Chapter

Protein-Based Functional Gels as Fat Replacers in the Elaboration of Meat Products

Carina Fernández, Ricardo Fogar, Fabiana Rolhaiser, Cecilia Toth, Melisa Britez and Mara Romero

Abstract

Fat is a crucial component in meat formulations since it directly influences the overall acceptability of the product. Given its multiple functions, fat substitution cannot be achieved by simply removing it. Consequently, some strategies related to product reformulation that allow to achieve a healthier profile while maintaining acceptable sensorial and technological characteristics have emerged. Specifically, the active approach uses gels as fat replacers that can imitate fat behavior. Colloid gels are advanced materials possessing three-dimensional networks with the ability to incorporate large amounts of water or oil due to their spatial structure and unique properties, including high surface area, porosity, and loading capacity. Their application in foods requires the use of food-grade ingredients with appropriate techno functionality, such as globular proteins. The amphiphilic nature of these polymers allows them to be converted into a three-dimensional network after the unfolding of their native structure during the gelation process. Thus, in this chapter, we expose a practical description of the primary concepts regarding using fat gel replacers, emphasizing protein-based ones. We also describe some recent research advances on the theme, including those from our research group.

Keywords: food reformulation, oleogels, emulgels, fat mimetics, protein gelation

1. Introduction

In Western diets, hamburgers, sausages, bologna, and frankfurters are some of the most consumed meat products. However, their saturated fat content usually ranges between 20 and 40%, making them questionable regarding health implications. As known, high saturated fat diets are associated with the development of chronic non-communicable diseases [1, 2], as well as with obesity [3], increased percentage of body fat [4], and diabetes. All these implications led educated consumers to demand healthier meat products.

The food industry became aware of the new demands and responded by offering an ever-increasing number of healthier meat products mainly based on food reformulation, such as reducing the fat content by totally or partially replacing it with another non-fat gel ingredient to obtain low-fat meat products. Similarly, meat products with an improved lipid profile could be obtained by incorporating unsaturated oils entrapped in gel matrices of different complexity instead of traditional fat sources (i.e., pork back fat). Nevertheless, fat content and type influence physicochemical and sensory attributes such as flavor, mouthfeel, juiciness, texture, handling, bite, and heat transfer; thus, fat replacement represents a technological challenge for providing meat products that satisfy consumers in a sensory and nutritional way.

Gel fat replacers are ingredients used in food products to replace fat without compromising on texture or taste. These replacers are typically made from a combination of water, protein, and carbohydrates that work together to mimic the mouthfeel and creaminess of fat. This approach can help design functional meat products, although it is important to consider the impact on sensory attributes and potential negative effects on health.

Research literature exhibits several studies conducted to evaluate the effect of different fat replacers, either as substitutes or as fat mimetics, that evidence the potential of this strategy to obtain meat products more following the nutritional recommendations from international organisms. Thus, in this chapter, we expose a practical description of the primary concepts regarding using fat gel replacers, emphasizing protein-based ones. We also describe some recent research advances on the theme, including those from our research group.

2. Fat and fat replacers in meat products

Fat is a crucial component in food formulations since it directly influences the overall acceptability of the product. Fat provides palatability and helps produce satiety or a feeling of contentment after eating. It mainly contributes to flavor, acting as a source or carrier for volatile compounds, whose release may be responsible for specific tastes. Besides, it influences the color by providing shine, opacity or even promoting browning; it modifies the heat transfer inside the food during cooking and affects the softness and juiciness of the products. Even the shelf life of meat products is influenced by fat content mainly due to the oxidation process implied.

Given the multiple functions of fat in the formulation, its substitution cannot be achieved by simply removing it. Consequently, some strategies related to product reformulation that allow to achieve a healthier profile while maintaining acceptable sensorial and technological characteristics have emerged. Specifically, the active approach uses fat replacers that can imitate fat behavior.

Fat replacers can be classified into two categories, i.e., fat substitutes and fat mimetics. On the one hand, fat substitutes possess functional characteristics of conventional fat molecules (e.g., triglycerides). They can directly replace conventional fat molecules, successfully maintaining the palatability of foods. On the other hand, fat mimetics are typically protein- or carbohydrate-based substances that can imitate some of the organoleptic and physical properties of conventional fat molecules. They are generally polar, water-soluble compounds, which contributes to the creaming sense of high-fat products. However, they cannot directly replace typical functional characteristics of fats, such as lipid-soluble flavor-carrying capacity [5].

In one way or another, these structures converge to offer a new range of products with improved nutritional profiles (i.e., without trans fats, low-fat, or high in polyunsaturated fatty acids). Nevertheless, the notable distrust by consumers for food ingredients that are extensively processed or of unknown origin led to the demand for healthier alternatives [6]. This fact implied a preference for fat mimetics over fat substitutes, with the gel approach considered the most practical due to the ability to retain the solid-like behavior while possessing a healthier fatty acid profile, solubilizing hydrophobic and hydrophilic components, and keeping thermodynamic stability [7]. In this regard, there is considerable interest in using natural carbohydrates and proteins as structuring agents [8].

Carbohydrate-based fat mimetics include gums, maltodextrins, and dextrins, polydextrose, cellulose derivatives, starch derivatives, and oat flour derivatives. These fat mimetics are directly added to the food formulation and stabilize water in a gel-like matrix, leading to increased viscosity and a creamy mouthfeel similar to high-fat products. However, they are incorporated in foods with typically high water activity, resulting in increased potential for microbial growth; hence, they could influence the shelf life. Besides, some of them can alter the flavor profile, such as cellulose-based mimetics associated with a decrease in the flavor intensity [9].

Protein-based fat mimetics are typically produced from heating aqueous dispersions of globular proteins, including egg, milk, whey, soy, or wheat proteins. Heat-coagulated proteins typically form large gel particles perceived as rough in the mouth. However, if a high shearing force is applied during heating, tiny spherical (diameter 0.1–2.0 mm diameter) protein gel particles are produced. These microparticles are too small to be perceived as individual rough particles in the mouth. Instead, they are perceived in the mouth and taste buds as similar to fat with a creamy, smooth texture [10].

Although different biopolymers have been used as gel base structures for fat replacers, globular animal and plant proteins have shown high compatibility with various foods mainly due to their techno-functionality and the perception of natural ingredients [8]. Also, proteins have inherent advantages over polysaccharides for hydrogel development since they contain several functional groups, including amino, carboxyl, hydroxyl, sulfhydryl, and phenolic groups, which can act as reactive sites for chemical modifications and cross-linking [11].

3. Protein-based colloid gels as fat mimetics

Fat mimetics is a general term that refers to protein-based materials that were physically or chemically modified to imitate the organoleptic or functional properties of natural fats and oils [12]. However, their application in fat replacement implied different drawbacks such as non-suitability for frying, less flavorful than the fats as they carry only water-soluble flavors, the possibility of antigen/allergic reactions, and their inability to replicate rheological properties such as plasticity. These inconveniences led to a new generation of materials, collectively named colloid gels, engineered to enhance the nutritional profile of lipid-based food products, primarily based on non-chemical transformations of liquids into solid or semi-solid structures which could mimic the physical properties and functionality of solid fats [13].

Colloid gels are advanced materials possessing three-dimensional networks with the ability to incorporate large amounts of water or oil due to their spatial structure and unique properties, including high surface area, porosity, and loading capacity. They are also defined as intermediate semisolid products between a solid and a liquid possessing both elasticity and viscosity characteristics [13]. Their application in foods requires the use of food-grade ingredients with appropriate techno functionality for designing functional and engineered colloids [14].

The amphiphilic nature of proteins allows them to be converted into a three-dimensional network after the unfolding of their native structure during the gelation process [15]. The gel network can be stabilized through non-covalent cross-links such as hydrophobic and electrostatic interactions and hydrogen bonds, and, thus, it is formed as a mesh able to retain liquids within its structure. This procedure can be triggered using physical (cooling, heating, high pressure), chemical (acidification and addition of salt), or enzymatic treatments [16]. Nevertheless, we focused on physical thermal treatment due to its higher scaling potential at industry levels.

In heat-induced gelation, the three-dimensional network is formed through interactions among the exposed hydrophobic amino acid residues after unfolding the heated polypeptide chains. Despite their source, heating protein dispersions above the denaturation temperature allow the extension of the native structure, followed by an aggregation process *via* covalent (S-S bonds) and non-covalent interactions (mainly hydrophobic interactions). Animal proteins are commonly heated at 75–95°C for 20–40 min [17, 18], whereas more extensive heating (80–95°C, ~30 min) is required for plant proteins due to their higher thermal stability [19, 20]. Depending on the properties of the continuous phase, hydrogels or emulgels will be formed.

4. Protein-based hydrogels and emulgels

Protein-based hydrogels and emulgels are soft solids of particular interest in designing functional foods due to their ability to retain water or emulsified lipid droplets within the gel matrix. They are obtained by heating the initial dispersion under the required conditions that lead to the formation of protein aggregates that will be reorganized during cooling to form the pore walls and reinforce the network through several junction points. The continuous phase will be retained inside the pore space, as shown in the simplified scheme of **Figure 1**.

The above-described process is direct for hydrogels (HGs) obtained from heating aqueous dispersion. However, emulgels (EGs) require a previous emulsifying step

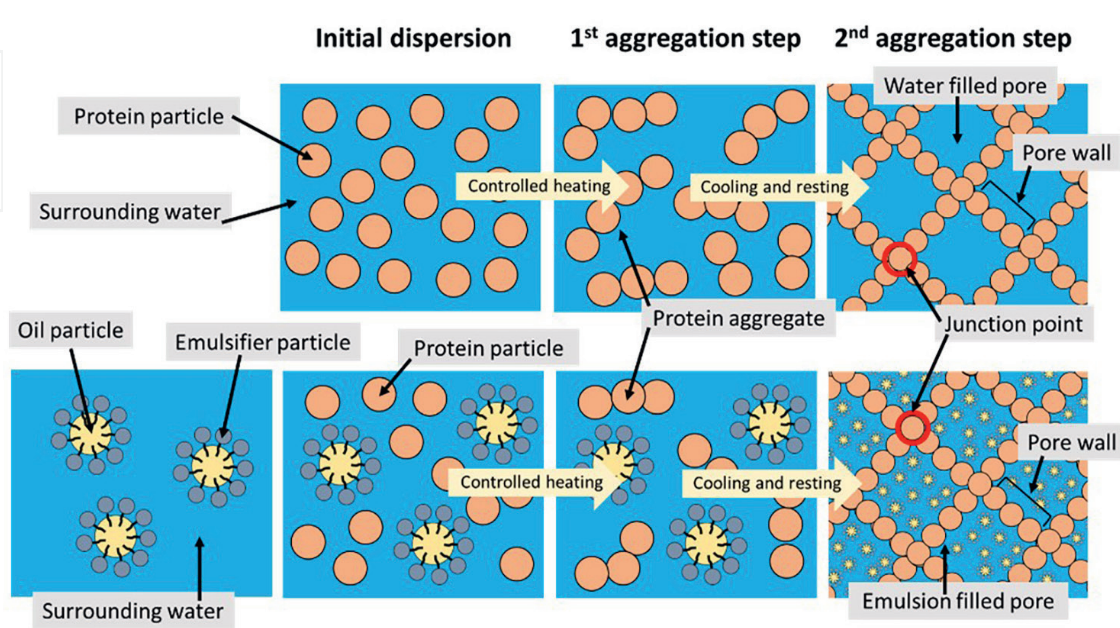

Figure 1.
Simplified schematization of heat-induced gelation of aqueous and emulsion systems.

under specific conditions depending on the emulsion characteristics (i.e., emulsion type; type, and proportion of components, among others). Either for HGs or EGs, gelation implies conformational changes in the protein structure, followed by an aggregation stage which leads to the formation of the three-dimensional gel network because of the interaction between aggregates, which determines the density of the gel network. In an aqueous medium, the aggregates undergo covalent (S-S bonds) and non-covalent interactions (mainly hydrophobic interactions). Contrary, in an oily medium, these aggregates undergo hydrophilic (protein-protein) and lipophilic (protein-lipid) interactions, increasing the gel strength when hydrophilic ones overcome hydrophobic interactions [21, 22].

Despite their origin, all proteins have the potential to form HGs and EGS due to their available functional groups such as amino and carboxyl in their structure, as reported in several studies. Although collagen and gelatin-based HGs are frequently described [10, 23, 24], inulin [25], chicken meat proteins [26], casein proteins [27], and egg white proteins [28] have shown suitability for food usages. However, thermally induced protein HGs cannot re-swell to their original volume after drying due to the increment of protein–protein interactions *via* hydrogen bonding and electrostatic and hydrophobic interactions occurring as a result of dehydration [29, 30]. In this regard, EGs partially overcome that drawback.

Emulgels are structured emulsions with concentrated oil-in-water emulsions where the interfaces around the dispersed oil droplets are structured with the help of emulsifier particles. The external phase of these biphasic systems is semisolid, which improves its thermodynamic stability, resulting in the formation of semisolid formulations with the combined controlled release of emulsions and thermodynamic stability of gels [31]. These composite systems are structurally either emulsion-filled gels (protein networks entrapping emulsion droplets) or emulsion particulate gels (networks of aggregated emulsion droplets), although distinguishing among them is not always accessible. Their composite structure is considered a hybrid network fabricated from a combination of these idealized structuring models [32].

Emulsion-filled gels can be obtained by (I) embedding an emulsion into a gel phase or a pre-gel polymer solution, followed by gelation [16], (II) gelling the continuous phase of an emulsion with a low or moderate oil content [33], and (III) using stimuli-responsive polymers in the formulation [34]. On the other hand, emulsion particulate gels can be obtained through (I) irreversible aggregation through altering pH and ionic strength, thermal processing [35], bridging flocculation [36] or enzyme treatment [37], (II) reversible aggregation [38] and (III) hetero aggregation [39]. In any of these cases, the physicochemical properties of the resulting emulgels depend on the fabrication method and the formulation, as reported in different studies that evidenced the technological advantages of EGs for improving the lipid profile of meat products, such as pork-skin-based EGs [40] and mainly whey proteins EGs [41–43].

Scientific literature also exhibits some reports about using proteins in combination with polysaccharides to develop composite-reinforced gels. For example, gellan gum has been used to modulate the technological functionality of egg white heat-induced gels for designing food formulations with anti-obesity properties [44]. Similarly, it was evaluated the combination of corn fiber gum-soy protein isolate to produce double-network gel-based food products with various texture profiles [45]. Likewise, EGs from soy protein isolate combined with carrageenan, chia flour, and inulin were added as a fat replacer in Bologna sausage with an enhanced fatty acid profile [46].

Nevertheless, blending proteins and carbohydrates is a strategy that reduces the clean label as it increases the list of ultra-processed ingredients nowadays widely criticized for their potentially adverse effects on health [47].

5. Edible oleogels

Although HGs and EGs represent a promising strategy as fat mimetics in meat products, oleogels appear as another structured system that better imitates solid fats, mainly due to their spread ability. Oleogels (OG) are defined as semisolid colloid systems with the hydrophobic continuous phase physically entrapped in a self-assembled network through a structuring agent, with technological properties similar to those of solid fats but with higher nutritional benefits [48]. The use of OGs has been advancing into experimental culinary kitchens since many years ago [49] because of their effects on texture and flavor release that allow the incorporation of novel structures within foods to provide functional properties such as taste, texture, appearance, or flavor [50].

Oleogels can be obtained through different approaches. The most common and straightforward method is the direct dispersion of hydrophobic gelators (e.g., waxes, fatty acids, monoglycerides, ethylcellulose, and colloidal silicon dioxide) into the pre-heated liquid oil. The blending procedure leads to a sol system that becomes a self-assembled network after cooling under specific conditions [51].

Given their hydrophilic behavior, proteins are unsuitable to gel oils through the direct method. Thus, an indirect approach can be used *via* stabilized oil-in-water emulsions that can be converted into OGs by cross-linking the interfacial protein layer and removing the water. This indirect procedure is commonly known as the emulsion-templated method, and it was applied for the first time in 2006 by Mezzenga et al. [52]. Compact gels or oil powders are obtained depending on the specific process followed to remove water, spray-drying, or freeze-drying [53].

Regardless of the obtaining method, the technological properties of the OGs depend on the characteristics of the formed network, which is especially important considering that these oleogels are developed to incorporate them in food matrices. Thus, changes in the acceptability of the product can occur. Regarding the use of Ogs as fat mimetics, most of the studies report the use of OGs obtained through direct dispersion, whereas a few describe the incorporation of emulsion-templated derived OGs [54–56].

6. Our contribution

Based on current knowledge, we take advantage of the emulsifier and gelling ability of different proteins and their strategic combinations for the development of fat mimetics useful in the elaboration of functional meat products. Considering the controversy on whether nanoparticles can be absorbed from the intestinal lumen in their intact form [57], we preferred the micro-scale particles. Besides, we recognize the importance of morphological and rheological analysis, nevertheless, we preferred the evaluation of textural, color, and syneresis properties by assuming them more connected to the consumers 'sensory perception, which allows us a more direct way to optimize the formulations.

We have first focused on studying bovine blood proteins since they can be easily collected and processed to obtain protein fractions that deliver specific functionality to various food products. This functionality includes relevant technological properties such as emulsifying and gelling capacity that motivated the use of blood-derived products as valuable ingredients, either by the food industry or as dietary supplements [58, 59]. The studies mainly concentrated on the use of bovine blood plasma (BBP) due to its lack of problems concerning developing undesirable flavors or colors [60–62]. On the contrary, only a few considered the techno-functionality or the nutritional value of the red cells fraction (RFC) mainly due to the dark color that it imparts [63, 64].

On one hand, we have developed hydro gelled colloidal systems from heating aqueous dispersions of BBP concentrates, which could be useful in the manufacture of low-fat meat products. These hydrogels were obtained by simply heating aqueous dispersions of bovine plasma proteins (BBP) alone or combined with red cell fraction (RCF) under controlled conditions, as shown in the simplified process of **Figure 2**. Both types of hydrogels exhibited appropriate textural and color properties, as well as no significant water release was observed. Although these properties were acceptable for both hydrogels, those obtained with the combination of BBP and RCF exhibited lower gel strength, which suggested a careful evaluation of the potential use, since the benefit of adding RCF implied an additional supply of heme iron, which imparts a healthier functionality apart from the technological one.

The lack of transparency of HGA and HGB resulted in an interesting issue since transparent gels were expected. According to previous reports, the opacity was attributed to different molecular rearrangements during the aggregation steps [65]. Given the neutral pH conditions (away from the isoelectric point of BBP and RCF), strand formation was favored during the primary aggregation. On the contrary, the surface charge of the proteins reoriented the arrangement during the secondary assembly, and tiny spheres were formed and aggregated while the junction points occurred. Thus, coarse-stranded opaque gels resulted.

On the other hand, BBP and RFC were used for developing unsaturated fatty acids vehicles since most of our work aims to create functional meat products. For this purpose, we have gelled flaxseed [65], soybean [66], and sunflower oil [67] as sources of unsaturated fatty acids *via* an emulsification step followed by a thermal treatment that triggered the gelation process. These experiments allowed us to better

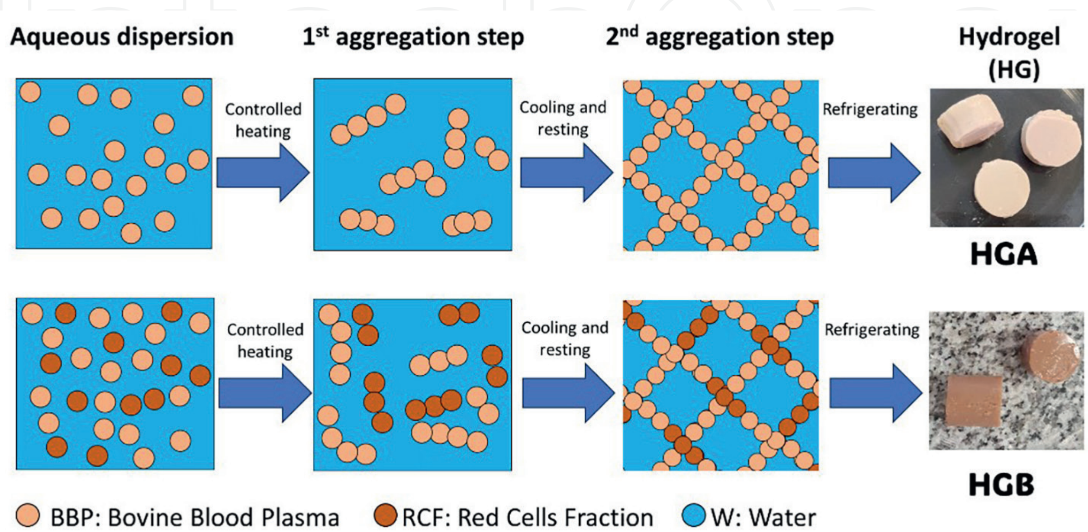

Figure 2.
Simplified representation of heat-induced hydrogels obtaining process.

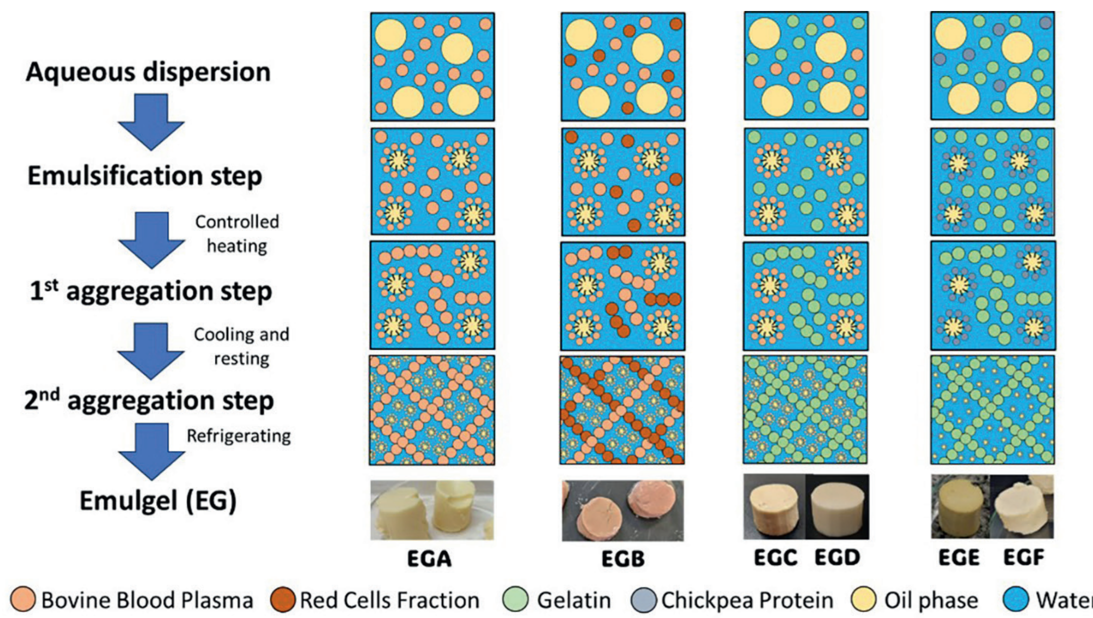

Figure 3.
Simplified schematization of emulges obtaining.

understand the behavior of the selected proteins as a function of the polarity of the oil medium and, thus, better evaluate the potential use of the gelled systems.

As seen in **Figure 3**, all fat mimetics were obtained through the emulsion-templated method. Thus, we required an emulsifier to stabilize the systems until the gelation was completed and a gelling agent to gel the whole mixture. Firstly, we used appropriate amounts of BBP to emulsify and gel flaxseed oil (FO), resulting in the EGA system (**Figure 3**). We also tested the effect of combining BBP with RFC to gel FO, and the EGB system was obtained. Despite the apparent different firmness, both EGs showed appropriate textural and syneresis behavior.

Similarly, we used appropriate amounts of BBP to emulsify and gel simple w/o emulsions containing soybean oil (SO) as an oily phase (EGC system). Nevertheless, BBP was insufficient to emulsify and gel double w/o/w emulsions and gelatin (G) was needed to obtain emulgel EGD. Considering that gel formation strongly depends on the type and extent of molecular interactions [68], we concluded that the lipid profile of SO diminished the dual performance of BBP as an emulsifier and gellant due to unfavored interactions that inhibited the formation of the junction points and so the polymeric network.

We also develop fat mimetics by using globular vegetal proteins. Chickpea proteins (CHP) were used in attending to the global food policy that shifted to place greater emphasis on more sustainable farming practices and protein sources [69]. CHP was used to obtain EGD and EGE (**Figure 3**) emulgels derived from FO and sunflower oil (SuO) o/w emulsions. Even if CHP could gel the o/w systems, the resulting emulgels were not self-supporting, and G was required.

Although significantly different from traditional fat sources (pork-back fat, bovine fat, and margarine), HGs and EGs exhibited appropriate color and textural properties that allow their use as fat replacers in food formulations. Similar conclusions were arrived regarding syneresis, which was acceptable in all cases despite the more or less open and strong gel mashes due to the different components of each system. I.e., the properties of each described gel depended on the type of gel (HG or EG), the type of

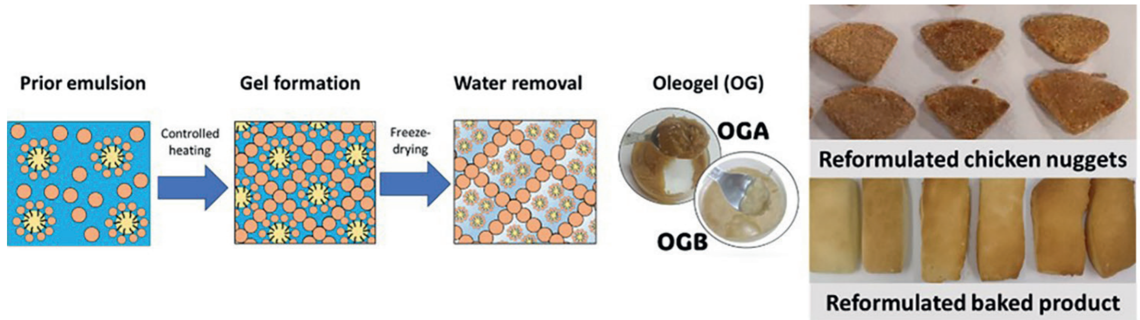

Figure 4.
Oleogels obtaining procedure and their use as fat replacers in food products.

emulsifier and gellant, the type of oil, and the interactions between all the components. Thus, HGs were harder and firmer than any of the EGs, mainly due to the presence of the oily phase, which implied a polarity-dependent behavior of the proteins.

Regarding oleogels, we only used BBP to obtain this structured system by removing water from the prior emulsion. We evaluated the properties and potential use of OGs derived from FO and SuO (OGA and OGB, respectively). As shown in **Figure 4**, freeze-drying removed almost all water and the main difference between OGA and OGB was due to the type of oil. FO yielded brighter gels, whereas SuO produced more yellow and opaque gels. Nevertheless, the oil retention ability of both gels was appropriate, and no oil losses were registered after 15 days of refrigerated storage [65, 70].

Even though we have compared the textural and mechanical properties of our HGs and EGs with traditional fat sources, we have not yet evaluated their behavior in meat matrices. In contrast, we evaluated the incorporation of OGA to replace chicken skin fat in functional chicken nuggets. Besides, we took advantage of the spreadability of OGB to replace bovine fat in the elaboration of a baked product, as shown in the right side of **Figure 4**.

7. Trends and challenges

Gel structures have also been shown to act as a protectant of biological activity by providing a stable environment for biomolecules such as enzymes, antibodies, and growth factors [71]. The gel matrix retains the biological activity of these biomolecules for extended periods, which can be useful in a variety of applications, such as drug delivery, tissue engineering, and biocatalysis. Thus, edible emulgels and oleogels developed by our research group hold great potential as a tool for preserving the biological activity of the entrapped unsaturated lipids and improving their efficacy in various applications.

Unsaturated lipids are a type of biomolecule that plays a crucial role in maintaining the structural integrity of cell membranes. They have one or more double bonds in their fatty acid chains, which give them a bent or kinked shape. This shape makes it more difficult for the unsaturated lipids to pack tightly together, resulting in a more fluid and flexible membrane [72, 73].

The biological activity of unsaturated lipids extends beyond their structural role. They also have essential signaling functions in the body. For example, some unsaturated lipids, such as eicosanoids, act as hormones that regulate inflammation, blood clotting, and blood pressure. Other unsaturated lipids, such as phospholipids, are involved in signaling processes that regulate cellular growth and differentiation. However, the biological activity of unsaturated lipids can be altered by external factors, such as diet and exposure to toxins. For instance, consuming a diet high in saturated fats can increase the amount of saturated lipids in cell membranes, leading to a more rigid and less flexible membrane. This can impair the function of membrane-bound proteins and lead to different health problems.

In this sense, we have used FO, a rich source of unsaturated fatty acids, primarily alpha-linolenic acid (ALA), an omega-3 fatty acid, and linoleic acid (LA), an omega-6 fatty acid. These fatty acids have a wide range of biological activities in the body, including anti-inflammatory, antioxidant, and cardioprotective effects [74]. FO has been shown to lower blood pressure, improve cholesterol levels, and reduce the risk of heart disease. Additionally, FO has been found to have potential benefits for skin health, brain function, and hormonal balance [75].

Considering that the biological activity of FO can be influenced by factors such as processing, storage, and cooking methods, it is important to handle FO properly to preserve its biological activity and maximize its health benefits. In this sense, the gel structure of emulgels and oleogels can act as a protectant against FO biological activity by providing a stable environment for the biomolecule, mainly preventing the oxidation process which can lead to the formation of harmful free radicals and degradation of the lipid molecule.

Thus, we began the evaluation of adding antioxidants to the gel matrix that scavenge free radicals and prevent lipid oxidation. Aligned with the natural approach, we have studied the incorporation of Surinam cherry (E*ugenia uniflora L.*) fruit extract, which improved the oxidative stability of the emulsified FO [76], although we have not yet evaluated the performance of the antioxidant extract when added to gelled systems.

8. Conclusions

Reducing the fat content of meat products by adding functional colloid gels represents a challenge to industries, since these ingredients can improve the texture and technological properties of meat products but also affect their nutritional value and overall acceptability. Regarding the improvement of lipid profile, confining oil droplets in biopolymer networks seems to be a promising strategy, given the runoff losses involved in the direct addition of unsaturated lipid sources, the low stability of liquid emulsified systems, and the ability of colloid gels for controlled released and thermodynamic stability. Besides, the ability of gel structures to protect the structure of the unsaturated lipids and, hence, to retain their biological activity during extended periods of time also represents an extra benefit. Although the choice of hydrogels, emulgels, or oleogels depends on the objective sought, the impact on the technological and sensory properties of the product must be considered. Considering the environmental impact and processing costs, further studies are required to develop eco-friendly technologies and low-cost raw materials (generally vegetable waste shells and animal by-products). Since reformulation involves modifying the food matrix and the appearance of new interactions between food ingredients, additional studies regarding nutrient bioaccessibility and bioavailability could complement technological and nutritional improvement.

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Author details

Carina Fernández*, Ricardo Fogar, Fabiana Rolhaiser, Cecilia Toth, Melisa Britez and Mara Romero
Institute for Research in Advanced Technological Processes (INIPTA), National Council for Scientific and Technical Research (CONICET), Chaco Austral National University (UNCAUS), Chaco, Argentina

*Address all correspondence to: carinafernandez@uncaus.edu.ar

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