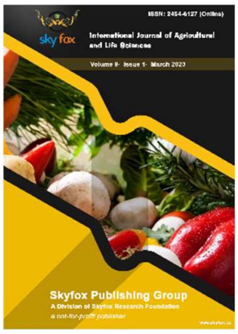

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REVIEW ARTICLE

Sterols and Phytosterols: A Review

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Abstract: Different classes of gelators molecules have been generating interest in recent years due to their specific nature and to their oil structuring capacity. To meet a particular food product's requirement, oil structuring agents or oleogelators should be capable of producing gel systems with a certain level of tailoring proficiency, which will allow them to serve as feasible alternatives as fat substitute drivers. Food engineering perceives that the principles of food formulation should always comprise food safety and quality concepts without disregarding consumer acceptance as a priority when developing a novel food product. It is thus extremely challenging to meet all criteria stating that in order to serve as an agent for oil structuring, these alternatives should be food grade, affordable, nonreactive with other ingredients, versatile, and prone to tailoring features. Within the design of novel food products, underestimating these considerations is certainly a recipe for disaster, since new food systems should offer very similar sensory perceptions for consumers in addition to healthier benefits.

Keywords: FAME, Saturated and unsaturated fats, Sterols and Phytosterols

INTRODUCTION

Saturated fats are the responsible constituents for food's characteristic mouthfeel and critical texture properties (i.e., hardness, plasticity, cohesion, etc.), which translate to foodstuffs as dissimilar as ice creams or meat products (Stortz et al. 2012). Apart from the interesting texture and mechanical properties, which can magnify oleogels' fittingness as fat mimicking materials, reducing saturated fatty acids in common foods has driven research within this field as the search for triacylglycerol like building blocks has intensified. Triacylglycerols (TAGs: triesters comprised of a glycerol bound to three fatty acid molecules) are intrinsically connected to fat crystallization behavior, melting point, and solubility properties, and they serve as the main functional lipid phase in foodstuffs. Here is one of the main issues for the global production of TAGs. Palm oil is the most widely traded vegetable oil, accounting for nearly 60% of worldwide oilseed exports (Carter et al. 2007; Ayompe, Schaafsma, and Egoh 2021). Its main advantage is the significantly higher production per hectare and its distinctive combination of fatty acids; however, the wide use of palm oil to produce triacylglycerols (TAGs) for functional fat/lipid constituents embodies several concerns among food manufacturing processes, since its production has become increasingly controversial because of environmental and social problems (e.g., deforestation, habitat and biodiversity loss, disruption of food chains, and air and water pollution) (Ayompe, Schaafsma, and Egoh 2021). Also, because of the improvement in testing methods, contaminants (e.g., glycidyl fatty acid esters, 3MCPD, and 2MCPD) have been detected in palm oil and palm oil fats (as well as in other oils and fats) because of the deodorisation stages of oil refining. As a consequence, the European Food Safety Authority (EFSA) expressed concerns (EFSA Journal 2016) setting the tone concerning consumers' exposure to such contaminants, managing potential risks and stating levels of exposure among distinct consumer age groups (i.e., formula consumption in infants, and margarine and pastries consumption in consumers ages three and above).

Recently, examples of oleogel applications for the development of novel food products with scalability potential has increased the overall global interest in these promising edible materials. Finding healthy replacements for common TAGs is driving the field. This chapter discusses the suitability of sterols (a subclass of steroid alcohols) to formulate oleogels and their derivative structures for use in developing fat mimetics within food formulation frameworks. Plant sterols, commonly referred to as phytosterols, exhibit a steroid structure composed by a hydroxyl group at position 3 and a side chain at position 17; they can have one or more double bonds along the steroid skeleton (Figure 2.2.1). Structurally, these phytosterols are closely related to stanols (which do not exhibit unsaturated bonds), which are normally considered part of the phytosterol group (Bot 2018).

Plant sterols can be obtained from numerous natural sources, namely vegetable oils, such as rapeseed oil, soybean oil, sunflower oil, and corn oil. Corn and rapeseed are the richest sources of sterols, averaging total sterol contents per 100 g of refined oil of, and 700750 and 750800 mg respectively for corn and rapeseed oil (Fernandes and Cabral 2007; Cantrill 2008). There are a few recent proposals involving lipase assisted procedures; however, on an industrial scale, phytosterols are commonly recovered using a refinement process called deo distillation that removes the flavor and odour from the oil (Maniet, Jacquet, and Richel 2019). Sterol recovery comes from the unsaponified fraction of oil distillates; the oil type will influence the phytosterol content obtained, which can range from 820%, resultant from 0.30.5% of the overall refined oil volume (Cantrill 2008). Phytosterols can also be obtained from crude tall oil, which is a byproduct of wood pulp manufacturing. In this extraction route, the heavy phase (pitch tall oil) has fewer volatiles (a major part of the unsaponifiables and a minor amount of fatty and rosin acids), and the sterol content may range from 515%. P-sitosterol is the most abundant plant sterol, and the commercial sources of tall oil and vegetable oil present a p-sitosterol percentage ranging from 3680% and 4352% (Cantrill 2008).

In regard to Y oryzanol, this is a complex molecule containing ferulate, sterol esters, and triterpene alcohols. It is found in rice bran and rice bran oil, being about 20% of the unsaponifiable fraction in rice bran oil (Srikaeo 2014). The yoryzanol content in rice bran oil can go slightly above 1% after oil refining, with an overall content of 20% in the unsaponified fraction (Narayan, Barhate, and Raghavarao 2006). In terms of y-oryzanol recovery, apart from solid liquid extraction and crystallization/precipitation methods, the supercritical fluid extraction is also an alternative methodology of high interest (Bran, King, and John 2004; Jesus, Grimaldi, and Hense 2010; Claycamp et al. 2014; Kayathi et al. 2021).

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Amid the studied sterol based oleogels, there is a remarkable similarity among the sterol based oleogels that are commonly produced using the Y oryzanol sitosterol binary system with regular fat; this is amongst the many interesting features that embodies their suitability for industrial food applications. A specific time dependent aggregation (that will be discussed later in section 2.2.2.2) at the molecular level will lead the phytosterols/oryzanol content to construct nanotubular conformations used for developing the well known macroscale transparent gels (Duffy et al. 2009). The fact that these components are deprived of any of the saturated fatty acids, which account for the crystallization process in the majority of other systems (i.e., natural waxes; mono and diglycerides; and sorbitol tristearates), epitomizes a convenient feature that could facilitate their use in making healthier food products. Given the ability to replace saturated fats with healthier possibilities, also having in mind oleogel technological properties and aptitude to serve as vehicles for bioactives has led food science to meaningfully investigate introducing these oleogels into food matrices (Kouzounis, Lazaridou, and Katsanidis 2017; Franco et al. 2019, 2020; Martins et al. 2019; Martins et al. 2020). This structuring system also seems to positively respond as a two way ingredient in the sense that it can answer to the need of having an oleogelator that; apart from being food grade, it can be likewise a functional ingredient. Thus, the combination between the abovementioned gelators meets both purposes nutritional and technological, since the value is added when it is incorporated into food systems (Lemus et al. 2014). Sterol based oleogels bear physiological effects on human diet. According to the European Food Safety Authority (EFSA), phytosterols, besides being approved for use in foodstuffs, have been proven to reduce the risk of coronary heart disease and to lower levels of LDL cholesterol (European Food Safety Authority 2012).

Y-ORYZANOL STEROLS SYSTEM

Apart from the abovementioned matters, this oleogel system is not as easy to comprehend and predict as some other systems based on crystalline or polymeric networks that employ mono or multicomponent systems. When the discussion is around mixed sterol based oleogels, we are usually referring to the predominantly studied y-oryzanol p-sitosterol binary system (Bot and Agterof 2006; Bot, Den Adel, and Roijers 2008; Bot et al 2009; Bot, den Adel, and Heussen 2010; Sawalha et al. 2013; Matheson and Dalkas et al. 2017; Matheson et al. 2017; Dalkas et al. 2018). Because multicomponent mixtures of TAGs combined with minor components are the main constituents of edible oils and fats, sterol based oleogels follow a non TAG structuring route by means of gelling fibrillar building blocks. The result is a highly stable oleogel that exhibits an elevated binding capacity (Floter et al. 2021). Normally, a solid fat mixture is characterized by its solid fraction content (i.e., the mass fraction of solid present at a certain temperature); this quality allows for a prediction on the material's selected physical properties (Himawan, Starov, and Stapley 2006). The solid fat content is the gelators' mass within the oleogel formulation, which in this case is namely the y-oryzanol and p-sitosterol content.

HYDRATE CRYSTALS

One of the interesting aspects about sterol based gelation is hydrate crystals' formation. Within the oryzanol sterols system, hydrate forms' formation is responsible for gel disturbance; this is a consequence of the lack of dispersibility of the water droplets in the gel matrix. Studies of emulsion based gels showed that tubular self assembled arrangement creation is fostered by reducing water activity and/or by developing emulsions using oils with low polarity. Bot et al. discovered that the presence of hydrates due to emulsification on sterol based gels leads to extremely soft gels (Bot et al. 2009; Bot, den Adel, and Heussen 2010). As discussed earlier in this chapter, modifications among fibrillar arrangements will likely occur when polar molecules are available (Figure 2.2.8A) in the vicinity of the ferulic acid moieties, since this will foster gel disturbance during solgel transition temperatures. Such disturbance in the fibrillar network was investigated by Matheson et al. using molecular dynamic simulations. This investigation explained the consequences of forming hydrogen bonds between water, and the hydroxyl group of sitosterol and the carbonyl group of oryzanol, wherein the gel structure would be compromised (Matheson and Koutsos et al. 2017). Similar behavior was observed as y-oryzanol and p-sitosterol mixtures were applied in structuring w/o emulsions. The tubular network did not shape completely, or it only partially formed with the tendency to endure disintegration alongside prolonged storage periods. To some degree, this ternary system produced an emulsion association with reduced viscoelastic (and optical) properties from the original binary gel. Sawalha et al. observed that emulsion based oleogels with a water activity below 0.9 are exceptionally stable (for at least one year), which displays clear evidence of tubules within its structure (Sawalha et al. 2012). Despite this, the authors showed that by keeping the water activity under 0.9, with the addition of sodium chloride, sterol hydration was prevented, thus maintaining the tubular structuring network. Molecular dynamics simulations indicated that using glycerol as a component in a ternary system (as a substitute for water) induced a successful association with water molecules; when these were maintained at a certain level, the capacity to shape the known tubular structure was increasingly stable (Matheson et al. 2018).

STRUCTURED EMULSION

As discussed earlier, Y-oryzanol and p-sitosterol hydrates can develop under distinct environmental conditions. It was reported that the presence of water will interfere with forming the self assembled fibrillar network, which will produce different types of building blocks. Recently, Pinto et al. developed water in oleogel structured emulsions using y-oryzanol and p-sitosterol. Due to the presence of a dispersed aqueous phase, hydrate crystalline forms (hemihydrates and monohydrates) of p-sitosterol developed at the water oil interface, thus modifying the hydrogen bonding network commonly generated between the p-sitosterol and oryzanol (Pinto et al. 2022). Hence, the influence of the oleogelator ratio on gel firmness would be more significant for emulsion based oleogels than for pure oleogels. However, it can highlight the feasibility of structured emulsion based systems toward the production of fat mimetic like structures, so that oleogel particle filled systems can be considered good fat substitution candidates in foods. With that in mind, a ternary system composed of an aqueous part, possibly a polymeric based hydrogel, could act similarly, producing an emulsified composition with some degree of viscoelasticity with an a polar fraction with sterol based fibrillar building blocks.

COMBINATIONS OF STEROLS

Aging polycrystalline networks with and without hydrates are some facets to consider with co crystallization methods using sterols and other oleogelators. Recent studies have focused on the combination of phytosterols and other oleogelators, which has escalated the development of numerous complex microstructures that include O/W, W/O, and bicontinuous assemblies (please see table 2.2.1). It is expected that in coming years, further exploration on the combination of oleo gelators involving sterols will translate to new scientific findings. Given this, mixtures of sterols and glycerides as well as the development of oleofoams or whipped oleogels, and the development of food bioinks for 3D printing are explored in this text.

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INDUSTRIAL PERTINENCE OF OLEOGELS

Defining strategies/guidelines related to developing products using sterol based oleogels is still a conundrum. Difficulties lie in openly identifying the ways in which building blocks behave in different oleogel systems and how their actions will translate in industrial applications where a greater range of elemental interactions makes such identification even harder. For instance, the effects of the gelator molecules on the stabilization of nutraceuticals (lipophilic and hydrophilic), can present consequences for the oleogels/bigels' physical stability due to potential crystal interactions between the bioactives and the sterol molecules during crystallization. It is straightforward to consider that most of the technological and fundamental qualities for fat mimicry, combined with

consumer preferred properties, usually occur in a hybrid system. On the other hand, overcoming regulatory concerns might present additional challenges because of the synergistic effects among distinct gelators.

With industry interest fostered by academic research, is essential to establish a more direct approach towards exploring food formulations and integrating the most appropriate oleogels and their derivatives. The suitability of oleogels' applications in foods will remain connected to the role of the structuring kinetics; this is a critical point for successful implementation and scalability. State transition in chocolate would be greatly influenced by oleogel structuring kinetics; chocolate manufacturing could be heavily targeted for this technological breakthrough because chocolate is constituted by a continuous phase organized by a blend of fats. In this case, merits could go from developing heat resistant chocolate (Stortz and Marangoni 2013), preventing surface fat bloom (as a consequence of oil migration) (Wendt et al. 2017), and into substituting expensive and poor yield cocoa butter (Espert et al. 2021). The role of the food matrix is somewhat elemental for effective industrial implementation in that variations on processing environmental conditions will be key for developing distinct food products. For example, lipid phase transformations (e.g., polymorphism) during the processing conditions (Bot and Floter 2013) will influence products like baked goods (confectioneries, pastries, or spreads), which embody a critical class of products where oleogel structuring kinetics will represent an elementary function (Fayaz et al. 2017; Espert et al. 2021; Selvasekaran and Chidambaram 2021). Oleogel kinetics is expected to play a secondary role in products with a semisolid phase (emulsified) core ingredient, like ice creams or the so called processed cold meats (Gao et al. 2021). As noted, the water activity of the food matrix will direct the oleogel applicability.y

since it will affect the fibrillar self assemblies' maintenance, which come from the oryzanolsterol system. Our perspective is that using phyto sterollike molecules as gelators in both hybrid and native gelling systems would facilitate the introduction of sterol based oleogel systems in the industrial food processing. It is known that molecular arrangements within sterol based oleogels can produce oleogels with different technological properties when compared to those shaped in a water free environment. The study of hydrate systems with these oleogelators is still underexplored. Future studies should increase knowledge of their applicability as fat replacers with the capability to mimic natural fat (providing similar structural configuration and supplementary quality wise characteristics) while being nutritionally healthier. The intrinsic lipid content of oleogels could represent a primary challenge when combined with aqueous food matrices; hence, developing emulsion based systems will surely enable their easy integration, while meaningfully reducing the fat saturation of certain products while expanding their potential toward integrating hydrophilic bioactive compounds. Food matrix compositions can be highly diverse and complex, and the interaction of already formed oleo gel structures with a different set of food compositions is yet to be understood. This represents an exciting technological challenge for the future.

CONCLUSION

Commonly, molecular engineering concepts are applicable toward the design and modulation of the functional and physicochemical properties of oleogel structures and their derivatives, thus improving the targeting of functionality toward very specific applications. The main goal of this chapter was to provide an overview about the suitability of sterol based oleogels to be used as fat mimicking materials in food products. It is greatly recognized that the most interesting oil structuring results for these kinds of oleogels are obtained by creating nanometric tubular structures using the p-sitosterol and Y-oryzanol combination (in equimolar proportion). This produces transparent and high mechanically resistant gels. Both p-sitosterol and Y-oryzanol are naturally sourced materials; they are highly compatible, and their availability is crucial for the material's price cutback and to scale oleogel production for food manufacturing. These structures are highly likely to arrest the oil phase, consuming relatively low amounts of oleogelator material wherein no chemical modifications are induced, which ensures the gelation mechanics' success. Features such as disintegration set temperature, mechanical properties, and even optical characteristics of this system are highly susceptible to tailoring actions through concentration effects. When non equimolar ratios of p-sitosterol and y-oryzanol are used, crystallites are produced. The role of phytosterols on lowering intestinal cholesterol absorption and of being pivotal elements for faecal removal of cholesterol are certainly advantageous features toward healthier food formulations. Identifying a food grade alternative to y-oryzanol within this binary system is still unmapped; that progress will lend a different perspective for the feasible applicability of this gelling system and similar ones as well.

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CONFLICTS OF INTEREST

"The authors declare no conflict of interest".