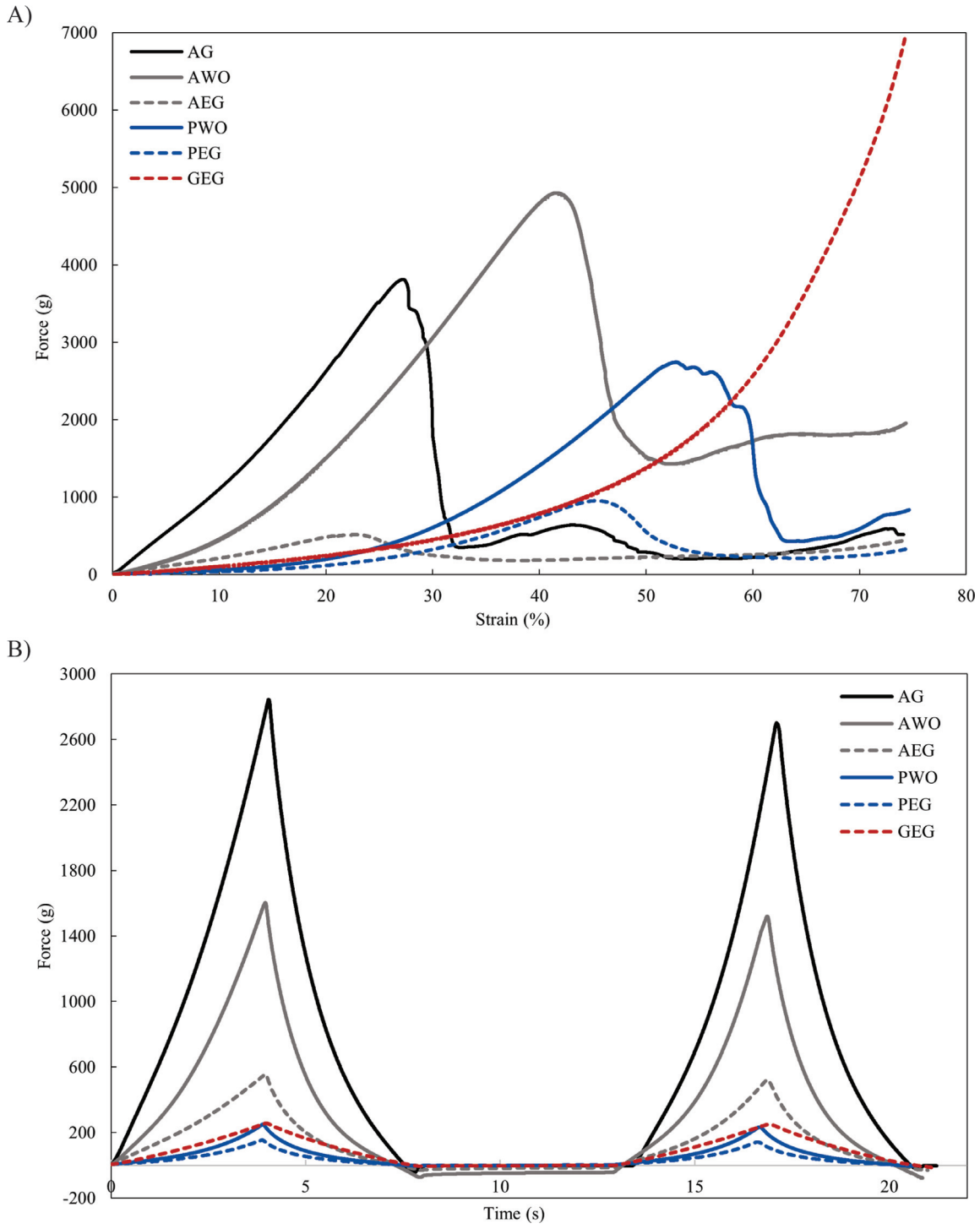

Fig. 1. A) Large scale deformation of the gels with texture analyzer. 75% strain single compression was applied. The curves are averages of 6–10 replicates (obtained from a single batch). B) Texture profile analysis of the gels with 20% strain. Each curve represents the average of 7–10 gels (obtained from a single batch). The abbreviations and compositions of the gels are given in Table 1.

of oil in the agar and pectin formulations resulted in a more brittle and weaker texture as the oil droplets behave as inactive fillers (Kim et al., 1997, 1999; Zhang et al., 2022). The emulsified oil droplets may provide an inactive filler effect with the continuous phase which results in a weaker polysaccharide gel network and provide possible failure zones for crack propagation (Dickinson, 2012; Dille, Draget & Hattrem, 2015; Sala, van de Velde, Stuart & van Aken, 2007). AEG showed significantly higher brittleness than PEG (Table 5).

GEG had the significantly highest maximum force among all gels, and it did not fail at 75% strain, pointing to the ductile texture of the gel (Fig. 1A, Table 5). The polysaccharide emulsion gels had a significantly lower maximum force, and they were significantly more brittle than GEG (Table 5). The brittleness of polysaccharide gels has been attributed to their high chain stiffness, as opposed to gelatin with long and flexible cross-links (Van Vliet & Walstra, 2017). Compared to AEG, although significantly different, PEG had strain at failure and maximum

Table 5
Parameters of large scale deformation (75% strain single compression) of the gels with texture analyzer. The results are given as the average of 6–9 replicates (obtained from a single batch) ± standard deviation. The abbreviations of the gels are given in Table 1. Different letters in superscript indicate statistically significant differences between gels. For all three parameters $p < 0.001$ between different gels.

	Young's modulus (kN/m^2)	Max force (g)	Strain at failure (%)
AG	552.4 ± 31.7 ^{e}	3439 ± 906 ^{b}	25 ± 5 ^{a}
AWO	182.0 ± 24.7 ^{d}	4993 ± 227 ^{c}	42 ± 1 ^{b}
AEG	95.0 ± 1.9 ^{c}	523 ± 39 ^{a}	22 ± 1 ^{a}
PWO	21.5 ± 1.1 ^{ab}	3299 ± 573 ^{b}	56 ± 4 ^{c}
PEG	12.6 ± 0.3 ^{a}	975 ± 100 ^{a}	46 ± 1 ^{b}
GEG	46.2 ± 3.7 ^{b}	7349 ± 150 ^{d}	> 75 ^{d}

force values closer to GEG, suggesting a more similar sensory perception. Young's modulus and strain at failure parameters were significantly different between the emulsion gels ($p < 0.001$), pointing to their different deformation characteristics.

Gelatin gels have a unique chewy texture that is familiar to consumers of gelatin-based jelly desserts, gummies, and confectionery (Schrieber & Gareis, 2007). This texture is difficult to be mimicked with polysaccharides since polysaccharide gels typically do not have a similar elastic/chewy texture (Haug, Draget & Smidsrød, 2004; Karim & Bhat, 2008). The texture of the gels was analyzed with standard TPA tests at three different strains (20%, 30%, and 75%) which represent a variety of strain at failure values obtained from the single compression test. These strain values also represent different anatomical components of mastication such as the tongue, hard palate and teeth (Arai & Yamada, 1993). It has been shown that different degrees of compression, *i.e.*, maximum strain, impact TPA parameters (Bourne & Comstock, 1981). Standard TPA parameters (hardness, adhesiveness, cohesiveness, resilience, springiness, and gumminess) were obtained for each gel at three different strains.

Fig. 1B shows TPA curves of the gels with 20% strain, which is below the strain at failure for all gels. The textural properties of the gels are shown in Table 6. Similar to the Young's modulus values, hardness of the gels was in the following order for agar: AG > AWO > AEG, and PWO > PEG for pectin gels with significant differences, pointing out to weaker gel structure due to the inactive filler effect (Farjami & Madadlou, 2019; Koç et al., 2019). Hardness, gumminess, and adhesiveness of the emulsion gels were in the following order: AEG > GEG > PEG, and AWO showed the significantly highest adhesiveness among all gels. Resilience, springiness, and cohesiveness parameters describe the structural integrity of the gel after the first compression. The springiness value was close to 100, and cohesiveness value was one for all gels since none of the gels fractured at this strain. The significantly lower resilience of the polysaccharide emulsion gels, compared to gelatin emulsion gel, indicated some structural damage, especially for AEG which had a strain at failure closer to 20% strain than PEG (Table 6). All parameters of the 20% strain TPA test (excluding cohesiveness) were significantly different between the gels ($p < 0.001$).

The only fractured gel during 30% strain TPA test was AEG, and therefore, its cohesiveness value was zero unlike the other gels which had a cohesiveness value of one (Supplementary Table 1). Consequently, it also had significantly lower resilience and springiness than the other gels ($p < 0.001$). Compared to 20% strain TPA test, the springiness and resilience of all gels decreased, pointing to structural damage. Similar to the 20% strain TPA test, polysaccharide emulsion gels had significantly lower hardness and gumminess compared to their WO gels, indicating the negative impact of the inactive filler effect on these parameters. The hardness and adhesiveness of the emulsion gels followed the same order as 20% strain TPA (Table 6, Supplementary Table 1). The 30% strain TPA curves of the gels are shown in Supplementary Figure 2. All parameters of the 30% strain TPA test (excluding cohesiveness) were significantly different between the gels ($p < 0.001$).

As expected, all polysaccharide gels fractured at 75% strain TPA test (reflected by the cohesiveness values). At this strain, GEG had a significantly higher hardness and gumminess than the polysaccharide emulsion gels (Supplementary Figure 3). Although it did not fracture at 75% strain, GEG's low resilience indicated structural damage. The hardness of GEG increased with increasing maximum strain of the TPA (Table 6, Supplementary Table 1, Supplementary Table 2). At 75% strain, the gel is close to its elastic limit, resisting deformation to a larger extent (Mazumder, Roopa & Bhattacharya, 2007). The presence of oil significantly decreased the hardness and gumminess of the polysaccharide gels, whereas it had little effect on the resilience, cohesiveness, and springiness. Although not significantly different, PEG had a slightly higher hardness than AEG. All parameters of the 75% strain TPA test (excluding cohesiveness) showed statistically significant differences between the gels ($p < 0.001$).

Both single compression and 75% strain TPA test compressed the gels to the same extent. However, maximum force and fracture strain results of the single compression test (0.1 mm/s) were lower than those obtained from the hardness results of the TPA test (1 mm/s). This may be due to the compression speed differences of the two tests. A slower compression rate gives the gel more time to relax and dissipate the applied force through friction between structural components of the gel at higher deformation speeds (Pons & Fiszman, 1996; Sala, Van Vliet, Stuart, Van Aken & Van de Velde, 2009). In previous studies, compression rates of 2–3 mm/s were suggested to be more physiologically relevant to mastication (Pons & Fiszman, 1996; Rosenthal, 2010).

The texture analyses indicated that at high strains (75%), the addition of sugar alcohols to the pure agar gel resulted in higher hardness, whereas lower strains (20 and 30%) showed the opposite. The addition of sugar alcohols resulted in a less brittle texture and further addition of oil to the agar formulation led to a lower hardness at all strains. Structure maintained in the gel after deformation was as followed: AEG > AWO > AG, suggesting that the addition of polyols and oil to a pure agar gel resulted in a texture with higher structural integrity (Supplementary Figure 4). AEG had a harder, firmer, and more brittle texture than GEG. The addition of oil weakened the pectin gels at all strains. PEG had lower hardness than both AEG and GEG. However, it was less brittle and had higher resilience, cohesiveness, and springiness than AEG, potentially making its mouthfeel more similar to GEG.

Table 6
Textural properties of the gels obtained with 20% strain TPA test. The results are given as the average of (7–10) gels (obtained from a single batch) ± standard deviation. The abbreviations and compositions of the gels are given in Table 1. Cohesiveness value for all the gels was 1 ± 0. Different letters in superscript indicate statistically significant differences between gels.

Gel	Hardness (g)	Adhesiveness (g·sec)	Resilence (%)	Springiness (%)	Gumminess
AG	2853 ± 175 ^{e}	−30 ± 24 ^{c}	65 ± 1 ^{c}	91 ± 1 ^{a}	2404 ± 160 ^{e}
AWO	1614 ± 53 ^{d}	−271 ± 83 ^{a}	61 ± 1 ^{b}	93 ± 1 ^{b}	1366 ± 50 ^{d}
AEG	558 ± 25 ^{c}	−101 ± 16 ^{b}	56 ± 1 ^{a}	96 ± 1 ^{c}	459 ± 20 ^{c}
PWO	251 ± 4 ^{b}	−9 ± 4 ^{c}	78 ± 1 ^{e}	99 ± 1 ^{d}	227 ± 4 ^{b}
PEG	155 ± 4 ^{a}	4 ± 2 ^{c}	76 ± 1 ^{d}	100 ± 0 ^{d}	139 ± 4 ^{a}
GEG	255 ± 11 ^{b}	−14 ± 7 ^{c}	92 ± 2 ^{f}	99 ± 1 ^{d}	249 ± 11 ^{b}

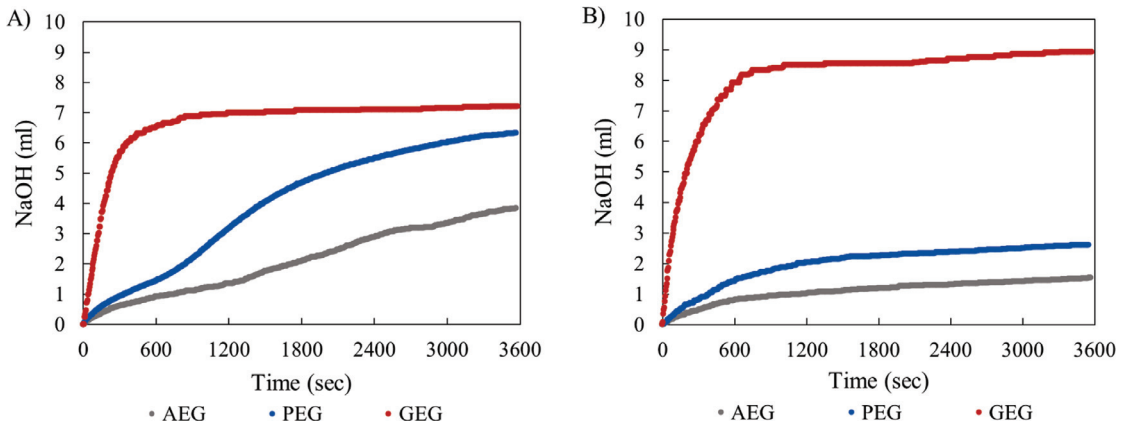

Fig. 2. Total NaOH consumption of agar (AEG), pectin (PEG) and gelatin (GEG) emulsion gels during *in vitro* lipolysis in A) gastrointestinal and B) intestinal stage. The curves show total NaOH consumption without subtracting the consumption of the corresponding control gels.

Table 7
The D[4, 3] and D[3, 2] droplet size averages of agar (AEG), pectin (PEG) and gelatin (GEG) emulsion gels. The D[4, 3] and D[3, 2] droplet size averages are given as the average of three replicate gels with five measurements ± standard deviation. The results of the *in vitro* lipolysis are given as the average of two experiments ± standard deviation. Different letters in superscript indicate statistically significant differences between groups.

	Droplet size averages		Initial lipolysis rate (μmol FFA/*sec*)	
	D[4, 3] (μm)	D[3, 2] (μm)	Gastrointestinal	Intestinal
AEG	13.2 ± 1.5 ^{a}	3.0 ± 0.8 ^{a}	2.75 ± 0.07 ^{a}	2.45 ± 0.07 ^{a}
PEG	31.5 ± 5.1 ^{b}	9.7 ± 1.7 ^{b}	4.30 ± 0.14 ^{a}	3.50 ± 1.13 ^{a}
GEG	1.0 ± 0.2 ^{c}	0.6 ± 0.0 ^{c}	24.85 ± 7.57 ^{b}	35.15 ± 6.29 ^{b}

Texture analyses demonstrated different characteristics of agar, pectin, and gelatin emulsion gels. These differences will impact the sensory perception, aroma release profile, and dissolution time of the emulsion filled gels (Sala et al., 2008). A lower pH of PEG (pH 4) may result in faster flavor release than AEG or GEG (pH 4.5) (Hansson, Andersson, Leufven & Pehrson, 2001). One of the most commonly used biopolymers in vegan confectionery is pectin, which may have a familiar texture for vegan consumers (Šeremet et al., 2020). In Asia, AEG may have higher consumer acceptance since agar has traditionally been used as a food ingredient for centuries (Sousa et al., 2021).

3.3. *Droplet size and* in vitro *lipolysis of emulsion gels*

The droplet size of an emulsion is known to influence the digestion, uptake, and bioavailability of TAGs (Dille, Baydin, Kristiansen & Draget, 2021a). Especially the surface mean diameter, *i.e.*, D[3, 2], is important since a lower average provides a larger surface area accessible for the digestive enzymes. For both D[3, 2] and D[4, 3], GEG had the smallest droplet size, whereas PEG had the largest (Table 7). Both droplet size averages were significantly different between emulsion gels ($p < 0.001$).

The lipolysis of the emulsion gels was studied through *in vitro* experiments. To evaluate the decrease in pH due to the experimental setup, gastrointestinal lipolysis was performed with a "blank" sample (water instead of a gel), as a background control. The total NaOH consumption was 0.40 ± 0.11 ml ($n = 3$). This indicates the background lipolysis reaction occurring, possibly due to the presence of lipid impurities in the bile extract or the lipolysis of pancreatin (Larsen, Sassene & Müllertz, 2011). During gastrointestinal lipolysis, the net NaOH consumption is calculated as the total consumption of the control gel (AC, PC, and GC) subtracted from the total consumption of the corresponding emulsion gel (AEG, PEG, and GEG) (Section 2.8). The control gels were only subjected to gastrointestinal lipolysis, *i.e.*, control gels have not been tested in the intestinal stage. In the gastrointestinal stage, GEG had the highest net consumption with 6.09 ± 0.44 ml, followed by PEG with 5.22 ± 0.44 ml and lastly AEG with 3.18 ± 0.34 ml (Fig. 2A). The total NaOH consumption in the intestinal stage was 8.93 ± 0.67 ml for GEG, followed by 2.61 ± 0.13 ml for PEG and 1.53 ± 0.01 ml for AEG (Fig. 2B). The reason for GEG having a higher total NaOH consumption in the intestinal stage compared to the gastrointestinal stage may be because of the free fatty acids released from the triacylglycerols (TAGs) in the SGF becoming deprotonated as the pH is raised to 7 before the intestinal stage. Therefore, less TAGs would be available in the intestinal stage to be hydrolyzed and release fatty acids that are detected by the pH-stat method since some of the TAGs were already hydrolyzed to diacylglycerols or monoacylglycerols in the gastric stage. The lipolysis curves of the gels are shown in Fig. 2.

In both gastrointestinal lipolysis and intestinal lipolysis, the titration curves of polysaccharide emulsion gels have not reached a plateau during 1 hour of titration. A higher total NaOH consumption might have been recorded for the gels if the intestinal titration was prolonged. A longer duration of titration could, however, have given more physiologically relevant results since the gastrointestinal transit time in humans is longer than 1 hour (Read, Al-Janabi, Holgate, Barber & Edwards, 1986; Worsøe et al., 2011).

The initial lipolysis rate of the emulsion gels followed the same order for both gastrointestinal and intestinal lipolysis: GEG > PEG > AEG. In both gastrointestinal ($p < 0.025$) and intestinal ($p < 0.005$) lipolysis, the initial rate of different emulsion gels was significantly different with GEG being different from the polysaccharide emulsion gels (Table 7). Larger oil droplets are lipolyzed at a slower rate compared to small oil droplets which provide a larger surface area for the digestive enzymes to adsorb to (Dille et al., 2021a). Although AEG had a smaller droplet size than PEG, its initial lipolysis rate was slower. The initial rate of AEG and PEG were lower in intestinal stage, compared to their relative rate in the gastrointestinal stage. However, the opposite was observed for GEG, with 1.4x faster initial lipolysis in the intestinal stage.

The photomicrographs of the emulsion gels after the gastric stage showed coalescence of oil droplets in AEG (Fig. 3A). Similar observations have been reported for citrem stabilized emulsions in the gastric stage at pH 1.3 and 3, and this was suggested to be due to the lowered emulsifying capacity of citrem with the loss of negative charges at lower pH values (Lamothe, Desroches & Britten, 2019, 2020; Sørensen et al., 2008). The photomicrographs of PEG indicated flocculated oil droplets (Fig. 3B). At low pH values, emulsions stabilized by soybean lecithin have been shown to destabilize due to reduced emulsifying capacity of the emulsifier (Comas, Wagner & Tomás, 2006; Lin & Wright, 2018; Lin, Wang, Li & Wright, 2014). Simultaneously, pectin may contribute to the stabilization of the emulsion, keeping the emulsion droplets in an aggregated but not coalesced state, because of bridging flocculation (Ngouémazong, Christiaens, Shpigelman, Van Loey & Hendrickx, 2015).

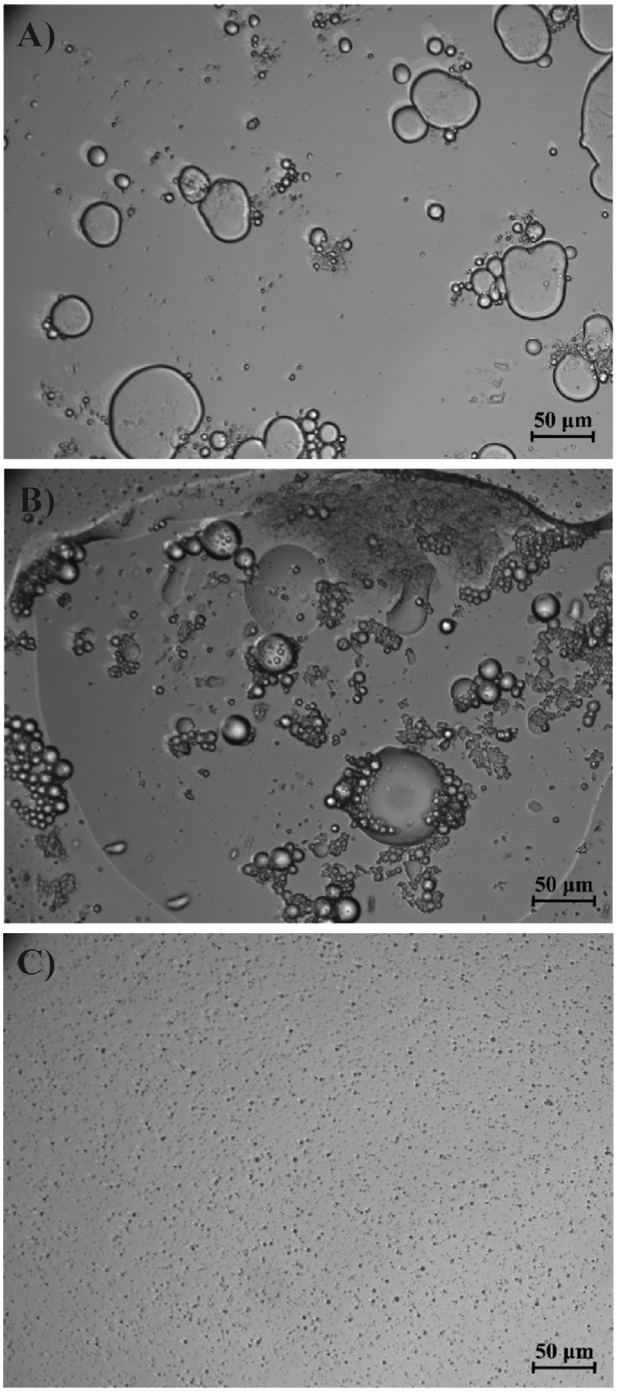

Fig. 3. Photomicrographs of A) agar, B) pectin, and C) gelatin emulsion gel after the gastric stage. 20x objective was used.

Coalescence and flocculation of oil droplets reduce the available substrate area for digestive enzyme adsorption and reduce the total extent and rate of lipolysis (Li et al., 2011; McClements, 2018). The lipolysis curves of gastrointestinal lipolysis of AEG and PEG show an increase at approximately 1000 s which was not observed for the intestinal lipolysis without a gastric pretreatment (Fig. 2). For PEG, this increase in NaOH consumption may be due to the increase of available substrate for the lipases in the intestinal stage at pH 7 which may disperse the flocs and create more available surface. It should also be noted that calcium ions are absent in the gastric stage. Calcium helps with the precipitation of FFAs, removing them from the surface of the oil droplets, and increasing the accessibility of the surface to the digestive enzymes (Minekus et al., 2014). Furthermore, the absence of calcium in SGF may mediate dissolution of the pectin network since calcium binds to pectin and facilitates its gelling (Fraeye, Duvetter, Doungla, Van Loey & Hendrickx, 2010). For AEG, the increase may be due to the gel remaining in the lipolysis medium for a longer period (two hours in the gastrointestinal stage, instead of one hour in the intestinal stage), with coalesced oil droplets being lipolyzed further, exposing a larger substrate area for the lipases. This would also promote shrinking of oil droplets due to lipase activity, further resulting in droplet release from the gel network.

It should be noted that *in vitro* lipolysis does not directly indicate the outcome of lipolysis *in vivo*. More complex *in vitro* lipolysis experiments with greater physiological relevance have been developed (Brodkorb et al., 2019), and the limitations of *in vitro* lipolysis experiments are well known (Ghorbani & Abedinzade, 2013). The current simplified setup serves a means to compare the lipolysis potential of emulsion gels, prepared with different biopolymers which contain the same amount of oil. Lower initial lipolysis rate, as well as lower total NaOH consumption for AEG and PEG in both gastrointestinal and intestinal stages, compared to GEG, point to limitations for the digestibility of oils in polysaccharide emulsion gels. These limitations may suggest potential lower absorption of polysaccharide emulsion gels *in vivo*, and consequently result in lower bioavailability. However, in the human body, shear forces in the stomach may result in a higher extent of mechanical deformation of the polysaccharide gels, resulting in smaller gel particles exposing a larger surface area available to lipases.

4. Conclusions

Emulsion gels using agar and pectin were prepared, characterized, and compared to a traditional gelatin emulsion gel. The higher melting temperature of agar emulsion gel may be advantageous in warm climates for enhanced stability of the final product, while it may pose difficulties in processability. The pectin emulsion gel had a more similar melting/gelling temperature to gelatin emulsion gel. The addition of sugar alcohols and oil to a pure agar gel decreased hardness and brittleness, potentially resulting in a more pleasant mouth-feel. Pectin emulsion gel had a softer texture than the agar emulsion gel, and a more similar texture to the gelatin emulsion gel. In the future, the textural properties of the emulsion gels should be further studied through a sensory panel using *e.g.*, quantitative descriptive analysis. *In vitro* lipolysis studies showed a lower extent of lipolysis and slower initial lipolysis rate with polysaccharide emulsion gels compared to the gelatin emulsion gel. Absorption and bioavailability of these three systems should be further studied using an *in vivo* model, and preferably in humans. The polysaccharide emulsion gels can also be used for other applications, as food gels or for the oral delivery of pharmaceuticals.

Ethical statement

The research presented does not involve any animal or human study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2022.100225.

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