Chapter 24
In Vitro Digestion of Lipid-Based Gels

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Abbreviations

CLSM	Confocal laser scanning microscopy
DAG	Diacylglyceride
EC	Ethyl-cellulose
FFA	Free fatty acid
GI	Gastrointestinal
HIPE	High internal phase Pickering emulsion
HMOG	High-molecular-weight oil gelator
HPLC	High-performance liquid chromatography
LMOG	Low-molecular-weight oil gelator
MAG	Monoacylglyceride
MCT	Medium chain triacylglyceride
PLM	Polarized light microscopy
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SGF	Simulated gastric fluid
SIF	Simulated intestine fluid
SSF	Simulated saliva fluid
TAG	Triacylglyceride

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24.1 Introduction

The food science community is currently working extensively to develop, study, and explore new mimetic systems aiming to replace animal-based products due to their recognized environmental drawbacks. These attempts have led to an increased interest in fat alternatives that will be combined with the protein matrix. Such alternatives are usually manufactured by replacing the animal-based fat with lipid-based gels such as oleogels, emulsion gels, or bigels. Such systems provide structural integrity similar to original animal-fat due to various structuring approaches while maintaining desirable nutritional profile and mouth feel sensation.

Fats and oils are macronutrients that can be classified based on the triacylglycerides (TAGs) content. Each TAG molecule is built of a triple-alcohol glycerol backbone attached to three fatty acids via an ester bond. Fatty acids can be divided into two major categories: saturated versus unsaturated. Saturated fatty acids typically exhibit higher melting temperatures compared to unsaturated fatty acids. Therefore, fats consist mostly of saturated fatty acids, while oils consist of unsaturated ones [1]. The solid texture presented by saturated fats is driven by the ability of the TAG molecules to self-assemble into lamella crystal structures termed nanocrystal platelets [2]. The platelets further associate through one-dimensional stacking to create larger-scale crystal clusters responsible for the final fat structure and properties [3]. This is a temperature-induced process that results in the crystallization of the solid TAG molecules into a crystal network that physically entraps the liquid TAG molecules. Solid fat properties and functionality are directly correlated to its structural building blocks from the nano- to the meso-scale crystal network [4]. Over the past few decades, the deleterious effects of trans and saturated fatty acids, such as an increased risk for coronary heart disease and a metabolic syndrome, have been well established. However, previous studies have shown that health risks are reduced most effectively when trans and saturated fatty acids are replaced with cis unsaturated fatty acids (usually found in vegetable oils) [5].

Targeting minimum saturated fat content while maintaining desirable textural and sensorial attributes led to the development of oleogels, oil-based gels, that consist of high unsaturated fatty acid content (up to 96%) with preferable solid texture. Oleogels are produced by mixing liquid oil with oil structuring agent that self-organize in various architectures to create a stable three-dimensional (3D) network that stabilizes the liquid oil [6]. Several strategies were previously proposed for oil structuring, depending on the type of oil structuring agent used, which can be classified into two major groups: low- and high-molecular-weight oil gelators (LMOGs and HMOGs). Most oil structuring strategies are based on LMOGs that mimic the natural ability of TAGs to self-assemble and crystallize to form an organized fat structure such as monoacylglycerides (MAGs), free fatty acids (FFAs), and waxes [7]. Less common are oleogel systems based on HMOGs, such as biopolymers and proteins. The incorporation of most proteins or polysaccharides directly in oil phase is not possible due to their hydrophilic nature. Therefore, indirect processes, such as emulsion templet and solvent exchange, are used [8, 9].

Several studies explored the incorporation of water phase into the oleogel phase forming a water-in-oleogel or an emulsion gel system [10–12]. Such systems aim to mimic natural butter or margarine systems composed of 20%wt. water [13] or to reduce the amount of total fat used. Other emulsion gel systems exploit the opposite phase behavior where oil-in-water gels are formed [14]. In these systems, the water phase is composed of a biopolymer-based network where oil droplets are embedded inside it. Previous studies have used polysaccharides such as alginate [15] and flaxseed gum [16] and proteins such as whey [17], chickpea [18], and ovalbumin [19].

A relatively new generation of fat mimetic system further advances the concept of water and oil phase combination by structuring both phases, thus producing biphasic gel system termed bigel. In this system, oleogel and hydrogel are combined into a single consistent matrix with unique physiochemical properties. The biphasic system properties can be altered based on each phase composition and the interphase behavior thus producing a versatile system that can be used in a wide range of applications. Previous studies examined the formulation of bigels using various techniques differing by the mixing scheme and conditions [20–25]. The hydrogel formulations included various biopolymers such as Guar gum [25], xanthan gum [26], alginate [20, 27], pectin [22], hydroxypropyl-methylcellulose [21], κ-carrageenan [28], agar–gelatin mixture [24], gelatin [29], and whey protein [30], while the oleogel formulations were based on low-molecular-weight crystalline materials such as waxes [20, 22, 27, 29], stearic acid [24, 30], sorbitan monostearate [25], γ-oryzanol/phytosterol [26], and glyceryl monostearate [22, 28].

Understanding the way these systems hydrolyze and break down under gastrointestinal (GI) conditions is a crucial step in order to utilize these systems in "real foods" and to further develop and improve them in the future.

24.2 The Digestion Routes for Lipids

Lipid digestion occurs along the gastrointestinal (GI) tract, which includes four distinct sub-stages or processes that run in a series comprising unique control gates from one stage to the other [31]. These stages include oral processing, gastric phase, intestinal stage, and fermentation, which occur in the mouth, stomach, small intestine, and large intestine or colon, respectively. Each stage applies different chemical and physical conditions on the food matrix or digesta aiming to maximize the nutrient breakdown and absorption [32].

The digestion process starts in the mouth where the food is broken down through mechanical processing, i.e., chewing, and mixed with saliva that consists of biopolymers such as mucin, enzymes such as amylase that catalyzes the breakdown of starch to produce glucose, and salts such as sodium and calcium. The mixture formed, termed the bolus, is swallowed and transferred into the stomach [31, 33]. In the stomach, the digesta is subjected to a strong acidic environment typically around pH 2 and peristaltic waves that are responsible for structure

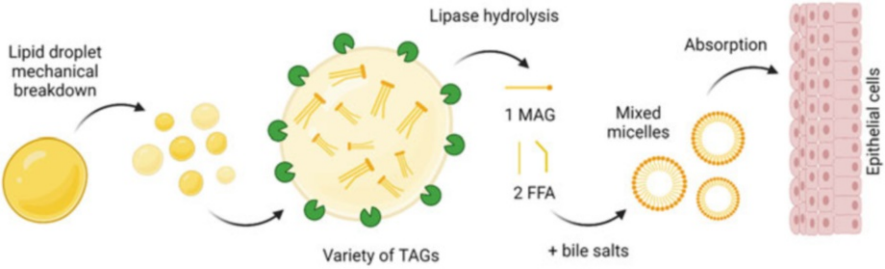

Fig. 24.1 Lipid digestion pathway in the gastrointestinal tract. FFA: Free fatty acid, MAG: monoacylglycerides, TAGs: triacylglycerides (Created with BioRender.com)

breakdown and mixing. Two enzymes are secreted in this stage: pepsin, which is responsible for protein hydrolysis, and gastric lipase, which is responsible for fat lipolysis [34]. The acidic partially broken down digesta is then moved into the intestine where the pH is immediately changed to neutral value, around pH 7, and pancreatin hydrolytic enzyme mixture is secreted in order to break down proteins (protease), fats (pancreatic lipase), and carbohydrates (amylase) [35]. In addition to the hydrolytic enzymes, bile salts are secreted to the intestine to emulsify the fat content, thus contributing to the fat lipolysis. Intestine peristaltic movements are subjected in order to promote content mixing and digesta transport along the intestine. Nutrient absorption mainly occurs in the intestine phase due to the large surface area produced by villi and microvilli structures on the intestine surface [31]. Any hydrolysis products not absorbed in this stage will move to the colon and likely consumed by colonic microbiota. The colon has an anaerobic environment where the microbiota (including probiotic bacteria) can be found. These organisms are responsible for a series of fermentation stages where undigested components such as fibers are broken down mainly to short chain fatty acids, which can be used as a source of energy, and sometimes methane [31, 36].

Lipid digestion is achieved in the stomach and intestine stages, where 10–30% of the fat is digested in the stomach while the rest is digested in the intestine [37]. Overall, lipid digestion involves the breakdown of TAGs into one MAG and two FFAs by three different lipase enzymes (Fig. 24.1). In the stomach, lipolysis is achieved by lingual and gastric lipases that are secreted in the mouth and stomach, respectively. They are only able to catalyze the hydrolysis of one of the fatty acids attached to the glycerol backbone, thus leading to the formation of one FFA and a diacylglyceride (DAG). DAGs are not able to transport through the mucus layer, thus additional breakdown stage is required. Both enzymes are acid stable while their activity rely on their ability to adsorb onto the lipid surface (they do not act with cofactors) [38]. Moreover, the activity of these lipases highly depends on the chain length of attached fatty acids, whereas they are more efficient for short and medium chain length TAGs than long chain length TAGs [37, 39]. In the small intestine pancreatic lipase with the help of a cofactor, co-lipase, catalyzes the hydrolysis of both sn-1 and sn-3 positions of TAGs, thus responsible for the majority of lipid digestion. These

byproducts are amphiphilic in nature and thus accumulate at the lipid interface leading to lipase inhibition. Bile salts are important biosurfactants responsible for the solubilization of these byproducts, thus eliminating them from the interface allowing the lipolysis to progress efficiently [38]. These processes lead to the formation of various phases including oil phase, crystalline phase that include calcium soaps, a "viscous isotropic" phase, and a micellar phase [40]. The micellar phase comprises of small and highly dispersed mixed lipid micelles and vesicles (4–8 nm) based on lipid hydrolysates (e.g., FFAs and MAGs), along with bile salts, cholesterol, and phospholipids, that solubilize hydrophobic compounds such as nutrients and drugs [41]. These entities serve as effective shuttles across the viscous unstirred water layer to the intestinal absorptive epithelial cells called enterocytes [42].

24.3 Parameters Affecting Lipid Digestion

The digestion of lipids is a complex process that involves the activity and performance of various entities and phases. Therefore, the process is controlled mainly by parameters such as the lipid phase structure and state, interface behavior, and enzyme activity.

The lipid phase structure and state refer to the TAGs content and type, supramolecular organization that can potentially alter the lipid state, i.e., liquid vs. solid, or macroscopic structures. More specifically, the molecular content of the lipid phase includes saturated, unsaturated fatty acids, or other lipidic components such as phospholipids; the molecular organization in crystal phases, membranes; and the bulk properties such as emulsion, emulsion gels, oleogels, or bigels [43]. Generally, it was shown that solid fats are digested more slowly than liquid oils although both comprise TAGs with different fatty acid content [44].

Lipid hydrolysis is an interfacial process; thus, controlling the physical and chemical properties of the interface as well as the enzyme activity can directly affect the lipid digestion process. Overall, the interface surface area can positively contribute to the lipolysis process while byproducts or bile salts occupying the interface can negatively affect the lipolysis [38]. In addition, the lipase activity along the GI tract plays a major role in the lipid hydrolysis [39]. As discussed above there are three main lipase enzymes responsible for the TAG hydrolysis: lingual, gastric, and pancreatic lipases. The activity of each enzyme can be controlled and manipulated based on its action and environment. Lingual and gastric activities rely on their ability to adsorb directly to the lipid surface and act in acidic environment. Therefore, their activities are mainly governed by the droplet surface area and the interface content and structure. Pancreatic lipase activity, on the other hand, is governed by the action of the cofactor, which allows better anchoring to the lipid interface. Thus, in this case its activity is controlled by the presence of the cofactor and the occupancy of the interface. During the intestine digestion stage FFAs and MAGs

occupy the interface, thus hindering the pancreatic lipase activity; thus, bile salts activity as solubilizing agents of the lipolysis byproducts is crucial [38].

24.4 Analysis of Lipid Digestion

Analysis of digestion process raises some challenges relating to the high complexity of the process and the various conditions applied on the food matrix. Investigating food digestion in real life using in vivo models (animals or humans) is complicated, expensive, and sometimes raises ethical issues [45, 46]. Therefore, in order to mimic real digestion conditions, which involves shear and grinding during mastication, dynamic enzyme and bile salts release, physical forces exerted on the digesta, and flow behavior, dynamic in vitro digestion systems were proposed [47]. Several dynamic models were proposed for digestion process, which can be classified into two main groups: mono-compartmental (simulate one compartment of the GI tract) or multicompartmental (simulate a cascade of several compartments) [46]. These models include physicochemical and mechanical processes and temporal changes in luminal conditions as occur in vivo [48]. On the other hand, the static in vitro digestion system allows easy analysis of the digestion process in three successive stages. It suffers from some limitations related to its static nature, which does not reproduce the dynamic processes occurring during human digestion such as gastric emptying, continuous changes in pH, and dynamic ingredients secretion [47, 48]. However, it offers simple, high-throughput, and cost-effective solution that can assist with the initial screening of food hydrolysis during development; thus, most studies up to date use this approach [47].

Over the years various research and review papers dealt with the issue of food in vitro digestion analysis using static model systems [47, 49, 50] and its relation to in vivo results [45]. Various studies implemented various conditions such as sample size, enzyme unit activity, and mixing scheme. Such variation between different publications hindered the ability to compare between studies and provide a reliable progress in the field. Therefore, a standardized procedure was recently published after extensive research and collaboration as part of the COST action INFOGEST [51]. The main protocol developed for static analysis of food digestion involves several important stages required in order to compare and reproduce data between studies [51] (Fig. 24.2). First, all enzymes' activities and bile concentration must be determined prior to analysis in order to maintain comparable conditions. The in vitro analysis should include three stages:

1. *Oral phase*: where food matrix is mixed with simulated saliva fluid (SSF) at 1:1 w/w ratio and incubated while mixing for 2 min at 37 °C and pH 7. SSF solution consists of 1.5 mM $CaCl_2$ and salivary amylase 75 U mL^{-1} (if required).
2. *Gastric phase*: where the oral bolus is mixed with simulated gastric fluid (SGF) at 1:1 v/v ratio and incubated while mixing for 2 h at 37 °C and pH 3. SGF solution consists of 0.15 mM $CaCl_2$, pepsin (2000 U mL^{-1}), and gastric lipase (60 U mL^{-1}).

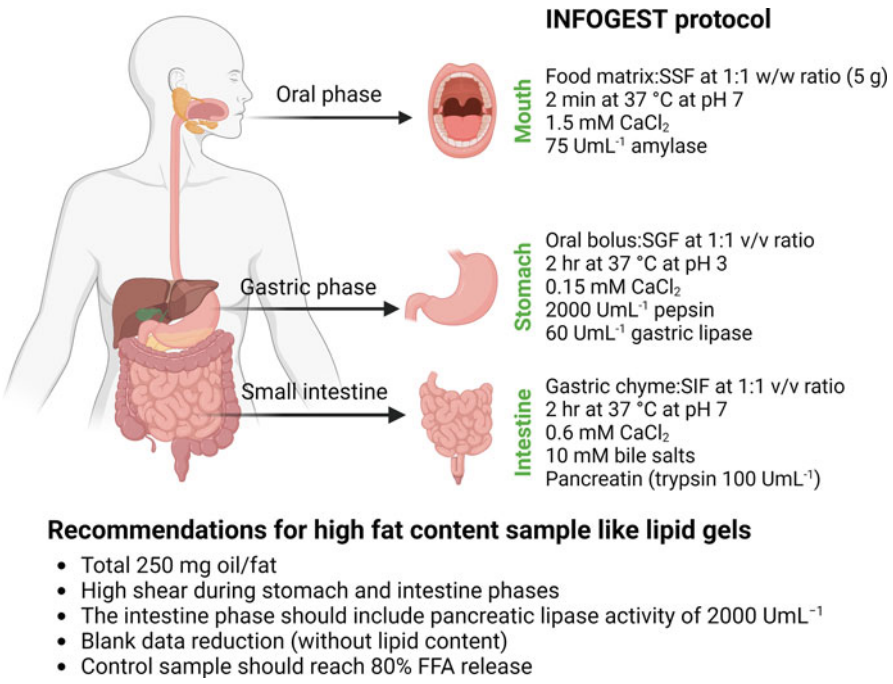

Fig. 24.2 The digestion process stages and conditions used in INFOGEST protocol and recommendation for high-fat lipid gels. (Created with BioRender.com)

3. *Intestinal phase*: where the gastric chyme is mixed with simulated intestine fluid (SIF) at 1:1 v/v ratio and incubated while mixing for 2 h at 37 °C and pH 7. SIF solution consists of 0.6 mM $CaCl_2$, 10 mM bile salts, and pancreatin (trypsin activity 100 U mL^{-1}, or for high-fat-containing foods a pancreatic lipase activity of 2000 U mL^{-1}).

The process analysis is performed based on the research requirements by sampling during the above stages. More specifically, parameters such as protein molecular weight, carbohydrate hydrolysis, particle size, and lipid lipolysis can be analyzed during digestion [52]. While handling lipid systems the main parameters that are usually examined are the FFA release or lipolysis progress, FFA content, droplet size, zeta potential, and micelle size [53].

The standardized protocol suffers from some limitations that can be related to its static nature, which is different from the dynamic natural process involving gradual changes in pH and controlled enzyme and salt secretion. However, the protocol also failed to relate to food systems with high fat content, such as oleogels, emulsion gels, and bigels. Recently, Sabet and coworkers [54] pointed out the limitation of the INFOGEST protocol with respect to oleogel systems and proposed a modification of

the protocol for such systems (Fig. 24.2). After thorough examination of various oleogel systems comprising ethyl-cellulose (EC) and various waxes (sunflower seed, rice bran, candelilla, carnauba, and berry waxes) using the in vitro static protocol suggested by INFOGEST and the same protocol with some modifications, important conclusions were obtained. To obtain consistent and reliable data, the authors propose to modify the protocol accordingly:

- The amount of sample should be modified with total oil/fat of maximum 250 mg over 40 mL of total digestion volume (summation of diluted sample, SSF, SGF, and SIF). Therefore, the oleogel/fat sample should be diluted with water to total 5 g sample and then SSF can be added as recommended in the INFOGEST protocol.
- Due to the high stickiness and low dispersibility of the oleogel/fat samples, it is recommended to use high shear during digestion. Moreover, the experiment should be conducted in the static titration chamber with controlled stirring, pH, and temperature to maintain reproducibility of the data and avoid errors.
- Proper blank data (without lipid content) should be subtracted from the data obtained with the lipid content.
- Reliable comparison between results should only be achieved when the control sample (with only oil phase) reaches at least 80% FFA release at the end of the intestinal phase.

24.5 Digestibility of Lipid-Based Gels

Oleogels, emulsion gels, and bigels were proposed for various food applications such as bioactive delivery, food 3D printing, confectionary products, bakery products, spreads, and meats [55]. All these applications involve human consumption and thus physiological breakdown of the lipid components in the GI system. Understanding the lipid lipolysis and the obtained byproducts is therefore crucial for further development of these systems in real food applications.

The first relation for the digestibility behavior was started with the analysis of the lipolysis of EC oleogels using various EC grades (i.e., molecular weight) under in vitro analysis test modulated from lipolysis of emulsions [56]. In this study, EC/canola oil oleogels were prepared with and without β-carotene where the % FFA and β-carotene release were examined. Various EC types and concentration were examined where a linear correlation between the β-carotene transfer and lipolysis extent was found suggesting the release of FFAs and MAGs contributes to the β-carotene micellization. A comparative analysis of the digestibility of various oleogel systems based on different oil structuring agents, which involves different gelation mechanism, was done using EC, MAGs and DAGs mixture (E-471), and β-sitosterol/γ-oryzanol mixture. The results show that in addition to gels' mechanical properties that alter its ability to physically break down, different structuring agents and gelation mechanisms exhibit significantly different susceptibility to digestive

lipolysis [57]. Moreover, lipid digestion can be altered using specific structuring agent and concentration in order to tune the susceptibility of a product to digestive degradation [58]. Such approach can be exploited to alter and control the release of hydrophobic nutraceuticals through network stability [59].

The digestibility of various lipid-based gel systems has drawn a lot of attention over the last 5 years, as can be seen in the sharp incline in the number of publications related to this topic (Tables 24.1 and 24.2). It seems that researchers revealed the importance of quantifying the sample functionality with respect to digestibility in addition to other mechanical, thermal, and structural material characterization

Table 24.1 Summary of previous studies using in vitro digestion models used to investigate the digestion and absorption of oleogel systems

Type of oil structuring agent	Oil gelation mechanism	Type of oil	Characterization techniques used for digestibility analysis	References
Ethyl-cellulose	Direct	Canola oil	%FFA release and β-carotene	[56]
E471 (mono- and diglyceride mixture), ethyl-cellulose, β-sitosterol + γ-oryzanol	Direct	Canola oil	%FFA release	[57]
β-sitosterol and lecithin	Direct	Corn oil	%FFA release and curcumin release	[60]
β-sitosterol + γ-oryzanol, saturated MAGs, and rice waxes	Direct	Sunflower oil	%FFA, particle size and zeta potential, and curcumin release	[59]
Candelilla wax	Direct	Nut oils (peanut, pine nut, and walnut oil)	β-carotene release	[61]
Ethyl-cellulose and waxes (sunflower seed, rice bran, candelilla, carnauba, and berry waxes)	Direct	Sunflower oil	FFA content by HPLC and %FFA released	[54]
Gelatin/gellan gum mixture	Indirect	MCT oil	Bioaccessibility of curcumin, %FFA release, and ex vivo everted gut sac-permeability	[62]
Alginate/gelatin	Indirect	Camellia oil	%FFA release	[63]
Hydroxypropyl-methylcellulose	Indirect	MCT oil	Curcumin release and % FFA release; in vivo tests were done by analyzing the curcumin concentration over time in rats	[64]

(continued)

Table 24.1 (continued)

Type of oil structuring agent	Oil gelation mechanism	Type of oil	Characterization techniques used for digestibility analysis	References
Alginate, carboxymethyl cellulose, and pectin	Indirect	MCT oil	Curcumin release and % FFA release	[65]
Soy protein-tannic acid	Indirect	Pine nut oil	%FFA release, lipolysis kinetic analysis, light and confocal microscopy, particle size and zeta potential, fat bioaccessibility (micellization), fatty acid composition, and oxidative products of the micellar fraction	[66]
Glycerol monostearate	Direct	Sunflower oil	%FFA release and lipolysis kinetics, CLSM and PLM, bioaccessibility of astaxanthin, and FFAs composition of micellar fraction	[67]
Soy protein isolate and glycerol monolaurate	Indirect	Soybean oil	%FFA release, particle size, and zeta potential	[68]
Whey protein isolate	Indirect	Sunflower or flaxseed oil	CLSM, protein digestibility, %FFA release, and micelle size distribution	[69]
MAGs and waxes (carnauba wax, beeswax, candelilla wax, rice bran wax)	Direct	High oleic sunflower oil	FFA release of particles, particle size, and zeta potential	[70]
Gelatin	Indirect	Camellia oil	%FFA release, particle size, and zeta potential	[71]
Diosgenin	Direct	Sesame oil, olive oil, and linseed oil	%FFA release	[72]
Bamboo shoot protein and soybean protein isolate	Indirect	Camellia oil	%FFA release, particle size, and zeta potential	[73]
Sorbitan tristearate and nanocellulose	Direct	Coconut oil	5-Aminosalicylic acid release	[74]

CLSM: confocal laser scanning microscopy; FFA: free fatty acid; HPLC: high-performance liquid chromatography; MCT: medium chain triacylglyceride; PLM: polarized light microscopy

Table 24.2 Summary of previous studies using in vitro digestion models used to investigate the digestion and absorption of emulsion gels and bigel systems

Type of oil structuring agent	System type	Type of oil	Characterization techniques used for digestibility analysis	References
Casein hydrogel embedded with milk fat or rapeseed oil	Emulsion gel (oil in hydrogel)	Milk fat or rapeseed oil	Physical breakdown (matrix degradation index) and %FFA release	[77]
Agar hydrogel embedded with soybean oil	Emulsion gel (oil in hydrogel)	Soybean oil	%FFA release, droplet size, and zeta potential during digestion	[78]
Alginate hydrogel embedded with MCT loaded with nobiletin	Emulsion gel (oil in hydrogel)	MCT	%FFA release, nobiletin release and bioaccessibility, and optical and fluorescent microscopy	[15]
κ-carrageenan hydrogel and monoglyceride oleogels	Bigel	Corn oil	β-carotene release	[28]
Chickpea protein cross-linked with transglutaminase embedded with corn oil	Emulsion gel (oil in hydrogel)	Corn oil	Protein breakdown using SDS-PAGE	[18]
Whey protein concentrate hydrogel and stearic acid with/without soy lecithin oleogel	Bigel	Soybean oil	Probiotic protection, % FFA release, and fatty acid content released during digestion	[17]
Whey protein and flaxseed gum hydrogel embedded with corn oil	Emulsion gel (oil in hydrogel)	Corn oil	Astaxanthin release and bioaccessibility	[16]
Ovalbumin hydrogel with sunflower oil	Emulsion gel (oil in hydrogel)	Sunflower oil	Protein digestibility	[19]
Lipophilic protein embedded with soybean oil	Emulsion gel (oil-in-hydrogel) and HIPE	Soybean oil	Particle size and zeta potential, CLSM, FFAs release, and lycopene bioavailability	[79]
Konjac glucomannan/gelatin hydrogel and stearic acid oleogel	Bigel	Soybean oil	%FFA release, quercetin release, and bioaccessibility	[80]
Ovalbumin hydrogel with different fillers embedded with sunflower oil	Emulsion gel (oil in hydrogel)	Sunflower oil	Protein digestibility	[81]
Myofibrillar protein with carboxymethyl cellulose embedded with soybean oil	Emulsion gel (oil in hydrogel)	Soybean oil	Protein digestibility and % FFAs release	[82]

CLSM: confocal laser scanning microscopy; FFA: free fatty acid; HIPE: high internal phase Pickering emulsion; MCT: medium chain triacylglyceride; SDS-PAGE: sodium dodecyl sulfate–polyacrylamide gel electrophoresis

analysis. According to Table 24.1, it is evident that most studies concentrated on the %FFA and drug release; however, more in-depth characterization analyses tools were also suggested such as lipolysis kinetic analysis, light and confocal microscopy, particle size and zeta potential, bioaccessibility (micellization), and fatty acid composition.

Over the last decade the combination of two phases to formulate biphasic lipid-based gels was suggested and explored due to the obvious benefits arising from the presence of two phases, which allows the ability to deliver hydrophilic and hydrophobic drugs, better spreadability, better water and oil binding capacities, protective environment for various drugs, and ability to manipulate the gel mechanical and thermal properties as well as drug release rate [14, 75]. With respect to digestibility process, these systems offer a complex breakdown process arising from the unique composition of each phase. The water phase usually includes water-soluble components that can gel or solidify it by the formation of a polymer-based network such as carbohydrates (polysaccharides, fibers, and starch) and proteins. Oil phase solidification includes low- or high-molecular-weight oil gelators such as waxes, FFAs, MAGs, polysaccharides, and proteins, using direct or indirect gelation routes [76]. Analysis of the in vitro digestibility of these systems usually includes %FFA release, protein hydrolysis, drug release, and microscopy observation analysis (Table 24.2).

24.6 Concluding Remarks

Progress in the field of food design leads to the formulation of various lipid-based gel systems aiming for various edible applications in food, pharmaceutical, and medicine. Such systems can improve food nutritional values by replacing harmful ingredients, deliver hydrophobic micronutrient and drugs through the natural lipid digestion routes thus improving their bioavailability, and protect them from the harsh environment of the stomach. Therefore, understanding the way such systems hydrolyze and break down along the GI tract is crucial. Increased interest in lipid gel systems and their digestibility behavior is evident from the increased amount of publications dealing with this issue in the last 5 years.

Recent standardized protocol for in vitro digestion analysis, the INFOGEST protocol, was published aiming to allow reproducibility and comparability of in vitro studies between different laboratories as well as to compare these data as much as possible to in vivo results. This protocol needs adjustment in order to fit digestion of high-fat food products such as lipid-based gels. Today, most studies dealing with lipid-based gels such as oleogels, emulsion gels, and bigels rely on the INFOGEST protocol with some modifications in order to address the high fat content in lipid gels.

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