

STRUCTURED PROTEIN PARTICULATE IN FIBRE OR SHEET FORM THAT FORMS
OLEOGELS AND MACROCOLLOIDS
FOR REPLACING FATS AND THICKENERS IN FOOD AND COSMETIC PRODUCTS

FIELD OF THE INVENTION

[0001] This patent disclosure relates generally to decreasing environmental impact by using plant-based ingredients to replace animal fats, tropical oils, and artificial thickeners. Compositions with superior performance characteristics are provided for use in food and personal care products.

RELATED APPLICATIONS

[0002] This PCT application claims the priority benefit of U.S. patent applications 63/451,645, filed March 13, 2023; 18/199,974, filed May 22, 2023; 18/473,245, filed September 25, 2023; and 63/552,668, filed February 13, 2024. This application is a continuation-in-part of U.S. patent application 18/199,974, filed May 22, 2023, which issued as U.S. Patent No. 11,896,687 on February 13, 2024.

[0003] The aforelisted patents and patent applications are hereby incorporated herein by reference in their entireties for all purposes.

BACKGROUND

[0004] Food security — the reliable access to safe, affordable, and nutritious food — is inextricably linked to a predictable climate and to healthy ecosystems. Extreme weather, droughts, fire, and disease that result from climate change are already threatening the production of food around the world. Unless we act decisively, these problems will worsen, and the poorest and most vulnerable members of the world community will suffer disproportionately.

[0005] According to the United Nations Foundation in 2021, the worldwide yield growth for wheat, maize, and other crops has been declining for years, due to extreme heat and droughts. By some estimates, unless climate is reversed, global yields of agricultural products could decline by as much as 30% by 2050. Despite decades of efforts to improve the global food supply, widespread hunger persists at staggering rates. According to a 2019 report, nearly 750 million people worldwide experience undernourishment or food insecurity — and the numbers continue to rise.

[0006] Farm animals are high energy consumers, and they produce huge volumes of greenhouse gasses, contributing to climate change. Use in foods of solid tropical oils (such as coconut and palm oil) also has an adverse environmental impact by causing tropical deforestation, which compromises biodiversity.

[0007] The impact of society on the environment can be mitigated in part by decreasing the world's reliance on animal derived fats and tropical oils in the manufacture of foods and other products. Innovators in the food industry are placing an emphasis on developing plant-based foods that mimic traditional foods such as meat, fish, eggs, and dairy products. In a similar fashion, the beautification industry is taking steps to reduce the content of animal derived ingredients in cosmetics.

[0008] Texture, flavor, and mouthfeel of meat and dairy substitutes, and the moisturizing and beautifying effects of cosmetics, are all partly a function of the thickeners and fats they contain. The improved ingredients provided in this disclosure can help develop more climate friendly foods and personal care products.

SUMMARY

[0009] This disclosure provides a protein dry particulate that has a defined structure with beneficial properties. Combined with aqueous solution, the particles form a macrocolloid with improved thickening properties. Combined with an oil, the particles form an oleogel with unique oil retention and release properties. The products are configured for use as a replacement for artificial ingredients, oils, structured fats, and tropical oils in food, food ingredients, cosmetics, and personal care products.

[0010] The dry particulate can be manufactured by a process that includes denaturing the protein in water, and freeze channeling to create dispersible microlayers. There are fibrils, sheets, and other shapes with a high aspect ratio which are substantially not interconnected, and therefore free-flowing. The dry particulate physically traps and structures the liquid in which it is suspended.

[0011] The oleogel of this disclosure has a solid, free standing, fat-like performance, whereby the oil is released under shear or upon heating, much like traditional animal-derived structured fat. As illustrated below, the oleogel has superior performance in food systems and in beauty care products. It releases oil upon cooking and chewing, thereby providing juiciness expected for fat-containing foods. The oleogel is spreadable, and forms an emulsion in aqueous liquid that is stable for at least six weeks without evidence of creaming.

Aspects of the technology

[0012] This disclosure provides a proteinaceous dry particulate that comprises mostly a protein that disperses and forms a structurant in either liquid oil or water. The protein can be a denatured protein, typically a protein isolate or mixture. Some proportion of the dry particulate has a solid microstructure that confers upon the product special properties. Typically, the microstructure comprises microparticles that have a median size of at least 10 μm in one or two dimensions. In the dry particulate, the microparticles pack together in some close arrangement, but are free flowing and substantially not interconnected when suspended and diluted in vegetable oil.

[0013] Accordingly, for the dry particulate, there are two aspects of the structure: the microparticles having particular shape and dimensions, which together constitutes the microstructure, and the loosely packed and dispersible assembly or aggregation of the microparticles, which constitutes a macrostructure.

[0014] When suspended and diluted gently in liquid oil or water, the macrostructure will generally disperse, but individual microparticles will remain substantially intact. In an aqueous liquid, the dispersed microparticles will generally form a macrocolloid, which may cause thickening or increase the viscosity of the liquid. When suspended in a liquid oil at high density, the microparticles may pack together, but when diluted, the microparticles will generally separate and can be characterized apart from each other.

[0015] In packed form, the microparticles obtained according to this disclosure will typically overlap. The term “overlap” in this context means that the microparticles can be packed together such that each microparticle is positioned past the middle of the microparticle beside or above it, and are thereby capable of forming an interleafed, intertwined, coplanar, or parallel arrangement. Roundish or pebble like microparticles cannot do this. The amount of overlap in close-packed spheres is ~26% of the diameter of each sphere. Overlap is enabled by an elongated or irregular shape of the microparticles. Each microparticle can be quantified by the aspect ratio of its dimensions. The “aspect ratio” is the ratio of a microparticle’s longest dimension to its shortest dimension. Spheres have an aspect ratio of 1. Microparticles in a dry particulate or an oleogel of this disclosure may have an aspect ratio of at least 2, 3, 5, or more. High aspect ratio microparticles may occur in the form of fibers, sheets, more irregular structures, or a combination thereof.

[0016] In packed or dispersed form, the microparticles according to this disclosure may be substantially not interconnected. When packed together, the microparticles may touch each other and may interconnect loosely with strands of material. However, such interconnections (if present) break apart easily when the microparticles are dispersed in water, or dispersed and diluted in liquid oil.

[0017] This disclosure provides oleogels and macrocolloids containing such microparticles, typically but not necessarily obtained by dispersing a dry particulate in liquid oil or water. The oleogels of this disclosure may be characterized independently according to their structural and physicochemical properties, by their method of manufacture, or by their properties when stored or when used to prepare emulsions, food products, personal care products, pharmaceuticals, and other industrial goods.

[0018] This disclosure provides foods, food ingredients, cosmetics, personal care products, pharmaceutical fillers and encapsulating means, and other industrial products that contain or are made using the dry particulate, macrocolloids, and oleogels referred to above.

[0019] This disclosure provides several procedures for manufacturing a dry particulate, a macrocolloid, or a protein oleogel. In one approach, the manufacturing process includes hydrating and solubilizing a mixed isolate of plant proteins in an aqueous solvent, typically forming a gel. The

protein (if water soluble or reactive) may be denatured by any suitable means, such as low (acidic) pH and/or heat. The usual subsequent step is freeze channeling the protein to form a protein powder comprising a matrix having a solid microstructure. Freeze channeling can be done, for example, by flash freezing and removing the water by vacuum. It is thought that the freeze channeling promotes arrangement of the denatured protein to form a desirable microstructure.

[0020] For making oleogel, the process continues by gently adding an oil or mixture of oils (optionally containing some water and other components) to the dried protein in a manner so as to disperse but not substantially triturate, fracture, or otherwise damage the microparticles until reaching a desired protein-to-oil ratio. The procedures put forth can be used to make the dry particulate, macrocolloids, and oleogels detailed above, or for any other suitable purpose.

[0021] This disclosure also provides methods for using the dry particulate, macrocolloids, and oleogels of this disclosure for particular purposes. For example, the dry particulate can be used for thickening, stabilizing, or altering the mouthfeel of a food product or ingredient. The protein oleogel can be used for altering the perceived firmness, juiciness, fattiness, and/or flavor of a food product or ingredient, or for increasing the creaminess and improving the therapeutic and beautifying function of a personal care product.

Advancement over previous technologies

[0022] The technology presented in this disclosure has important new features. The high aspect ratio of the microparticles in both the dry particulate and the oleogels of this disclosure contributes to their superior behavior when used in foodstuffs and other industrial products. The owners of this technology hypothesize that the high aspect ratio of the microparticles enable them to be suspended close together in a liquid oil, constituting an effective structurant — but because they are substantially not interconnected, the microparticles may slide past each other, giving the oleogel a lubricious or creamy feel, conferring superior oil retention and release properties.

[0023] As described below, the microstructures in previous protein oleogels do not have these features. Prior art microstructures are either large solid blocks and honeycombs, or small roundish pebble-like microparticles. This is an important difference from what is described here. A solid microstructure will give food a gritty consistency, whereas roundish microparticles will release oil readily upon storage or early in the cooking process.

Unity of invention

[0024] The dry particulate and the oleogels of this disclosure, while claimed independently, share common inventive features relating to the solid microstructure: for example, the ability of the microparticles to overlap when packed together, microparticles having a high aspect ratio, and the feature that the microparticles are substantially not interconnected and/or free flowing. These features

imbue the products with the special properties when used in foodstuffs and other products, as put forth below and in the appended claims.

[0025] Whereas the products of this disclosure are not constrained to a particular method of manufacture, the processes in this disclosure provide a suitable and convenient way of obtaining dry particulate preparations and oleogels with special properties. The freeze channeling and other methodologies put forth in this disclosure promote the formation of the solid microstructure in the dry particulate and the oleogel.

Terms, ranges, and patent terminology

[0026] A protein particulate or dry particulate of this disclosure is a preparation of solid proteinaceous particles that are dispersible in an aqueous liquid to form a macrocolloid — and/or in a liquid oil to form an oleogel. A preparation of proteinaceous particles comprises mostly protein that is denatured or otherwise configured so that it forms at least in part a dispersion (rather than a solution) in the liquid in which it is suspended.

[0027] A “macrocolloid” consists mostly of particles that typically range from 0.1 to 100 μm in diameter suspended in water or aqueous liquid. Macrocolloids may have a discernible structure, such as a gel network, foam structure, or emulsion droplets. The particles in macrocolloids may be aggregated or dispersed within a continuous medium. The structure may stabilize the protein in the liquid, thereby rendering the particles less prone to settling. Macrocolloids exhibit macroscopic properties such as viscosity, elasticity, and flow behavior due to the interactions between large particles. Examples include gels, emulsions, foams, and suspensions used as food additives, skincare products, and drug delivery systems.

[0028] The term “oleogel” as used in this disclosure has its ordinary meaning. It generally refers to a semisolid or solid-like material that is formed by a solid network of self-assembled molecules or oleogelators such as proteins or other polymers, which are able to immobilize liquid oil in within their structure, resulting in a semisolid or gel-like consistency. A semisolid has a pliable viscous consistency that is spreadable but does not pour in a narrow stream. The network provides structural stability to the liquid oil phase, allowing it to maintain its form and texture at room temperature. When cooked, the network typically remains such that the oleogel softens and/or release a proportion of the oil contained therein. A protein oleogel may be characterized as a structured protein dispersed, distributed in, or encompassing a liquid oil phase, or as a composition or colloidal system where an oil or oil mixture is dispersed within and/or structured by the protein. These descriptions have similar meanings and (depending on context) are generally interchangeable.

[0029] The oil-protein compositions of this disclosure can be characterized or defined either: (1) according to the microstructure of the protein contained therein; (2) according to the method of manufacture; or (3) according to the performance properties of the composition once prepared. The compositions can also be characterized by any two of these criteria: microstructure and manufacture,

microstructure and performance, or microstructure and performance; or by all three criteria in combination. Similarly, the methods of this disclosure can be characterized or defined by the steps that are taken, and/or by the characteristics of the oleogel produced thereby, such as the microstructure of the oleogel and/or the performance of the oleogel, immediately after production and/or incorporated into a food or cosmetic product.

[0030] The term “protein oleogel” refers in this disclosure to a composition in which a protein is dispersed in a liquid oil phase, or an oil that is dispersed in and permeates a protein framework. The protein oleogels provided in this disclosure often have a total protein or oleogelator protein to oil ratio of at least 2:98 by weight, up to 40:60 by weight. More typically, they have protein to oil ratios of 2% to 40% up to 20% to 80%, 5% to 95% up to 15% to 85%; or an average of 5% to 95% by weight. The oleogel may be characterized as a protein oleogel of at least 30%, 50%, 70%, or 90% of the composition that is not oil is protein, and/or if the oleogel has been manufactured using a protein isolate as a primary ingredient.

[0031] The oleogelator protein that gives the oleogel its structure is typically but not necessarily a protein mixture or isolate. Alternatively or in addition, the protein used to structure the oleogel may consist essentially of or contain single or multiple proteins made by recombinant expression. Suitable mixed isolates are those that contain a median protein mol. wt. between 5 and 75 kDa, or about 10 to 50 kDa, optionally with an acidic isoelectric point ($\text{pH} \leq 5$). Exemplary is a protein isolate from potatoes, comprising patatins and/or protease inhibitors, such as the Solanic®200 or Solanic®300 fractions made by Avebe (Veendam, the Netherlands). See WO 2018/183770.

[0032] The oil can be any oil or oil mixture that is liquid at room temperature or upon heating. It may comprise fatty acids or other fatty structures in unsaturated or saturated form. Examples are provided in a later section of this disclosure. The oleogel composition may include other solid components or solutes such as crystallizable carbohydrates, maltodextrins, or polysaccharide derivatives that contribute to the oleogel, for example, by helping to form or stabilize the protein microstructure. The composition is typically produced and/or stored in a form that is substantially free of water or aqueous solvent: that is, less than 3% (wt/wt), preferably less than 1%. It may then be combined with aqueous solvents, oils, solids, other components, and combinations thereof for the purpose of analysis or in the course of manufacturing an industrial product therefrom.

[0033] The protein in a dry particulate, macrocolloid or oleogel of this disclosure is often but not necessarily a plant protein. Alternatively or in addition, the protein may be an animal derived protein, or a protein that is produced naturally by microorganisms. The protein is typically denatured. This means at a minimum that the protein is configured so that it forms some kind of suspension, macrocolloid, dispersion or other heterogenous phase when combined with a liquid, rather than a solution. This typically implies that the protein no longer has its quaternary structure, tertiary structure, and/or secondary structure which is present in their native state. As a consequence, the protein generally loses enzymatic activity or other innate function. Denaturation may be achieved by

application of some external stress or compound, such as a strong acid or base, a concentrated inorganic salt, an organic solvent such as alcohol, agitation, radiation, heat, or a combination thereof. The protein in the oleogel may be denatured during isolation from a plant before manufacture of the oleogel, and/or during the course of the oleogel manufacturing process (for example, by decreasing the pH and heating the solubilized protein isolate). Denatured proteins tend to be less soluble in aqueous solvents and liquid oils, increasing their tendency to form a stable microstructure in an oleogel.

[0034] At least some of the protein in the dry particulate or oleogel creates or exists as a microstructure that tends to reduce viscosity and/or increase hardness of the oil in which it is dispersed at room temperature. At least 10%, 20%, 30%, 50%, 70%, or 90% of the protein content in the macrocolloid or oleogel (wt/wt) may be part of the microstructure. The contents of the microstructure that is protein (wt/wt) is at least 30%, 50%, 70%, or 90% protein, with most of the rest typically being other components of the protein isolate used to manufacture the oleogel.

[0035] The microstructure has dimensions and properties that imbue the macrocolloid or oleogel with beneficial thickening or oil holding and releasing features described below and in the sections that follow. For example, the oleogel has a solid but pliable or a spreadable texture at room temperature. It may retain the oil when stored at 4°C or at room temperature. The term “spreadable” means that it can be spread freely and fairly evenly on an ordinary slice of bread using an ordinary butter knife at room temperature without inordinate effort, and without substantially deforming the bread slice. The oleogel fluidizes gradually upon heating, and/or releases some but not all of the oil upon cooking (typically between 20% to 30% and 60% to 80%). This contrasts with saturated and hydrogenated fats, which are solid at room temperature but melt quickly once heated to a transition point.

[0036] The oil retaining ability of an oleogel at different temperatures can be assessed by determining a melt curve, as illustrated in FIG. 4. Depending on its intended use, the melt curve of the oleogel has a descending slope that is not as steep as the slope for coconut oil. The oleogel melt curve may demonstrate that the oleogel retains some but not all of the oil when heated to typical cooking temperatures (for example, 160 to 200°C; or 300 to 425°F), indicated by a curve that bottoms out or continues only a gradual downward slope at these temperatures. The remaining oil may be between 20 to 80%, 10 or 30 to 60%, 25% to 75%, or 40 to 80% of the oil in the oleogel composition before heating.

[0037] Typically, the microstructure comprises particles that are at least 5, 10, or 20 µm in size in one or two dimensions or in all three dimensions. When suspended and diluted in vegetable oil, the microparticles are substantially not interconnected or irreversibly intertwined. This means that pieces of microstructure that are more than 50 or 200 µm in any dimension are rare, comprising less than 10% or 2% of the microstructure by weight. In this configuration, the particles can pass or slide by and between each other freely. The oleogel (including its suspended microstructure) will be free

flowing if sufficiently diluted. The nature and shape of the microstructure and its component particles can be assessed by diluting the oleogel by 5, 10, 20, 50, or 100-fold vegetable oil, for example, using the dilution protocol provided later in this disclosure, and then observing the microstructure by light microscopy, scanning electron microscopy, or another appropriate visualization technology.

[0038] For example, the microstructure may contain fibrils having a median size that is at least 10, 20, or 30 μm in length, typically at least 0.5 μm but less than 2, 3, or 4 μm in diameter. The term “fibril” refers to any shape that is long and skinny: it may or may not be rod shaped, ribbon shaped, hollow, or fiber-like. The fibrils may be free standing, branched, and/or clustered or entangled to form aggregates. For branched structures, length is defined as the longest linear span of the structure in three dimensions.

[0039] Alternatively or in addition, the microstructure may contain sheets. The sheets may have a median size that are at least 10 or 20 μm in length and width, typically at least 0.2 or 0.5 but less than 2, 3, or 4 μm in thickness. They may be folded, ruffled, or compacted together. Alternatively or in addition, the microstructure may have particles of rounded, faceted, or indeterminate shape. Such particles may be derived from the protein isolate used to make the oleogel, they may be generated during manufacture of the oleogel, or they may be formed by grinding, sonicating, or otherwise dispersing a larger, less malleable, or more solid or interconnected microstructure initially produced during manufacturing. To provide desirable oil retention and release properties, the particles are often designed to flow past each other by having a high median aspect ratio (diagonal length to thickness) of at least 2, 3, 5, 7, 10, or 15.

[0040] The oleogels having the composition described above, manufactured as described below, or having desirable properties put forth throughout this disclosure can be used in the preparation of food products or ingredients, or cosmetic or personal care products. This can reduce the dependency of such products on animal-derived fats and other renderings, thereby mitigating at least some of the effects of climate change.

[0041] Descriptive terms used as a guideline for proportional amounts of a component or preparation are as follows: “some” means at least 10% or 20%, “most” means more than 50%, “substantially all” means at least 90%, wherein the component(s) that constitute the remaining 10% or less do not substantially affect performance characteristics of the mixture or product. Proportional amounts are given in units of (wt/wt), unless stated otherwise.

[0042] Particular embodiments, aspects, properties, and features of the dry particulate and oleogels of this disclosure, their manufacture and use, are described and exemplified in the sections that follow.

Trademark

[0043] Oleogels having characteristics described herein and/or manufactured by a process put forth herein may be referred to in this disclosure and elsewhere under the name OleoPro™, a trademark of Shiru Inc., Alameda, CA.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] FIG. 1 shows light micrographs of two different oleogel preparations having plant protein microstructures in accordance with this disclosure. The scaling bar in the bottom of each panel represents 20 μm in length. Panel (A) shows a preparation that was made by flash freezing denatured plant protein in a 1% (wt/wt) solution, followed by drying and combining with high oleic acid sunflower seed oil. The field shows fibrils, branched fibrils, and fibril clusters or entanglements. Panel (B) shows another preparation made substantially the same way, except that the denatured protein solution was flash frozen at a concentration of 5% (wt/wt). This field comprises protein sheets. The upward sloping lines are folds in the sheets and embedded fibrils.

[0045] The images were obtained by diluting each of the two oleogel preparations 1 to 10 in vegetable oil. Both types of microstructure comprise particles that are at least 10 μm in one or two dimensions, but are substantially not interconnected and free flowing. This contributes to the oil structuring and retention properties that make these oleogels suitable as replacements for animal derived fats and oils.

[0046] FIGS. 2A and 2B are SEM images of dry particulate preparations made using potato protein and whey, respectively. Each row of FIG. 2A is a separate preparation. Left and right images in each row are magnification levels 200x and 1000x. The images show ruffled or ridged plates and intermittent fibrillar features with few interconnections. The two rows of FIG. 2B are two interior regions of the same particle aggregate at 200x and 1000x magnification. There are loosely connected plates with fibrillar features and a high aspect ratio.

[0047] FIG. 3 is a consolidation of micrographs adapted from publications reporting previous protein oleogels. The images from refs. (A), (C), (D) show mesh and honeycomb type microstructures. The images from refs. (B), (E), and (F) show pebble-like solids and microaggregates that are round or ellipsoid in shape or aggregates thereof, thereby having an aspect ratio (length to thickness) that is no more than two. None of the previous protein oleogels has anything like the fibrils and sheets shown in FIG. 1 — and as a consequence, have inferior oil holding and release properties.

[0048] FIG. 4A is a flow chart that provides an overview of procedural steps that can be used for optimizing a manufacturing an oleogel according to this disclosure. FIG. 4B is a flow chart that illustrates one possible way of manufacturing an oleogel having the microstructure shown in FIG. 1. The starting protein isolate is hydrated and solubilized, and then acidified and heated to denature the protein. A clear stranded gel is formed. This is blended, flash frozen, and dried to form a powder.

Oil is added to the prepared powder gradually to preserve the inherent microstructure and form the oleogel.

[0049] FIG. 4C is a melt curve that compares two determinations for each of two different protein oleogel preparations according to this disclosure made with coconut oil. Curves obtained for several oleogel preparations are gently downward sloping, showing gradual release of a substantial proportion of oil as they are heated to cooking temperatures. The curves do not descend all the way to zero, because not all of the oil is released. There was some residual oil in the protein structure after heating.

[0050] FIG. 5 is a quantitative analysis of the oleogel microstructures shown in FIG. 1. Details A1, A2, A3, and A4 are representative fibrils or ribbons that were measured by comparing the image details with the 20 μm scale bar. Total length from end to end (including branches) ranged from 12 to 35 μm . Diameter ranged from 1.7 to 3.0 μm . Aspect ratio (length to diameter) ranged from 9 to 21. Details B1, B2, B3, and B4 are representative plates of different shapes. Length or height ranges from 19 to 88 μm ; width ranges from 18 to 42 μm . Given a thickness of 2 μm , the aspect ratio (length to thickness) ranged from 10 to 44.

[0051] FIG. 6A shows the five-point scale for subjectively assessing gel characteristics. Protein oleogels prepared in the manner described scored as follows: Hardness = 2, lubricity = 4.5, smoothness = 5, and adhesiveness = 1. FIG. 6B presents the subjective data of these criteria as a spider plot. The solid line marked "Shiru oleogel 1" was made by the manufacturing process outlined above. High values for lubricity and smoothness are prominent.

[0052] FIG. 7 is a flowchart suitable for iteratively and empirically optimizing process control variables (right column) by measuring sensory properties using internal benchmarks (left column) and material properties (middle column).

[0053] FIGS. 8A and 8B show features of burger patties made with protein oleogel. FIG. 8(A) shows weight loss during cooking and hardness measured mechanically. FIG. 8(B) shows perceived firmness, juiciness, and fattiness assessed by a panel of trained volunteers.

[0054] FIG. 9 is a scale used to evaluate the patties by sensory criteria.

[0055] FIG. 10 is an image showing the spreadable texture of a protein oleogel manufactured in accordance with this disclosure.

[0056] FIG. 11 provides two photographic images comparing test patties made with coconut oil or with protein oleogel when heated to cooking temperature. Oil oozed and bubbled away from the patty made with coconut oil (left), but not from the patty made with protein oleogel (right).

[0057] FIG. 12 shows protein oleogel according to this disclosure that is incorporated as discrete layers into a plant-based replacement product for bacon.

[0058] FIG. 13 shows the visual appearance of three preparations of spreadable olive oil. The product is an oleogel containing only pure virgin olive oil, potato protein as an oleogelator, and trace ingredients to adjust flavoring and color.

[0059] FIG. 14 shows the visual appearance of a chocolate nut spread. Inclusion of 1% protein dry particulate on the right prevents oil separation that was observed in the control sample on the left.

[0060] FIG. 15 shows the effects of including other elements in an oleogel preparation on browning properties.

[0061] FIG. 16 shows temperature-dependent rheology of macrocolloids of this disclosure, compared with methylcellulose, and a pure recombinant gelation causing protein designated P44548.

[0062] FIG. 17 demonstrates the viscosifying effect of protein dry particulate combined with an aqueous liquid to form a macrocolloid. Viscosity increases when salt is added.

[0063] FIG. 18 shows the appearance of a dry particulate-structured emulsion, made by adding liquid oil to a macrocolloid.

[0064] FIG. 19 shows the appearance of uncooked plant-based meat doughs prepared using methylcellulose (left) or dry particulate (right) as a cold-binding agent. They exhibited a uniform, smooth surface and a relatively light coloration, compared with meatballs containing no cold binding agent (center).

[0065] FIG. 20 is an image of a cosmetic cream containing 48% water, 47% oleogel, 4% olive oil, plus other trace ingredients. Left to right are freshly prepared cream, a preparation stored for 10 days at ambient temperature, and a cream stored 10 days at 50°C. All preparations retained a thick, unseparated and indistinguishable appearance and texture.

DETAILED DESCRIPTION

[0066] Described in the following sections are preparations of dry particulate, macrocolloids, and oleogels that have a microstructure that confers beneficial properties.

[0067] Oleogels of this disclosure are stable and have excellent mechanical properties, making them ideal for use in products that require a semisolid or a solid but meltable consistency. The dry particulate and oleogels are capable of large-scale manufacturing, and have superior properties for inclusion in processed foods, cosmetics, and personal care products.

Interrelationship between microstructure, method of manufacture, and functional properties

[0068] The protein dry particulate and oleogels of this disclosure can be characterized in terms of any one of three types of features put forth below — either alone, or in combination with one or both of the other features. (1) the protein microstructure; (2) the method of preparation or manufacture; and (3) the physical properties and sensory characteristics of foods and other products made therefrom.

[0069] Although these features may be characterized separately, they are operationally interrelated. The properties of the macrocolloids and oleogels of this disclosure are a property of the

structure of the preparation of dry particulate, which in turn is a function of or influenced by the methodology used their manufacture.

Previous oleogels and their use

[0070] An *organogel* is a class of gel composed of a liquid organic phase within a structured network. An *oleogel* is an organogel with an oil as the organic phase. Oleogels are lipophilic liquid and solid mixtures, in which solid lipid materials (oleogelators) (<10 wt%) entraps and solidifies bulk liquid oil (typically a mixture of edible fatty acids) by ways of the network of oleogelators in the bulk oil.

[0071] A commonly used structurant for oleogels is ethyl cellulose: a polysaccharide manufactured from wood pulp. It is a semi crystalline derivative of cellulose that can be introduced into edible oils by direct dispersion. Its gel forming ability is attributed to its hydrophobic nature and semicrystalline characteristics. To induce gelation, ethyl cellulose is heated to 130°C (beyond its glass transition temperature). Subsequent cooling forms rigid intermolecular interactions linked by hydrogen bonds, creating a three-dimensional entangled network from one-dimensional polymer strands that is responsible for entrapment of oil. F. Manzoor et al., Food Hydrocolloids for Health, Vol 2:Dec. 2022.

[0072] E.I. Du Pont De Nemours and Company appetizingly describes ethyl cellulose this way: Ethocel™ polymers are water-insoluble thermoplastic polymers; they can therefore be employed for a wide variety of functions. They are used for rheology modification, film formation, binding, water barriers, and as time-release agents. Ethocel™ can also be effectively used as a sacrificial binder as they exhibit clean burn out.

[0073] Oleogels are internally structured, and can be used to replace structured oils commonly used in processed foods, particularly animal fats. The field of oleogel research has been very active in recent years, generating products with desirable properties like thermal resistance, texture, and structural stability. Depending on the matrix underlying the oleogel, food products have been shown to resemble textural attributes of products conventionally made with conventional hardstock fat, to affect nutritional qualities, to demonstrate high physical and oxidative stability, and to exhibit a high oil binding capacity.

[0074] An excellent review article by C. Park and F. Maleky (Front. Sustain. Food Syst. 4:139, 2020) provides an overview of oleogels that have been incorporated into various types of food products. Such food products are shown below in TABLE 1. The reader is referred to the Park article for the cited publications that describe the products listed in the table and the types of oleogels they incorporate.

TABLE 1 | Examples of oleogel applications in food product formulation.

Food products	Liquid oil type	Organogelator type
MEAT PRODUCTS		
Frankfurter	Soybean; canola; sunflower	Rice bran wax; ethylcellulose; γ -oryzanol and phytosterol
Meat patties	Linseed; sesame	Oryzanol and β -sitosterol; beeswax
DAIRY PRODUCTS		
Cream cheese and processed cheese products	High oleic soybean; soybean;	Rice bran wax; ethylcellulose and sunflower wax
Ice cream	High oleic sunflower; sunflower	Rice bran wax; γ -oryzanol and phytosterols
SPREADS		
Margarine	Soybean	Sunflower wax, rice bran wax, and candelilla wax
Spread	Sunflower; virgin olive and hazelnut	Shellac wax; beeswax and sunflower wax
CONFECTIONARIES		
Chocolate paste	Sunflower; pomegranate seed and palm	Shellac wax; monoglyceride, beeswax, and propolis wax
Chocolate	Sunflower; hydrogenated palm kernel	γ -oryzanol and β -sitosterol; ethylcellulose
Filling	Rice bran and palm; canola	Beeswax; hydroxypropyl methylcellulose and methylcellulose
PASTRIES		
Cookie	Refined hazelnut; canola; rice bran oil	Beeswax and sunflower wax; candelilla wax; bleached rice bran wax
Cake	Sunflower; cotton and high oleic sunflower	Beeswax, candelilla wax, and rice bran wax; carnauba wax
OTHER APPLICATIONS		
Oleogels as carriers of bioactive compounds	High oleic sunflower; canola	Beeswax with β -carotene; ethylcellulose with β -carotene

[0075] None of the oleogels listed in TABLE 1 are made with protein as the oleogelator. The owners and inventors of the technology put forth in this disclosure have developed protein oleogels that have superior properties to replace animal fats and other components in food and cosmetic products.

Unique microstructure of the oleogels of this disclosure

[0076] An oleogel according to this disclosure can be characterized as having a microstructure with certain observable features.

[0077] FIG. 1 is a pair of light micrographs of oleogel preparations produced by optimized freeze channeling, drying, and oil dispersion. The scaling bar in the bottom of each panel is 20 μm in length. Panel (A) shows a preparation that has been diluted to 1% in oil. The field shows fibrils and fibril clusters or entanglements. The fibrils are 30 to 50 μm in length and 1 to 3 μm in diameter. The fibrils may be solid (rod-like) or hollow tubes with varying degrees of branching. Individual branches or unbranched structures are commonly 10-50 μm in length.

[0078] In addition to the fibrils, microstructures in the form of tetragonal or irregular sheets can also be observed. Panel (B) shows a preparation that has been diluted to 1% in oil following flash-freezing and freeze-drying of a 5% w/v protein solution. Representative sheet structures exhibit a wide range of estimated planar dimensions, on the order of 10 μm x 10 μm (length x width) to greater than 10 μm x 100 μm . The sheets may be less than 2 μm , 1 μm , or 0.5 μm thick. Often, as shown in Panel (B), the sheets appear as ruffled or textured with embedded ridge-like features.

[0079] Typically, the microstructure represents greater than 50% of oleogelator protein or total protein in the composition. Water insoluble microstructures typically comprise ~50% of the freeze-dried protein material before the oil is added. This can be determined by dispersing the processed protein powder in water, centrifuging at 14 x g at 24°C for 30 min, and measuring the soluble protein content in the supernatant using the bicinchoninic acid assay (Thermo Fisher Scientific). The protein in the composition is predominantly associated with the microstructure when dispersed in oil.

Differences between the microstructure shown here and the microstructure of previous protein oleogels

[0080] The microstructure shown here is new for protein oleogels: it differs in important ways from microstructures observed in oleogels previously made using protein as the oleogelator.

[0081] FIG. 3 is a consolidation of optical images, confocal micrographs, and SEM micrographs adapted from the following prior publications:

- (A) *Protein oleogels from protein hydrogels via a stepwise solvent exchange route.* Auke de Vries et al., *Langmuir*. 2015 Dec 29;31(51):13850-9.
- (B) *Controlling agglomeration of protein aggregates for structure formation in liquid oil: A Sticky Business.* Auke de Vries et al., *ACS Appl. Mater. Interfaces* 2017, 9, 11, 10136–10147.
- (C) *Structural characterization of oleogels from whey protein aerogel particles.* S. Plazzotta et al., *Food Res Int.* 2020 Jun;132:109099.
- (D) *Structural characterisation and absorption capability of whey protein aerogels obtained by freeze-drying or supercritical drying.* L. Manzocco et al., *Food Hydrocolloids*, Vol 122, Jan. 2022, 107117.
- (E) *Protein oleogels prepared by solvent transfer method with varying protein sources.* A. Feichtinger, E. Scholten et al., *Food Hydrocolloids*, Vol 132, Nov. 2022, 107821.
- (F) *Formation of protein oleogels via capillary attraction of engineered protein particles.* S.-S. Wang et al., *Food Hydrocolloids*, Vol 133, Dec. 2022, 107912

[0082] The images in FIG. 3 are described in the aforelisted references as follows:

- (A) SEM micrographs of whey protein isolate (WPI) at two different magnifications.
- (B) SEM micrographs of WPI particles shown at two different magnifications.
- (C) Optical micrographs of a freeze-dry WPI aggregate (upper panel), contrasted with aggregates produced using super-critical drying (lower panel).
- (D) SEM micrographs showing continuous WPI aerogel produced by freeze-drying (upper panel) or supercritical drying (lower panel).
- (E) Confocal microscopy of WPI of aqueous microgel pellets (upper panel) and corresponding oleogel (lower panel) made with WPI.
- (F) Confocal laser scanning microscopy (CLSM) of protein aggregates after homogenization by a stator-rotor dispenser (upper panel) or ball milling (lower panel).

[0083] TABLE 2 compares each of the references (Col. 1) on the basis of oil structuring mechanism, the starting protein, the colloidal form of the protein after heating, and the morphology of the respective microstructure observed in oil (Col. 5).

TABLE 2: Microstructure of previously published protein oleogels

Ref.	Oil structuring mechanism	Initial protein input	Colloidal form of the protein	Microstructure morphology (in oil)
(A)	hydrogel solvent exchange	WPI (Whey protein isolate)	fine-stranded or aggregate hydrogel mesh network	"mesh": smooth (<100 nm) to coarse mesh structure of original hydrogel
(B)	direct dispersion of structured freeze-dried particles	WPI	~150 nm heat-set protein aggregate/microgel particles	pseudo-spherical "pebbles": (~50-100 um) porous agglomerates for freeze-dry procedure
(C)	direct dispersion of structured freeze-dried/supercritical CO ₂ -dried particles	WPI	heat-set protein microgel particles	"mesh": 300-700 nm porous aerogel particles (drying method dependent)
(D)	direct dispersion of structured freeze-dried/supercritical CO ₂ -dried particles	WPI	hydrogel mesh network	"mesh": porous aerogel scaffold
(E)	direct dispersion of structured, solvent dried particles	WPI (Whey), EPI (Egg), PPI (Pea), SPI (Soy), Solanic® 200 PoPI (Potato)	~100 nm - 10 um heat-set protein microgels/aggregates (protein dependent)	pseudo-spherical "pebbles" (assumed spherical for sizing w/ Mie Theory)
(F)	ball-mill dispersion of protein particles and water	WPI, Tannic-Acid treated zein (hydrophilic treatment)	~200 nm spherical zein aggregates prepared by "bottom-up liquid-liquid dispersion (anti-solvent precipitation)"; ~150 nm heat-set WPI aggregates (de Vries et al. 2017)	spherical aggregates, capillary bridged with water

[0084] As is evident in FIG. 3, the mesh type microstructures (A), (C), and (D) and the pebble type microstructures (B), (E), and (F) of previous perspirations are unlike the fibrils and sheets for the oleogels of this disclosure shown in FIG. 1. Not to imply any limitation on the invention described and claimed herein, the makers of this invention suppose that differences in the preparation account for the differences in the protein microstructure. The oleogels of this disclosure benefit from optimized freeze channeling, drying, and oil dispersion. For example, in ref. (A), water is removed by solvent exchange to avoid agglomeration. Too much agglomeration may generate microstructures that are continuously interconnected as a solid mass, or in a rigid honeycomb shape.

[0085] The microstructure in turn is believed to affect the working properties of a microgel that contains it. Not to imply any limitation on the invention described and claimed in this patent application, oleogels having an underlying pebble type microstructure tend to be semi soft at room

temperature and melt completely upon heating. On the other hand, oleogels having an underlying microstructure in the form of a large solid mesh or honeycomb tend to be solid at room temperature, and remain solid on heating. The data presented in this disclosure demonstrates that the Goldilocks optimum is a microstructure that consists mostly of fibrils, sheets, and similar structures that have a high aspect ratio: they are sizeable in one or two dimensions, but substantially flat without extensive interconnectedness. Protein oleogels with these microstructures are pliantly solid or of spreadable consistency at room temperature, and release some but not all of their oil content gradually during cooking. The melt curve for a protein oleogel with this type of microstructure is discussed in a later section of this disclosure.

[0086] A theoretical elaboration for the relationship of microstructure to functional properties is as follows. A high surface area provided by a high aspect ratio of dry particulated protein structures can seed fat crystal nucleation or otherwise contribute to the alignment of aliphatic chains of unsaturated fatty acids, thus increasing intermolecular forces in the oil and leading to semisolid materials properties. Oleogels produced by adding oil directly to the initial protein isolate without denaturing or drying produces a suspension with poor oil holding and non-standing (non-solid) structures. Besides size and shape of the dry particulated proteins and microgels, rigidity of the particles may influence the mouthfeel because of their tribological (frictional) properties.

Overview of manufacturing process for making protein dry particulate

[0087] With few exceptions, protein isolate mixtures by themselves are sparingly soluble in water or aqueous solvents. Oleogels having protein as the oleogelator are usually made by a multi-step process.

[0088] Previously, some protein oleogels were made using an emulsion template approach, high internal phase Pickering emulsions (HIPE's) are formed whereby an emulsion is first prepared using protein as an emulsifier, followed by stripping off the aqueous phase. Alternatively, protein oleogels were made via solvent exchange. First, a hydrogel is prepared by dispersing the hydrophilic protein in water followed by heat treatment so that hydrophobic groups of globular proteins are exposed. This establishes hydrophobic interactions and results in strong physical and covalent interactions linking proteins via disulphide bridge formation. After the network has formed, water is removed in a stepwise manner using organic solvents with medium polarity to avoid any coalescence-induced disruption of the protein network. After completely replacing water with solvent, oil is induced into the system, resulting in an oleogel with less than 1% water. F. Manzoor et al., Food Hydrocolloids for Health, Vol 2:Dec. 2022.

[0089] The owners and makers of this disclosure have developed a particular strategy and methodology for preparing a dry particulate with beneficial properties. The procedure when empirically optimized promotes formation of an effective oleogel microstructure, with beneficial properties ensuing therefrom. The following sequence of steps is recommended to the reader.

1. Hydrate and solubilize the starting protein isolate into purified water;
2. Make the dissolved protein moderately acidic (pH of 2 to 4);
3. Denature the protein with heat below boiling temp for a modest period of time (such as 30 min), which may form a clear stranded gel;
4. Blend the denatured protein using a high shear mixer such as an immersion blender or overhead stirrer;
5. Flash freeze (for example, by spraying into liquid N₂);
6. Dry the frozen protein in a manner that doesn't disturb the emerging microstructure (for example, by lyophilization) to form a powder.

If the dry particulate is used to make a protein oleogel, the next part of the procedure is to add an oil gradually and gently to the prepared powder in a manner that maintains the preferred microstructure of the particles, including a high aspect ratio.

[0090] FIG. 4A is a flow chart that provides a scheme for evaluating and adjusting aspects of this procedure. With the objective of forming an oleogel that has the sensory and performance characteristics of this disclosure, the following adaptations of the preparation process may be beneficial.

Selection of the starting protein isolate. Beneficial are isolates that may have an initial microstructure of some kind and/or begin to form a microstructure early in the process — such as forming a clear stranded gel referred to in step (3) above.

Optimized conditions for formation of the gel from the denatured protein. Having a fine-stranded nano-structure may impact the morphologies and related physical properties of microstructures in the drying process. Adjusting the pH to between 2 to 4 helps. Other factors affecting structure formation that can be empirically optimized include salt concentration, temperature cycle, timing, and other details of this part of the process.

- *Optimized freeze channeling.* Instantly freezing the gel made from denatured protein helps minimize ice crystal size. Drying the frozen preparation in a vacuum forms microchannels as passageways for water removal. These events promote microstructure formation and consolidation, and preserve microstructures already beginning to form. Spraying the denatured protein into liquid nitrogen and lyophilizing at low pressure is effective. Depending on circumstances, a pellet freezer may be used for scale-up, such as a GEA brand nitrogen freezer. Another option is microwave assisted freeze drying, which reduces drying time and production costs compared with standard vacuum lyophilization.
- *Incorporating oil into the dried powder gradually with optimized shear.* The oleogel imaged in FIG. 1 was obtained by adding oil into the powder dropwise. Possible alternatives for scale-up include spraying, dripping, or otherwise adding oil into the powder and using a

paddle mixer to knead, or by tumble mixing. The oil is added to the microstructure with a calibrated (empirically optimized) amount of shearing.

Manufacturing process to obtain a dry structured dry particulate

[0091] The particulate obtained after drying can be manufactured and distributed as a dry food additive, without further processing. The dried product can be added to an oil by the consumer, it can be suspended in water, or it can be added directly to a food product (for example, as a thickening agent) during manufacture or cooking. When added to water or an aqueous liquid, the dried dry particulate will generally form a macrocolloid having a substantially smooth, emulsion-like organoleptic character. The term “organoleptic” refers to the sensory properties or characteristics of a substance that can be perceived by the senses, particularly taste, smell, appearance, texture and/or mouthfeel.

Properties and uses of the dry particulate in commercial products

[0092] Dry particulate preparations of this disclosure comprise proteins that are not water soluble. As a consequence, when added to an aqueous liquid, they form a dispersion, suspension, or macrocolloid that may alter and improve properties of the product.

[0093] In an ordinary protein suspension, solid protein particles are dispersed throughout a liquid medium. However, they typically do not form stable networks, and settle out of the suspension over time due to gravity. A protein macrocolloid is a colloidal system where protein aggregates form larger, stable structures dispersed in a liquid medium. Protein aggregates are distributed more evenly throughout the medium, and are less prone to settling out. The protein molecules in a macrocolloid interact with each other to form stable networks or aggregates, which contribute to the macroscopic properties of the system, such as viscosity, texture, and stability.

[0094] Types of colloidal structures are the following:

Sol: Colloidal protein particles are dispersed throughout a continuous liquid medium. The colloidal particles are small enough to remain suspended and do not settle out over time.

Sol particles are typically on the order of nanometers to micrometers in size;

Gel: A gel is a colloidal system in which a three-dimensional network of interconnected colloidal particles forms within a liquid medium. The network structure gives gels a semi-solid or jelly-like consistency. Gels can be formed by aggregation, cross-linking, or entanglement of colloidal particles;

Emulsion: Protein macrocolloids have emulsifying properties that enable them to stabilize emulsion droplets by reducing interfacial tension between immiscible phases;

Foam: A foam is a colloidal system composed of gas bubbles dispersed within a liquid or solid medium. The colloidal structure of foam involves the arrangement of gas bubbles, which may be stabilized by surfactants or proteins, within the continuous phase.

[0095] Macrocolloids made using the dry particulate of this disclosure can be used to replace thickening agents currently used in processed foods, cosmetics, and other products: particularly animal sourced ingredients, allergens, and artificial ingredients, such as methylcellulose derivatives. Some commercially valuable properties of macrocolloids in processed food products include the following:

Texture enhancement: Macrocolloids contribute to the desired texture of foods, providing attributes such as creaminess, thickness, and smoothness that improve the mouthfeel and overall sensory experience;

Viscosity control: Macrocolloids influence the viscosity of food products, providing thickening or thinning effects by controlling the flow behavior and consistency of a product;

Stabilization: Help prevent phase separation, sedimentation, or syneresis in food formulations;

Moisture retention: Helps prevent drying out, extends shelf life, and enhances the juiciness and succulence of the product;

Structural support: Helps maintain food product shape, volume, and integrity during processing and handling;

Flavor encapsulation: Protects flavors, aromas, and volatile compounds in food products from degradation and dispersion, thereby improving stability.

[0096] Sheets of macrocolloids can contribute to the texture of food products by forming layers or coatings. Macrocolloid sheets can act as barriers to moisture, gases, and flavors, helping to retain freshness and extend the shelf life. Sheet-like structures of macrocolloids can stabilize emulsions by forming interfacial layers between oil and water phases. Macrocolloids can serve as scaffolds or frameworks that hold other ingredients in place. In baked goods, sheets of macrocolloids may contribute to the formation of flaky layers or crusts during baking, while in fried foods, they can influence the crispness and texture of the exterior coating.

[0097] Macrocolloid fibers can form networks or matrices that contribute to the thickening and gelation of food formulations, helping to create a smooth, creamy texture and improve mouthfeel. In baked goods, fibers may contribute to the formation of a fibrous crumb structure, while in meat products, they can influence the texture and mouthfeel of the final product during cooking. Macrocolloid fibers can also act as fillers or binders in food formulations, helping to improve the

texture, consistency, and yield of processed foods. They provide bulk and volume to products like meatballs, sausages, and meat substitutes, enhancing their sensory attributes and nutritional profile.

[0098] In cosmetics, macrocolloids can enhance texture, improve stability, retain moisture, control viscosity, cause thickening, gelling, or film formation, and improve sensorial experience.

Modes of combining the oil with the protein to make an oleogel

[0099] In general terms, the mixing of oil into the protein preparation is done in a manner whereby particles of solid protein are lifted into suspension and separated from one another, gently breaking apart microparticles that are loosely associated but not structurally interconnected. The objective is to minimize triturating or substantially damaging the individual microparticles that have an optimal size and aspect ratio.

[0100] The mode of adding the oil to the protein powder can be continuous or discontinuous. For gentle manual or automated paddle mixing methods, partial oil additions are carried out in plurality of tranches, each comprising adding a portion of the oil, and mixing the portion into the oil into the protein before adding more. For example, the combination can be mixed in between each addition for 1 to 5 min, with complete oil incorporation achieved over a total of least 10 or 20 min, up to 30 or 60 min or more. Alternatively, the oil may be dripped, sprayed or flowed into the powder on a continuous basis with continuous or intermittent mixing: for example, over a period of at least 10 or 20 min, up to 30 or 60 min or more.

[0101] When using a stirring apparatus, the shape and pliability of the blade is chosen to optimize maintenance of the microstructure. To mix by stirring around a vertical axis, a hydrofoil type blade may provide the gentlest agitation with the least shear. The blade profile creates nearly uniform flow with the minimum rpm and power input, and is especially effective for materials that can be damaged by higher shear. For example, large diameter hydrofoils of one-third of the vessel diameter driven at low rpm may work well. Marine style propellers and axial flow turbine impellers may be used as an alternative. The chosen mixing speed used (in rpm) is slow and gentle, increasing the time required to complete the procedure but lessening impact on individual particles of the microstructure.

[0102] Alternatively, when using a tumble mixer, the size of the chamber is chosen to match the lot size of the preparation, decreasing the distance and impact of falling in the downward part of the rotation. Again, the mixing speed (in rpm) is slow and gentle, increasing the time required to complete the procedure but lessening impact on individual particles of the microstructure.

[0103] In some circumstances (depending on the protein source and the desired outcome), the protein powder can be added to the oil rather than the other way around. However, this requires that the buoyancy of the dry protein in the oil be overcome, often implying more vigorous mixing, which imposes greater shear. A third alternative is to combine the full amount of oil with the protein in a

single step. This is followed by gentle mixing under slow tumbling or gentle flow that helps preserve the microstructure: for example, over mixing times of 10 or 20 min or more.

[0104] The mode of mixing is selected from amongst these alternatives and developed empirically to impose an optimized amount of shear, thereby creating an oleogel comprising protein particles dispersed in the oil that have a microstructure with a high aspect ratio. Too much shearing of the protein during oil incorporation will unnecessarily triturate or grind the particles to a smaller median size and rounder shape that is less capable of structuring the oleogel to have desirable oil release properties. Shearing is a function of shear stress and the time over which the shearing is applied. Shear stress depends on the manner of combining the oil into to the powder and the equipment used.

[0105] If the dried protein preparation comprises a microstructure that is already mostly in the form of fibrils, sheets, or other particles with a high aspect ratio, the amount if shear is calibrated to minimize impact on individual particles, breaking apart loose interconnections between particles, but without reducing the median diameter of the particles by two-fold or more, or decreasing the median aspect ratio of the particles by two-fold or more. If instead the dried protein comprises a microstructure that is mostly in the form of larger solid, meshed, or honeycombed blocks, the amount of shear is calibrated differently. In this case, the objective is to break apart the blocks of protein in such a way as to generate and maintain particles that have a median size and a median aspect ratio that structures the oleogel to have desirable oil release properties.

Detailed protocol for making dry particulate and oleogel

[0106] FIG. 4B is a flow chart of the current method used at Shiru to prepare oleogels having desirable characteristics and properties. Putting this in terms of a Betty Crocker® style recipe, the procedure is as follows.

[0107] Ingredients:

- 1) protein isolate (for example, potato protein fraction Solanic®300 from Royal Avebe, Veendam, Netherlands)
- 2) baking soda
- 3) vacuum bag
- 4) filtered water
- 5) food safe liquid nitrogen
- 6) edible vegetable oil (for example, high oleic acid sunflower seed oil).

[0108] Procedure:

[0109] *Hydration and solubilization:* Measure out 190 g of potato protein isolate powder into one or two large containers. Add 2.8 L of filtered water to the protein. Stir at 600 rpm on a magnetic stir plate with a large magnetic stir bar for at least 20 min; or until the solution is no longer opaque,

with no visible chunks of the protein powder. The resulting suspension will be transparent and tinted brown.

[0110] *Creating a protein aggregate.* Prepare a sous vide (a vacuum pack) of the hydrated protein using, for example, equipment from Anova (Lewis, Delaware). This is done as follows: dress a sous vide container in a jacket to decrease heat losses during the temperature ramp and gelation cycle. Fill the sous vide container to 9 to 10 quarts of liquid. Set the temperature to 92°C, and begin preheating.

[0111] Adjust the pH of the hydrated protein to 4 by adding baking soda, monitoring with a calibrated pH probe. The baking soda is added in small (< 0.5 g) increments, allowing about two min between each addition for the pH to equilibrate. Once the pH has been adjusted and stabilized, pour the acidified protein mixture into sous vide vacuum bags, and seal using the vacuum sealer. This typically generates five bags of about 500 mL each.

[0112] Place the bags in the heated liquid in the sous vide container. Start timing when the liquid reequilibrates to 92°C (the gelation temperature). Maintain at this temperature for 30 min. Then remove the sous vide bags from heat, and immerse the bags in ice cooled water for 10 to 20 min. A clear stranded gel will form.

[0113] *Creating a high shear mix:* Combine clear gels from the sous vide bags in a cambro (or other large plastic food safe container). Add 500 mL of filtered water to the gel mass. Begin shearing with a high shear mixer or an immersion blender (such as a Breville Control Grip Immersion Blender, combined with an overhead stirrer such as the IKA Microstart set to at least 10,000 rpm). The resulting solution will be homogenous throughout. It will appear opaque due to incorporation of air pockets upon mixing. If a foam forms on top, allow time for the foam to settle.

[0114] *Flash freezing in liquid nitrogen (LN2).* Clean and rinse a mister spray apparatus (such as a HeritageQ™ brand Food Grade mister) with a food safe sanitizing solution and water. Fill a cryogenic container with liquid nitrogen. Spray the high shear protein mix directly into the liquid nitrogen, while breaking ice dry particulate that may form using a strainer. Once the container is full, collect frozen beads using a mesh, and pour the beads into labeled containers. Repeat spraying and collecting as necessary. The frozen beads may be stored at this point, or placed directly into freeze dryer trays.

[0115] *Freeze drying / lyophilization:* Load the frozen material from the preceding step into trays, and place them in the lyophilization apparatus (such as a Harvest Right freeze dryer). Begin the drying cycle. When samples reach room temperature, and the pressure is lower than 200 MT, remove the dried protein from the freeze dryer. Disperse into a powder if necessary. Moisture at this stage should typically be no more than about 5 percent.

[0116] *Oil incorporation:* Delicately place the freeze-dried protein into large beakers or cambro. Begin to add the oil gradually, stirring between additions with a paddle or rubber spatula. Combine by performing multiple tranches or cycles of adding oil into the powder, and mixing

between each addition. For example, add about 25% of the oil at a time, using smaller amounts towards the final addition. Alternatively, the oil can be added to the protein gradually by dripping or spraying with continual mixing. To produce oleogel having the microstructure shown in FIGS. 1A and 1B, the oil was added discontinuously with mixing in between of 1 to 5 min, for a total time to complete the combining of between 10 and 30 min.

[0117] Knead the final mixture (for example, by hand or using a rubber spatula) until the mass is homogenous. This forms an oleogel dough with no visible heterogeneity of dryness or oiliness.

Nature of the protein used to make the dry particulate or oleogel

[0118] Mixed protein preparations for making dry particulate and oleogels according to this disclosure may have one or more of the following properties:

high solubility (well over 5% (wt/vol)) in low ionic strength aqueous buffers or pure water.
Isolates having a low median mol. wt or that have been hydrolyzed tend to be more soluble;
consistent (lot-to-lot) higher-order protein structure for robust and reproducible heat-onset aggregation and gelation;
an appropriate isoelectric point (pI) so that the protein remains soluble during the low pH denaturation step;
when heated, the protein forms a clear, fine-stranded and relatively homogeneous hydrogel network.

[0119] High aqueous solubility in the relevant processing conditions facilitate formation of relatively homogeneous clear gel intermediates, which may subsequently yield relatively homogeneous protein structures and oleogels with superior texture and oil-holding characteristics.

[0120] Mixed protein preparations with high solubility or dispersibility in low-ionic strength solutions (aqueous, organic, or binary solvent mixtures) are generally amenable to freeze-structuring: particularly protein dispersions lacking the dense micron- or multi-micron-scale amorphous aggregates common to many conventional plant protein dry ingredients. Such proteins may be prepared from a botanical source by micro-filtration, and/or dried by methods intended to maximize their dispersibility in solution (For example, optimized spray drying and/or dry milling or sieving). Solution-state protein inputs, (protein solutions not subjected to drying prior to the freeze-structuring process) may work well.

[0121] Proteins that are suitable for testing for the manufacture of oleogels or structured particles according to this disclosure include the following:

Concentrated, solution-state potato protein isolate, shipped and received as solution directly from a commercial potato isolate manufacturer: for example, Solanic® 300, which is commercially available from Royal A`vebe in the Netherlands;

A soy protein isolate, microfiltered and available as both a highly dispersible powder and liquid solution;

A high-solubility, chemically modified, pea protein isolate; commercially available as a powder for dairy alternatives and viscosifying applications;

A high-solubility, hydrolyzed pea protein isolate with gelling functionality;

A high-solubility canola protein isolate with gelling functionality;

Plant proteins, either single proteins or mixtures, produced by heterologous expression and precision fermentation.

[0122] Other potential candidates include RUBISCO, processed as a dry powder or liquid stream from plant leaves, which has high solubility and robust gelling characteristics; and zein, gluten, or other prolamins, used in an organic solvent or binary solvent.

[0123] Non-plant proteins can be used to generate morphologically similar (but not identical) macrostructures via rapid-freeze channeling. For example,

whey protein isolates and concentrates, optionally varying in the ratio of major protein constituents (β -lactoglobulin, α -lactalbumin, bovine serum albumin) to fine-tune interprotein chemical (disulfide) bonding, intermediate aggregate/gel densities, and long-range dry particulate structures to achieve a wide range of textural outcome;

dry particulated whey proteins with high dispersibility in solution;

other high solubility/dispersibility egg or dairy proteins;

hydrolyzed proteins with high solubility and gelling propensity;

non-plant proteins, either single proteins or mixtures, produced by heterologous expression and precision fermentation.

[0124] As an alternative to mixed protein isolates, some aspects of this disclosure may be implemented using a recombinantly expressed protein or mixture thereof as the principal source of protein, or as an additive. U.S. Patent No. 11,439,159 (Hume et al., Shiru Inc.) provides information on how to select individual proteins with a target function, and how to express and test the selected proteins. Particular recombinant gelation proteins are provided in PCT/US2023/075601.

[0125] In some contexts, the methodology provided in this disclosure can be used to produce oleogels or particulates made from materials other than protein. The oleogelator in this category includes functionalized celluloses such as methylcellulose, food-grade gums such as gellan,

carrageenan, and agar, and other food-grade fibers or polysaccharides such as citrus fiber, maltodextrin, pectin, B-glucans, konjac starch, alginate, and chitosan.

Nature of the oil used to make the oleogel

[0126] The oil phase of the oleogel can be any type of oil or a blend of oils suitable for the intended purpose. For use in foods, cosmetics, and pharmaceuticals, the oil will be suitable for human consumption. In this context, the oil is typically a mixture of fatty acids and/or fatty acid esters wherein the lipids are primarily saturated, monounsaturated, polyunsaturated, or a combination thereof, and typically not hydrogenated. Examples of suitable oils include canola oil, soybean oil, sunflower oil, olive oil, palm oil, and coconut oil. Suitable oils for personal care products may or may not be characterized as edible, as long as they are safe when ingested or topically applied on frequent occasions. Different oils may pack differently, which in turn may affect the properties of the oleogel product.

[0127] Physical properties and/or sensory properties of an oleogel of this disclosure can be altered and optimized empirically by testing and selecting from a variety of starting protein isolates, by testing and selecting the oil, by adjusting the protein to oil ratio, and by adjusting the method of preparation. In principle, the oleogel can be formulated to have a range of desired properties and consistency, from a soft smooth consistency (for example, for use in spreadable foods and cosmetics), to a firm consistency that releases oil or stays solid when cooked (for example, for use in plant-based meat substitutes).

Optional protein ingredients or additives to promote microstructure formation

[0128] The detailed protocol provided above promotes formation of high aspect ratio (HAR) microstructure by rapid freezing, followed by freeze channeling to remove the water. To form oleogel from the dried protein, the oil is added gently to preserve the structural features.

[0129] Other manners of drying the denatured protein and adding oil can also be used. Certain additives promote the formation of HAR microstructure that gives the oleogel a desired oil release profile,

[0130] For example, the solubilized or suspended protein may be treated to promote crosslinking between smaller particles before the solution or suspension is flash frozen and dried. Suitable agents include the enzyme transglutaminase, which catalyzes protein crosslinking between glutamyl and lysyl residues in the protein, and certain oxidative enzymes. Other potentially suitable enzymes include laccase, tyrosinase, and peroxidase. M. Motoki et al., Trends Food Sci Technol 9(5):204-210, 1998; N.S. Sulaiman et al., Int Food Res J 29(4):723-739, 2022. The amount of crosslinking agent and reaction time are titrated so that the protein in the preparation forms a loose

association of particles of an appropriate size, which can then be dispersed optimally during the oil incorporation phase.

[0131] Formation of fibrils and related structures can also be promoted by adding additional components to the protein preparation at an appropriate time during the procedure. For example, a maltodextrin or other polysaccharide can be used to create a glycoconjugate in the form of fibers, optionally by applying a physical process such as needleless ultraspinning. M Gibis et al., Appl. Sci. 2021, 11:7896-7909.

[0132] As an alternative or in addition to freeze-structuring, other upstream routes to microstructuring of proteins include alternative solvent evaporation processes and bulk-spinning or film-casting methods. Y. Shen et al., ACS Nano 2021 15 (4), 5819-5837; DOI: 10.1021/acsnano.0c08510.

[0133] Other potential additives to include in the preparation of a suitably structured dry protein dry particulate include the following.

[0134] *Solubility modifiers (at the thermal gelation step)*: small molecules known to enhance protein solubility (small amounts of salt, sugars, or other osmolytes) can promote fine-tuned gel network structures and exert subtle impacts on the oil-holding and shear-sensitivities of final dry-particle structures. These types of molecules may be natively present in certain input ingredients, or intentionally added/optimized to achieve the desired properties.

[0135] *Colloidal stability modifiers (for the homogenized microgel mixture)*: desirable charge-shielding or surface interactions with small molecules or other biopolymers (proteins or polysaccharides) can reduce undesired agglomeration prior to freezing. This can reduce the inclusion of low-aspect-ratio agglomerate inclusions that disrupt the freeze-channeled high-aspect ratio structure and lead to sub-optimal shear-sensitivity or oil holding properties.

[0136] *Colloidal structure modifiers (such as enzymes)*: chemical or enzymatic modification of the homogenized, colloidal microgel particles can be used to reduce or enhance specific intermolecular interactions within and between colloidal protein particles. This can be used to adjust the properties (such as viscosity) of the solution immediately prior to freezing, as well as fine-tune surface properties and the porosity or density of the dry particulate structure. Partial enzymatic hydrolysis can be used for viscosity reduction or to reduce the overall extent of covalent bonding and/or steric entanglement between particles prior to freezing. Enzymatic crosslinking can be used to increase viscosity and the overall extent of covalent bonding. In addition to fine-tuning the properties of the process intermediates and dry particulate structures this can also have an impact on the textural attributes of the final oil-incorporated system.

[0137] Alternatively or in addition, microstructure in a dry particulate or oleogel can be promoted by using an oleogelator ingredient that already has a layered structure. Homogeneous cellular macromaterials are often built from repeating units that comprise particles with a high aspect ratio (HAR). For example, fungal mycelium (a macroscopic aerogel) comprises fungal hyphae

(typically 1-10 μm in diameter or more). Following cultivation by liquid or solid-state fermentation), fungal mycelium is dried, and a porous network of loosely associated HAR hyphae fibrils is preserved in the dried state. Dry mycelium particles or dry mycelium cubes, each containing porous networks of HAR microparticles, may be soaked in oil, then shear-dispersed and homogenized to produce an oleogel.

Fine-tuning properties of the microstructure

[0138] Once the high aspect ratio (HAR) fibrils or sheets have been formed, the oil may be added slowly to preserve the structure as much as possible. However, if the user wishes to work with HAR particles of more modest dimension, the microstructure particles can be broken up to any extent desired — for example, mixing before or after oil addition with more shear, or putting the particles through a brief grinding and/or screening process — thereby fracturing fibrils and sheets, reducing their longest dimension. Alternatively, the fracturing can be done to the dry particulate by jet, blade, or ball milling — or to the assembled oleogel by milling or homogenization.

[0139] Other properties of the microstructure can be adjusted by adjusting the protein source and/or the additives in the mixture prepared for drying. The protein preparation may be functionalized via bioconjugation, enzymatically modified (partially hydrolyzed), or further fractionated to modify solubility and tune surface chemistry of the preparation when used to manufacture aggregated and dry particulated forms. For example, adjusting the number of reactive thiols in the protein mixture may alter long-range (covalent) bonding between proteins in the microstructures, further impacting the final dry particulate density/morphology and shear-sensitivity.

[0140] Another means to adjust properties of the microstructure is to modify the drying process: for example, the freeze rate and/or packing density of the protein solids. The freezing rate determines the size and morphology of the ice-crystal inclusions responsible for forcing the protein into the highly interconnected parent structure that contains high-aspect-ratio sheets and fibrils. Freeze rate modifications can be made by: 1) changes to the solute/solvent system that significantly change the freezing temperature or freeze rate of the solution (such as alternative solvents, antifreeze compounds or antifreeze proteins); 2) changes to the physical drop size, feed rate, and feed temperature of the solution delivered to the cryogenic bath/surface; 3) modification of the cryogenic liquid (alternative liquid cryogens or stirring/agitation of the cryogen bath); or 4) replacement of liquid the bath with a cryogen chilled metal surface with high thermal-conductivity.

Image analysis for characterizing microstructure

[0141] Dry particulates and oleogel preparations according to this disclosure can be characterized visually by light microscopy, confocal microscopy, scanning electron microscopy (SEM), or other technique that visualizes microparticles in the micron range. Particle aggregates in a

dry particulate can be imaged as a whole. Individual microparticles can be characterized by imaging them separately, and thereafter determining their shape and dimensional measurements.

[0142] The light micrographs shown in FIG. 1 were taken from a protein oleogel made according to the protocol provided below. The oleogel was dispersed in liquid vegetable oil that was substantially free of water: 0.1 grams of the oleogels mixed with the liquid oil for 1 minute using a benchtop vortexer (Vortex® Genie 2) on its highest setting. The resulting suspension was directly imaged on a light microscope using an oil immersion 100x microscope objective.

[0143] FIGS. 2A and 2B are images obtained by scanning electron microscopy (SEM) for dry particulate preparations made using potato protein and whey, respectively. Individual dry particle aggregates were gently cross-sectioned using a razor blade to access the particle interior. Samples were attached to an SEM stub/stage with double sided copper tape and sputter coated with 15 nm of platinum. Imaging was performed using a FEI Scios dual-beam Scanning Electron Microscope in low vacuum mode.

[0144] Each row of FIG. 2A is a separate preparation of dry particulate made from potato protein. Left and right images in each row are magnification levels 200x and 1000x. Row 1 was obtained from a standard bench-top preparation in which a 5% (wt/vol) homogenized gel slurry was flash-frozen and dried. The images include plates and ruffled or ridged plates that include intermittent fibrillar features. There are few interconnections. Long axes of these structures is generally >100 µm; plate thicknesses is of the order of 1 µm. Rows 2 and 3 were obtained from larger-scale preparations using pilot-scale manufacturing equipment, in which a 5% (wt/vol) homogenized gel slurry was flash-frozen and dried. Loosely connected high aspect ratio structures are shown. There are also less ordered fibrils but still loosely connected irregular structures, <100 µm in length.

[0145] The images in FIG. 2B were obtained from a particulate made with whey protein instead of potato protein. Solubilized whey protein isolate (6% wt/vol) was adjusted to pH 6.5 prior to heat denaturation and gelation, producing an optically clear gel intermediate, which was then freeze dried. Rows 1 and 2 are two interior regions of the same particle aggregate at 200x and 1000x magnification. There are loosely connected plates with fibrillar features and a high aspect ratio. The cilia like structures may constitute rare interconnections between plates.

[0146] It is a hypothesis of this disclosure that the plates and fibrils appearing in the protein oleogels of this disclosure are obtained by dispersing the larger structures shown in the SEM images so that individual plates and fibrils separate. If the dry particulate is combined with a liquid oil gently with minimal shearing, the few interconnections between microparticles will mostly break apart, but the sheets and fibrils will mostly remain intact.

[0147] The dilution protocol used to obtain FIG. 1 constitutes an assay assessing the character and median aspect ratio of microparticles — either in an oleogel, or the dry particulate. To prepare the particulate for analysis, a protein suspension is made by gently adding and incorporating a liquid oil, so as not to overly triturate or shatter the individual microparticles. The oleogel or the protein

suspension is then diluted in liquid oil (such as vegetable oil) to separate the microparticles sufficiently so that they may be individually characterized and measured. The amount of dilution that is required depends on the ratio of protein to oil in the oleogel or protein suspension, and the size of particles, and is empirically determined. Dilution of the oleogel to a ratio of 1:2, 1:5, 1:10, 1:20, 1:50, or 1:100 or a range therewithin (in terms of oleogel to final suspension, wt/vol) may be appropriate. Suspension and dilution of a dry particulate preparation to a ratio of 1:50, 1:100, 1:200, 1:500, or 1:1,000, or a range therewithin (in terms of particulate to final diluted suspension, wt/vol) may be appropriate.

Image analysis of FIG. 1

[0148] The superior oil retention and release properties of the oleogels of this disclosure are believed to be a function of the microstructure embedded in the oil. The solid microstructures of some of the previous protein oleogels results in the oleogel being hard, unspreadable, and resistant to releasing the oil upon heating or shearing. The pebble or granular microstructures of some of the previous protein oleogels results in the oleogel liquifying at low temperature, and being unstable upon storage or when emulsified in water. Oleogels having a microstructure of larger particles will often be gritty and have an unsatisfactory mouthfeel.

[0149] FIG. 5 quantifies some of the features that can be seen in the micrographs shown in FIG. 1. The fibrils shown in insets A1, A2, A3, and A4 (left) were characterized by outlining resolved edges of the structures (center) and representing their features in a line drawing (right). Measurements were taken using a pixel-to-micron conversion derived from the embedded 20 μm scale bar. The selected structures are micron-scale protein tubes, fibers, or ribbons with a characteristic diameter or thickness (T) defining their narrowest dimension, which ranges from 0.5 to 5, typically 1 to 3 microns.

[0150] Some of the fibrils in this preparation constituted a single linear segment with a defined length (L). Other fibrils were more complex structures comprising several linear segments of length (L) joined at branch points (indicated by circles in the line drawing). The maximum length (ML) of each branched structure was calculated here as the sum of the length of its most parallel linear segments, which ranged from 10 to 100 μm . to be larger than 10 microns. The aspect ratio (AR) of each microstructure was calculated as its maximum (ML) length divided by its diameter or thickness (T). Aspect ratios as high as 10 to 100 were frequently observed.

[0151] The sheet-type structures shown in insets B1, B2, B3, and B4 were irregular plates, having a characteristic width (W) and height (H) determined as the longest apparent axes of an idealized two-dimensional polygonal structure. In this preparation, W and H ranged from 10 to 100 microns. The thickness (T) of a plate is assumed to be no larger than the thickness of fibrils observed in the same preparation or micrograph: in this instance, about 1 to 3 (average of 2) microns. Plates may or may not have one or more associated or embedded fiber, tube, or ribbon-like features. Such

features are suggested by the coincident termination at the edges of the larger continuous structure. Plate to plate interconnections (“ridges”) or plate to fibril interconnections (“seams”) often extended along the width or height of a plate. These extended interconnections are indicated in insets B1, B2, B3, and B4 as dotted lines. Each of the plates may be flat, bucked, or folded.

[0152] The high aspect ratio (due to the low thickness of the fibrils or sheets) helps to maximize the effective oil absorption capacity of the micro structurant (the oil to protein ratio), while maintaining a semi-solid, lubricating system that lacks the grit associated with dispersing low aspect ratio protein particles in oil.

[0153] The percentage of total protein or denatured protein in the preparation that is in the microstructure is typically 20% to 100%, with higher percentages (40%, 60%, or more) representing a more efficient use of the protein content for structuring and stabilizing the oil. Additional protein, carbohydrate, or other components may be included, for example, to catalyze or promote formation of the microstructure, to stabilize or preserve the oleogel during storage, as a pharmaceutical ingredient, and/or to increase nutritional value.

[0154] Ratio of protein to oil in a protein oleogel preparation are typically 2:98 to 40:60 (wt/wt). Ratios at or above 25:75 will generally be less creamy, and accordingly will be less desirable in many contexts. Lower ratios (5:95, or 3:97 to 10:90) are generally more efficient use of the protein and therefore more cost effective in food production. Lower ratios are also appropriate for use with highly saturated oils, such as coconut oil and palm oil used in baking, and mango butter used in cosmetics. Saturated oils generally are solid or semi-solid at room temperature, but may benefit from incorporation into an oleogel to improve melting and/or oil retention characteristics.

Assessing physical properties of protein oleogels

[0155] Besides microstructure, protein oleogels can be assessed by physical criteria. This enables the user to compare oleogels made via modified procedures, and iteratively adjust the process and reassess the oleogels obtained thereby.

[0156] FIG. 4C is a melt curve that compares the behavior of coconut oil with two preparations of protein oleogel (determined each in duplicate). Traditional (non-protein) oleogels are commonly able to hold onto oil but don’t release it, resulting in a material that is waxy or plastic-like in consistency. In plots of this type, this would show as a mostly horizontal line near the top. Protein oleogels having a rigid mesh or honeycomb structure would show the same sort of horizontal melt curve. In contrast, unstructured coconut oil melts completely at low temperatures, showing as a steeply descending curve that goes to zero. Protein oleogels having a meshwork that consists mostly of pebble shapes or other small particles would show a similar melt curve.

[0157] The oleogels of this disclosure achieve a happy compromise. They are pliable solid or spreadable at room temperature, and release oil upon heating and/or shearing. Curves for several oleogel preparations in FIG. 4C are downward sloping, showing release of a substantial proportion of

oil they contain at typical cooking temperatures. The curves do not descend all the way to zero, because not all of the oil is released. There was some residual protein material after-heating to cooking temperature ($160^{\circ}\text{C} \equiv 320^{\circ}\text{F}$).

[0158] Oleogel texture can be objectively measured using an analysis apparatus such as the AMETEK™ Brookfield CTX texture analyzer. Texture profile analysis (TPA) is done via a double compression test to 50% deformation at 0.5 mm per sec. using a 5 kg load cell. The readout is the peak force during first compression reported in Newtons (N). Total work (mJ), chewiness (N), gumminess (N), springiness, cohesiveness, and adhesiveness (mJ), can also be determined. Hardness tends to be the most differentiating measurement. Oleogels can also be characterized according to the effect on viscosity at room temperatures or upon heating.

Assessing emulsifying properties of protein oleogels

[0159] Emulsifying properties were determined at several stages during the oleogel production process. The relative proportions of oil, water, and protein were maintained for all samples. A solution of native potato protein foamed but did not form a stable emulsion and separated quickly. A gelled potato protein solution showed some ability to emulsify, but a substantial portion of free oil remained, and the mixture separated into two phases. However, dry particulated protein obtained after denaturing and freeze drying produced a firm, stable, bright white emulsion.

[0160] The protein oleogels of this disclosure create stable emulsions without the need for additional stabilizers. At overall use rates of 40 to 60%, the oleogel can be combined with water using medium to high shear. The oil-in-water emulsion formed thereby is stable for at least four or eight weeks at ambient temperature, with no evidence of phase separation.

[0161] Emulsions may be destabilized by any one of four different mechanisms: creaming or sedimentation, flocculation, coalescence, and Ostwald ripening. Creaming occurs when the emulsion separates due to a density difference where the lighter oil droplets rise to the surface. Sedimentation follows the same mechanism but occurs typically in water-in-oil emulsions where denser water droplets accumulate on the bottom of the emulsion. Creaming or sedimentation can be hindered by having a high viscosity continuous phase. Flocculation occurs when the emulsion droplets aggregate and thereby form larger units. Coalescence occurs when smaller droplets merge together forming a larger droplet. This results from droplets coming in contact with each other, rupturing the interfacial film and leading to phase separation. Ostwald ripening occurs when the smaller drops first dissolve in the continuous phase, and then coalesce into larger drops to reach thermodynamically more stable state. The oleogels of this disclosure are resistant to all such forms of destabilization.

Assessing sensual properties of oleogels

[0162] Sensory properties of oleogels can be assessed systematically while still essentially a composition of protein and oil. A standardized method for assessing lubricity, smoothness, hardness, and adhesiveness is as follows. The determination is done by human volunteers using thumb and index fingers. TABLE 3 outlines the protocol.

TABLE 3: Subjective tests for sensory attributes of fats, oils, and oleogels

Attribute	Definition	Method to test	Low benchmark (scale value)	High benchmark (scale value)
Hardness	Force required to push into a sample	Gently press with index finger	Lard (1)	Tallow (5)
Lubricity or oiliness	Ability to reduce friction. Slippery, amount of liquid oil	Rub oleogel between index finger and thumb until melted	Tallow (3)	Coconut oil (5)
Smoothness	Opposite of gritty, has a smooth texture, it does not feel grainy or sandy when rubbed or spread	Rub oleogel with thumb and index finger and feel the number or size of particles	Lard (3)	Coconut oil (5)
Adhesiveness/ Stickiness	How the sample adheres to a surface	Place sample between thumb and index finger and separate. Higher resistance means higher stickiness	Coconut oil (1)	Tallow (2.5)

[0163] FIG. 6A shows the five-point scale for each of the four values. Each scale is benchmarked using lard, butter, shortening, coconut oil, and tallow, as shown. For a protein oleogel preparation prepared according to the protocol set forth above, the values were as follows: Hardness = 2, lubricity = 4.5, smoothness = 5, adhesiveness = 1. These values are especially appropriate for food preparation: the oleogel preparation tested here is not hard or sticky, but is smooth and lubricating.

[0164] FIG. 6B presents the test data as a spider plot. The solid line marked “Shiru oleogel 1” was made by the optimized process outlined above. The high values for lubricity and smoothness are prominent. The line with sort dashes marked “Shiru oleogel 2” was an early product made with a different process that did not include pH adjustment, denaturation, or immersion blending.

[0165] FIG. 7 is a flowchart for iteratively and empirically optimizing process control variables (right column) by measuring sensory properties using internal benchmarks (left column) and material properties (middle column).

Assessing properties of foods made with protein oleogels

[0166] The sensory properties of oleogels can also be determined systematically when combined with other ingredients in the manner of food preparation. Products made using different oleogel preparations can be compared with each other and with products made with more traditional structured fats and oils.

[0167] A protein oleogel made according to the protocol put forth above was compounded into a cooked patty. The ingredients are listed in TABLE 4. In this sort of test, use of a flavoring agent is optional. Flavoring was not used here, so that the volunteer test subjects could focus on texture and other subjective features.

TABLE 4: Ingredients for test patties

Ingredient	Percent (wt/wt)
Textured pea protein (TVP)	19
Salt	0.8
Water	38
Methylcellulose	1.5
Soy protein isolate	3
Fat (oleogel or other oil)	15
Water	19
Cornstarch, native	3

[0168] The procedure for preparing the patties was as follows: Soak TVP in first portion of water and salt for 30 min. Disperse methylcellulose and soy protein in melted coconut oil, and add additional water to form an emulsion. Add cornstarch and emulsion to soaked TVP and mix until incorporated. Cool at 40°F for 2 hours. Form into 25 g patties and bake at 375°F for 14 min.

[0169] Two controls were prepared with an oil instead of the oleogel. The positive control was 15% coconut oil, and the negative control was 5% coconut oil baked for an additional 8 min.

[0170] FIG. 8A are graphs of the cooking loss (percent weight loss after baking) and hardness. The hardness was assessed using a Brookfield CTX texture analyzer at 40% compression, internal temperature of 50-70°C. Patties made with oleogel were compared with two controls: patties made instead with 15% coconut oil, (the positive control), and patties made with just 5% coconut oil, baked for an additional 8 min (the negative control). Compared with the positive control, the oleogel reduced cooking loss from an average of 17% to 13.6%. The hardness of the oleogel (5.5 N) was comparable with hardness of coconut oil (6.3 N).

[0171] FIG. 9 shows a scale used to evaluate the patties by sensory criteria. Qualitative descriptive analysis (QDA) was done using a panel of 24 trained volunteers to assess burger patties

for firmness, juiciness, and fattiness. The test was done blinded, so the panel members did not know which of the patties were made with the oleogel, and which were made with coconut oil. Each of the criteria were benchmarked using other products. Benchmark patties were made with oleogel, or with low (5%, Shiru B), medium (15%, Shiru A), and high (20%, Shiru C) levels of coconut oil.

[0172] FIG. 8B shows the results. Firmness, juiciness, and fattiness scores for patties made with oleogel (“Shiru alt fat”) were assessed by the panel as similar to the positive control. There was a significant difference from the negative control, demonstrating the sensitivity of the responses.

[0173] FIG. 10 shows the spreadable texture of a preparation of protein oleogel as manufactured according to this disclosure. This is anhydrous oleogel: it looks more like a cream but isn’t wet. When mixed with water, the oleogel forms an emulsion that is shinier and has softer edges.

d[0174] FIG. 11 compares the cooking of test patties made with coconut oil or with protein oleogel. The oil melted and bubbled away from the patty made with coconut oil (left). In contrast, oil in the oleogel patty mostly stayed within the patty (right).

Commonly used fats and oils in foods that can be replaced with oleogels

[0175] The oleogels of this disclosure can be used *mutatis mutandis* to replace structured fats and tropical oils, especially those that are solid, semisolid, or spreadable at room temperature, but transition to a liquid or something softer when heated: For example, beef tallow, pork back fat, lard, other meat rendered fats and extracts, coconut oil, palm oil, hydrogenated oil, margarine, other types of shortening, and butter.

Food products incorporating protein oleogels

[0176] The oleogels of this disclosure can be used any food product that currently has a substantial oil and/or fat content. Protein oleogel may be used as a partial or complete substitute for animal fats, for fats that are considered nutritionally unsatisfactory, or for fats that are difficult or expensive to produce. Alternatively, the user may wish to use the oleogel in a food product simply because the oleogels of this disclosure have superior properties. Any of the foods listed in TABLE 1 above can be prepared using an oleogel according to this disclosure in place of the oleogel specified in the cited reference.

[0177] Incorporation of oleogels into a food product or production method may be empirically optimized by the user. As a guide, the user may wish to start by adapting a known recipe or manufacturing process for a particular processed food product by replacing entirely or in part one or more oils, fats, or oily structures in the recipe or process with roughly an equal or equivalent mass of oleogel. For foodstuffs such as marbled meat or bacon, the process may involve seeding a scaffold structure with pockets of meat-like protein and meat-like fat to visually and textually mimic the animal product.

[0178] The user may incorporate oleogels into food products at a mass ratio that is appropriate for the optimal texture they require. This will depend on the other ingredients in the product, whether the product will be heated, and the particular protein oleogel in use.

[0179] In general, any concentration of between 1% and 90% (wt/wt) of dry food ingredients may be suitable. A range of 3% or 5% to 80% is more typical. Typical working ranges for various types of food products are as follows:

- plant-based ground meat replacement: 4 to 25% (wt/wt) of product formulation;
- vegan cake: 10 to 25% (wt/wt);
- dairy products: 2 to 50%;
- whipped topping: 15 to 45%.

[0180] The protein oleogel preparations of this disclosure can be used to make plant derived replacement foods for meat products, dairy products, and baked goods that are normally made with animal fats or tropical oils. The protein oleogels can also be used in foods traditionally made with nonsaturated plant oils with the objective of improving structure, oil release properties, or mouthfeel.

[0181] Detailed recipes for using protein oleogel in the making of plant based beef-like products, sausages, plant-based chicken nuggets, vegan vanilla cake, cookies, and plant based ice-cream are provided in U.S. Patent 11,896,687, to which this disclosure claims priority.

Spreadable olive oil made as an oleogel

[0182] The technology of this disclosure can be used to make spreadable olive oils. Current commercial “olive oil spreads” are made with a mixture of oils, such as olive oil mixed with palm, canola, and flax oils, combined with water and an emulsifier such as lecithin. A superior product can be made as an embodiment of the technology described here, containing just pure olive oil, a protein structurant, and trace amounts of flavor enhancers. The spreadable olive oil of this disclosure has superior flavor and mouthfeel.

[0183] Preparations of spreadable olive oils made in the Shiru food laboratories are shown in TABLE 5.

TABLE 5: Spreadable olive oil preparations

	Ingredients	Result
<u>Dry particulate Batch "A"</u>		
A1	6% dry particulate 92% olive oil	good flavor slight grittiness flows but structured salty finish
A2	5% dry particulate 10% mango butter 83% olive oil	clean olive oil flavor flows slightly at room temp Richer than olive oil alone
<u>Dry particulate Batch "B"</u>		
B1	3% dry particulate 95% olive oil	rich, smooth, clean flavor soft but not fully flowing
B2	5% dry particulate 93% olive oil	holds particles spreadable very smooth rich, slightly bitter
B3	7% dry particulate 91% olive oil	rich texture melts but lingers (most preferred texture) slight bitterness
<u>Flavored olive oil</u>		
C1	5% dry particulate 10% mango butter 83% olive oil	clean olive oil flavor flows slightly at rom temp richer than olive oil alone
C2	same using truffle infused oil	mustard color strong flavor could mix with neutral oil thicker than olive oil (good spread)
Cd	same using chili infused oil	bright orange color good spicy flavor slightly flowable

[0184] The procedure was as follows. Batch A dry particulate was produced from a 5% (wt/vol) homogenized potato protein isolate gel slurry using commercial pilot-scale equipment. Batch B dry particulate was produced from a 1% (wt/vol) homogenized potato protein isolate gel slurry using bench-scale methods. The oil used was extra virgin olive oil. The spread was made by incremental additions of the oil to protein dry particulate with gentle mixing and stirring. After 24 hours, baking soda and flour salt were added as flavor enhancers.

[0185] Both batches produced excellent olive oil spread products. Batch B preparations all had acceptable outcomes. Batch A preparations contained more dry particulate. The addition of saturated fat in the form of 10% mango butter (A2) provided additional structuring. Preparations C1, C2, and C3 were made using Batch A dry particulate and extra virgin olive oil that had been flavored. The distinct color and flavor obtained using truffle and chili infused oils demonstrate that small molecules with desirable organoleptic properties can be easily incorporated.

[0186] FIG. 13 shows the visual appearance of preparations B1, B3, and C2. A higher content of dry particulate (B3) increases the firmness and shape forming of the spread. The use of truffle infused oil darkens the color. Further refinements in ingredients and procedure can be made to enhance desirable attributes, such as a smooth texture, fatty mouthfeel, and product versatility.

Other spreadable foods

[0187] Peanut oil spread was made in the Shiru laboratories containing 5 to 10% protein dry particulate, 1 to 2% baking soda, up to 1% flour salt, 65 to 93% peanut oil, and up to 30% saturated fat (mango butter, cocoa butter, or coconut oil). The procedure was as follows: Oil and fat was added incrementally to dry particulate with gentle stirring until all oil was added. The combination was set aside for 24 hours for the oil to incorporate, following which baking soda and flour salt were added.

[0188] Spread formulations can also be produced by combining the protein dry particulate with an oil-rich mixture of natural fats, oils, proteins, carbohydrates, and other components of a ground nut preparation. This helps stabilize the product, which prevents separation and oil loss. A stabilized almond butter was made in the Shiru laboratories using 0.5% dry particulate as follows: Smooth almond butter was warmed to 200°F, then mixed with the dry particulate until homogeneous. Thirty two gram samples of the almond butter with or without the dry particulate were stored in a conical flask 40°C for six days. The almond butter by itself lost 2.0 g oil, but the dry particulate stabilized preparation lost only 1.3 g.

[0189] Chocolate nut spread was made in the Shiru laboratories using 61% hazelnut spread, 17% powdered sugar, 8.5% Ghirardelli cocoa powder, 1% vanilla, and 12% sunflower oil. Up to 1% protein dry particulate was added by heating the nut butter to 200°F. Oil separation was determined in the same manner as the almond butter.

[0190] FIG. 14 shows the results. The control sample containing no dry particulate (left) had a 2 to 5 μm layer of separated oil. The sample containing the dry particulate (right) did not separate.

Meat alternative products containing an oleogel

[0191] When using a protein oleogel as a fat substitute in meat alternative products, it is often appropriate to adjust textures, flavors, and outcomes of cooking to match the natural meat product.

[0192] FIG. 15 shows the browning of oleogel preparations containing different additives. The oleogel was 95% fat mixture (high oleic acid sunflower oil and mango butter) with 5% protein oleogelator (wt/vol). Row 1: oleogel only, oleogel plus glycerin, oleogel plus monodiglycerides, oleogel plus lecithin, and oleogel plus arginine. Row 2: oleogel emulsion (1% water) plus trisodium phosphate, emulsion plus citrus fiber, emulsion plus xanthan gum, oleogel containing sodium bicarbonate, and emulsion alone. The outcomes were as follows:

medium browning (sodium bicarbonate, xanthan gum, and citrus fiber samples),
intense browning (glycerin, diglyceride, lecithin, and sodium phosphate samples), and
structural/textural changes (lecithin and trisodium phosphate).

[0193] A plant based bacon alternative was made in the Shiru laboratories by layering a protein meat base with an oleogel. The meat base was made with 74% extra firm tofu (Azamaya), 15% soy protein (FarBest), 7% potato starch (Bob's Red Mill), 1.4% vegetarian pork (Givaudan), 1% kappa carrageenan, plus natural flavorings and color ingredients. The ingredients were blended until smooth, and placed in a -20°C refrigerator for 1 h. It was then sliced lengthwise into 1 cm sheets, and layered with sheets of oleogel. The combined product was frozen and sliced.

[0194] FIG. 12 (right side) shows the combined product, frozen and sliced into bacon rashers. The left side shows the product pan fried until brown.

[0195] Other foodstuffs made in the Shiru laboratory were as follows. based sausage or frankfurter: 16 to 20% texturized vegetable protein, 14 to 20% oleogel, about 38% water, 5% lava bean protein, flavors and colors, in a vegetarian casein. Plant based fois gras: A fois gras flavored nut or tofu preparation (50 to 70% cashew or silken protein base with appropriate flavoring), combined with 30 to 50% oleogel.

Making and storing preparations of the dry particulate

[0196] A dry particulate preparation according to this disclosure can be used not just as a structurant in oleogels. It may be added to food preparations directly in dry form as a thickener or stabilizer.

[0197] Dry particulate preparations for use as a dry ingredient or for preparing a macrocolloid are prepared in substantial the same way as dry particulate used to make oleogels. The dry particulate typically is an assemblage of microparticles packed into larger particles, beads, or aggregates. This constitutes the macrostructure of the preparation, which breaks down when dispersed in an oil or aqueous liquid into separate individual high aspect microparticles, such as those shown in FIG. 1.

[0198] The bulk material may consist essentially of an ensemble of porous, irregular hemispherical dry bead-like macrostructures and/or flakes, about 0.1 mm to 10 mm in diameter, as determined by mesh-sieve analysis. The solid volume of each particle may be reduced by presence of open pores. The macrostructures (both in bulk and on an individual particle basis) absorb both polar liquids (water) and non-polar liquids (oil) when passively soaked.

[0199] The physical structure of the dry particulate at the level of macroscopic aggregation may be tuned by varying air incorporation prior to freeze channeling. Typically, some amount of air is incorporated into the viscous homogenized protein hydrogel slurry prior to freezing consequent to physical manipulation, such as mixing, heating, cooling, and spraying. Air bubbles remaining in the protein slurry when it is frozen may produce small spherical occlusions in the dry macrostructure. The air content can intentionally be increased, for example, by agitation, using a high speed or immersion blender, or by sparging (bubbling of air or gas through the solution). High levels of aeration of the protein slurries used in the preparation of dry particulate (for example, 5% vol/vol) can be used to generate stable foams that can be frozen and dried. The presence of the air content may also act at the microstructure level to promote formation of high aspect ratio microparticles.

[0200] A preparation of dry particulate does not require immediate solubilization or formulation. It can be stored for long-term use by sealing under gentle vacuum. For smaller, bench-scale quantities, this can be done using a table top vacuum bag sealer (Weston Pro-2600 or equivalent). Rigid vacuum containers or drums can be used for larger quantities of material. Moisture content may be determined by heating a small (<2 g) sample of powder to 160°C on a halogen-heated gravimetric moisture analyzer. A moisture content of 2 to 9% is generally useful for prolonging shelf-life and ensuring consistent dispersibility in oil or water. Care should be taken not to apply excessive force when vacuum is applied that would collapse particle structure.

Assessing physical properties of protein dry particulate and macrocolloids

[0201] A hallmark property of macrocolloids of this disclosure is that they tend to have increased viscosity, especially at high salt concentration.

[0202] FIG. 17 demonstrates this effect. Dry particulate made from potato protein was combined with deionized water in 2 mL microcentrifuge tubes. The dry particulate was gently dispersed using repeat inversions on a tube rotator (30 to 60 min). The resulting solution had a pH of 4. The 1.25% dry particulate solution (Panel A, left tube) appears substantially transparent, whereas the 2.5% dry particulate solution (Panel A, right tube) appears slightly turbid but homogeneous. The turbidity is attributable to the presence of protein particles that remain dispersed in solution.

[0203] Panel B demonstrates the impact of adding salt (sodium phosphate) to a final concentration of 20 mM. A previously free-flowing, low viscosity aqueous solution (left) forms a gel-like slurry (coacervate) with relatively high viscosity as evidenced by its lack of flow in an inverted tube (right). There was also a substantial change in opacity, which indicates formation of larger dry

particulate aggregates or networks. When the solution was vigorously mixed for a prolonged period, it became free flowing again (shear-thinning behavior). This suggests that the association between proteins in the dry particulate network is relatively weak and reversible.

[0204] Dry particulate coacervates can be prepared by adding one part of a 5 x concentrated salt solution (20 to 100 mM sodium phosphate and/or 50 to 300 mM NaCl). Adding salts in powdered or crystalized form produces the same effect. The dynamic salt-dependent gel-like network formation in macrocolloids may be used in food and cosmetic formulations.

[0205] FIG. 16 shows temperature-dependent rheology of macrocolloids of this disclosure, compared with methylcellulose, and a pure recombinant gelation causing protein designated P44548. Small-amplitude oscillatory shear (SAOS) rheology was determined across a heating progression (25°C to 75°C) and a cooling progression (75°C to 50°C). This mimics heating to a typical internal temperature of a cooked burger (75°C) and its temperature at “hot bite,” following a period of cooling (50°C) as indicated at the “end points.”

[0206] A 2% solution of dry particulate exhibited an initial storage modulus (G') comparable with that of 2% methylcellulose. However, in contrast to methylcellulose, the storage modulus decreased as the temperature ramp progressed, dropping to approximately 0 Pa for the remainder of the temperature cycle (cooling data not shown). By adding a heat-onset gelling protein to the solution (2% P44588), the dry particulate demonstrated an initially high G' (40 Pa) with heat-onset gelation behavior (increasing G' as a function of increasing temperature). The two-component system more closely recapitulated the rheometric profile of methylcellulose than either the dry particulate or P44588 alone. Meltback of the 2% methylcellulose (decreasing G' during the 75°C to 50°C cooling progression) is not commonly observed in the cooling behavior of most protein gels.

Food products incorporating protein dry particulate

[0207] Macrocolloids made from the dry particulate in water can be used to prepare oil-in-water emulsions. Methylcellulose is commonly incorporated into plant-based meat doughs and batters as an emulsion. A macrocolloid stabilized emulsion can be used in its place.

[0208] FIG. 18 is an image of a representative dry particulate-structured emulsion. It was prepared as follows: One to 10% dry particulate was mixed in water for 30 min with gentle, occasional agitation to hydrate (for example, with a spoon). The aqueous dispersion was further homogenized at medium shear using an immersion mixer. Oil was added, repeating the medium-shear mixing.

[0209] FIG. 19 shows the appearance of uncooked meat doughs prepared using dry particulate as a cold-binding agent. Plant-based meatballs were prepared in a manner similar to the test patties in FIG. 11. Hand-formed meatballs made using 2% dry particulate (right) exhibited a uniform, smooth surface and a relatively light coloration. This appeared similar to the meatballs formed using

methylcellulose (left). The meatballs containing no cold binding agent (center) were more loosely formed, showing a rougher texture and darker surface.

Regulatory approval of dry particulate and oleogels as ingredients in processed food products

[0210] After a particular dry particulate or oleogel formulation has been identified for further development as a food ingredient, the user will assure that all regulatory requirements are met before beginning commercial distribution. For example, new food additives and products thereof for distribution in the U.S. may be subject to premarket approval by the Food and Drug Administration (FDA). The new additives are “generally recognized as safe” (GRAS) if there is generally available and accepted scientific data, information, or methods indicating it is safe, optionally corroborated by unpublished scientific data. A notification sent to FDA’s Office of Food Additive Safety for approval includes a succinct description of the substance (chemical, toxicological and microbiological characterization), the applicable conditions of use, and the basis for the GRAS determination. The FDA then evaluates whether the submitted notice provides a sufficient basis for a GRAS determination.

Use of dry particulate and oleogels in cosmetics and beauty products

[0211] The dry particulate and oleogels of this disclosure can be tested as replacements for any one or more of the various thickeners, lubricants, emulsifiers, emollients, and other oily and creamy components that are commonly used as components of cosmetics and personal care products.

[0212] Cosmetics typically contain a combination of the following core ingredients: water, emulsifier, preservative, thickener, emollient, color, fragrance and pH stabilizers. Purified water forms the basis of almost every type of cosmetic product.

[0213] Emulsifying agents keep hydrophilic and hydrophobic components of a preparation from separating. Many cosmetic products are based on emulsions — small droplets of oil dispersed in water or small droplets of water dispersed in oil. Emulsifiers are added to change the surface tension between the water and the oil, producing a homogeneous and well-mixed product with an even texture. Emulsifiers frequently used in cosmetics include polysorbates, laureth-4, and potassium cetyl sulfate. Preservatives are added to cosmetics to extend their shelf life and prevent the growth of microorganisms such as bacteria and fungi, which can spoil the product and possibly harm the user.

[0214] Thickening agents are used to give products an appealing consistency and facilitate use. Lipid thickeners work by imparting their natural thickness to the formula. Examples include cetyl alcohol, stearic acid and carnauba wax. So-called naturally derived thickeners are polymers that absorb water, causing them to swell up and increase the viscosity of a product. Examples include hydroxyethyl cellulose, guar gum, xanthan gum and gelatin. Mineral thickeners absorb water and

oils to increase viscosity, but give a different result to the final emulsion than the gums. Popular mineral thickeners include magnesium aluminum silicate, silica and bentonite.

[0215] Emollients soften the skin of the user by preventing water loss. They are used in a wide range of lipsticks, lotions and cosmetics. A number of different natural and synthetic chemicals work as emollients, including beeswax, olive oil, coconut oil and lanolin, as well as petrolatum (petroleum jelly), mineral oil, glycerin, zinc oxide, butyl stearate and diglycol laurate. Coloring agents and pigments are used in many cosmetics to accentuate or alter a person's natural coloring. Mineral ingredients can include iron oxide, mica flakes, manganese, chromium oxide and coal tar. Natural colors can come from plants, such as beet powder, or from animals, like carmine, often used in red lipsticks. The two most common organic pigments are lakes and toners. Fragrances are often added to liquid and cream cosmetics to improve their appeal.

[0216] Tallow is an animal product with a long history of use to soothe and moisturize skin. It is often a component of cosmetics, personal care products, and soap. Tallow is a rendered form of beef or mutton fat, primarily made up of triglycerides, including a combination of saturated, monounsaturated, and polyunsaturated fatty acids. Reformulating cosmetics to remove animal derived materials can include replacement of one or more of components such as tallow, lanolin, squalene, and/or other oils and oil-related chemicals and materials in any combination.

[0217] The microparticulate or oleogel will be present in a cosmetic or personal care product typically at a concentration of 0.5% to 80%, 1% to 60%, or 2 to 20% of oleogel by weight of the final product, depending on the nature of the product and the desired properties. This includes but is not limited to personal care products such as creams, lotions, and balms.

[0218] Contemplated is a body butter and a mineral sunscreen, having the formulations shown in TABLE 6.

TABLE 6: Cosmetic formulations		
Ingredients (% (wt/wt))	Body butter	Sunscreen
oleogel	67 %	76 %
avocado oil	33 %	
aloe vera gel		15 %
powdered zinc oxide		8 %
essential oil (fragrance)	0.2 %	0.2 %

[0219] Potential performance advantages of the body butter include decreased water loss, increased barrier function, activated protein absorption and activation, and an apparent anti-aging effect. Potential performance advantages of the sunscreen include active stabilization, increased water resistance to UV irradiation, and extended bioactive activity. Selection of particular ingredients and the amounts used may be adjusted by the user to generate a product having a desired texture, fragrance, color, stability, washability, moisturizing capability, and other factors.

[0220] For example, mango butter is lightweight, non-greasy and non-comedogenic (not pore-clogging). It is antibacterial, can help nourish the acne-prone skin and reduce production of sebum. Mango and shea butter can be adapted for use in cosmetics by incorporating it into an oleogel of this disclosure as part of an oil mixture at a low protein to oil ratio of 2:98 to 5:95 (wt/wt).

[0221] FIG. 20 is an image of a cosmetic cream made under Shiru's direction using an oleogel preparation that was 90% sunflower oil and 10% oleogelator protein. The cream contained 48% water, 47% oleogel, 4% olive oil, plus trace amounts of preservatives, an antioxidant, and a chelating agent. The image shows the appearance of a freshly prepared cream (left), a cream stored 10 days at ambient temperature (middle), and a cream stored 10 days at 50°C (right). Long-term stability of the product was confirmed: all creams retained a thick, unseparated, and indistinguishable appearance and texture.

Regulatory approval of dry particulate and oleogels as ingredients in personal care products

[0222] In the context of this disclosure, the term "personal care product" generally means any article intended to be rubbed, poured, sprinkled or sprayed on, introduced into or otherwise applied to any surface or part of the human body for cleansing, beautifying, promoting attractiveness or altering the appearance, and any item intended for use as a component thereof. In the context of this disclosure, the oleogel may be a component of a product or ingredient that is a compounded liquid, cream, gel, emulsion, colloid, powder, or dissolvable solid, optionally used in combination with a dispensing agent or personal care device.

[0223] Some personal care products and ingredients are regulated by the Food and Drug Administration as cosmetics. The Federal Food, Drug and Cosmetic Act (FD&C Act) defines cosmetics as "articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance." Included in this definition are products such as skin moisturizers, perfumes, lipsticks, fingernail polishes, eye and facial makeup preparations, shampoos, permanent waves, hair colors, toothpastes, and deodorants, as well as any material intended for use as a component of a cosmetic product.

[0224] Some personal care products and ingredients meet the FDA definitions of both cosmetics and drugs. This may happen when a product has two intended uses. For example, a shampoo is a cosmetic because its intended use is to cleanse the hair. An antidandruff treatment is a drug because

its intended use is to treat dandruff. Consequently, an antidandruff shampoo is both a cosmetic and a drug, because it is intended to cleanse the hair and treat dandruff. Among other cosmetic/drug combinations are toothpastes that contain fluoride, deodorants that are also antiperspirants, and moisturizers and makeup marketed with sun-protection claims. Such products must comply with the requirements for both cosmetics and drugs.

Use of dry particulate and oleogels in pharmaceutical products

[0225] Dry particulates and oleogels of this disclosure can be used as part of a pharmaceutical or nutraceutical product, for example, by combining with an effective dose of one or more pharmaceutically active agents or nutritional ingredients, optional components such as a pharmaceutically compatible preservative, and a pharmaceutically or nutraceutically compatible excipient, lubricant, diluent, or packing material. By way of illustration, the product may be in the form of a capsule or a measurable semisolid for oral administration, or a cream or ointment for topical administration. The oleogel will be present at a concentration of 0.5% to 50% or 2 to 20% by weight of the final product. The user may wish to adjust the salt and pH of the product so as to stabilize the oleogel and its role in the composition.

[0226] A drug or pharmaceutical product is a composition that contains at least one active agent that requires regulatory approval and provides pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of man or animals. A nutraceutical product is any substance or ingredient that is promoted as providing a health benefits, but not regulated by the Food and Drug Administration in the U.S.

[0227] FDA approval of a drug requires that the drug's effects have been tested for safety and efficacy in clinical trials or their equivalent, and reviewed by the FDA's Center for Drug Evaluation and Research (CDER). The drug is approved if it is determined to provide benefits that outweigh its known and potential risks for the intended population.

Use of dry particulate and oleogels in other industrial products and processes

[0228] The structured dry particulate and oleogels of this disclosure can be used as substitutes in other manufactured products that comprise thickeners and solid, semisolid, or structured oils and lubricants.

[0229] By way of illustration, oleogels can be used as a component of motor fuels and lubricants; in the print industry for applying to metal print plates to provide a resistance to acid etching; as an additive to the substrate used in polymer banknotes; in the manufacture candles and other solid fuel sources for heat or light production; in lubrication of steam-driven piston engines in locomotives and steamship engines, in which they are resistant to expulsion; in the steel rolling

industry to provide the required lubrication as the sheet steel is compressed through the steel rollers; in the lubrication of rifles and other artillery; as a flux for soldering; or in the production and storage of textiles, for example, to strengthen and lubricate yarns mounted on looms and for textile finishing.

Prior patent publications

CN 113261594 B (South China Ag. U.) — A rice bran protein oil gel.

CN 114190443 A (South China Inst. Technol.) — Preparing an oleogel comprising dispersing protein powder into oil by using a ball milling technology .

EP 3011836 A1 (Sholten) (abandoned) — Protein-stabilized oleogels made by solvent exchange

US 2022/0295811 A1 (Sholten) — Procedure for producing a protein oleogel by suspending protein in an oil, then adding water very very very slowly.

US Patent No. 4,734,287 (N. Singer, John Labatt Ltd.) — Protein product base

US Patent No. 8,940,354 (Marangoni, Mars Inc.) — Edible oleogel comprising an oil, ethylcellulose and a surfactant.

US Patent No. 9,655,376 (Ergun, Dow Chemical) — A continuous process for preparing an oleogel from ethylcellulose and an oily feed material

US Patent No. 10,874,115 (Perez Gallardo, Sigma Alimentos) — Edible oleogel comprising an oil or mixture of oils, grease or mixtures of fats, and a structuring agent of a distilled monoglyceride of saturated fatty acid

WO 2022/031172 (Camilleri, BFLike BV) — Oleogel made by cross-linking a hydrocolloid of oil and water using protein.

Incorporation by reference

[0230] Each and every publication and patent document cited in this disclosure is hereby incorporated herein by reference in its entirety for all purposes to the same extent as if each such publication or document was specifically and individually indicated to be incorporated herein by reference.

Interpretation and implementation of this technology

[0231] Although the technology described above is illustrated in part by certain concepts, procedures, information, and working examples, the claimed invention is not limited thereby except with respect to the features that are explicitly referred to or otherwise required. Theories that are put forth in this disclosure with respect to the formation and behavior of microstructure, other underlying modes of production, action, and assessment of various products and components thereof are provided

for the interest and possible edification of the reader, and are not intended to limit practice of the claimed invention.

[0232] While the dry particulate and oleogels of this disclosure were developed by Shiru primarily for use in the manufacture of food and cosmetics, they may be used in any other context for any reason. Discussion in this disclosure about the microstructure of dry particulate, macrocolloids, and oleogels does not limit the practice of the invention, the compositions of matter, or the methods claimed below except where explicitly stated or otherwise required. For example, oleogels of this invention made by the manufacturing processes put forth herein, or having the beneficial properties put forth herein, may or may not have a particular microstructure. Oleogels of this invention comprising a particular microstructure may or may not be made by a particular process. The reader may use any aspect of the technology put forth in this disclosure for any suitable or desirable purpose.

[0233] While the invention has been described above with reference to the specific examples and illustrations, changes can be made and components may be substituted to adapt the technology a particular context or intended use as a matter of routine development and optimization and within the purview of one of ordinary skill in the art, thereby achieving benefits of the invention without departing from the scope of what is claimed below and equivalents thereof.

CLAIMS

Products

1. A proteinaceous dry particulate that comprises mostly a protein that disperses and forms a structurant in either liquid oil or water,
 wherein the protein is a substantially denatured plant protein isolate or mixture,
 wherein at least 20% of the dry particulate has a solid microstructure,
 wherein the microstructure comprises microparticles that have a median size of at least 10 μm in one or two dimensions that substantially overlap in the dry particulate, but are free flowing and substantially not interconnected when suspended and diluted in vegetable oil.
2. The proteinaceous dry particulate of claim 1, wherein the particles suspended in an oil phase form an oleogel that has a substantially smooth, emulsion-like organoleptic character.
3. The proteinaceous dry particulate of claim 1, wherein the particles in a hydrated state form a macrocolloid that has a substantially smooth, emulsion-like organoleptic character.
4. A macrocolloid preparation, comprising a protein structurant according to claim 1 dispersed in an aqueous phase.
5. A protein oleogel comprising a protein structurant dispersed in an oil phase,
 wherein the oleogel has a protein-to-oil ratio between 2:98 and 20:80 (wt/wt),
 wherein the protein is a substantially denatured plant protein isolate or mixture,
 wherein at least 20% of the protein dispersed in the liquid oil phase has a solid microstructure,
 wherein the microstructure comprises microparticles that have a median size of at least 10 μm in one or two dimensions that substantially overlap when packed together, but are free flowing and substantially not interconnected when diluted in vegetable oil.
6. The oleogel of claim 5, wherein the microstructure instills the oleogel with the property of being solid or semisolid at room temperature, and releasing some but not all of the oil when cooked,
 thereby qualifying the oleogel as suitable as a replacement for animal fats and tropical oils in food products.

7. The product of any preceding claim, wherein the microstructure is mostly in the form of microparticles having a median aspect ratio (length to thickness) of at least 3 or at least 5.
8. The product of any preceding claim, wherein the microstructure comprises at least 20% fibrils and/or sheets.
9. The product of claim 8, wherein the microstructure comprises fibrils having a median size that is at least 20 μm in length but less than 4 μm in diameter.
10. The product of claim 8, wherein the microstructure comprises sheets having a median size that is at least 10 μm in length and width, but less than 2 μm in thickness.
11. An oleogel according to any of claims 4 to 10, wherein most of the oil in the oleogel is a vegetable oil that comprises mostly monosaturated or polyunsaturated fatty acids, or a mixture thereof.
12. An oleogel according to any of claims 4 to 10, which retains the oil it contains at room temperature, and releases between 20% and 80% of the oil when heated to 160°C.
13. An oleogel according to any of claims 4 to 10, which forms an emulsion when combined 1:1 with an aqueous liquid, wherein the emulsion is stable for at least four weeks at room temperature, with no evidence of creaming or phase separation.
14. An oleogel according to any of claims 4 to 10, which has a spreadable consistency when at room temperature.
15. An oleogel according to any of claims 4 to 10, in the form of a stored oil or fat replacement that comprises substantially no aqueous liquid.
16. An oleogel according to any of claims 4 to 10, in the form of an oil or fat replacement that makes up at least 5% (wt/wt) of a processed food product.
17. The oleogel of claim 16, wherein the processed food product is a hamburger patty or other meat product, or a plant-based substitute therefor.
18. The oleogel of claim 16, wherein the processed food product is a spreadable olive oil, a nut butter, or a chocolate spread.

19. An oleogel according to any of claims 4 to 10, in the form of an oil or fat replacement that makes up at least 5% (wt/wt) of a cosmetic or personal care product.
20. The oleogel of claim 19, wherein the cosmetic or personal care product is in the form of a cream, ointment, or lotion.

Methods of manufacture

21. A process for manufacturing a protein oleogel according to any of claims 4 to 10, the process comprising:
 - a) hydrating and solubilizing a mixed isolate of plant proteins in an aqueous solvent, thereby forming a gel;
 - b) denaturing protein in the gel by heating above 80°C at a pH at or below 4, and then cooling;
 - c) freeze channeling the protein from step (b) to form a protein powder comprising a matrix of denatured proteins having a solid microstructure; and
 - d) gradually adding an oil or mixture of oils gradually to the powder with a calibrated shear that disperses but does not triturate the solid microstructure until reaching a desired protein-to-oil ratio;thereby producing a protein oleogel comprising said oil dispersed in a protein microstructure that comprises particles with defined characteristics that are substantially not interconnected,
wherein the oleogel formed thereby is solid or semisolid at room temperature, and releases some but not all of the oil when heated to cooking temperature (160°C).
22. The process of claim 21, wherein the denaturing and cooling in step (b) forms a clear stranded gel.
23. The process of claim 21, wherein the freeze channeling in step (c) is done by immersing the protein in liquid nitrogen and drying in a vacuum.
24. The process of claim 21, wherein the oil is added to the protein powder in step (d) in at least four tranches with sheared mixing in between.

25. The process of claim 21, wherein the oil is added to the protein powder in step (d) by spraying or dripping the oil into the protein powder and mixing the oil with the powder on a continuing basis over a period of at least 10 min.

Properties

26. The process of claim 21, wherein the oleogel produced thereby forms an emulsion when combined 1:1 with an aqueous liquid, wherein the emulsion is stable for at least four weeks at room temperature with no evidence of creaming or phase separation.
27. The process of claim 21, wherein the oleogel produced thereby has a spreadable consistency when at room temperature.
28. The process of any of claims 21, wherein the oleogel produced thereby retains the oil it contains at room temperature, and releases between 20% and 80% of the oil when heated to 160°C.

Food and cosmetic products

29. The process of claim 21, further comprising manufacturing a food product using said oleogel in place of one or more animal derived fats or oils.
30. The process of claim 29, wherein the food product is a hamburger patty or other meat product, or a plant-based substitute therefor.
31. The process of claim 29, wherein the food product is a spreadable oil, a chocolate spread, or a baked product.
32. The process of claim 21, further comprising manufacturing a cosmetic product using said oleogel in place of one or more animal derived fats or oils.
33. The process of claim 33, wherein the cosmetic or personal care product is in the form of a cream, ointment, or lotion.

Use of dry particulate or oleogel

34. A method of thickening, stabilizing, or altering the mouthfeel of a food product or ingredient, comprising manufacturing the food product or ingredient to include a protein dry particulate according to claim 2 or 3.
35. A method of altering the perceived firmness, juiciness, fattiness, or flavor of a food product or ingredient, comprising manufacturing the food product or ingredient to include a protein oleogel according any of claims 4 to 10.