

FAT TISSUE SUBSTITUTES AND METHODS OF MAKING THE SAME

The present application claims priority to Singapore patent application number 10202102676Y titled "FAT TISSUE SUBSTITUTE AND METHODS OF MAKING THE SAME" filed
5 on 16 March 2021 which is incorporated by reference herein in its entirety.

Background of the invention

This invention relates to methods and compositions to produce plant-protein-based and
10 cellulose-based fat tissue substitutes (or fat tissue mimics) as described herein. Plant protein-based fat tissue mimics are prepared by sequentially blending plant protein into a water/ice mix, then blending with flavouring compounds, micronutrients, functional ingredients and vegetable oils, and followed by heat or/and enzymatic treatment. Cellulose-based fat mimics are prepared by sequentially blending cellulose derivatives into a water/ice mix, then
15 blending with flavouring compounds, micronutrients, functional ingredients and vegetable oils to form a flavour encapsulated oil-in-water emulsion. The plant protein-based fat tissue mimics can be applied into many types of meat analogues, from minced meat to steak, while cellulose-based fat tissue mimics are suitable for burger patty and minced meat mimic, such as vegetarian/vegan meat ball, dumpling and rice dumpling. They can also be used in hybrid
20 products that contain both plant protein and animal meat. Once cooked, the fat tissue mimics have high resemblance to animal fat tissues. It can slowly release flavouring substances and partly release fats to improve the texture and juiciness of meat analogues.

Meat alternatives are proliferating recently due to the growing demand for protein and
25 increasing concerns about food security, food safety, animal fare and sustainability. Therefore, the replacement of meat protein with alternative protein has become an important research topic. However, the taste, texture and nutritional profile of meat alternatives are still known to be different from meat.

30 Fat plays a major role in the quality of meat or meat analogue. Animal fat tissues are composed of liquid and solid fats incorporated in connective tissue. The unique structure endows animal fat tissue elastic and melting properties that give rise to a comminution

behaviour during processing, and provide meat products with juiciness, tenderness, mouthfeel, and flavour release.

It is a monumental challenge to mimic animal fat tissues using non-animal ingredients.

5 Directly replacing animal fat tissues with vegetable oils usually negatively affect juiciness and texture, as the rheological and thermal properties of vegetable oils may differ significantly in their physicochemical properties from animal fat tissues. In recent years, many attempts have been made to mimic the animal fat tissues.

10 Among them, gels formed by polysaccharides (such as konjac glucomannan and carrageenan) combined with other food additives have been widely studied to mimic fat tissue. They provide satisfactory springiness, cohesiveness, chewiness, and hardness, but were inferior in juiciness and mouthfeel due to the low-fat content and non-releasable property of the fat.

15 Another approach is the formation of oleogel using ethyl cellulose. This is usually achieved by dispersing the derivatized cellulose molecule in oil at temperature above its glass transition temperature and subsequently cooling the polymer solution below its gel point. Ethyl cellulose molecules form a highly interconnected network which is filled with entrapped oil. This method is effective in reducing the consumption of both saturated and *trans* fat.

20 However, the glass transition typically occurs at approximately 130 °C and above, increasing with the polymer molecular weight. The high processing temperature makes the invention unsuitable for temperature-sensitive flavouring compounds and nutrients, such as polyunsaturated fatty acids. The high cost of ethyl cellulose also hinders the application of the invention in food industry.

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To better mimic the elasticity and melting properties of animal fat tissue, Johannes Dreher et al used canola oil mixed with <30 wt% of fully hydrogenated canola oil, which is hot-emulsified with a soy protein isolate suspension (8 wt%). This fat tissue mimic formed contains 70 wt% lipid while displaying melting and elasticity properties. A similar approach was reported in
30 US10,039,306 B2, which uses buffered plant-protein solutions and vegetable oils to form emulsion gels.

One of the disadvantages of the protein-vegetable oil inventions discussed above is the tofu-like mouthfeel due to the soft texture. Its overall hardness is significantly lower than animal fat tissue, such as pork fat tissue. Another disadvantage is the strict requirement on proteins such as protein isolates or isolated protein fractions.

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Hence, there is a need for an improved animal fat tissue substitute or mimic.

The listing or discussion of an apparently prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

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Any document referred to herein is hereby incorporated by reference in its entirety.

Summary of the invention

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In an aspect of the invention, there is provided a method of producing a plant cellulose-based fat tissue substitute, the method comprising:

- (a) adding a plant cellulose material to water to form a cellulose solution;
 - (b) blending the cellulose solution with oil to form an emulsion; and
 - (c) cooling the emulsion to form the fat tissue substitute,
- wherein the blending of step (b) is carried out at a temperature between 30°C to 50°C.

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In various embodiments, the emulsion is cooled to temperature below room temperature to form the fat tissue substitute.

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In various embodiments, the cellulose is selected from the group consisting of methyl cellulose, and hydroxypropyl methylcellulose.

In various embodiments, the amount of cellulose in the cellulose solution is between 1wt% to 4wt% of the cellulose solution.

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In various embodiments, the oil is selected from the group consisting of soybean oil, peanut oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter, *Malania oleifera* oil, palm olein, palm stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof.

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In various embodiments, the method further comprising adding an additive to the oil to form an oil mixture before adding the oil mixture to the cellulose solution, wherein the additive is selected from the group consisting of a flavouring compound, a micronutrient, and combinations thereof.

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In various embodiments, the flavouring compound is selected from the group consisting of a sweetener, salt, spice, lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat flavour, duck flavour, goose flavour, pigeon flavour, rabbit flavour, deer flavour, horse flavour, bacon flavour, ham flavour, cured sour meat flavour, monosodium glutamate, inosine monophosphate, guanosine monophosphate, hydrolysed vegetable protein and yeast extracts.

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In various embodiments, the micronutrient is selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a carotenoid, and combinations thereof.

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In various embodiments, the flavouring compound is present in an amount of about between 0.07 wt% to 4 wt% of the oil mixture.

In various embodiments, the micronutrient is present in an amount of about between 0.01 wt% to 0.07 wt% of the oil mixture.

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In various embodiments, the amount of oil present in the oil mixture is about between 95.9 wt% to 100 wt% of the oil mixture.

In another aspect of the invention, there is provided a fat tissue substitute obtained by or obtainable from the method according to an aspect of the invention.

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In yet another aspect of the invention, there is provided an emulsion for a meat substitute product, the meat substitute product is capable of releasing oil slowly during cooking, the emulsion comprising water, at least 0.6 wt% of a plant cellulose material, 50 wt% to 90 wt% of a lipid, a flavour compound, and a micronutrient.

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In various embodiments, the water in the emulsion is present in an amount of about between 9.4 wt% to 49.4 wt% of the emulsion.

In various embodiments, the lipid is present in an amount of about between 50 wt% to 75 wt% of the emulsion.

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In various embodiments, the lipid is an oil selected from the group consisting of soybean oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter, *Malania oleifera* oil, palm olein, palm stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof.

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In various embodiments, the flavour compound is selected from the group consisting of a sweetener, salt, a spice, lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat flavour, duck flavour, goose flavour, pigeon flavour, rabbit flavour, deer flavour, horse flavour, bacon flavour, ham flavour, cured sour meat flavour, monosodium glutamate, inosine monophosphate, guanosine monophosphate, hydrolysed vegetable protein and yeast extracts, and the flavour compound is present in an amount of about between 0.05 wt% to 3 wt% of the emulsion.

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In various embodiments, the micronutrient is selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a carotenoid, and combinations thereof, and the micronutrient is present in an amount of about between 0.01 wt% to 0.05 wt% of the emulsion.

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In another aspect of the invention, there is provided a meat substitute product comprising an emulsion according to an aspect of the invention.

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In another aspect of the invention, there is provided a method of producing a plant protein-based fat tissue substitute, the method comprising:

- (a) blending a plant protein and a polysaccharide with water to form a plant protein-polysaccharide solution; and
- 5 (b) blending the plant protein-polysaccharide solution with oil to form an emulsion or an emulsion gel; and
- (c) incubating the emulsion and/or emulsion gel with an enzyme to form the plant protein-based fat tissue substitute.

- 10 In various embodiments, the emulsion is incubated with the enzyme for about 0.25 to 24 hours at about 4°C to 60°C to form the emulsion gel, and the enzyme is selected from the group consisting of sulfhydryl oxidase, laccase, peroxidase, tyrosinase, and transglutaminase.

In various embodiments, the method further comprising the following steps after step (c):

- 15 (d) heating the emulsion gel at a temperature about 60°C to 90°C for about 20 to 60 minutes; and
- (e) cooling the emulsion gel to form the plant protein-based tissue substitute.

- 20 In various embodiments, the method further comprises adding an additive to the plant protein-polysaccharide solution, wherein the additive is selected from the group consisting of salt, a polysaccharide, a lecithin, and combinations thereof.

- 25 In various embodiments, the salt is present in an amount of about 0.1 wt% to 2wt% of the plant protein-polysaccharide solution and the salt is selected from the group consisting of calcium carbonate, calcium chloride, potassium chloride, sodium chloride, and combinations thereof.

- 30 In various embodiments, the polysaccharide is selected from the group consisting of carrageenan, xanthan, konjac flour, locust bean gum, guar gum, gellan gum, alginate, agar, pectin, gum acacia, curdlan, a cellulose derivative, and combinations thereof.

In various embodiments, the lecithin is selected from the group consisting of a soya lecithin, a sunflower lecithin, a rapeseed lecithin, and combinations thereof.

5 In various embodiments, the lecithin is present in an amount of about between 0.16 to 0.33 wt% of the plant protein/protein-polysaccharide solution.

10 In various embodiments, the xanthan is present in an amount of about between 0.5 to 2 wt%; the carrageenan is present in an amount of about between 1 to 5 wt%; and the curdlan is present in an amount of about 1 to 5 wt% of the plant protein-polysaccharide solution.

15 In various embodiments, the oil is a low saturated fatty acid vegetable oil selected from the group consisting of soybean oil, peanut oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter, *Malania oleifera* oil, palm olein, palm stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof.

20 In various embodiments, the method further comprises adding and mixing an additive to the oil prior to blending the oil with the plant protein-polysaccharide solution to form an emulsion, wherein the additive is selected from the group consisting of a flavouring compound, a micronutrient, and combinations thereof.

25 In various embodiments, the flavouring compound is selected from the group consisting of a sweetener, salt, spice, lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat flavour, duck flavour, goose flavour, pigeon flavour, rabbit flavour, deer flavour, horse flavour, bacon flavour, ham flavour, cured sour meat flavour, monosodium glutamate, inosine monophosphate, guanosine monophosphate, hydrolysed vegetable protein and yeast extracts.

30 In various embodiments, the micronutrient is selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a carotenoid, and combinations thereof.

In various embodiments, the flavouring compound is present in an amount of about between 0.07 to 4 wt% of the oil mixture.

5 In various embodiments, the micronutrient is present in an amount of about between 0.01 to 0.07 wt% of the oil mixture.

In various embodiments, the amount of plant protein-polysaccharide solution is about between 25 to 90 wt% of the cooled emulsion gel.

10 In various embodiments, the amount of plant protein present in the plant protein-polysaccharide solution is about 5 wt% to 15 wt% of the plant protein-polysaccharide solution.

15 In various embodiments, the amount of oil present in the cooled emulsion gel is about 10 wt% to 75 wt% of the cooled emulsion gel.

In another aspect of the invention, there is provided an emulsion gel for a meat substitute product, the meat substitute being capable of releasing oil slowly during cooking, the emulsion gel comprising water, 1.2 wt%-13.5 wt% of a plant protein material, a polysaccharide, 10 wt%-75 wt% of a lipid, a flavour compound and a micronutrient.

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In various embodiments, the water in the emulsion gel is present in an amount of about between 11.5 wt% to 88.8 wt% of the emulsion gel.

25 In various embodiments, the polysaccharide is selected from the group consisting of carrageenan, xanthan, konjac flour, locust bean gum, guar gum, gellan gum, alginate, agar, pectin, gum acacia, curdlan, a cellulose derivative, and combinations thereof, and the polysaccharide is present in an amount of between 0.15 wt% to 4.5 wt% of emulsion gel.

30 In various embodiments, the lipid is present in an amount of about between 10 wt% to 75 wt% of the emulsion gel and is a vegetable oil selected from the group consisting of soybean oil, peanut oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil,

palm oil, olive oil, algae oil, cocoa butter, *Malania oleifera* oil, palm olein, palm stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof.

5 In various embodiments, the flavour compound is selected from the group consisting of a sweetener, salt, spice, lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat flavour, monosodium glutamate, inosine monophosphate, guanosine monophosphate, hydrolysed vegetable protein and yeast extracts, and the flavour compound is present in an amount of about between 0.01 wt% to 3 wt% of the emulsion gel.

10 In various embodiments, the micronutrient is selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a carotenoid, and combinations thereof, and the micronutrient is present in an amount of about between 0.01 wt% to 0.05 wt% of the emulsion gel.

15 In various embodiments, the emulsion gel further comprises lecithin, wherein the lecithin is present in about 0.05 wt% to 0.3 wt% of the emulsion gel.

In various embodiments, further comprises transglutaminase, wherein the transglutaminase is present in an amount of about at least 0.4 wt% of the emulsion gel.

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In yet another aspect of the invention, there is provided a meat substitute product comprising an emulsion gel according to an aspect of the invention.

Detailed description of the invention

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In a first aspect of the invention, there is provided a method of producing a plant cellulose-based fat tissue substitute, the method comprising: (a) adding a plant cellulose material to water to form a cellulose solution; (b) blending the cellulose solution with oil to form an emulsion; and (c) cooling the emulsion to form the fat tissue substitute, wherein the blending

30 of step (b) is carried out at a temperature between 30°C to 50°C.

The emulsion formed from the above method may be the fat tissue substitute, which may also be referred to here as a fat tissue mimic(s).

Advantageously, the fat tissue substitute obtained from the methods of the present invention is capable of releasing oil slowly during cooking. In the method of the first aspect of the invention, the emulsion is able to form a hydrogel when heated during cooking and the oil is slowly released during the gel formation. The cooking loss of the present cellulose-based fat tissue substitute were found to be lower than pure vegetable oils, which indicated a slower release of the oil. As will be seen and described in Figure 4, the size of the oil droplets in the mixture is uniform and small (about 15µm on average), resulting in the properties of the present invention.

In various embodiments, the method comprises cooling the emulsion at a temperature below that of room temperature to form the fat tissue substitute. In various embodiments, the room temperature in Singapore is about 25°C on average. As such, any suitable temperature may be selected to form fat tissue substitute.

In various embodiments, the cellulose may be any plant cellulose material or its derivative. In particular, in various embodiments, the cellulose may be selected from the group consisting of methyl cellulose, and hydroxypropyl methylcellulose. Such cellulose material may be commercially obtained.

In various embodiments, the amount of cellulose in the cellulose solution is between 1 wt% to 4 wt% of the cellulose solution. In various embodiments, the amount of cellulose present is 2 wt% when the cellulose is methyl cellulose, while the amount of cellulose present is 3 wt% when the cellulose is hydroxypropyl methyl cellulose.

In various embodiments, the oil may be a pure natural oil, a modified oil, or a combination thereof. The oil may include hydrogenated oils, inter-esterified oils and fractionated oils. In various embodiments, the oil is a vegetable oil and may be selected from the group consisting of soybean oil, peanut oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter, *Malania oleifera* oil, palm olein, palm

stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof. Preferably, the oil is selected such that its solid fat content is relatively high at room temperature to mimic the mouthfeel of animal fat.

- 5 In various embodiments, the method further comprises adding an additive to the oil to form an oil mixture before adding the oil mixture to the cellulose solution, wherein the additive is selected from the group consisting of a flavouring compound, a micronutrient, and combinations thereof. In various embodiments, the amount of oil mixture and cellulose solution are present in an amount of 10 wt% to 50 wt% and 50 wt% to 90 wt% respectively.

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- In various embodiments, the flavouring compound may be selected from the group consisting of a sweetener, salt, spice, lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat flavour, duck flavour, goose flavour, pigeon flavour, rabbit flavour, deer flavour, horse flavour, bacon flavour, ham flavour, cured sour meat flavour, monosodium glutamate, inosine monophosphate, guanosine monophosphate, hydrolysed vegetable protein and yeast extracts. In various embodiments, the flavouring compound may be present in an amount of about between 0.07 wt% to 4 wt% of the oil mixture.
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- In various embodiments, the micronutrient may be selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a carotenoid, and combinations thereof. In various embodiments, the micronutrient may be present in an amount of about between 0.01 wt% to 0.07 wt% of the oil mixture.
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- In various embodiments, the amount of oil present in the oil mixture is about between 95.9 wt% to 100 wt% of the oil mixture.
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- In a second aspect of the invention, there is provided an emulsion obtained or obtainable by the method set out in the first aspect of the invention. The emulsion may be used for a meat substitute product, the meat substitute product is capable of releasing oil slowly during cooking.
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In various embodiments, the emulsion comprises water, at least 0.6 wt% of a plant cellulose material, 50 wt%-90 wt% of a lipid, a flavour compound, and a micronutrient.

5 In various embodiments, the water is present in an amount of about between 9.4 wt% to 49.4 wt% of the emulsion.

In various embodiments, the lipid is present in an amount of about between 50 wt% to 75 wt% of the emulsion.

10 In various embodiments, the lipid may be a vegetable oil selected from the group consisting of soybean oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter, *Malania oleifera* oil, palm olein, palm stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof.

15 In various embodiments, the flavour compound may be selected from the group consisting of a sweetener, salt, spice, lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat flavour, inosine monophosphate, guanosine monophosphate, and hydrolysed vegetable proteins.

20 More particularly, the flavours may include but not limited to the following:

1. 2-Methylfuran-3-thiol, Non-2(E)-enal, Deca-2(E),4(E)-dienal, bis(2-methyl-3-furyl) disulphide, 2-Acetyl-l-pyrroline, 4,5-Dimethylthiazole, Trimethylthiazole for beef flavour;
2. 2-Pentylfuran, Hexanal, 1-Octen-3-ol, Methylpyrazine, 2,6-Dimethylpyrazine, 4,5-Dimethylthiazole, 2-Acetylthiazole and bis(2-methyl-3-furyl) disulphide for pork
25 flavour; or
3. 2-Methyl-3-furanthiol, bis(2-Methyl-3-furyl)disulphide, 2-Furfurylthiol, 2,5-Dimethyl-3-furanthiol, 3-Mercapto-2-pentanone, 2-trans-4-trans-Decadienal, Pyrazines, γ -Dodecalactone, Hexanal for chicken flavour.

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In various embodiments, a flavour enhancer may be added. Such enhancers include any substance which when present in a food accentuates the taste of the food without

contributing any flavour of its own. Here, flavour enhancers include but not limited to monosodium glutamate (MSG) and yeast extracts.

In various embodiments, the flavour compound is present in an amount of about between
5 0.05 wt% to 3 wt% of the emulsion.

In various embodiments, the micronutrient is selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a carotenoid, and combinations thereof.

10 In various embodiments, the micronutrient is present in an amount of about between 0.01 wt% to 0.05 wt% of the emulsion.

The flavour compounds and micronutrients of the present invention may be commercially obtained. For example, the flavour compounds may be obtained from flavours houses such
15 as Givaudan and Takasago. The hydrolysed vegetable proteins may also be commercially available. Also, the use of these flavour compounds, flavour enhancers and micronutrients may also be applied to the protein-based fat tissue substitute that will be described later below.

20 In a third aspect of the invention, there is provided a meat substitute product comprising an emulsion according to the second aspect of the invention.

In a fourth aspect of the invention, there is provided a method of producing a plant protein-based fat tissue substitute, the method comprising: (a) blending a plant protein and a
25 polysaccharide with water to form a plant protein-polysaccharide solution; (b) blending the plant protein-polysaccharide solution with oil to form an emulsion or an emulsion gel; and (c) incubating the emulsion and/or emulsion gel with an enzyme to form the plant protein-based fat tissue substitute.

30 In various embodiments, the method further comprising the following steps after step (c): (d) heating the emulsion gel at a temperature of about 60°C to 90°C for about 20 to 60 minutes; and (e) cooling the emulsion gel to form the plant protein-based tissue substitute.

The emulsion and/or emulsion gel is incubated with the enzyme for about 0.25 to 24 hours at about 4°C to 60°C to form the emulsion gel, and the enzyme is selected from the group consisting of sulfhydryl oxidase, laccase, peroxidase, tyrosinase, and transglutaminase.

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The plant protein of the present invention may be any suitable protein isolated or concentrated from plants. The isolated or concentrated plant protein must be able to form a gel structure after enzymatic crosslinking and heating. The plant proteins of this invention may contain soy protein concentrates and isolates, legume proteins (proteins from pea, lentil, lupine, chickpea, faba bean, mung bean and other types of beans), potato protein, zein, peanut protein, hemp protein, seed proteins (proteins from sunflower, rapeseed, quinoa, chia, pumpkin and other types of seeds) and leaf proteins (such as RubisCo). These proteins are concentrated and/or isolated from its matrix by various known methods.

15 In various embodiments, the plant protein of the present invention are soy protein isolates and concentrates that are commercially obtained. Advantageously, the present invention does not require the protein to have high purity. Any protein isolate and concentrate may be suitable for use in the method of the present invention. Protein isolates usually have higher protein content (>85%) than protein concentrates (>70%).

20 In various embodiments, incubating the emulsion and/or the emulsion gel with an enzyme results in an emulsion gel (in the case when an emulsion is incubated with the enzyme) and also strengthens (i.e. increase the hardness of) the emulsion gel (in the case when an emulsion gel is incubated with the enzyme).

25 In various embodiments, after the heating step of step (d), the resultant emulsion gel is cooled to a temperature that is at or below room temperature. By "room temperature", it is meant to include the Singapore Standard CP 13 (Code of Practice for mechanical ventilation and air-conditioning in buildings) which specifies an indoor temperature as one that is maintained between 22.5°C and 25.5°C. Having said that, the method includes cooling the emulsion gel sufficiently at a suitable temperature until said emulsion gel firms up into a solid structure.

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In various embodiments, the method further comprises adding an additive to the plant protein-polysaccharide solution, wherein the additive is selected from the group consisting of a salt, a further polysaccharide, a lecithin, and combinations thereof.

- 5 In various embodiments, the salt is present in an amount of about 0.1 wt% to 2 wt% of the plant protein-polysaccharide solution and is selected from the group consisting of calcium carbonate, calcium chloride, potassium chloride, sodium chloride, and combinations thereof.

- 10 In various embodiments, the polysaccharide may be selected from the group consisting of carrageenan, xanthan, konjac flour, locust bean gum, guar gum, gellan gum, alginate, agar, pectin, gum acacia, curdlan, a cellulose derivative, and combinations thereof. The xanthan may be present in an amount of about between 0.5 wt% to 2 wt% of the plant protein-polysaccharide solution, the carrageenan is present in an amount of about between 1 wt% to 5 wt% of the plant protein-polysaccharide solution, and the curdlan is present in an amount
15 of about 1 wt% to 5 wt% of the plant protein-polysaccharide solution.

- In various embodiments, the lecithin may be selected from the group consisting of a soya lecithin, a sunflower lecithin, a rapeseed lecithin, and combinations thereof. The lecithin may be present in an amount of about between 0.16 wt% to 0.33 wt% of the plant protein-polysaccharide solution.
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- In various embodiments, the oil may be an oil having low saturated fatty acids. The vegetable oil may be a pure natural oil, a modified oil, or a combination thereof. The oil may be hydrogenated oils, inter-esterified oils and fractionated oils. Still further, the vegetable oil
25 may be selected from the group consisting of soybean oil, peanut oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter, *Malania oleifera* oil, palm olein, palm stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof.

- 30 In various embodiments, the method further comprises adding and mixing an additive to the oil prior to blending the oil with the plant protein-polysaccharide solution to form an

emulsion, wherein the additive is selected from the group consisting of a flavouring compound, a micronutrient, and combinations thereof.

The flavouring compound may be selected from the group consisting of a sweetener, salt, spice, lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat flavour, duck flavour, goose flavour, pigeon flavour, rabbit flavour, deer flavour, horse flavour, bacon flavour, ham flavour, cured sour meat flavour, inosine monophosphate, guanosine monophosphate, and hydrolysed vegetable proteins.

More particularly, the flavours may include, but not limited to, the following:

1. 2-Methylfuran-3-thiol, Non-2(E)-enal, Deca-2(E),4(E)-dienal, bis(2-methyl-3-furyl) disulphide, 2-Acetyl-l-pyrroline, 4,5-Dimethylthiazole, Trimethylthiazole for beef flavour;
2. 2-Pentylfuran, Hexanal, 1-Octen-3-ol, Methylpyrazine, 2,6-Dimethylpyrazine, 4,5-Dimethylthiazole, 2-Acetylthiazole and bis(2-methyl-3-furyl) disulphide for pork flavour; or
3. 2-Methyl-3-furanthiol, bis(2-Methyl-3-furyl)disulphide, 2-Furfurylthiol, 2,5-Dimethyl-3-furanthiol, 3-Mercapto-2-pentanone, 2-trans-4-trans-Decadienal, Pyrazines, γ -Dodecalactone, Hexanal for chicken flavour.

In various embodiments, a flavour enhancer may be added. Such enhancers include any substance which when present in a food accentuates the taste of the food without contributing any flavour of its own. Here, flavour enhancers include but not limited to monosodium glutamate (MSG) and yeast extracts.

In various embodiments, the flavouring compound is present in an amount of about between 0.07 wt% to 4 wt% of the oil mixture.

In various embodiments, the micronutrient may be selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a carotenoid, and combinations thereof.

In various embodiments, the micronutrient is present in an amount of about between 0.01 wt% to 0.07 wt% of the oil mixture.

5 In various embodiments, the amount of plant protein-polysaccharide solution is about between 25 wt% to 90 wt% of the cooled emulsion gel.

10 In various embodiments, the amount of plant protein component present in the plant protein-polysaccharide solution is about 5 to 15 wt% of the plant protein-polysaccharide solution.

In various embodiments, the amount of oil present in the cooled emulsion gel is about between 10 wt% to 75 wt% of the formed emulsion gel.

15 In a fifth aspect of the invention, there is provided an emulsion gel obtained or obtainable by the method set out in the fourth aspect of the invention. The emulsion gel may be used for a meat substitute product, the meat substitute is capable of releasing oil slowly during cooking, the emulsion gel comprising water, 1.2 wt% -13.5wt% (or a narrower range 2.4 wt%-3.6 wt%) of a plant protein material, a polysaccharide, 10-75 wt% of a lipid, a flavour compound and a micronutrient.

20 In various embodiments, the water is present in an amount of about between 11.5 wt% to 88.8 wt% of the emulsion gel.

25 In various embodiments, the plant protein is present in an amount of about between 1.2 wt% to 13.5 wt% of the emulsion gel.

30 In various embodiments, the polysaccharide may be selected from the group consisting of carrageenan, xanthan, konjac flour, locust bean gum, guar gum, gellan gum, alginate, agar, pectin, gum acacia, curdlan, a cellulose derivative, and combinations thereof.

In various embodiments, the polysaccharide is present in an amount of between 0.15 wt% to 4.5 wt% of emulsion gel.

In various embodiments, the lipid is present in an amount of about between 10 to 75 wt% of the emulsion gel.

- 5 In various embodiments, the lipid may be a vegetable oil selected from the group consisting of soybean oil, peanut oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter, *Malania oleifera* oil, palm olein, palm stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof.

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In various embodiments, the flavour compound may be selected from the group consisting of a sweetener, salt, spice, lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat flavour, monosodium glutamate, inosine monophosphate, guanosine monophosphate, hydrolysed vegetable protein and yeast extracts.

15

In various embodiments, the flavour compound is present in an amount of about between 0.01 wt% to 3 wt% of the emulsion gel.

- 20 In various embodiments, the micronutrient may be selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a carotenoid, and combinations thereof.

In various embodiments, the micronutrient is present in an amount of about between 0.01 wt% to 0.05 wt% of the emulsion gel.

- 25 In various embodiments, the emulsion gel further comprises lecithin, wherein the lecithin is present in about 0.05 wt% to 0.3 wt% of the emulsion gel.

In various embodiments, the emulsion gel further comprises transglutaminase, wherein the transglutaminase is present in an amount of about at least 0.4 wt% of the emulsion gel.

30

In a sixth aspect of the invention, there is provided a meat substitute product comprising an emulsion gel according to the fifth aspect of the invention.

By "emulsion", it is meant to refer to a mixture of two or more liquids in which one is present as droplets, of microscopic or ultramicroscopic size, distributed throughout the other. Emulsion refers to mixed systems that should better be characterized as solutions or
5 suspensions.

By "emulsion gel", it is meant to refer to a gel which is formed from a stable emulsion incorporated into a gelled continuous phase. It is a combination of an emulsion dispersion and a gel phase.
10

The protein-based emulsion gel is made of protein assemblies and oil droplets. The stress bearing strands are formed by the proteins and protein-stabilised oil droplets alike. The cellulose-based emulsion is an oil-in water emulsion stabilized by cellulose molecules.

15 The following documents are incorporated by reference herein in their entirety to further elaborate and explain what is meant by "emulsion" and "emulsion gel".

• <https://arxiv.org/pdf/2007.11843.pdf> "Rheology of protein-stabilised emulsion gels envisioned as composite networks. 2 - Framework for the study of emulsion gels".
20

• <https://www.sciencedirect.com/science/article/abs/pii/S2213329121000629>
"Rheology of oil-in-water emulsions stabilised by native cellulose microfibrils in primary plant cell dispersions"

25 In order that the present invention may be fully understood and readily put into practical effect, there shall now be described by way of non-limitative examples only preferred embodiments of the present invention, the description being with reference to the accompanying illustrative figures.

30 In the Figures:

Figure 1. Force-time plot of two cyclic uniaxial compression test of ESSSC-C.

Figure 2. Representative CLSM micrograph of soy protein-based emulsion gel (A) and pork backfat (B).

5 **Figure 3.** A spider plot of the sensory scores of the patties with or without fat or fat tissue mimic. LE:100% lean; WK: 80 wt% lean + 20 wt% Wilkote 2610; MW-2: 80 wt% lean + 20 wt% methyl cellulose-based fat tissue mimic.

Figure 4. Representative CLSM micrograph of cellulose-based emulsion (MW-2).

10

To overcome the deficiencies identified in the prior art, the invention relates to two methods and compositions, one is plant protein-based and the other one is cellulose-based, to produce the fat tissue substitutes. Compared to the prior art, the invention has the following advantages:

15

Protein/polysaccharide-based fat tissue substitute:

20

1. The requirement on protein quality is not as strict. Not only protein isolates but also protein concentrates can be used to form the emulsion gel with vegetable oils.

25

2. Compared to existing art where the pH level of the protein isolate solution needs to be adjusted to 7.0 by addition of 1 M HCl, no buffer solution is needed to adjust the pH level of the protein isolate solution of the present invention.

30

3. The preparation condition is carried out at less harsh conditions (for example, at lower temperatures $\leq 90^{\circ}\text{C}$) comparing to oleogel inventions ($\geq 130^{\circ}\text{C}$), so thermal-sensitive flavouring components and nutrients (such as poly-unsaturated fatty acids) can be encapsulated in the fat mimic for improved stability during storage and released upon cooking or heat treatment.

4. The addition of food polysaccharides (including carrageenan, xanthan, konjac flour, locust bean gum, guar gum, gellan gum, curdlan, alginate, agar, pectin, gum acacia,

and cellulose derivatives). These additives increase the emulsion gel's hardness, making it closer to the hardness of real animal fat tissue.

- 5 5. Oils used in this invention can be pure natural oils, modified oils, or a mixture of natural and modified oils. Natural oils and fats that can be used in this invention include, but not limited to, soybean oil, peanut oil, canola/rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter, and *Malania oleifera* oil (rich in Nervonic Acid). Modified oils include hydrogenated oils, inter-esterified oils and fractionated oils, such as palm olein and palm stearin, shea olein and shea butter, and 1,3-dioleoyl-2-palmitoylglycerol (OPO). Vegetable oils that are low in saturated fatty acids usually present better results.
- 10
6. The present invention requiring gelling of the plant / polysaccharide system provides the advantage of providing a fat substitute having a consistency closer to actual adipose tissue because the product obtained by the present method has a higher oil content compared to the traditional fat replacer or other fat substitutes. Its composition is closer to real adipose tissue. Therefore, it better simulates the property of adipose tissue by slow releasing of oil and flavour compounds which will improve the juiciness and flavour.
- 15
7. This invention is ideal for delivery of lipophilic micronutrients such as Vitamin A, Vitamin D and carotenoids.
- 20

Cellulose-based fat tissue substitute:

- 25
1. When incorporated in meat alternatives, this fat tissue substitute is able to slowly release vegetable oils when cooking at high temperature to increase juiciness while maintaining a firm and succulence texture.
- 30
2. The preparation condition is much milder ($\leq 50^{\circ}\text{C}$) comparing to oleogel inventions ($\geq 130^{\circ}\text{C}$), so thermal-sensitive flavouring components and nutrients (such as poly-unsaturated fatty acids) can be encapsulated.

3. Cellulose derivatives that can be used include, but not limited to, methyl cellulose, and hydroxypropyl methylcellulose.
- 5 4. Oils used in this invention can be pure natural oils and fats, modified oils, or a mixture of natural oils and modified oils. Natural oils and fats that can be used in this invention include, but not limited to, palm oil, cocoa butter, cocoa butter equivalent, shea olein and shea butter. Other oils include, but not limited to, soybean oil, peanut oil, canola/rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, can be used together with
- 10 the above mentioned oils and fats.

Example

Methods and compositions to produce protein/polysaccharide-based and cellulose-based fat

15 tissue mimics are described herein.

One part of the invention relates to protein-polysaccharide-based fat tissue mimic composition comprising of flavours, nutrients and vegetable oils encapsulated in a protein-polysaccharide emulsion gel invention; wherein the protein-polysaccharide emulsion gel

20 comprises a combination of plant protein, polysaccharides, lipids, flavours, micronutrients and enzyme.

Proteins used in this invention can be plant protein concentrate, protein isolate or a combination of protein isolate and concentrate.

25

Food polysaccharides used in this invention can be one or more gums selected from carrageenan, xanthan, konjac flour, locust bean gum, guar gum, curdlan, gellan gum, alginate, agar, pectin, gum acacia, and cellulose derivatives.

30 Lipids used in this invention can be pure natural oils, modified oils, or a mixture of natural and modified oils. Natural oils and fats that can be used in this invention include, but not limited to, soybean oil, canola/rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm

oil, olive oil, algae oil, coco butter, and Malania oleifera oil (rich in Nervonic Acid). Modified oils include hydrogenated oils, inter-esterified oils and fractionated oils, such as palm olein and palm stearin, shea olein and shea butter, and 1,3-dioleoyl-2-palmitoylglycerol (OPO).

- 5 Lecithins including, but not limited to, soya lecithin and sunflower lecithin can also be used in this invention.

Vegetable oils that are low in saturated fatty acids usually present better results. Flavours include lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat
10 flavour and flavour enhancer.

Micronutrients include, but not limited to, vitamin A, vitamin D, vitamin E and vitamin K and carotenoids.

- 15 The enzyme is an edible enzyme can catalyse protein crosslinking. Frequently used enzymes are sulfhydryl oxidase, laccase, peroxidase, tyrosinase, and transglutaminase.

The fat tissue mimics can comprise a protein-polysaccharide solution, which accounts for about 25 wt%-90 wt% (or a narrower range 25 wt%-50 wt %) of the fat tissue mimic. Protein-polysaccharide solution is an aqueous solution where protein concentrates/isolates and
20 polysaccharides are dissolved in. "25 wt%-90% of the fat tissue mimic" implies that the weight percentage of water, protein and polysaccharide is 25 wt%-90 wt%. Protein component only refers to protein concentrates/isolates that are in dry form. The protein component in the emulsion (containing water, fats et al) is 1.2 wt%-13.5 wt%. Besides, the invention contains
25 fats, which account for about 10 wt%-75 wt% (w/w, or a narrower range 50 wt%-75 wt%) of the fat tissue mimic. Additionally, the mimic comprises flavour components, which accounts for 0.01 wt%-3 wt% of the fat tissue mimic. In some cases, micronutrients are encapsulated at dosage about 0.01 wt%-0.05 wt%. In some embodiments, the protein component comprises about 1.2 wt%-13.5 wt% of the fat tissue mimic. In some cases, lecithin comprises
30 about 0.05 wt%-0.3 wt% of the fat tissue mimic. In some embodiments, xanthan was added to improve the chewiness of the fat tissue mimic, wherein xanthan accounts for 0.15 wt%-0.6 wt% of the fat tissue mimic. In some cases, carrageenan was used to ameliorate the texture

of fat tissue mimic, wherein carrageenan accounts for 0.3 wt%-1.5 wt% of the fat tissue mimic. In some embodiments, curdlan was added to increase the hardness of the mimic, wherein curdlan accounts for 0.3 wt%-1.5 wt% of the fat tissue mimic. The gelation of fat tissue mimic can be induced by using one or more approaches, such as using transglutaminase or gelling via heating and cooling cycle. In some embodiments, the fat tissue mimic can comprise transglutaminase, which accounts for at least 0.4 wt% of emulsion gel.

Preparing protein/polysaccharide-based fat tissue mimics comprises the steps of:

1. Mixing flavours and micronutrients in vegetable oil to form a lipid premix.
2. Blending protein and polysaccharides with water to give a fully rehydrated mixture.
3. Homogenizing the lipid phase with the protein-polysaccharide solution to form an oil-in water emulsion.
4. Adding transglutaminase to the above emulsion and keeping it at 4°C -60°C for 0.25-24 hours.
5. Then heating the emulsion gel formed after treatment or incubation with the enzyme to a temperature ranging from 60°C-90 °C for 20-60 min.
6. Finally, cooling the emulsion gel to room temperature to obtain the final product.

Another part of the invention related to cellulose-based fat tissue mimic composition comprising of vegetable lipids, flavours, and micronutrients encapsulated in cellulose derivative emulsion invention; wherein the cellulose derivative can be methyl cellulose or hydroxypropyl methylcellulose. Lipids used in this invention can be pure natural oils, modified oils, or a mixture of natural and modified oils. Oils and fats that can be used in this invention include, but not limited to, palm oil, cocoa butter, cocoa butter equivalent, shea olein and shea butter.

The fat tissue mimics can comprise a cellulose solution, which accounts for about 10 wt%-50 wt%, (or a narrower range 25 wt%-50 wt%) of the fat tissue mimic. Cellulose solution is an aqueous solution where cellulose is dissolved in. "10 wt%-50 wt% of the fat tissue mimic" implies that the weight percentage of water and cellulose is 10 wt%-50 wt%. Cellulose component only refers to cellulose that is in dry form. The cellulose component in the fat tissue mimic is at least 0.6 wt% while the amount of water is 9.4 wt% to 49.4 wt%. Besides,

the invention contains oil/fat, which accounts for about 50 wt%-90 wt% (or a narrower range 50 wt%-75 wt%) of the fat tissue mimic. Additionally, the mimic comprises flavour component, which accounts for 0.05 wt%-4 wt% of the fat tissue mimic. In some cases, micronutrients are encapsulated at a dosage about 0.01 wt%-0.05 wt% .

5

Preparing cellulose-based fat tissue mimic comprises the steps of:

1. Mixing flavours and micronutrients in vegetable oil to form a lipid premix.
2. Dispersing cellulose derivative in water until homogenous mixture is reached.
3. Homogenizing the lipid phase with the cellulose solution to form an oil-in-water
10 emulsion at a temperature between 30°C-50°C.
4. Cooling the emulsion to obtain the final product.

Protein/polysaccharide-based fat tissue mimic:

15 **Procedure for the preparation of plant protein-based fat tissue mimics**

Preparing lipid premix. The oil phase was prepared by adding flavouring agents, such as beef tallow flavour, beef fat flavour and lard flavour, to soybean oil at 0.1 wt% - 1 wt% depending on the type of targeted animal fat.

- 20 **Preparing soy protein-based emulsion gel as fat tissue mimic (ES-A).** Soy protein (soy protein isolate, 5 wt%-15 wt%) powder was dispersed in water. Then the lipid premix (50 wt%-75 wt%) was homogenized with the soy protein solution to form an oil-in water emulsion. After that, 200 mg TG/g protein was added to the emulsion. The resulting mixture was incubated at 4°C for 12 h to obtain the fat tissue mimic which can be further shaped into any
25 configurations depending on the processing requirements such as mince, flakes, slices, or cubes.

- Preparing soy protein-based emulsion gel as fat tissue mimic (ESSJ-A).** Soy protein (soy protein concentrate Wilcon SJ, 5 wt%-15 wt%) powder was dispersed in water and stirred
30 overnight on a magnetic stirrer to ensure complete protein hydration. After that, the lipid premix (50 wt%-75 wt%) was homogenized with the soy protein solution to form an oil-in

water emulsion. Then, 200 mg TG/g protein was added to the emulsion. The resulting mixture was incubated at 4°C for 12 h to obtain the final product.

Preparing soy protein-based emulsion gel as fat tissue mimic (ESSJ-B). Soy protein (soy protein concentrate Wilcon SJ, 5 wt%-15 wt%) powder was dispersed in water and stirred overnight on a magnetic stirrer to ensure complete protein hydration. After that, the lipid premix (50 wt%-75 wt%) was homogenized with the soy protein solution to form an oil-in water emulsion. Then, 200 mg TG/g protein was added to the emulsion. Sample was incubated at 4°C for 12 h followed by further incubation at 90°C for 30 min. After cooling down to room temperature, the final product was obtained.

Preparing soy protein-based emulsion gel as fat tissue mimic (ESSS-A). Soy protein (soy protein concentrate Wilcon SS, 5 wt%-15 wt%) powder was dispersed in water and stirred overnight on a magnetic stirrer to ensure complete protein hydration. After that, the lipid premix (50 wt%-75 wt%) was homogenized with the soy protein solution to form an oil-in water emulsion. Then, 200 mg TG/g protein was added to the emulsion. The resulting mixture was incubated at 4°C for 12 h to obtain the final product.

Preparing soy protein-based emulsion gel as fat tissue mimic (ESSS-B). Soy protein (soy protein concentrate Wilcon SS, 5 wt%-15 wt%) powder was dispersed in water and stirred overnight on a magnetic stirrer to ensure complete protein hydration. After that, the lipid premix (50 wt%-75 wt%, w/w) was homogenized with the soy protein solution to form an oil-in water emulsion. Then, 200 mg TG/g protein was added to the emulsion. Sample was incubated at 4°C for 12 h followed by further incubation at 90°C for 30 min. After cooling down to room temperature, the final product was obtained.

Preparing lecithin-soy protein-based emulsion gel as fat tissue mimic (ESL-A). Soy protein (soy protein isolate, 5 wt%-15%) powder was dispersed in water and stirred overnight on a magnetic stirrer to ensure complete protein hydration. After that, lecithin was added to give a final concentration of 0.16 wt%. The lipid premix (50 wt% -75 wt%) was then homogenized with the soy protein solution to form a concentrated oil-in water emulsion. Subsequently,

200 mg TG/g protein was added to the emulsion. The resulting mixture was incubated at 4°C for 12 h to obtain the final product.

Preparing lecithin-soy protein-based emulsion gel as fat tissue mimic (ESL-B). Soy protein (soy protein isolate, 5 wt%-15 wt%) powder was dispersed in water and stirred overnight on a magnetic stirrer to ensure complete protein hydration. After that, lecithin was added to give a final concentration of 0.33 wt%. The lipid premix (50 wt%-75 wt%) was then homogenized with the soy protein solution to form a concentrated oil-in water emulsion. Subsequently, 200 mg TG/g protein was added to the emulsion. The resulting mixture was incubated at 4°C for 12 h to obtain the final product.

Preparing xanthan-soy protein-based emulsion gel as fat tissue mimic (ESX-A). Soy protein (soy protein isolate, 5 wt%-15 wt%) powder was dispersed in water and stirred overnight on a magnetic stirrer to ensure complete protein hydration. After that, xanthan was added to a final concentration of 0.5 wt%. The lipid premix (50 wt%-75 wt%) was then homogenized with the soy protein solution to form a concentrated oil-in water emulsion. Subsequently, 200 mg TG/g protein was added to the emulsion. The resulting mixture was incubated at 4°C for 12 h to obtain the final product.

Preparing xanthan-soy protein-based emulsion gel as fat tissue mimic (ESX-B). Soy protein (soy protein isolate, 5 wt%-15 wt%) powder was dispersed in water and stirred overnight on a magnetic stirrer to ensure complete protein hydration. After that, xanthan was added to a final concentration of 1.5 wt%. The lipid premix (50 wt%-75 wt%) was then homogenized with the soy protein solution to form a concentrated oil-in water emulsion. Subsequently, 200 mg TG/g protein was added to the emulsion. The resulting mixture was incubated at 4°C for 12 h to obtain the final product.

Preparing curdlan-soy protein-based emulsion gel as fat tissue mimic (ESSSCU-D). Soy protein (soy protein concentrate Wilcon SS, 5 wt%-15 wt%) powder was dispersed in water and stirred overnight on a magnetic stirrer to ensure complete protein hydration. After that, curdlan was added to a final concentration of 4 wt%. The lipid premix (50 wt%-75 wt%) was then homogenized with the soy protein solution to form a concentrated oil-in water emulsion.

To induce gelation via protein crosslinking, 200 mg TG/g protein was added. Sample was incubated at 4°C overnight followed by further incubation at 80°C for 30 min. After cooling down to room temperature, the final product was obtained.

- 5 **Preparing carrageenan-soy protein-based emulsion gel as fat tissue mimic (ESSSC-C).** Soy protein (soy protein concentrate Wilcon SS, 5 wt%-15 wt%) powder was dispersed in water and stirred overnight on a magnetic stirrer to ensure complete protein hydration. After that, carrageenan was added to a final concentration of 4 wt%. The lipid premix (50 wt%-75 wt%) was then homogenized with the soy protein solution to form a concentrated oil-in water
10 emulsion. To induce gelation via protein crosslinking, 200 mg TG/g protein was added. Sample was incubated at 4°C overnight followed by further incubation at 80°C for 30 min. After cooling down to room temperature, the final product was obtained.

General procedures for the characterization of the plant protein-based fat tissue mimics

- 15 **Texture profile analysis (TPA).** The textural properties of fat tissue mimics were evaluated using a texturometer. Each sample was cut into cube (1 × 1 × 1 cm) and subjected to a two-cycle compression test. The sample was placed on a sample retaining plate and compressed axially with a cylindrical probe (35 mm in diameter) to 50% of their original height in two consecutive compression cycles. The test velocity was 2 mm·s⁻¹ and the trigger force was 5.0
20 g. Each sample was analysed in triplicate. Hardness, cohesiveness, springiness and chewiness were calculated using the instrument's software based on the generated force-time curves.

- Processing property evaluation of the plant protein-based fat tissue mimics.** A vegan pork patty model was used to evaluate the cooking properties of the fat tissue mimics. The patties
25 consist of 80 wt% lean mimics and 20 wt% fat portion, without the addition of other ingredients. The fat portion was pork backfat, vegetable oil, or plant protein-based fat tissue mimic, respectively. All the ingredients were ground and shaped to obtain mini patties of approximately 10 g. Three mini-patties from each group were pan-fried at 170 °C for 1 min on each side and then cooled to 25 °C for 1 h before weighing. The weight of each patty was
30 measured before and after cooking. Cooking loss was defined as:

$$\text{Cooking loss (\%)} = (\text{Raw weight} - \text{Cooked weight}) / (\text{Raw weight}) \times 100$$

Microstructure. Nile red and fluorescein isothiocyanate (FITC) were used to label the oil droplets and protein, respectively. First, the emulsions were cut into slices of 1 cm × 1 cm × 0.5 cm. After that, slices were immersed in a Nile Red-FITC dye solution comprising of 0.003% FITC and 0.02% Nile Red for 15 min followed by rinsing in deionised water for 5 min. Slices were stored in moist conditions up to 3 h prior to image using a confocal laser scanning microscope (CLSM, FV1000, Olympus Corporation, Tokyo, Japan) equipped with a 20×0.70 NA objective. FITC and Nile Red were visualized via excitation wavelength of 488 nm and 543 nm, and emission wavelength of 497-553 nm and 553-693 nm, respectively. Images were taken at a resolution of 1,024 × 1,024 pixel (sample size 212.1 × 212.1 μm) using the Fluoview software (Olympus Corporation, Tokyo, Japan). Pork backfat was used as a positive control in this study.

Results and discussion

15

Composition and characterization of the plant protein-based fat tissue mimics. The representative formulations of plant protein-based fat tissue mimic are listed in Table 1 below. Among them, ES-A, ESSJ-A, ESSJ-B, ESSS-A, and ESSS-B were examples of soy protein-based fat tissue mimics, which were produced by soy proteins from different sources. ESL-A and ELS-B were lecithin-soy protein-based fat tissue mimic; ESX-A and ESX-B were examples of xanthan-soy protein-based fat tissue mimics; ESSSCU-D was a representative example of curdlan-soy protein based fat tissue mimic; ESSSC-C was a representative example of carrageenan-soy protein-based fat tissue mimics.

25 Table 1. Representative formulations of the plant protein-based fat tissue mimics

Samples	Soybean oil	Soybean protein	Xanthan gum	Curdlan	Carrageenan	Soy lecithin	Transglutaminase	Water
ES-A	69.5	3.6	-	-	-	-	0.7	26.2
ESSJ-A	69.5	3.6	-	-	-	-	0.7	26.2
ESSJ-B	69.5	3.6	-	-	-	-	0.7	26.2
ESSS-A	69.5	3.6	-	-	-	-	0.7	26.2
ESSS-B	69.5	3.6	-	-	-	-	0.7	26.2

ESL-A	69.5	3.6	-	-	-	0.05	0.7	26.25
ESL-B	69.5	3.6	-	-	-	0.1	0.7	26.2
ESX-A	69.5	3.6	0.15	-	-	-	0.7	26.05
ESX-B	69.5	3.6	0.3	-	-	-	0.7	25.9
ESSSCU-	69.5	3.5	--	1.2	-	-	0.7	25.1
D								
ESSSC-C	69.5	3.5	-	-	1.2	-	0.7	25.1

Textural profile analysis of the plant protein-based fat tissue mimics. A representative force-time curve obtained from the double compression test was shown in Figure 1. Based on the curve, sample's hardness, cohesiveness, springiness and chewiness can be determined. Sample's hardness is defined as the maximum force necessary for the compression. Cohesiveness represents the fraction of work required to compress the sample a second time after an initial first compression (A_2/A_1). The value thus indicates whether an irreversible loss of structural integrity and elasticity may occur after the primary compression. The smaller the cohesiveness, the greater the irreversible structural damage. Springiness is indicative of how well a sample is able to regain their initial form after being compressed, and as such yield insight into how elastic a sample is. The greater the springiness, the more elastic the material. Chewiness is defined as the product of gumminess and springiness, which equals to the product of hardness $\times$ cohesiveness $\times$ springiness. Chewiness is the most difficult to measure precisely, as mastication involves compressing, shearing, piercing, grinding, tearing, and cutting along with adequate lubrication by saliva at body temperature.

Results of the above samples are shown in Table 2. Both soy protein isolates and soy protein concentrates can be used to make fat tissue mimics. Comparison between the textural results of ESSJ-A and ESSJ-B showed that heating after the enzyme treatment can significantly increase the hardness of the gels. Similar results were observed on ESSS-A and ESSS-B. Furthermore, the hardness of the fat tissue mimics also increases with the addition of polysaccharides, such as xanthan, curdlan or carrageenan, while decreases in the presence of lecithin (data not shown). The best result was obtained when carrageenan was used together with soy protein concentrate (ESSSC-C). Based on one of the literature reviews (ref 7), the

hardness of the real pork backfat (215.16 ± 71.10 N) was not on par with the best formulation (13.93 ± 2.15 N) by less than one-tenth of its targeted hardness. Although the hardness of ESSC-C (846.04 ± 42.43 g) is still lower than that of the real pork backfat (1863.14 ± 299.96 g), it was evident that this experiment could close up the gap from one-tenth to one-third of its targeted hardness. All the fat tissue mimics have the same cohesiveness as the pork fat tissue. No significant difference in springiness was observed between the fat tissue mimic and pork fat tissue. Similar to hardness, the chewiness of fat tissue mimic is influenced by the presence of polysaccharides and lecithin.

Table 2. Textural properties of the plant protein-based fat tissue mimics

Sample	Hardness (g)	Cohesiveness	Springiness (%)	Chewiness
ES-A	250.04 ± 20.53^a	0.858 ± 0.008^a	91.66 ± 1.11^a	195.97 ± 15.69^a
ESSJ-A	277.73 ± 15.19^a	0.87 ± 0.021^a	92.94 ± 1.88^a	217.54 ± 9.39^a
ESSJ-B	320.03 ± 7.94^b	0.845 ± 0.009^a	92.46 ± 1.07^a	250.05 ± 6.37^b
ESSS-A	262.47 ± 26.08^a	0.854 ± 0.016^a	94.36 ± 1.75^a	211.31 ± 19.15^a
ESSS-B	338.98 ± 13.45^b	0.868 ± 0.011^a	92.90 ± 1.22^a	273.49 ± 14.41^b
ESL-A	264.80 ± 2.25^a	0.853 ± 0.005^a	89.17 ± 1.82^a	201.33 ± 5.59^a
ESL-B	204.72 ± 10.32^c	0.842 ± 0.006^a	91.64 ± 1.61^a	156.71 ± 7.24^c
ESX-A	237.66 ± 6.28^a	0.851 ± 0.002^a	92.13 ± 0.40^a	186.40 ± 5.27^a
ESX-B	339.02 ± 9.12^b	0.839 ± 0.019^a	89.99 ± 1.65^a	255.93 ± 10.63^b
ESSSCU-D	605.82 ± 21.00^d	0.843 ± 0.002^a	95.20 ± 1.60^a	486.07 ± 17.22^d
ESSC-C	846.04 ± 42.43^e	0.802 ± 0.016^a	93.18 ± 0.52^a	632.57 ± 47.11^e
Pork fat tissue	1863.14 ± 299.96^f	1.036 ± 0.198^a	90.421 ± 4.21^a	1777.34 ± 442.6^f

Values followed by the same small letter within the same column are not significantly different ($P > 0.05$) according to *t* test

The annotations “a-e” refer to the significant difference among groups. For example, the annotations in the hardness of ES-A and ESSJ-A are “a”, which indicates there is no significant difference between these two groups. The hardness of ES-A and ESL-B was labelled by different letters, which means these two groups have significant difference.

Processing properties of the plant protein-based fat tissue mimics. Similar to pork fat tissue, the fat tissue mimics of the present invention were observed as small white particles in the mini patties during the entire cooking process. On the contrary, pores were found on the surface and in the interior of the patties in the vegetable oil group due to the completely melting and release of oil during cooking.

Juiciness is an important eating quality attribute in meat. It is related to the water content and intramuscular fat content of meat. Many parameters affect the juiciness of meat, such as the raw meat quality, pH, water holding capacity, and the cooking procedure. The mechanism is still far from understood. What has been known is that the parameters influence the cooking loss, and part of the effect on juiciness might be due to this effect. In beef, juiciness and cooking loss are negatively correlated, implying that a high cooking loss results in low juiciness. In pork, the correlation is more sophisticated.

In this invention, the cooking loss reflects the property of fat or fat mimic when other parameters are the same. Table 3 lists the cooking losses of the pork patties with pork fat tissue, vegetable oil and a representative plant protein-based fat tissue mimic. Patties that contain soy protein-based fat tissue mimic have cooking losses that are similar to that of the animal fat tissue group, while lower than that of the vegetable oil group.

Table 3. Cooking losses of the pork patties with pork fat tissue, vegetable oil and a plant protein-based fat tissue mimic

Samples	Cooking loss (%)	Samples	Cooking loss (%)
Animal fat tissue	23.77±2.21 ^a	ESL-A	21.65±1.73 ^a
Vegetable oil	36.96±0.44 ^b	ESX-B	21.13±1.34 ^a
ES-A	24.76±3.61 ^a	ESSSC-C	21.13±2.33 ^a

Values followed by the same small letter within the same column are not significantly different ($P > 0.05$) according to *t* test.

Microstructure of the plant protein-based fat tissue mimics. A representative CLSM image of soy protein-based emulsion gel (Figure 2 (A)) showed that dispersed oil droplets (red) were

embedded in continuous protein phase (yellow). A three-dimensional protein network was observed. This structure highly resembled the microstructure of pork backfat as shown in Figure 2 (B). It effectively retarded oil releasing during cooking compared with that of pure fat.

5

Cellulose-based fat tissue mimics:

Procedure for the preparation of cellulose-based fat tissue mimics

Preparing lipid premix. The same procedure described in the plant protein-based fat tissue
10 mimic session was used.

Preparing methyl cellulose-based emulsion as fat tissues mimics (MW-2). Methyl cellulose (1 wt%-4 wt%) was dispersed in water. Then, the lipid premix (50 wt%-75 wt%), which was Wilkote 2610 (refined palm kernel and soyabean oil), was mixed with the methyl cellulose
15 solution in a kitchen mixer to form a concentrated oil-in-water emulsion. The mixing was carried out at a temperature between 30-50°C. Finally, the emulsion was incubated at 4°C until solidification to obtain final product.

Preparing methyl cellulose-based emulsion as fat tissues mimics (MSHPK). Methyl cellulose
20 (1 wt%-4 wt%) was dispersed in water. Then, the lipid premix (50 wt%-75 wt%), which consists of 80 wt% hydrogenated palm kernel oil and 20 wt% shea olein, was mixed with the methyl cellulose solution in a kitchen mixer to form a concentrated oil-in-water emulsion. The mixing was carried out at a temperature between 30-50°C. Finally, the emulsion was incubated at 4°C until solidification to obtain final product.

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Preparing methyl cellulose-based emulsion as fat tissues mimics (MSAHPK-1). Methyl cellulose (1 wt%-4 wt%) was dispersed in water. Then, the lipid premix (50 wt%-75 wt%), which consists of 90 wt% hydrogenated palm kernel oil and 10 wt% safflower oil, was mixed with the methyl cellulose solution in a kitchen mixer to form a concentrated oil-in-water
30 emulsion. The mixing was carried out at a temperature between 30-50°C. Finally, the emulsion was incubated at 4°C until solidification to obtain final product.

Preparing methyl cellulose-based emulsion as fat tissues mimics (MFHPK-1). Methyl cellulose (1 wt%-4 wt%) was dispersed in water. Then, the lipid premix (50 wt%-75 wt%), which consists of 90 wt% hydrogenated palm kernel oil and 10 wt% flaxseed oil, was mixed with the methyl cellulose solution in a kitchen mixer to form a concentrated oil-in-water emulsion. The mixing was carried out at a temperature between 30-50°C. Finally, the emulsion was incubated at 4°C until solidification to obtain final product.

Preparing hydroxypropyl methylcellulose-based emulsion as fat tissue mimics (E4M-1). Methocel™ E4M (1 wt%-4 wt%) was dispersed in water. Then, the lipid premix (50 wt%-75 wt%), which was Wilkote 2610, was mixed with the cellulose solution in a kitchen mixer to form a concentrated oil-in-water emulsion. The mixing was carried out at a temperature between 30-50°C. Finally, the emulsion was incubated at 4°C until solidification to obtain final product.

Preparing hydroxypropyl methylcellulose-based emulsion as fat tissue mimics (K100M-1). Methocel™ K100M (1 wt%-4 wt%, w/w) was dispersed in water. Then, the lipid premix (50 wt%-75 wt%), which was Wilkote 2610, was mixed with the cellulose solution in a kitchen mixer to form a concentrated oil-in-water emulsion. The mixing was carried out at a temperature between 30-50°C. Finally, the emulsion was incubated at 4°C until solidification to obtain final product.

Procedure for the characterization of the cellulose-based fat tissue mimics

Evaluating the thermal property of the cellulose-based fat tissue mimic using differential scanning calorimetry (DSC). The melting behaviour of the cellulose-based fat tissue mimics was analysed using a differential scanning calorimeter. Five milligrams of each sample were weighed into a 50 µL aluminium pan, and the pan was then sealed with aluminium cover lids. The sample was held at 15 °C for 5 min, then heated to 90 °C with a heating rate of 5°C/min, and finally kept at 90°C for 5 min. the onset melting temperature, peak melting temperature, and melting enthalpy was calculated using the Pyris Series Data Analysis software provided by the measurement manufacturer.

Processing property evaluation of the cellulose-based fat tissue mimic. The method was similar to the procedure described in the plant protein-based fat tissue mimic session. A vegan beef patty model was used to evaluate the cooking properties of the fat tissue mimic. The patties consist of 80% lean mimics and 20% fat portion.

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Microstructure of the cellulose-based fat tissue mimic. Nile red was used to label the oil droplets. First, the emulsions were cut into slices of 1 cm × 1 cm × 0.5 cm. After that, slices were immersed in 0.02% Nile Red solution for 5 min followed by rinsing in deionised water for 1 min. Slices were stored under moist condition for up to 3 h prior to image using a confocal
10 laser scanning microscope (CLSM, FV1000, Olympus Corporation, Tokyo, Japan) equipped with a 20 × 0.70 NA objective. Nile Red was visualized via excitation wavelength of 543 nm, and emission wavelength of 553-693 nm. Images per sample were taken at a resolution of 1,024 × 1,024 pixel (sample size 212.1 × 212.1 μm) using the Fluoview software (Olympus Corporation, Tokyo, Japan).

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Particle size distributions were determined by a combination of CLSM and image analysis using the image analysis software, ImageJ 1.52a. Images were first converted to an unsigned 16-bit grayscale and then converted to a binary format using the default auto threshold. Next, noise was reduced by a median filter (radius 0.1 pixel), individual particles separated by
20 watershed segmentation, and finally counted by the “Analyze Particles” tool. Particles on the edges of analyzed images and particle below a size of 1.2 μm were excluded to avoid misinterpretations. Individual particle diameter was calculated from the determined droplet area using Eq. (1) under the presumption that droplets were spherical:

$$d = 2 \times (A/\pi)^{0.5} \quad (1)$$

25 Sphericity of droplets can be expected at the used oil droplet concentration and was confirmed by CLSM images. From the obtained droplet size distributions, the Sauter mean diameter (d_{32}) was calculated as

$$d_{32} = 6/S_V \quad (2)$$

where S_V is the specific surface area:

$$30 \quad S_V = S_{\text{total}}/V_{\text{total}} \quad (3)$$

Results and discussion

Composition of the cellulose-based fat tissue mimic. The representative formulations of cellulose-based fat tissue mimic were listed in Table 4.

5 Table 4. Representative formulations of the cellulose-based fat tissue mimics

Ingredients	MW-2	MSHPK	MSAHPK-1	MFHPK-1	K100M-1	E4M-1
Water	29.4	29.4	29.4	29.4	29.4	29.4
Methyl cellulose	0.6	0.6	0.6	0.6	-	-
Methocel™K100M	-	-	-	-	0.6	-
Methocel™ E4M	-	-	-	-	-	0.6
Wilkote 2610	70	0	-	-	70	70
Hydrogenated palm kernel oil	-	56	63	63	-	-
Shea olein	-	14	-	-	-	-
Safflower oil	-	-	7	-	-	-
Flaxseed oil	-	-	-	7	-	-

Among them, MW-2 was example of methyl cellulose-based fat tissue mimic. K100M-1 and E4M-1 were examples of hydroxypropyl methylcellulose-based fat tissue mimics, which were produced by hydroxypropyl methylcellulose with different degree of substitutions.

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Characterization of the cellulose-based fat tissue mimic. The processing property of MW-2 was similar to animal fat tissues. It was observed as small translucent white particles in the patties after pan-frying. In addition, the cooking loss of MW-2 (22%, w/w) was the same as animal fat tissue and lower than that of vegetable oil group (37%, w/w). The result of sensory evaluation was shown in Figure 3. Fat tissue mimic significantly ameliorates the appearance, tenderness as well as juiciness of the patty, which in turns improve the overall acceptability. The melting behaviour of the oil-in-water emulsion was determined by DSC. the onset melting temperature, peak temperature and melting enthalpy was 29.30°C, 35.91°C and 52.58 J/g, respectively.

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Microstructure of the cellulose-based fat tissue mimic. A representative CLSM image of methyl cellulose-based fat tissue (MW-2) was shown in Figure 4. The lipid phase was stained

with Nile red. The image revealed a narrow size range of the oil droplets in MW-2, from 3 to 45 μm with a d_{32} of $15.4 \pm 1.6 \mu\text{m}$.

5 Whilst there has been described in the foregoing description preferred embodiments of the present invention, it will be understood by those skilled in the technology concerned that many variations or modifications in details of design or construction may be made without departing from the present invention.

CLAIMS

1. A method of producing a plant cellulose-based fat tissue substitute, the method
5 comprising:
 (a) adding a plant cellulose material to water to form a cellulose solution;
 (b) blending the cellulose solution with oil to form an emulsion; and
 (c) cooling the emulsion to form the fat tissue substitute,
 wherein the blending of step (b) is carried out at a temperature between 30°C to 50°C.
- 10 2. The method according to claim 1, wherein the emulsion is cooled to temperature below room temperature to form the fat tissue substitute.
3. The method according to any one of claims 1 or 2, wherein the cellulose is selected
15 from the group consisting of methyl cellulose, and hydroxypropyl methylcellulose.
4. The method according to any one of the preceding claims, wherein the amount of cellulose in the cellulose solution is between 1wt% to 4wt% of the cellulose solution.
- 20 5. The method according to any one of the preceding claims, wherein the oil is a selected from the group consisting of soybean oil, peanut oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter, *Malania oleifera* oil, palm olein, palm stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof.
- 25 6. The method according to any one of the preceding claims, further comprising adding an additive to the oil to form an oil mixture before adding the oil mixture to the cellulose solution, wherein the additive is selected from the group consisting of a flavouring compound, a micronutrient, and combinations thereof.
- 30 7. The method according to claim 6, wherein the flavouring compound is selected from the group consisting of a sweetener, salt, spice, lard flavour, beef fat flavour, beef tallow

flavour, lamb tallow flavour, chicken fat flavour, duck flavour, goose flavour, pigeon flavour, rabbit flavour, deer flavour, horse flavour, bacon flavour, ham flavour, cured sour meat flavour, monosodium glutamate, inosine monophosphate, guanosine monophosphate, hydrolysed vegetable protein and yeast extracts.

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8. The method according to claim 6, wherein the micronutrient is selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a carotenoid, and combinations thereof.

10 9. The method according to claim 6, wherein the flavouring compound is present in an amount of about between 0.07 wt% to 4 wt% of the oil mixture.

10. The method according to claim 6, wherein the micronutrient is present in an amount of about between 0.01 wt% to 0.07 wt% of the oil mixture.

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11. The method according to claim 6, wherein the amount of oil present in the oil mixture is about between 95.9 wt% to 100 wt% of the oil mixture.

12. A fat tissue substitute obtained by or obtainable from the method according to any one of claims 1 to 11.

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13. An emulsion for a meat substitute product, the meat substitute product is capable of releasing oil slowly during cooking, the emulsion comprising water, at least 0.6 wt% of a plant cellulose material, 50 wt% to 90 wt% of a lipid, a flavour compound, and a micronutrient.

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14. The emulsion according to claim 13, wherein water is present in an amount of about between 9.4 wt% to 49.4 wt% of the emulsion.

15. The emulsion according to any one of claims 13 or 14, wherein the lipid is present in an amount of about between 50 wt% to 75 wt% of the emulsion.

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16. The emulsion according to any one of claims 13 to 15, wherein the lipid is an oil selected from the group consisting of soybean oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter, *Malania oleifera* oil, palm olein, palm stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof.

17. The emulsion according to any one of claims 13 to 16, wherein the flavour compound is selected from the group consisting of a sweetener, salt, a spice, lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat flavour, duck flavour, goose flavour, pigeon flavour, rabbit flavour, deer flavour, horse flavour, bacon flavour, ham flavour, cured sour meat flavour, monosodium glutamate, inosine monophosphate, guanosine monophosphate, hydrolysed vegetable protein and yeast extracts, and the flavour compound is present in an amount of about between 0.05 wt% to 3 wt% of the emulsion.

18. The emulsion according to any one of claims 13 to 17, wherein the micronutrient is selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a carotenoid, and combinations thereof, and the micronutrient is present in an amount of about between 0.01 wt% to 0.05 wt% of the emulsion.

19. A meat substitute product comprising an emulsion according to any one of claims 13 to 18.

20. A method of producing a plant protein-based fat tissue substitute, the method comprising:

- (a) blending a plant protein and a polysaccharide with water to form a plant protein-polysaccharide solution;
- (b) blending the plant protein-polysaccharide solution with oil to form an emulsion or an emulsion gel; and
- (c) incubating the emulsion and/or emulsion gel with an enzyme to form the plant protein-based fat tissue substitute.

21. The method according to claim 20, wherein the emulsion and/or the emulsion gel is incubated with the enzyme for about 0.25 to 24 hours at about 4°C to 60°C, and the enzyme is selected from the group consisting of sulfhydryl oxidase, laccase, peroxidase, tyrosinase, and transglutaminase.
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22. The method according to claims 20 or 21, wherein the method further comprising the following steps after step (c):
- (d) heating the emulsion gel at a temperature about 60°C to 90°C for about 20 to 60 minutes; and
- 10 (e) cooling the emulsion gel to form the plant protein-based tissue substitute.
23. The method according to any one of claims 20 to 22, further comprising adding an additive to the plant protein-polysaccharide solution, wherein the additive is selected from the group consisting of salt, a polysaccharide, a lecithin, and combinations thereof.
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24. The method according to any one of claims 20 to 23, wherein the salt is present in an amount of about 0.1 wt% to 2wt% of the plant protein-polysaccharide solution and the salt is selected from the group consisting of calcium carbonate, calcium chloride, potassium chloride, sodium chloride, and combinations thereof.
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25. The method according to any one of claims 20 to 24, wherein the polysaccharide is selected from the group consisting of carrageenan, xanthan, konjac flour, locust bean gum, guar gum, gellan gum, alginate, agar, pectin, gum acacia, curdlan, a cellulose derivative, and combinations thereof.
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26. The method according to claim 23, wherein the lecithin is selected from the group consisting of a soya lecithin, a sunflower lecithin, a rapeseed lecithin, and combinations thereof.
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27. The method according to claim 26, wherein the lecithin is present in an amount of about between 0.16 to 0.33 wt% of the plant protein/protein-polysaccharide solution.

28. The method according to claim 25, wherein the xanthan is present in an amount of about between 0.5 to 2 wt%; the carrageenan is present in an amount of about between 1 to 5 wt%; and the curdlan is present in an amount of about 1 to 5 wt% of the plant protein-polysaccharide solution.

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29. The method according to any one of claims 20 to 28, wherein the oil is a low saturated fatty acid vegetable oil selected from the group consisting of soybean oil, peanut oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter, *Malania oleifera* oil, palm olein, palm stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof.

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30. The method according to any one of claims 20 to 29, further comprising adding and mixing an additive to the oil prior to blending the oil with the plant protein-polysaccharide solution to form an emulsion, wherein the additive is selected from the group consisting of a

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31. The method according to claim 30, wherein the flavouring compound is selected from the group consisting of a sweetener, salt, spice, lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat flavour, duck flavour, goose flavour, pigeon flavour, rabbit flavour, deer flavour, horse flavour, bacon flavour, ham flavour, cured sour meat

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flavour, monosodium glutamate, inosine monophosphate, guanosine monophosphate, hydrolysed vegetable protein and yeast extracts.

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32. The method according to claim 30, wherein the micronutrient is selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a carotenoid, and combinations thereof.

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33. The method according to claim 30, wherein the flavouring compound is present in an amount of about between 0.07 to 4 wt% of the oil mixture.

34. The method according to claim 30, wherein the micronutrient is present in an amount of about between 0.01 to 0.07 wt% of the oil mixture.

35. The method according to any one of claims 20 to 34, wherein the amount of plant protein-polysaccharide solution is about between 25 to 90 wt% of the cooled emulsion gel.
- 5 36. The method according to any one of claims 20 to 35, wherein the amount of plant protein present in the plant protein-polysaccharide solution is about 5 wt% to 15 wt% of the plant protein-polysaccharide solution.
37. The method according to any one of claims 20 to 36, wherein the amount of oil
10 present in the cooled emulsion gel is about 10 wt% to 75 wt% of the cooled emulsion gel.
38. An emulsion gel for a meat substitute product, the meat substitute is capable of releasing oil slowing during cooking, the emulsion gel comprising water, 1.2 wt%-13.5 wt% of a plant protein material, a polysaccharide, 10 wt%-75 wt% of a lipid, a flavour compound and
15 a micronutrient.
39. The emulsion gel according to claim 38, wherein water is present in an amount of about between 11.5 wt% to 88.8 wt% of the emulsion gel.
- 20 40. The emulsion gel according to any one of claims 38 or 39, wherein the polysaccharide is selected from the group consisting of carrageenan, xanthan, konjac flour, locust bean gum, guar gum, gellan gum, alginate, agar, pectin, gum acacia, curdlan, a cellulose derivative, and combinations thereof, and the polysaccharide is present in an amount of between 0.15 wt% to 4.5 wt% of emulsion gel.
- 25 41. The emulsion gel according to any one of claims 38 to 40, wherein the lipid is present in an amount of about between 10 wt% to 75 wt% of the emulsion gel and is a vegetable oil selected from the group consisting of soybean oil, peanut oil, canola oil, rapeseed oil, rice bran oil, sunflower oil, flaxseed oil, coconut oil, palm oil, olive oil, algae oil, cocoa butter,
30 *Malania oleifera* oil, palm olein, palm stearin, shea olein, shea butter, 1,3-dioleoyl-2-palmitoylglycerol (OPO) and combinations thereof.

42. The emulsion gel according to any one of claims 38 to 41, wherein the flavour compound is selected from the group consisting of a sweetener, salt, spice, lard flavour, beef fat flavour, beef tallow flavour, lamb tallow flavour, chicken fat flavour, monosodium glutamate, inosine monophosphate, guanosine monophosphate, hydrolysed vegetable protein and yeast extracts, and the flavour compound is present in an amount of about
5 between 0.01 wt% to 3 wt% of the emulsion gel.

43. The emulsion gel according to any one of claims 38 to 42, wherein the micronutrient is selected from the group consisting of vitamin A, vitamin D, vitamin E, vitamin K, a
10 carotenoid, and combinations thereof, and the micronutrient is present in an amount of about between 0.01 wt% to 0.05 wt% of the emulsion gel.

44. The emulsion gel according to any one of claims 38 to 43, further comprising lecithin, wherein the lecithin is present in about 0.05 wt% to 0.3 wt% of the emulsion gel.
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45. The emulsion gel according to any one of claims 38 to 44, further comprising transglutaminase, wherein the transglutaminase is present in an amount of about at least 0.4 wt% of the emulsion gel.

20 46. A meat substitute product comprising an emulsion gel according to any one of claims 38 to 45.