

METHOD FOR IMPROVING THE TEXTURE OF A DAIRY-SUBSTITUTE PRODUCT

FIELD OF THE INVENTION

The invention relates to a method for improving the texture of an edible composition, said
5 method comprising a step of incorporating a casein composition comprising casein in the
sole form of non-animal origin to an edible composition.

BACKGROUND OF THE INVENTION

The impact of dairy products on environment

10 Environmental and ethical concerns related to food products of animal origin have raised
over the last decades. The burden of animal breeding for a 7 billion population has become
increasingly high. The environmental consequences are severe in terms of anthropogenic
greenhouse gas (GHG) emissions, water consumption, pollution by effluents, land
occupation, deforestation, and others.

15 Animal farming contributes to climate change by emitting greenhouse gases such as carbon
dioxide, methane, and nitrous oxide. These gases trap heat in the atmosphere and cause
global warming. According to a report by the Food and Agriculture Organization of the
United Nations (FAO), animal agriculture is responsible for about 14.5% of human-caused
greenhouse gas emissions, which is more than the emissions from all global transportation
20 (Rotz (2017) Modeling greenhouse gas emissions from dairy farms. J Dairy Science, Volume
101, pages 6675–6690).

According to a report by the Food and Agriculture Organization (FAO) of the United Nations
(FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, *Greenhouse Gas
Emissions from the Dairy Sector. A Life Cycle Assessment*), the global dairy sector alone
25 contributes about 4% of the total human-induced greenhouse gas emissions. This includes
emissions from feed production, enteric fermentation (the digestive process of ruminant
animals), manure management, processing and transportation of milk and dairy products.
The main greenhouse gases emitted by the dairy sector are carbon dioxide (CO₂), methane
(CH₄), and nitrous oxide (N₂O). Methane is a particularly potent greenhouse gas, with a
30 global warming potential 28 times higher than CO₂ over a 100-year period.

Global Pasture already represented in 2000 about 30 million km² (nearly the size of Africa),
(Ramankutty, N., A. T. Evan, C. Monfreda, and J. A. Foley (2008), Farming the planet: 1.

Geographic distribution of global agricultural lands in the year 2000, Global Biogeochem. Cycles, 22, GB1003). Land use for dairy production can lead to deforestation, biodiversity loss, soil erosion, and desertification. It can also compete with other land uses, such as food crops, biofuels, or natural habitats.

- 5 Livestock industries also have a very strong impact on water consumption, (Sultana et al (2014). Comparison of water use in global milk production for different typical farms. Agricultural Systems, Volume 129, pages 9-21; Ercin and Aldaya (2012) The water footprint of soy milk and soy burger and equivalent animal products. Ecological Indicators, Volume 18, pages 392-402), and drain about 8% of global water supply, largely due to feed production
- 10 (Schlink et al (2010), Water requirements for livestock production: a global perspective; rev. Sci. tech. 29:603-619). A related environmental impact of dairy production is eutrophication, which is the enrichment of water bodies with excess nutrients, such as nitrogen and phosphorus. These nutrients can come from fertilizers used for feed crops, manure from animals, or wastewater from processing facilities. Eutrophication can also affect human
- 15 health by contaminating drinking water sources or causing harmful algal blooms.

- Animal farming also causes environmental problems such as deforestation, water pollution, soil degradation, and biodiversity loss. But in addition, animal welfare has become an increasingly important concern. The scale-up of meat and dairy production and processing has turned it into an intensive industrial process, which is increasingly perceived as ethically
- 20 not acceptable.

While the extensive use of food of animal origin is raising very serious environmental and ethical concerns, there is in the sale time a growing demand for food. According to estimates by the Food and Agriculture Organization (FAO), by 2050, food production, including meat, will have to increase by 70% to feed a world population of 10 billion.

- 25 *Food transition and the alternative protein*

A transition to a diet less rich in animal-based products can reduce the recourse to cattle breeding.

- Animal-free substitutes for meat and dairy are products that mimic the taste, texture, and nutritional value of animal products, but are made from plant-based or other non-animal
- 30 ingredients. They are often referred to as vegan or plant-based alternatives.

In the case dairy products, such alternative can also address other issues, related to health, such as lactose intolerances, allergies (Mousan and Kamat (2016) Cow's Milk Protein Allergy. Clin Pediatr (Phila), Volume 55(11) pages 1054-63.; Manuyakorn and Tanpowpong (2018)

Cow milk protein allergy and other common food allergies and intolerances. *Paediatr Int Child Health*, Volume 39(1), pages 32-40), and others, such as a high content in saturated fatty acids, known to have a potential negative impact on health. (Micha and Mozaffarian (2010) Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: A fresh look at the evidence. *Lipids*, Volume 45, pages 893–905; Jakobsen et al (2009) Major types of dietary fat and risk of coronary heart disease: A pooled analysis of 11 cohort studies. *Am. J. Clin. Nutr*; Volume 89, pages 1425–1432; Nettleton et al (2017) Saturated fat consumption and risk of coronary heart disease and ischemic stroke: A science update. *Ann Nutr Metab* Volume 70, pages 26–33). Lactose intolerance is due to the lack of lactase, the enzyme that degrades lactose in the stomach and small intestine, and result in an accumulation of lactose in the colon, and its digestion by bacteria (Ugidos-Rodríguez et al (2018) Lactose malabsorption and intolerance: a review. *Food Funct*, Volume 9(8), pages 4056-4068). It is a very common feature in humans, is genetically determined, and results in the need for a dairy-free diet. In a dairy-substitute product, this composition could be modulated, to avoid lactose, and saturated fatty acids, and even, allergenic proteins.

However, plant-based dairy-substitute products have often proven to fall very short from really mimicking the conventional dairy products both in terms of organoleptic and nutritional properties, and notably in terms of protein composition.

Caseins

Indeed, replicating dairy texture, taste while preserving nutritional composition only with plant-based dairy-substitute products is a challenging task. For example, plant-based dairy-substitute products are by definition void of animal proteins, and therefore, do not contain caseins, milk's major protein, and a hallmark of most dairy products. Caseins represent more than 80% of cow's milk proteins, but bring also important functionalities such as curdling, melting and stretching. Mimicking both their nutritional input and texturing role is typically challenging.

The four major caseins, alpha-S1-, alpha-S2-, beta-, and kappa-caseins, represent more than 80% of cow's milk proteins, and nearly all of cheese proteins, since the other milk proteins (commonly named "whey proteins") such as beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin, and immunoglobulins are removed in whey after curdling. The solubility of caseins highly depends on pH, temperature and salt concentration (Post et al. (2012) Effect of temperature and pH on the solubility of caseins: Environmental influences on the dissociation of caseins. *J. Dairy Sci.* Volume 95 : pages 1603–1616), but in milk, they are not found as soluble proteins, but organized in micelles, resulting in a colloidal structure. Casein

micelles are roughly spherical particles ranging from 50 to 600 nm in diameter, with an average diameter of around 200 nm (de Kruif (1998), Supra-aggregates of casein micelles as a prelude to coagulation J Dairy Sci, Volume 81 pages 3019-3028; de Kruif et al. (2012) Casein micelles and their internal structure. Advances in Colloid and Interface Science, 5 Volume 171–172, pages 36-52). The evolution of this colloid into a curd is at the basis of cheese production (Gillis JC, Ayerbe A, Le fromage 4ème edition, Lavoisier - Technique Et Documentation 20 avril 2018). It is believed that κ -casein has a key role as a stabilizer of the micelle structure.

Caseins can be isolated from milk as sodium, calcium or potassium caseinate, by 10 neutralization after acid-mediated or rennet-mediated coagulation (Sarode et al. (2016) Methods of Manufacture. In: Caballero, B., Finglas, P., and Toldrá, F. (eds.) The Encyclopedia of Food and Health vol. 1, pp. 676-682. Oxford: Academic Press) The different individual caseins (α -S1-, α -S2-, β -, or κ -caseins) can also be purified individually from bulk casein preparations, based on their different physico-chemical 15 properties, using membrane filtration at low temperature (Murphy and Fox (1991) Fractionation of sodium caseinate by ultrafiltration. Food Chem. Volume 39 Pages 27–38; Ward and Bastian (1996) A method for isolating β -casein. J. Dairy Sci. Volume 79, pages 1332–1339; Huppertz et al. (2006). A method for the large-scale isolation of β -casein. Food Chem. Volume 99, pages 45–50; Lamothe et al. (2007). Short communication: Extraction of 20 β -casein from goat milk. J. Dairy Sci. Volume 90, pages 5380–5382; O'Mahony, et al. (2007). Purification of β -casein from milk. US Pat. No. 0104847 A1) or selective precipitation (Law and Leaver (2007) Methods of extracting casein fractions from milk and caseinate and production of novel products. Hanna Research Institute, assignee. WO Pat. No. 03003847; Post et al. (2009). β -Casein as a bioactive precursor—Processing for purification. Aust. J. 25 Dairy Technol. Volume 64, pages 84–88; Post and Hinrichs (2011) Large-scale isolation of food-grade β -casein. Milchwissenschaft, Volume 66, pages 361–364).

Precision fermentation

In order to more closely match dairy products compositions, with no recourse, or less recourse to animal-product, specific compounds can also be produced by precision 30 fermentation. Production by fermentation is based on the growth of bacteria or fungi producing a compound of interest in a fermenter, usually followed by the recovery and purification of the compound of interest. The making of proteins by fermentation is a process that has been used in the food industry, with one of the first examples being recombinant chymosin, the first artificially produced enzyme to be registered and allowed by the US Food

and Drug Administration and represents today a large part (more 80% in the US today) of the rennet market. (Food Biotechnology in the United States: Science, Regulation, and Issues". U.S. Department of State. Retrieved 2006-08-14).

Several studies have described the production by fermentation in various microorganisms of milk's specific molecules, including caseins and whey proteins such as beta-lactoglobulins, , and the use or potential use to make animal-free dairy substitutes, together with plant-based other ingredients:

- WO1991008675 describes the production of recombinant proteins for infant formula
- WO2016029193 describes the general principle of using recombinant milk's protein in food applications, gives a formula for cream and milk.
- WO2018039632, describes the mixing of fermentation caseins and plant-based proteins.
- WO2020219596 describes the production of recombinant beta-lactoglobulin
- WO2020223700 describes the use of alpha-S1 and kappa casein, without beta casein, to make micelles, and curds for cheese and yoghourts applications.
- WO2022098835 describes the mixing caseins from different animals, and their use for making micelles, curds and cheese substitutes.
- WO2022098853 describes the mixing of alpha-S1 and kappa casein caseins to make micelles, one being recombinant, and their use for making micelles, curds and cheese substitutes
- WO2022038601 describes general methods to make micelles.
- WO2022239000 describes the production of beta lactoglobulin in *Pichia pastoris*, and products made thereof.
- WO2023133417 describes the mixing of recombinant caseins and lipids made by fermentation, wherein the lipid is not necessarily recombinant, and describes production processes for casein and lipids.
- WO2022058573 describes cheese substitutes made from recombinant casein.
- WO2022253816 describes a method to produce caseins by precision fermentation, and the use of these caseins for making cheese substitutes.

Problem to be solved

Plant-based dairy-substitute products are largely available on the market today. They generally have the advantage of being lactose free and display a low cholesterol rate. These include notably substitutes for milk, cheese, yoghurt, ice-creams. These products can more or less closely mimic the conventional dairy products in terms of aspect, taste, nutritional values, and functionalities (in the case of ingredient, pizza cheese for example). However, as of today, they generally failed to fully convince the consumers.

Indeed, and as discussed above, mimicking taste, texture, and composition are three different challenges, and solving all of them is a complex process. In addition, solving them one by one may require the use of several ingredients and additives, thereby significantly increasing process complexity, manufacturing costs, as well as the list of ingredients.

In particular, commercial dairy-substitute products texture is often obtained with additives, including texturing agents. Such texturing agents are often found on the list of ingredients of vegan cheese, and are very commonly used in plant-based yoghurt (Montemurro, et al. Plant-Based Alternatives to Yogurt: State-of-the-Art and Perspectives of New Biotechnological Challenges. Foods 2021, 10, 316).

Texturing agents are food additives that are added to food products. They can be derived from natural sources, such as plants, animals, and seaweeds, or from synthetic sources, such as polymers and phosphates.

Texturing agents are widely used in the food industry to enhance the quality and appeal of various food products, such as bakery, confectionery, dairy, meat, beverages, and others, and improve their texture, stability, mouthfeel, and appearance. They can help to increase the creaminess, thickness, viscosity, pulpiness, and smoothness of food products. They can also help to extend the shelf life of food products by preventing microbial growth, reducing syneresis, providing freeze/thaw stability, and suspending ingredients in solution.

Texturing agents are important for meeting the consumer expectations and preferences for different food products. They can also help to create novel and innovative food products with unique textures and sensations. For example, texturing agents can be used to create crispy coatings, chewy candies, gummy bears, creamy yogurts, and fluffy cakes. Texturing agents can also be used to modify the texture of existing food products to appeal to a wider range of consumers or to differentiate from competitors.

Texturing agents can be derived from natural sources, such as plants, animals, and seaweeds, or from synthetic sources, such as polymers and phosphates. Some of the major texturing agents for food are:

- 5 • Hydrocolloids: These are water-soluble polysaccharides or proteins that form gels or viscous solutions when added to water. They can act as thickeners, stabilizers, gelling agents, or emulsifiers. Some examples of hydrocolloids are agar, alginate, carrageenan, gelatin, pectin, xanthan gum, and guar gum.
- 10 • Emulsifiers: These are substances that help to form and stabilize emulsions, which are mixtures of two immiscible liquids, such as oil and water. Emulsifiers can reduce the surface tension between the liquids and prevent them from separating. Some examples of emulsifiers are lecithin, mono- and diglycerides, polysorbates, and sucrose esters.
- 15 • Stabilizers: These are substances that help to maintain the consistency and structure of food products, such as preventing ice crystals from forming in frozen desserts or preventing syneresis (water loss) in yogurt. Stabilizers can also improve the texture and mouthfeel of food products by increasing their viscosity or elasticity. Some examples of stabilizers are cellulose derivatives, gums, starches, and proteins.
- 20 • Dough conditioners: These are substances that improve the quality and performance of doughs, such as enhancing their elasticity, extensibility, strength, volume, and shelf life. Dough conditioners can also modify the texture and appearance of baked products, such as making them softer, fluffier, or crispier. Some examples of dough conditioners are enzymes, gluten, oxidizing agents, reducing agents, and yeast.
- 25 • Phosphates: These are salts or esters of phosphoric acid that have various functions in food products, such as buffering, sequestering, chelating, emulsifying, stabilizing, and leavening. Phosphates can also improve the texture and quality of food products by enhancing their water retention capacity, binding capacity, solubility, gel strength, and color. Some examples of phosphates are sodium phosphate, potassium phosphate, calcium phosphate, and sodium hexametaphosphate

30 Consumer's perception about food additive is often negative (Osaili et al. (2023) Consumers' knowledge and attitudes about food additives in the UAE. PLoS ONE 18(3):e0282495; Miao et al (2020) Decreasing consumers' risk perception of food additives by knowledge enhancement in China. Food Quality and Preference 79:103781). Acceptance is often linked with the concept of naturality, with additive being considered as unnatural (Bearth et al. The

consumer's perception of artificial food additives: Influences on acceptance, risk and benefit perceptions (2014) Food quality and preferences 38, 14-23).

In addition, long lists of ingredients are increasingly considered as negative criteria (Kim, Michelle & Chen, Tianqi & Gershon, Rachel & scott, sydney & kupor, daniella & Trudel, Remi. (2022). Less is More (Natural): The Effect of Ingredient Quantity Framing on Consumer Preferences and Naturalness Perceptions).

Therefore, there is a need for new solutions for improving texture of edible composition and notably dairy-substitute products.

10 SUMMARY OF THE INVENTION

There is a need for alternative natural food-additive for improving the texture of edible compositions, such as plant-based edible composition.

We found that a small amount of non-animal casein made by precision fermentation was sufficient to improve the texture of plant-based edible composition, such as plant-based dairy-substitute products. For example, the texturing properties of this casein could be used to achieve dairy-like products, while adding a small amount can significantly improve texture while bringing additional protein content.

A first object of the present invention relates to a use of a casein composition for obtaining a second edible composition from a first edible composition, said second edible composition having an improved texture compared to the first edible composition, wherein said casein composition comprises casein in the sole form of non-animal origin.

A second object of the present invention relates to a method for improving the texture of a first edible composition, said method comprising a step of incorporating a casein composition comprising casein in the sole form of non-animal origin to a first edible composition thereby obtaining a second edible composition, said second edible composition having an improved texture compared to the first edible composition.

A third object of the present invention relates to a method for preparing a second edible composition having an improved texture, comprising the step of incorporating a casein composition comprising casein in the sole form of non-animal origin into a first edible composition, thereby obtaining a second edible composition having an improved texture compared to the first edible composition.

A fourth object of the present invention relates to a second edible composition having an improved texture comprising:

- a) a first edible composition, and
- b) a casein composition comprising casein in the sole form of non-animal origin,

5 wherein the second edible composition comprises proteins in the sole form of non-animal origin,

wherein the second edible composition comprises from 0.1 % to 10 % of casein of non-animal origin, and

10 wherein the second edible composition has preferably a pH below pH 7, such as below 6, such as below 5. In one embodiment, the second edible composition of the invention has a pH from pH 3 to pH 6.9, such as from pH 3 to pH 5, such as from pH 4 to pH 5, such as pH 4.5. In another embodiment, the second edible composition of the invention has a pH from pH 5 to pH 6, such as pH 5.8. In another embodiment, the second edible composition of the invention has a pH from pH 6 to pH 6.9, such as pH 6.8.

15 The following embodiments apply to each of the four objects of the invention.

In some embodiments, the second edible composition comprises from 0.1 % to 10 % of casein of non-animal origin, such as from 0.1 % to 2.5 % of casein of non-animal origin.

20 In some embodiments, the second edible composition comprises from 0.1 % to 10 % of casein of non-animal origin, such as from 2.5 % to 5 % of casein of non-animal origin or from 5 % to 10 % of casein of non-animal origin.

In some embodiments, the viscosity of the second edible composition is increased by at least 25% relatively to the viscosity of the first edible composition.

In some embodiments, the viscosity of the second edible composition is increased by at least 100% relatively to the viscosity of the first edible composition.

25 In some embodiments, the second edible composition comprises from 0.1 % to 10 % of casein of non-animal origin and the viscosity of the second edible composition is increased by at least 50% relatively to the viscosity of the first edible composition, such as at least 100% relatively to the viscosity of the first edible composition.

30 In some embodiments, the first edible composition is a beverage having a viscosity of less than 50 cP at 20°C.

In some embodiments, the first edible composition is a mixture of ingredients suitable for preparing a yogurt, said mixture having a viscosity from 40 cP to 250 cP at 4°C, such as from 40cP to 100 cP.

In some embodiments, the first edible composition is a beverage having a viscosity of less
5 than 3 cP at 20°C.

In some embodiments, the casein of non-animal origin represents at least 25 % w/w of the casein composition.

In some embodiments, the casein composition is in a powder form, such as a spray-dried casein composition.

10 In some embodiments, the casein composition is in a powder form, such as a spray-dried casein composition, and the casein of non-animal origin represents at least 50 % w/w of the casein composition, such as at least 75% w/w of the casein composition.

In some embodiments, the casein of non-animal origin is substantially free of phosphorylation.

15 In some embodiments, the casein of non-animal origin is produced in bacteria, such as *E. Coli*.

In some embodiments, at least 90% of the casein of the casein composition is non-micellar casein.

In some embodiments, the second edible composition has a pH below or equal to pH 7.

20 In some embodiments, the second edible composition has a pH from pH 4 to pH 5.5.

In some embodiments, the second edible composition has a pH from pH 5.5 to pH 6.9.

In some embodiments, the first edible composition is a plant-based edible composition, preferably the first edible composition is a dairy-substitute product made from plant milk.

In some embodiments, the first edible composition is a dairy-substitute product, and the
25 second edible composition is a dairy-like product. For example, the dairy-substitute product may be a milk-substitute or a dairy-substitute product derived from a milk-substitute, such as a yogurt-substitute, a cheese-substitute, or a buttercream-substitute.

In some embodiments, the first edible composition is a plant-based edible composition, preferably the first edible composition is a dairy-substitute product prepared from plant milk.

In some embodiments, the improved texture is selected from increased creaminess, increased density, increased thickness, increased viscosity, increased pulpiness, increased smoothness, or combinations thereof.

5 In some embodiments, the second edible composition contains from 0.1 % to 10 % of casein of non-animal origin.

In some embodiments, the second edible composition has a pH below pH 7, such as from pH 3 to pH 5, or from pH 4 to pH 5, or from pH 5 to pH 6, or from pH 6 to pH 6.9.

10 In some embodiments, the casein of non-animal origin is one, two or three casein(s) selected from the group consisting of alpha-S1 casein, alpha-S2 casein, beta casein and kappa casein.

In some embodiments, the casein of non-animal origin is alpha-S1 casein, beta casein, a mixture of alpha-S1 casein and beta casein, a mixture of alpha-S1 casein and alpha-S2 casein, or a mixture of beta casein, alpha-S1 casein and alpha-S2 casein.

15 In some embodiments, the casein composition does not comprise at least one casein protein selected from the group consisting of alpha-S1 casein, alpha-S2 casein, beta casein and kappa casein.

In some embodiments, the casein composition does not comprise kappa casein.

In some embodiments, the casein of non-animal origin is mixture of alpha-S1 casein and beta casein.

20 In some embodiments, at least 50% of the casein of the casein composition is caseinate and/or acid casein, such as 1, 2, 3 or 4 caseinate selected from the group consisting of sodium caseinate, calcium caseinate, potassium caseinate and magnesium caseinate and/or acid casein.

25 In some embodiments, at least 50 % of the casein of the casein composition is sodium caseinate.

In some embodiments, the second edible composition has protein content from 0.5 % to 40 %.

In some embodiments, the second edible composition has lipid content from 0 % to 50 %.

30 In some embodiments, the second edible composition comprises from 0.1 % to 10 % of casein of non-animal origin, such as from 0.1 % to 9 %, such as from 0.1 % to 8 %, such as from 0.1 % to 7 %, such as from 0.1 % to 6 %, such as from 0.1 % to 5 %, such as from

0.1 % to 4 %, such as from 0.5 % to 10 %, such as from 0.5 % to 9 %, such as from 0.5 % to 8 %, such as from 0.5 % to 7 %, such as from 0.5 % to 6 %, such as from 0.5 % to 5 %, such as from 1 % to 10 %, such as from 1 % to 9 %, such as from 1 % to 8 %, such as from 1 % to 7 %, such as from 1 % to 6 %, such as from 1 % to 5 %, such as from 1 % to 4 %, such as from 2 % to 10 %, such as from 2 % to 9 %, such as from 2 % to 8 %, such as from 2 % to 7 %, such as from 2 % to 6 %, such as from 2 % to 5 %, such as from 2 % to 4 %, such as from 3 % to 10 %, such as from 3 % to 9 %, such as from 3 % to 8 %, such as from 3 % to 7 %, such as from 3 % to 6 %, such as from 3 % to 5 %, such as from 3 % to 4 % of casein of non-animal origin, such as about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10% of casein of non-animal origin.

In some embodiments, the first and second edible compositions are free of texturing agents selected from starches, modified starches, gums [e.g., xanthan gum, bean gum, guar gum, gum arabic, gum ghatti, gum karaya, gum tragacanth, gellan gum], hydrocolloids [e.g., guar, acacia, locust bean gum, xanthan, gellan, carrageenan, cellulose, carboxymethyl cellulose, microcrystalline cellulose, methylcellulose, hydroxypropyl methyl cellulose, hydroxypropyl cellulose, pectin, low methoxyl pectin, gelatin, agar, furcellaran, dextran, or combinations thereof.

20 DETAILED DESCRIPTION

Definitions

In the context of the invention, the term “(w/w)” means weight/weight, on the total weight (mention is made when the quantities are evaluated on dry matter).

In the context of the invention, the term “edible composition” means a food product. In the context of the invention, the term “second edible composition” means an edible composition (first edible composition) supplemented with a casein composition comprising casein in the sole form of non-animal origin. The edible composition may be a dairy-substitute product or a dairy-like product.

In the context of the invention, the term “dairy product” means food product made from (or containing) dairy milk. A dairy product is preferably chosen from the group consisting in milk, cheese, yogurt, and cream.

In the context of the invention, the term “dairy-substitute product” means a product designed to mimic the sensory characteristics and functional properties of a dairy product,

while being made from ingredients of non-animal origin. Dairy-substitute products are formulated from non-animal sources, typically plant-based sources, to mimic taste, texture, and/or functionality of their dairy counterparts. Dairy-substitute product can be used in various culinary applications and serve as alternatives to traditional dairy products.

- 5 In the context of the invention, the term "milk-substitute" means a liquid that resembles dairy milk but being made from ingredients of non-animal origin. For example, a milk-substitute has one or more of the following: substantially similar (or similar) color, taste, nutritional content, and other quality as dairy milk. A milk-substitute is formulated from non-animal sources, typically plant-based sources. Typically, a milk-substitute includes its main
- 10 ingredients (but water) from plant-based sources, and is commonly known as a "plant-based milk substitute". However, depending on the national/regional specific regulatory rules, a "plant-based milk-substitute" may be marketed as a "plant-based beverage" (e.g., almond-based beverage) or a "plant milk" (e.g., coconut milk). For example, in the European Union, "plant-based milk substitutes" for sales cannot be labeled as "milk", except for coconut milk
- 15 which corresponds to a specific product. Examples of "plant-based milk-substitutes" according to the invention, which are marketed in Europe without using the term "milk", are disclosed in the Examples. In the present description, we will use the terms "milk-substitute", "plant-based milk" or "plant-milk", even if some national/regional specific regulatory rules prohibit the term "milk" for plant-based beverages, for the sake of simplicity.
- 20 The term "dairy milk" as used herein refers to a white fluid secreted by the mammary glands of female mammals. Dairy milk consists of an emulsion of fat in an aqueous solution comprising proteins (e.g., casein, albumin), sugars, inorganic salts, and other ingredients. Suitable mammals from which dairy milk can be obtained include but are not limited to cow, sheep, goat, buffalo, donkey, horse, camel, yak, water buffalo, human, and other mammals.
- 25 Dairy milk obtained from cow typically contains around 3.5% fat (whole cow milk). Fat levels can be reduced to standardized levels to obtain different grades of cow milk that comprise from 0% to about 75% by weight of the fat present in whole cow milk, including but not limited to 2% cow milk (cow milk comprising 2% by weight of fat), 1% cow milk (cow milk comprising 1% by weight of fat), and skim cow milk (cow milk comprising no fat).
- 30 In the context of the invention, the term "yogurt-substitute" means a food that resembles dairy yogurt but being made from ingredients of non-animal origin. For example, a yogurt-substitute has one or more of the following: substantially similar (or similar) color, taste, nutritional content, and other quality as dairy yogurt. A yogurt-substitute is formulated from non-animal sources, typically from plant-based sources, such as from plant-based milk-

substitute, and eventually from plant-based oil. Typically, a yogurt-substitute includes its main ingredients from plant-based sources, such as from plant-based milk-substitute.

In the context of the invention, the term "cheese-substitute" means a cheese that resembles dairy cheese but being made from ingredients of non-animal origin. For example, a cheese-substitute has one or more of the following: substantially similar (or similar) color, taste, nutritional content, and other quality as dairy cheese. A cheese-substitute is formulated from non-animal sources, typically from plant-based sources, such as from plant-based milk-substitute and/or from plant-based oil. Typically, a cheese-substitute includes its main ingredients from plant-based sources, such as from plant-based milk-substitute and/or from plant-based oil. In the context of the invention, the cheese-substitute encompasses fresh cheese-substitute, soft-cheese substitute, semi-soft cheese-substitute and hard-cheese substitute.

In the context of the invention, the term "cream-substitute" means a cream that resembles dairy cream but being made from ingredients of non-animal origin. For example, a cream-substitute has one or more of the following: substantially similar (or similar) color, taste, nutritional content, and other quality as dairy cream. A cream-substitute is formulated from non-animal sources, typically from plant-based sources, such as from plant-based milk-substitute and/or from plant-based oil. Typically, a cream-substitute includes its main ingredients from plant-based sources, such as from plant-based milk-substitute and/or from plant-based oil. The cream-substitute includes whipped cream-substitute.

In the context of the invention, the term "dairy-like product" means a dairy-substitute product supplemented with a casein composition comprising casein in the sole form of non-animal origin. In the context of the invention, a dairy-like product has improved similarity in texture to a dairy product compared to a dairy-substitute product.

In the context of the invention, the term "plant oil" or "plant-based oil" or "vegetable oil" means oil extracted from plant source.

In the context of the present invention, the term "casein" designates one or more casein selected from the group consisting in alpha-S1-, alpha-S2-, beta-, or kappa-casein.

In the context of the present invention, the term "casein composition" designates a composition which contains casein. Such casein composition can be solid or liquid, and contains at least one casein as defined above. Such solid casein composition is preferably in a powder form, such as a spray-dried casein composition. Such casein composition can result from the purification of casein from the production of caseins by microbial cultures and

subsequent purification. Such casein composition can comprise other compounds, notably calcium, other proteins, lipids, carbohydrates and others. A casein composition produced by a microbial culture (microbial fermentation) is a casein composition comprising casein in the sole form of non-animal origin.

5 In the context of the present invention, the term "of non-animal origin" means a compound or composition which has not been directly derived from an animal, produced from animal cells in culture, or isolated from animal products such as milk. Proteins produced in a microorganism by precision fermentation are thus "of non-animal origin" even though some products derived from animals, such as bacto peptone, or even waste from the dairy
10 industry, can be used in the fermentation medium of the microorganism. Therefore, in the context of the invention, a protein that is naturally produced in animals will be called of non-animal origin when it is produced in a microorganism (such as bacteria or yeast) or in non-animal cells, such as in plant cells, even though its sequence or structure may be identical to the sequence or structure of the protein that would be isolated from animal.

15 In the context of the present invention, the term "animal-free" means a compound or composition which has not been derived from an animal, from animal cells in culture, or from animal products such as milk, and whose production process does not involve any feedstock or additive of animal origin.

In the context of the present invention, the term "fermentation" designates the process used
20 for the production of a compound of interest, comprising the steps of growing a cellular culture producing the compound of interest in a fermenter, and recovering the compound of interest. In the case of microbial fermentation, bacteria or fungi (such as yeasts) are used for production.

In the context of the present invention the term "recombinant" means that the organism or
25 microorganism, is genetically modified so as to contain a nucleic acid molecule encoding at least a protein, as compared to a wild-type or non-modified organism or microorganism.

In the context of the present invention, the term "texture" encompasses creaminess, density, thickness, viscosity, pulpiness, smoothness, or combinations thereof. An improved texture, such as increased creaminess, increased density, increased thickness, increased viscosity,
30 increased pulpiness, increased smoothness, or combinations thereof. In the context of the present invention, the increased texture is preferably an increased viscosity, because the viscosity of an edible composition is easy to measure by a skilled person. For example,

viscosity can be characterized with a viscometer or a rheometer, as detailed in the present description.

The terms "glycosylation" and "glycosylated" as used herein refer to the attachment to proteins of glycan groups (i.e., monosaccharides, disaccharides, polysaccharides, linear
5 glycans, branched glycans, glycans with galf residues, glycans with sulfate and/or phosphate residues via C-linkage, N-linkage, or O-linkage, or via glypiation or phosphoglycosylation. Non-limiting examples of such glycan groups include D-glucose, D-galactose, D-mannose, L-fucose, N-acetyl-D-galactose amine, N- acetyl-D-glucose amine, N- acetyl-D-neuraminic acid, galactofuranose, phosphodiesteres, acetylglucosamine, acetylgalactosamine, and sialic acid.

10 The terms "phosphorylation" and "phosphorylated" as used herein refer to the attachment to proteins of phosphate groups. Phosphorylation of caseins is an important posttranslational modification for casein from animal origin occurring after the synthesis of the polypeptide chains in the Golgi apparatus of the mammary epithelial cell under the action of protein kinases. These kinases phosphorylate Ser or Thr by recognizing the tripeptide sequence
15 Ser/Thr-X-Glu/SerP/Asp, where X represents any AA residue and P indicates phosphorylation.

Use and method of the invention

A first object of the present invention relates to the use of a casein composition for obtaining a second edible composition from a first edible composition, said second edible composition
20 having an improved texture compared to the first edible composition, wherein said casein composition comprises casein in the sole form of non-animal origin.

Preferably, the present invention relates to the use of a casein composition for obtaining a dairy-like product from a dairy-substitute product, said dairy-like product having an improved texture compared to the dairy-substitute product, wherein said casein composition comprises
25 casein in the sole form of non-animal origin.

A second object of the present invention relates to a method for improving the texture of a first edible composition, said method comprising a step of incorporating a casein composition comprising casein in the sole form of non-animal origin to a first edible composition thereby obtaining a second edible composition, said second edible composition having an improved
30 texture compared to the first edible composition.

Preferably, the present invention relates to a method for improving the texture of a dairy-substitute product, said method comprising a step of incorporating a casein composition comprising casein in the sole form of non-animal origin to a dairy-substitute product thereby

obtaining a dairy-like product, said dairy-like product having an improved texture compared to the dairy-substitute product.

A third object of the present invention relates to a method for preparing a second edible composition having an improved texture, comprising the step of incorporating a casein composition comprising casein in the sole form of non-animal origin into a first edible composition, thereby obtaining a second edible composition having an improved texture compared to the first edible composition.

It is understood that the improved texture, according to the first, second and third object of the present invention, results from the incorporation of the casein composition comprising casein in the sole form of non-animal origin to the first edible composition.

The following embodiments, unless otherwise specified, relates to the first, second and third objects of the invention.

Casein composition

In the context of the present invention, a casein composition comprising casein in the sole form of non-animal origin is used.

In some embodiments, the casein of non-animal origin represents more than 50% of the proteins of the casein composition comprising casein in the sole form of non-animal origin. In a preferred embodiment, the casein of non-animal origin represents at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the proteins of the casein composition. The amount of proteins can be measured by any method known in the art. The casein composition comprising casein in the sole form of non-animal origin may contain other components than casein, such as other proteins, carbohydrate and/or lipids. This is particularly true when said casein composition results from a culture of recombinant microorganisms expressing casein (microbial fermentation).

In some embodiments, casein of non-animal origin represents the major part of dry weight of the casein composition, for example casein of non-animal origin represents at least 25 % w/w of the casein composition, for example at least 50% w/w of the casein composition, such as at least 60% w/w, at least 70% w/w, at least 75% w/w, at least 80% w/w, at least 85% w/w, at least 90% w/w, or at least 91% w/w, at least 92% w/w, at least 93% w/w, at least 94% w/w, at least 95% w/w, at least 96% w/w, at least 97% w/w, at least 98% w/w, or at least 99% w/w of the casein composition. The percentage of casein in the casein

composition corresponds to the mass of casein divided by the total dry mass of the casein composition.

In some embodiments, the casein composition is a spray-dried casein composition.

In some embodiments, the casein of non-animal origin is one, two or three casein(s)
5 selected from the group consisting of alpha-S1 casein, alpha-S2 casein, beta casein and kappa casein.

In some embodiments, the casein of non-animal origin is alpha-S1 casein, beta casein, a mixture of alpha-S1 casein and beta casein, or a mixture of alpha-S1 casein and alpha-S2 casein, or a mixture of beta casein, alpha-S1 casein and alpha-S2 casein.

10 In some embodiments, the casein composition comprising casein in the sole form of non-animal origin does not comprise at least one casein protein selected from the group consisting in of alpha-S1 casein, alpha-S2 casein, beta casein and kappa casein.

In some embodiments, the casein composition comprising casein in the sole form of non-animal origin does not comprise kappa casein.

15 In some embodiments, the casein composition comprising casein in the sole form of non-animal origin contains at least one casein selected from the group consisting of alpha-S1 casein, alpha-S2 casein, beta casein and kappa casein.

In some embodiments, the casein composition comprising casein in the sole form of non-animal origin contains at least two caseins selected from the group consisting of alpha-S1
20 casein, alpha-S2 casein, beta casein and kappa casein.

In some embodiment, the casein composition comprising casein in the sole form of non-animal origin contains at least three caseins selected from the group consisting of alpha-S1 casein, alpha-S2 casein, beta casein and kappa casein.

In some embodiments, at least one casein of non-animal origin selected in the group
25 consisting of alpha-S1 casein, alpha-S2 casein, beta casein and kappa casein is not present in the casein composition comprising casein in the sole form of non-animal origin. Preferably, kappa casein is not present in the casein composition comprising casein in the sole form of non-animal origin. In some embodiments, the casein composition comprising casein in the sole form of non-animal origin contains only beta casein. In some other embodiments, the
30 casein composition comprising casein in the sole form of non-animal origin contains only beta and alpha-S2 caseins. In some other embodiments, the casein composition comprising casein in the sole form of non-animal origin contains only beta and alpha-S1 caseins. In

some other embodiments, the casein composition comprising casein in the sole form of non-animal origin contains only beta, alpha-S1 and alpha-S2 caseins.

In some embodiments, the casein composition comprising casein in the sole form of non-animal origin contains only beta casein and alpha-S1 casein, preferably with a ratio beta
5 casein to alpha-S1 casein from 80:20 to 20:80, such as from 70:30 to 30:70, such as from 60:40 to 40:60, for example about 60:40 or about 50:50 or about 40:60.

According to the invention, the amino acid sequence of alpha-S1 casein may be SEQ ID NO: 2, and the amino acid sequence of beta casein may be SEQ ID NO: 4.

Indeed, the inventors have shown that it is possible to improve the texture of an edible
10 composition, such as a dairy-substitute product, with casein of non-animal origin, without kappa casein, so that the inventors did not try to reconstitute micelle formation or check whether the solution actually presents micelles.

This property is particularly interesting because the skilled person in the art knows that micelles are complex structures that are desirable for improving the texture of an edible
15 composition with a casein composition and would therefore not expect to be able to form an edible composition having such an improved texture using non-micellar casein. In fact, the inventors have shown that casein of non-animal origin without kappa casein, as defined in the present disclosure (e.g., alpha-S1 casein, beta casein, a mixture of alpha-S1 casein and beta casein, a mixture of alpha-S1 casein and alpha-S2 casein, or a mixture of beta casein,
20 alpha-S1 casein and alpha-S2 casein), is able to improve the texture of an edible composition without forming or being incorporated into micelles.

Therefore, in some embodiments, at least 50% of the casein of the casein composition is non-micellar casein such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as
25 at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is non-micellar casein.

In some other embodiments, at least 50% of the casein of the casein composition is micellar casein such as at least 60%, such as at least 70%, such as at least 80%, such as at least
30 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is micellar casein.

In some embodiments, at least 50% of the casein of the casein composition is caseinate, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%,
5 such as at least 99%, such as 100% of the casein of the casein composition is caseinate. In a preferred embodiment, almost 100% of the casein of the casein composition is caseinate. Caseinate may be 1, 2, 3 or 4 caseinates selected from the group consisting of sodium caseinate, calcium caseinate, potassium caseinate and magnesium caseinate.

In some embodiments, at least 50% of the casein of the casein composition is 1, 2, 3 or 4
10 caseinates selected from the group consisting of sodium caseinate, calcium caseinate, potassium caseinate and magnesium caseinate, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%,
15 such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is 1, 2, 3 or 4 caseinates selected from the group consisting of sodium caseinate, calcium caseinate, potassium caseinate and magnesium caseinate.

In some embodiments, at least 50% of the casein of the casein composition is sodium caseinate, such as at least 60%, such as at least 70%, such as at least 80%, such as at least
20 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is sodium caseinate. Sodium caseinate is particularly interesting for preparing a yogurt-substitute or a cheese-substitute. This was unexpected because calcium caseinate is usually used for preparing yogurt and cheese because casein of animal origin, which is phosphorylated, can
25 form phosphocalcic bridges having texturing properties. In fact, phosphorylation allows caseins to interact with calcium phosphate to form large colloidal structures called casein micelles. For casein essentially free of phosphorylation, there is no such bridges and interactions, and the impressive texturing property of casein of non-animal origin in the form of sodium caseinate was unexpected.

30 In some embodiments, at least 50% of the casein of the casein composition is calcium caseinate, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least

98%, such as at least 99%, such as 100% of the casein of the casein composition is calcium caseinate.

In some embodiments, at least 50% of the casein of the casein composition is potassium caseinate, such as at least 60%, such as at least 70%, such as at least 80%, such as at least
5 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is potassium caseinate.

In some embodiments, at least 50% of the casein of the casein composition is magnesium
10 caseinate, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is magnesium caseinate.

15 In some embodiments, at least 50% of the casein of the casein composition is a mixture of 2, 3 or 4 caseinates selected from the group consisting of sodium caseinate, calcium caseinate, potassium caseinate and magnesium caseinate, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at
20 least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is a mixture of 2, 3 or 4 caseinate selected from the group consisting of sodium caseinate, calcium caseinate, potassium caseinate and magnesium caseinate, such as a mixture of sodium caseinate and calcium caseinate.

In some embodiments, at least 50% of the casein of the casein composition is acid casein,
25 such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is acid casein.

In some embodiments, at least 50% of the casein of the casein composition is a mixture of
30 caseinate and acid casein, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein

composition is a mixture of caseinates and acid casein. In these embodiments, the caseinate may be 1, 2, 3 or 4 caseinates selected from the group consisting of sodium caseinate, calcium caseinate, potassium caseinate and magnesium caseinate.

5 In some embodiments, the casein of the casein composition is a mixture of calcium caseinate and sodium caseinate. For example, the ratio calcium caseinate and sodium caseinate is from 80:20 to 20:80, such as from 70:30 to 30:70, such as from 60:40 to 40:60, for example about 60:40 or about 50:50 or about 40:60.

10 In some embodiments, there is no need to add any texturing agent in the casein composition for improving the texture of an edible composition. Therefore, the casein composition can be substantially free of texturing agents other than the casein of non-animal origin.

Casein of non-animal origin can be produced, by fermentation technologies. Several studies have described the production of milk's constitutive proteins or homologues by fermentation in various microorganisms (Goda et al (2000) Recombinant expression analysis of natural and synthetic bovine alpha-casein in *Escherichia coli*. Appl Microbiol Biotechnol, Volume 54, pages 671-676; Kim et al (1997) High-level expression of bovine beta-lactoglobulin in *Pichia pastoris* and characterization of its physical properties. Protein Eng, Volume 10(11), pages 1339-45; Kim et al (2005) Production of human caseinomacropeptide in recombinant *Saccharomyces cerevisiae* and *Pichia Pastoris*, J Ind Microbiol. Biotechnology, Volume 32, pages 402-408; Choi BK and Jimenez-Flores R (2001) Expression and purification of Glycosylated Bovine b-casein (L70S/P71S) in *Pichia Pastoris*. J Agric Food Chem, Volume 49, pages 1761-1766; Viaene et al (1991) Efficient expression of bovine alpha-lactalbumin in *Saccharomyces cerevisiae*. Eur J Biochem, Volume 202(2), pages 471-7; US6,232,094; WO2013/148328; US2010/0223682; US5,942,274; WO2018039632 or plant cells (US20100119691, WO2022253816).

25 In some embodiments, the casein of non-animal origin from the casein composition comprising casein in the sole form of non-animal origin is produced by fermentation of a recombinant microorganism. For example, said casein is produced by fermentation of recombinant microorganism, such as fungus (yeast) or bacterium. In a preferred embodiment, said casein is produced by fermentation of a recombinant microorganism chosen among *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Pichia Pastoris*, or *Trichoderma reesei*, or in a microorganism chosen in the *Saccharomyces*, *Kluyveromyces*, *Pichia*, *Zygosaccharomyces*, *Candida*, or *Trichoderma* genus of fungi.

Such recombinant microorganism can be obtained by the introduction of a nucleic acid molecule encoding one or several caseins, alone or as part of a vector. The nucleic acid molecules can further comprise expression control sequences operably linked to the polynucleotide comprised in the nucleic acid molecule. The term "operatively linked" or "operably linked", as used throughout the present description, refers to a linkage between one or more expression control sequences and the coding region in the polynucleotide to be expressed in such a way that expression is achieved under conditions compatible with the expression control sequence. Regulatory elements ensuring expression in fungi as well as in bacteria, are well known to those skilled in the art. They encompass promoters, enhancers, termination signals, targeting signals and the like. Promoters for use in connection with the nucleic acid molecule may be homologous or heterologous with regard to its origin and/or with regard to the gene to be expressed. Suitable promoters are for instance promoters which lend themselves to constitutive expression. However, promoters which are only activated at a point in time determined by external influences can also be used. Artificial and/or chemically inducible promoters may be used in this context. For genetically modifying bacteria or fungi, the polynucleotides encoding one or several caseins can be introduced into plasmids

Thus, in accordance with the present invention, a recombinant organism or microorganism is produced by genetically modifying fungi or bacteria comprising introducing the above-described polynucleotides, nucleic acid molecules or vectors into a fungus or bacterium. An overview of different expression systems is for instance contained in Baghban et al. Yeast Expression Systems: Overview and Recent Advances. Mol Biotechnol. 2019 May;61(5):365-384; Yang and Zhang. Engineering strategies for enhanced production of protein and bio-products in *Pichia pastoris*: A review. Biotechnol. Adv. 2018 Jan-Feb;36(1):182-195.; Mojzita et al. Gene expression engineering in fungi. Curr. Opin. Biotechnol. 2019 Oct;59:141-149.; Gómez et al. Alternative Eukaryotic Expression Systems for the Production of Proteins and Protein Complexes. Adv Exp Med Biol. 2016;896:167-84.; Freudl. Signal peptides for recombinant protein secretion in bacterial expression systems. Microb Cell Fact. 2018 Mar 29;17(1):52.; Cui et al. Exploitation of *Bacillus subtilis* as a robust workhorse for production of heterologous proteins and beyond. World J Microbiol Biotechnol. 2018 Sep 10;34(10):145.; Chen. Bacterial expression systems for recombinant protein production: *E. coli* and beyond. Biotechnol Adv. 2012 Sep-Oct;30(5):1102-7; Deckers et al, Genetically Modified Micro-Organisms for Industrial Food Enzyme Production: An Overview. Foods 2020, 9, 326. The host cell is cultured in nutrient media meeting the requirements of the particular

host cell used, in particular in respect of the pH value, temperature, salt concentration, aeration, antibiotics, vitamins, trace elements etc.

In a preferred embodiment, casein of non-animal origin is prepared as detailed in Example 1.

Caseins are known in the art. One can cite, as illustrations, the caseins that are present in

- 5 Uniprot or Genbank. Examples of such caseins are given in the Table 1 below.

Uniprot	casein type	origin
P02662	alpha-S1	<i>Bos taurus</i>
A0A3Q1MJE5	alpha-S1	<i>Bos taurus</i>
<u>A0A3Q1NG86</u>	alpha-S1	<i>Bos taurus</i>
P02663	alpha-S2	<i>Bos taurus</i>
P02666	beta	<i>Bos taurus</i>
P02668	kappa	<i>Bos taurus</i>
P18626	alpha-S1	<i>Capra hircus</i>
P33049	alpha S2	<i>Capra hircus</i>
P33048	beta	<i>Capra hircus</i>
P02670	kappa	<i>Capra hircus</i>
P04653	alpha-S1	<i>Ovis aries</i>
P04654	alpha-S2	<i>Ovis aries</i>
P11839	beta	<i>Ovis aries</i>
P02669	kappa	<i>Ovis aries</i>
O62823	alpha-S1	<i>Bubalus bubalis</i>
B6VPY3	alpha-S2	<i>Bubalus bubalis</i>
B7VGH4	beta	<i>Bubalus bubalis</i>
P11840	kappa	<i>Bubalus bubalis</i>

Table 1: examples of casein types from cow (*Bos taurus*), goat (*Capra hircus*), sheep (*Avis aries*) and buffalo (*Bubalus bubalis*).

- 10 Such casein usually includes a signal peptide, which is not present in the mature protein. It is possible to use, in the casein composition, fragments of casein proteins (in particular the mature caseins for which signal peptide is not present), or proteins that are homologous to natural caseins and having casein-like properties. Typically, casein made by precision fermentation will have an additional methionine as first amino acid, a consequence of the

expression of the protein from a classical expression system, wherein the translation initiation codon codes for a methionine. For example, alpha-S1 casein has the amino acid sequence SEQ ID NO: 2 and beta casein has the amino acid sequence SEQ ID NO: 4.

In some embodiments, the casein of non-animal origin from the casein composition comprising casein in the sole form of non-animal origin is:

- identical to one of the above sequences, or
- at least n % identical to one of these sequences, with n being an integer from 80 to 100, such as 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99.

Alpha-S1 casein, alpha-S2 casein, beta casein and kappa casein of animal origin are highly phosphorylated milk proteins. In bovine milk, alpha-S1 casein has 2 common phosphorylation isoforms: alpha-S1 casein bearing 8 phosphorylations (α S1-CN-8P) and alpha-S1 casein bearing 9 phosphorylations (α S1-CN-9P); alpha-S2 casein is present with isoforms from 10P to 13P; beta casein is usually present with 5P and occasionally with 4P; and kappa casein is present with 1P to 2P and occasionally with 3P. Phosphorylation is one of the key factors responsible for the stabilization of calcium phosphate nanoclusters in casein micelles and the internal structure of the casein micelles.

In some embodiments, the casein of non-animal origin has non-native phosphorylation pattern. A "native phosphorylation pattern" means a phosphorylation pattern of casein from animal origin. For example, the casein of non-animal origin completely lacks or is substantially reduced in phosphorylation as compared to animal-derived casein. In a preferred embodiment, the casein of non-animal origin is substantially reduced in phosphorylation as compared to animal-derived casein, for example the casein of non-animal origin can be substantially free of phosphorylation. The term "substantially free of phosphorylation" means that the level of phosphorylation of casein of non-animal origin is at most 1% the level of phosphorylation of casein of animal origin (i.e., casein naturally occurring in animal milk). The level of phosphorylation can be measured by any method known in the art, such as by Western-Blot or by LC/MS.

In a preferred embodiment, the casein of non-animal origin is a mixture of alpha-S1 casein (e.g., having the amino acid sequence SEQ ID NO: 2) and beta casein (e.g., having the amino acid sequence SEQ ID NO: 4) which is substantially reduced in phosphorylation as compared to alpha-S1 casein and beta casein in bovine milk, preferably the mixture of alpha-S1 casein and beta casein of non-animal origin is substantially free of phosphorylation. In particular, the level of phosphorylation of said mixture of alpha-S1 casein and beta casein of

non-animal origin is at most 1% of the level of phosphorylation of alpha-S1 casein and beta casein in bovine milk.

In some embodiments, the casein of non-animal origin has non-native glycosylation pattern. A "native glycosylation pattern" means a glycosylation pattern of casein from animal origin.

5 For example, the casein of non-animal origin completely lacks or is substantially reduced in glycosylation as compared to animal-derived casein. In a preferred embodiment, the casein of non-animal origin is substantially reduced in glycosylation as compared to animal-derived casein, for example the casein of non-animal origin can be substantially free of glycosylation. The term "substantially free of glycosylation" means that the level of glycosylation of casein
10 of non-animal origin is at most 1% the level of glycosylation of casein of animal origin (i.e., casein naturally occurring in animal milk). The level of glycosylation can be measured by any method known in the art, such as by LC/MS.

In a preferred embodiment, the casein of non-animal origin is substantially reduced in phosphorylation and glycosylation as compared to animal-derived casein, for example the
15 casein of non-animal origin can be substantially free of phosphorylation and substantially free of glycosylation. It is known that dairy caseins, notably alpha-S1, alpha-S2 and beta caseins, are phosphorylated; in addition, kappa caseins are glycosylated (Walstra *et al.* (2006) Dairy Science and Technology. Taylor and Francis Group, Boca Ranton, USA; Martin *et al.*, (2003) Non bovine caseins quantitative variability and molecular diversity. In: Fox PF and
20 Mc Sweeney PLH. Advances in Dairy Chemistry – Proteins, Vol1, Springer, New-York, 227-310). Caseins phosphorylations play an important role in the interactions with calcium and in the structure of dairy casein micelles. Such post-translational modifications (PTMs) will be different in casein of non-animal origin, which are made in microorganisms. For example, in bacteria, PTMs will be much less frequent than in eukaryotic cells (Macek *et al.* (2019)
25 Protein post-translational modifications in bacteria. Nat Rev Microbiol 17, 651–664). In non-animal eukaryotic cells (e.g., protists, fungi, plants), high levels of PTMs may be observed, but with different patterns as compared with mammalian cells. It is also known that kappa caseins also play an important role in the formation of dairy casein micelles.

The fact that improving texture of an edible composition, such as dairy-substitute product,
30 can be achieved in the absence of post-translational modifications, or with low levels of post-translational modifications and in the absence of kappa caseins was particularly unexpected.

First edible composition (e.g., Dairy-substitute product)

In the context of the present invention, a casein composition comprising casein in the sole form of non-animal origin is incorporated to a first edible composition.

In some embodiments, the first edible composition is a dairy-substitute product.

- 5 In one embodiment, the dairy-substitute product provided herein is a milk-substitute.

In another embodiment, the dairy-substitute product provided herein is a substitute of food products commonly derived from dairy milk. Examples of products commonly derived from dairy milk include, but are not limited to, cooking milk, sweetened condensed milk, flavored milk, yoghurt, cheese, smoothies, shakes, coffee whiteners, coffee creamers, infant
10 formulas, weight loss beverages, nutritional supplemental beverages, clinical nutrition beverages, powdered beverages, and frozen confections (for example, ice cream, soft ice cream, frozen yoghurt, sundae, pudding, whipped topping) or combinations thereof. The dairy-substitute product provided herein may be prepared for human or animal consumption. The dairy product analogs may be used for various purposes, including, but not limited to,
15 feeding and delivery of active ingredients (for example, vitamins, minerals, nutrients, therapeutics).

In some embodiments, the dairy-substitute product is a milk-substitute or a dairy-substitute product made from a milk-substitute. Examples of a dairy-substitute product made from milk-substitute include, but are not limited to, a yogurt-substitute, a cheese-substitute or a
20 cream-substitute.

In some embodiments, the milk-substitute is a plant milk (or plant-based milk). A plant milk may be prepared from almond, coconut, cashew, macadamia nut, walnut, hazelnut, pistachio, pecan, soy, oat, rice, hemp, pea, flax, quinoa, sunflower seed, banana, or a combination thereof. Said plant milks are commonly called almond milk, coconut milk,
25 cashew milk, macadamia milk, walnut milk, hazelnut milk, pistachio milk, pecan milk, soy milk, oat milk, rice milk, hemp milk, pea milk, flax milk, quinoa milk, sunflower seed milk, banana milk, or a combination thereof.

In some embodiments, the plant milk comprises almond milk or coconut milk, or the combination thereof.

- 30 Methods for preparing plant milk are known in the art. In one example, the plant milk is almond milk, which can be made by grinding almonds in a blender with water, then straining out the almond pulp with a strainer or cheesecloth. Almond milk can also be made by adding water to finely ground almonds, almond butter or cream. In another example, the plant milk

is coconut milk, which can be made by grating the white inner flesh of a brown coconut and mixing the shredded coconut meat with a small amount of water in order to suspend the fat present in the grated meat. The grating process can be carried out manually or by comminution, a process used to facilitate the grating.

- 5 The plant milk may also be a combination of one, two or more different types of plant-based milks produced from other plants sources mixed together.

In some embodiments, the first edible composition, e.g., the dairy-substitute product, comprises lipids. Lipids play a role in the organoleptic sensation. However, edible compositions with reduced fat content (low fat) or even with no fat have been produced
10 during the last decades, in order to address health concerns. In the present invention, the lipids that are contained in the first edible composition are lipids of non-animal origin, such as lipids extracted from plant. The lipid content can be any lipid content commonly used in edible composition since it is not an essential parameter of the present invention. Lipid content may be adjusted, in quantity and quality, in order to mimic the sensory
15 characteristics of a dairy product.

In some embodiments, the first edible composition (e.g., the dairy-substitute product) has lipid content from 0 % to 50 %, such as from 0 % to 40 %, from 0 % to 30 %, from 0 % to 20 %, from 0 % to 10 %, from 1 % to 50 %, from 1 % to 40 %, from 1 % to 30 %, from 1 % to 30 %, from 1 % to 20 %, from 1 % to 10 %. Lipid content in a product corresponds to
20 the mass of lipids in the product divided by the total mass of the product. Lipid content of a food product may be determined by a variety of methods, including, but not limited to, AOAC International reference method AOAC 954.02.

The lipid content of the first edible composition (e.g., of the dairy-substitute product) usually depends on the nature and amount of the ingredients used to prepare it, for example it
25 depends on the nature and amount of plant milk, and eventually of plant oil used to prepare said first edible composition. For example:

- In milk-substitute, fat content may vary from 0% in a totally fat-free product, to 30%. Fat content in a milk-substitute corresponds to the mass of lipids in the milk-substitute divided by the total mass of the milk-substitute.
- 30 - In yogurt-substitute, fat content may vary from 0% in a totally fat-free product, to 20%. Fat content in a yogurt-substitute corresponds to the mass of lipids in the yogurt-substitute divided by the total mass of the yogurt-substitute.

- In cheese-substitute, fat content may vary from 0% in a totally fat-free product, to 50%. Fat content in a cheese-substitute corresponds to the mass of lipids in the cheese-substitute divided by the total mass of the cheese-substitute.
- In cream-substitute, fat content may vary from 0% in a totally fat-free product, to 50%. Fat content in a cream-substitute corresponds to the mass of lipids in the cream-substitute divided by the total mass of the cream-substitute.

In some embodiments, the first edible composition (e.g., the dairy-substitute product) comprises plant oil. Plant oil brings lipids to the dairy-substitute product. Examples of plant oils include, but are not limited to, sunflower oil, coconut oil, mustard oil, peanut oil, canola oil, corn oil, cottonseed oil, flax seed oil, olive oil, palm oil, rapeseed oil, safflower oil, sesame oil, soybean oil, almond oil, beech nut oil, brazil nut oil, cashew oil, hazelnut oil, macadamia nut oil, mongongo nut oil, pecan oil, pine nut oil, pistachio nut oil, walnut oil, avocado oil, grape oil, camelina oil, or a combination thereof.

In certain embodiments, the first edible composition (e.g., the dairy-substitute product) may comprise plant butter, such as cocoa butter.

In some embodiments, the first edible composition (e.g., the dairy-substitute product) comprises carbohydrates. Carbohydrates play a role in the organoleptic sensation. However, edible compositions (e.g., dairy-substitute products) with reduced carbohydrate content (low carbohydrate) or even with no carbohydrate have been produced during the last decades, in order to address health concerns. In the present invention, the carbohydrates that are contained in the first edible composition are carbohydrate of non-animal origin, such as carbohydrate extracted from plant. The carbohydrate content can be any carbohydrate content commonly used in edible compositions since it is not an essential parameter of the present invention. Carbohydrate content may be adjusted, in quantity and quality, in order to mimic the sensory characteristics of a dairy product.

In some embodiments, the first edible composition (e.g., the dairy-substitute product) has carbohydrate content from 0 % to more than 50 %, such as from 0.1 % to 50 %, from 0.1 % to 40 %, from 0.1 % to 30 %, from 0.1 % to 20 %, from 0.1 % to 15 %, from 0.1 % to 10 %, from 0.1 % to 5 %, from 1 % to 5 %.

In some embodiments, the first edible composition (e.g., the dairy-substitute product) has carbohydrate content from 0 % to more than 10 %, such as from 0 % to 9 %, from 0 % to 8 %, from 0 % to 7 %, from 0 % to 6 %, from 0 % to 5 %, from 0 % to 4 %, from 0 % to 3 %, from 0 % to 2 %, from 0 % to 1 %. Carbohydrate content in a product corresponds to

the mass of carbohydrates in the product divided by the total mass of the product. Carbohydrate content of a product may be determined by a variety of methods, including, but not limited to, high performance liquid chromatography.

5 Examples of suitable carbohydrates include, but are not limited to, sucrose, glucose, fructose, mannose, steviolosides, or combinations thereof. In certain embodiments, examples of suitable carbohydrates may be selected from one or more of the following: sucrose, glucose and fructose. The first edible composition (e.g., the dairy-substitute product) comprises carbohydrates of non-animal origin. Carbohydrates of non-animal origin may be glucose, saccharose or fructose. Glucose, saccharose and fructose can be obtained from
10 plants.

In a preferred embodiment, the first edible composition (e.g., the dairy-substitute product) does not comprise lactose.

In some embodiments, the first edible composition (e.g., the dairy-substitute product) may also contain other ingredients of non-animal origin. The other ingredient content can be any
15 ingredient commonly used in edible compositions (e.g., in dairy-substitute products) since it is not an essential parameter of the present invention. Non limiting "other ingredients" include, but are not limited to:

- Antioxidants (e.g., rosemary, spearmint, ascorbic acid, sodium ascorbate, Maillard browning products [melanoidins], BHA, BHT, TBHQ, propyl gal late, tocopherols, vitamin A, vitamin E,
20 carotenoids, flavonoids or combinations thereof. Antioxidant content may be between about 0.01 % and about 10%, between about 0.05% and about 5%, or between about 0.1 % and about 2% by weight.

- Vitamins (e.g., vitamin B 12, vitamin D, vitamin C, vitamin A, vitamin E, vitamin B, vitamin K, thiamine, riboflavin, pyridoxine, carotenoids (e.g., beta-carotene, zeaxanthin, lutein,
25 lycopene), niacin, folic acid, pantothenic acid, biotin, choline, inositol) or combinations thereof.

- Emulsifiers (e.g., lecithin, carrageenan, cellulose gum, cellulose gel, starch, gum arabic, xanthan gum, mono- and diglycerides, propylene glycol monoesters, sodium stearoyl-2-lactylate, polysorbate 60, polysorbate 80, lecithin, hydroxylated lecithin, or combinations
30 thereof. Emulsifier content may be between about 0.01 % and about 10%, between about 0.05% and about 5%, or between about 0.5% and about 2% by weight.

- Stabilizing agents (e.g., starches and modified starches, gums [e.g., xanthan gum, bean gum, guar gum, gum arabic, gum ghatti, gum karaya, gum tragacanth, gellan gum],

- hydrocolloids [e.g., guar, acacia, locust bean gum, xanthan, gellan, carrageenan, cellulose, carboxymethyl cellulose, microcrystalline cellulose, methylcellulose hydroxypropyl methyl cellulose, hydroxypropyl cellulose, pectin, low methoxyl pectin, gelatin, agar, furcellaran, dextran, or combinations thereof. Stabilizing agent content may be between about 0.1 % and about 5%, between about 0.5% and about 3%, between about 0.7% and about 1.5% by weight. The stabilizing agent enhance physical properties by imparting viscosity or mouthfeel properties, stabilize and/or suspend insoluble materials and prevent separation or settling of ingredients.
- 5
- Preservatives (e.g., potassium sorbate, sorbic acid or combinations thereof).
- 10
- Buffering agents that prevent undesired creaming or precipitation upon addition of the milk-substitute into hot, acidic environments (e.g., when added to a hot beverage such as coffee; e.g., monophosphates, diphosphates, sodium mono- and bicarbonates, potassium mono- and bicarbonates, potassium phosphate, dipotassium phosphate, potassium hydrophosphate, sodium bicarbonate, sodium citrate, sodium phosphate, disodium phosphate, sodium hydrophosphate, sodium tripolyphosphate or combinations thereof).
- 15
- Minerals (e.g., chloride, sodium, calcium, iron, chromium, copper, iodine, zinc, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, aluminum, soluble mineral salts, slightly soluble mineral salts, insoluble mineral salts, chelated minerals, mineral complexes, non- reactive minerals such as carbonate minerals, reduced minerals, ammonium or combinations thereof).
- 20
- Antimicrobial agents.
 - Dietary fibers (e.g., oat fiber, barley fiber, rye fiber, or a combination thereof). Dietary fiber content may be between about 0% and about 40%, between about 1 % and about 20%, or between about 1.5% and about 5% by weight.
- 25
- Amino acids (e.g., essential amino acids, such as arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and/or valine, amino acid salts, amino acid chelates or combinations thereof).
 - Salts (e.g., sodium citrate, sodium chloride, potassium citrate, potassium phosphate, dipotassium phosphate or combinations thereof. For example, salts are used to enhance
- 30
- flavor.
 - pH-adjusting agents (e.g., organic pH adjusting agents, inorganic pH adjusting agents, food grade acids [e.g., acetic, lactic, hydrochloric, phosphoric, citric, tartaric, malic, glucono,

deltalactone, gluconic acid], basic pH adjusting agent [e.g., disodium diphosphate, potassium hydroxide] or combinations thereof).

- Binding agents (e.g., carrageenan, cellulose gum, cellulose gel, starch, maltodextrin, gum arabic, xanthan gum or combinations thereof; between about 0.01 % and about 10%,
5 between about 0.05% and about 5%, or between about 0.1 % and about 2% by weight).
- Prebiotics (e.g., fructooligosaccharides, galactooligosaccharides); and
- Biotics (e.g., Bifidobacterium spp., Clostridium spp., Bacteroides spp. Enterococcus faecalis, E. coli, Enterobacter cloacae, Klebsiella pneumoniae, Staphylococcus epidermidis, Staphylococcus haemolyticus, Lactoferrin or combinations thereof).
- 10 - Ferments. For example, the first edible composition, such as a dairy-substitute product, may contain bacteria as ferments that can be used for fermentation, such as any lactic acid bacteria from plant sources, e.g., *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Lactobacillus brevis*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Bifidobacterium*, *Lactobacillus acidophilus*, or mixture thereof.
- 15 - Flavoring agents.

In some embodiments, the first edible composition is free of texturing agents selected from starches, modified starches, gums [e.g., xanthan gum, bean gum, gear gum, gum arabic, gum ghatti, gum karaya, gum tragacanth, gellan gum], hydrocolloids [e.g., guar, acacia,
20 microcrystalline cellulose, methylcellulose hydroxypropyl methyl cellulose, hydroxypropyl cellulose, pectin, low methoxyl pectin, gelatin, agar, furcellaran, dextran, or combinations thereof.

The Applicant has shown that the increase in viscosity obtained with a first edible composition in liquid form (i.e., a liquid composition) is particularly impressive. For liquids,
25 viscosity corresponds to the concept of thickness. Viscosity is defined scientifically as a force multiplied by a time divided by an area. Thus its SI units are usually newton-seconds per square meter (N.s/m²), pascal-seconds (Pa.s) or centipoise (cP). The poise is often used with the metric prefix centi- because the viscosity of water at 20 °C (standard conditions for temperature and pressure) is almost exactly 1 centipoise. A centipoise is one hundredth of a
30 poise, or one millipascal-second (mPa.s) in SI units (1 cP = 10⁻³ Pa.s = 1 mPa.s). The viscosity may be determined, for example, using a viscometer or a rheometer. The viscosity values in the present description may be, for example, on shear rate of 100 s⁻¹ or on shear rate of 50 s⁻¹. (e.g., a viscosity of less than 50 cP at 20°C on shear rate of 100 s⁻¹).

Therefore, the first edible composition is preferably a liquid composition. For example, the first edible composition is a beverage having a viscosity of less than 3 cP at 20°C, such as a viscosity of less than 2.9 cP at 20°C, a viscosity of less than 2.8 cP at 20°C, a viscosity of less than 2.7 cP at 20°C, a viscosity of less than 2.6 cP at 20°C, a viscosity of less than 2.5 cP at 20°C, a viscosity of less than 2.4 cP at 20°C, a viscosity of less than 2.3 cP at 20°C, a viscosity of less than 2.2 cP at 20°C, a viscosity of less than 2.1 cP at 20°C, or a viscosity of less than 2 cP at 20°C.

In other embodiments, the first edible composition is a beverage having a viscosity of less than 50 cP at 20°C.

- 10 In other embodiments, the first edible composition is a mixture of ingredients suitable for preparing a yogurt, said mixture having a viscosity from 40 cP to 250 cP at 4°C, such as from 40 cP to 100 cP at 4°C.

Second edible composition (e.g., Dairy-like product)

- 15 In the context of the invention, a second edible composition is a first edible composition in which a casein composition comprising casein in the sole form of non-animal origin has been added in order to improve the texture of the first edible composition. The second edible composition has improved texture compared to the first edible composition from which it is prepared.

- 20 For example, a dairy-like product is a dairy-substitute product in which a casein composition comprising casein in the sole form of non-animal origin has been added in order to improve the texture of the dairy-substitute product. The dairy-like product has improved texture compared to the dairy-substitute product from which it is prepared.

- In some embodiments, the improved texture is selected from increased creaminess, increased density, increased thickness, increased viscosity, increased pulpiness, increased smoothness, or combinations thereof.

In a specific embodiment, the improved texture is increased viscosity.

- In some embodiments, viscosity of the second edible composition (e.g., of the dairy-like product) is increased by at least 5%, such as by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 150%, at least 200%, at least 250%, at least 300%, at least 350%, at least 400%, at least 450%, at least 500%, at least 600%, at least

700%, at least 800%, at least 900%, at least 1000%, relatively to the viscosity of the first edible composition (e.g., relatively to the viscosity of the dairy-substitute product).

The Applicant has surprisingly shown that the viscosity obtained by the addition of casein of non-animal origin is generally higher than the viscosity obtained by the addition of casein of animal origin. For example, the viscosity obtained with a mixture of alpha-S1 casein (e.g.,
5 having the amino acid sequence SEQ ID NO: 2) and beta casein (e.g., having the amino acid sequence SEQ ID NO: 4) of non-animal origin described in the present description is much higher as compared to casein from bovine milk.

The increase in viscosity is particularly impressive for a second edible composition containing
10 from 0.1% to 10%, such as from 2.5 % to 10 % w/w of casein, in particular from 5% to 10% w/w of casein. Therefore, in some embodiments, the second edible composition comprises from 0.1 % to 2.5 % of casein of non-animal origin and the viscosity of the second edible composition is increased by at least 50% relatively to the viscosity of the first edible composition. The term "from 0.1 % to 2.5 %" encompasses "from 0.5 % to 2.5 %",
15 "from 1 % to 2.5 %", "from 1.5 % to 2.5 %", and "from 2 % to 2.5 %". In some other embodiments, the second edible composition comprises from 2.5 % to 10 % of casein of non-animal origin and the viscosity of the second edible composition is increased by at least 100% relatively to the viscosity of the first edible composition. The term "from 2.5 % to 10 %" encompasses "from 3 % to 10 %", "from 3.5 % to 10 %", "from 4 % to 10 %", and
20 "from 4.5 % to 10 %". In some other embodiments, the second edible composition comprises from 5 % to 10 % of casein of non-animal origin and the viscosity of the second edible composition is increased by at least 300% relatively to the viscosity of the first edible composition. The term "from 5 % to 10 %" encompasses "from 5.5 % to 10 %", "from 6 % to 10 %", "from 6.5 % to 10 %", "from 7 % to 10 %", "from 7.5 % to 10 %", "from 8 % to
25 10 %", "from 8.5 % to 10 %", "from 9 % to 10 %", and "from 9.5 % to 10 %". In some embodiments, the second edible composition (e.g., the dairy-like product) provided herein comprises from 0.1 % to 10 % of casein of non-animal origin, such as from 0.1 % to 9 %, such as from 0.1 % to 8 %, such as from 0.1 % to 7 %, such as from 0.1 % to 6 %, such as from 0.1 % to 5 %, such as from 0.1 % to 4 %, such as from 0.5 % to 10 %, such as from
30 0.5 % to 9 %, such as from 0.5 % to 8 %, such as from 0.5 % to 7 %, such as from 0.5 % to 6 %, such as from 0.5 % to 5 %, such as from 1 % to 10 %, such as from 1 % to 9 %, such as from 1 % to 8 %, such as from 1 % to 7 %, such as from 1 % to 6 %, such as from 1 % to 5 %, such as from 1 % to 4 %, such as from 2 % to 10 %, such as from 2 % to 9 %, such as from 2 % to 8 %, such as from 2 % to 7 %, such as from 2 % to 6 %, such as

from 2 % to 5 %, such as from 2 % to 4 %, such as from 3 % to 10 %, such as from 3 % to 9 %, such as from 3 % to 8 %, such as from 3 % to 7 %, such as from 3 % to 6 %, such as from 3 % to 5 %, such as from 3 % to 4 % of casein of non-animal origin, such as about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10% of casein of non-animal origin. Casein content in a product corresponds to the mass of casein in the product divided by the total mass of the product (i.e. w/w).

The inventors have surprisingly shown that the texturizing properties of a casein of non-animal origin, such as a mixture of alpha-S1 casein and beta casein of non-animal origin, is higher than the texturizing properties of a casein of animal origin. This is particularly interesting for preparing edible compositions having an improved texture with a relatively low amount of casein of non-animal origin.

For example, viscosity of the second edible composition comprising from 0.1 % to 10 % of casein of non-animal origin, such as a mixture of alpha-S1 casein and beta casein of non-animal origin, is increased by at least 100% compared to the viscosity of the first edible composition. Said viscosity may be at least 200%, at least 300%, at least 400%, at least 500%, at least 600%, at least 700%, at least 800%, at least 900%, at least 1000%, at least 1100%, at least 1200%, at least 1300%, at least 1400%, at least 1500%.

In some other embodiments, the second edible composition is a yogurt having a viscosity from 300 cP to 2000 cP at 4°C, such as from 500 cP to 2000 cP at 4°C, such as from 1000 cP to 2000 cP at 4°C.

In some other embodiments, the second edible composition is a cream cheese having a viscosity from 2000 cP to 5000 cP at 10°C.

These texturizing properties are particularly higher for dairy-like products at a concentration of casein of non-animal origin from 2.5% to 10%, in particular at a concentration of casein of non-animal origin from 5% to 10%.

It has to be understood that the first edible composition is free of casein, e.g., it does not even contain casein of non-animal origin. The casein in the second edible composition is therefore only brought by the casein composition added to the first edible composition.

The second edible composition (e.g., the dairy-like product) provided herein has higher amounts of protein compared to the first edible composition (e.g., to the dairy-substitute product) from which it is prepared. This increased amount of protein results from the addition of the casein composition.

In some embodiments, the dairy-like product provided herein may contain less, similar, or larger amounts of protein than the analogous dairy product. For example, the dairy-like product provided herein may have a protein content from 0.5 % to 40 %, such as from 0.5 % to 30%, from 0.5 % to 20 %, from 0.5 % to 15%, from 0.5 % to 15 %, from 1 % to 10 %
5 %
5 %, from 2 % to 10 %, from 2% to 8%, from 1 % to 3%, from 2% to 5%, from 3% to 7%. In some embodiments, the dairy-like product comprises 0.1 %, 0.5%, 1 %, 1.5%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14% 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, or 40% of protein. Protein content corresponds to
10 the mass of protein in the product divided by the total mass of product. Protein content of a food product may be determined by a variety of methods, including, but not limited to, AOAC International reference methods AOAC 990.03 and AOAC 992.15, and combustion analysis (ISO 14891 :2008).

In some embodiments, the composition comprising casein in the sole form of non-animal
15 origin is free or substantially free of lipids. Therefore, in some embodiments, the lipids contained in the second edible composition (e.g., in the dairy-like product) are those contained in the first edible composition (e.g., in the dairy-substitute product) from which it is prepared. In some embodiments, the second edible composition (e.g., the dairy-like product) has lipid content from 0 % to more than 50 %, such as from 0 % to 40 %, from 0
20 % to 30 %, from 0 % to 20 %, from 0 % to 10 %, from 1 % to 50 %, from 1 % to 40 %, from 1 % to 30 %, from 1 % to 30 %, from 1 % to 20 %, from 1 % to 10 %.

In some embodiments, the ratio of protein to lipid in the second edible composition (e.g., in the dairy-like product) is about 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, or 10:1.

In some embodiments, the composition comprising casein in the sole form of non-animal
25 origin is free or substantially free of carbohydrates. Therefore, in some embodiments, the carbohydrates contained in the second edible composition (e.g., in the dairy-like product) are those contained in the first edible composition (e.g., in the dairy-substitute product) from which it is prepared.

In some embodiments, the second edible composition (e.g., the dairy-like product) has
30 carbohydrate content from 0 % to more than 50 %, such as from 0.1 % to 50 %, from 0.1 % to 40 %, from 0.1 % to 30 %, from 0.1 % to 20 %, from 0.1 % to 15 %, from 0.1 % to 10 %, from 0.1 % to 5 %, from 1 % to 5 %.

In some embodiments, the second edible composition (e.g., the dairy-substitute product) has carbohydrate content from 0 % to more than 10 %, such as from 0 % to 9 %, from 0 % to 8 %, from 0 % to 7 %, from 0 % to 6 %, from 0 % to 5 %, from 0 % to 4 %, from 0 % to 3 %, from 0 % to 2 %, from 0 % to 1 %.

5 In some embodiments, the second edible composition (e.g., the dairy-like product) provided herein may have similar, or substantially similar, colors as analogous dairy products, and/or similar, or substantially similar color to the first edible composition (e.g., to the dairy-substitute product) from which it is prepared. The color may be determined, for example, using a colorimeter or spectrophotometer.

10 In some embodiments, the second edible composition (e.g., the dairy-like product) provided herein may have similar flavors as analogous dairy products, and/or similar, substantially similar or superior flavor to the first edible composition (e.g., to the dairy-substitute product) from which it is prepared. The flavor can be evaluated, for example, by various methods, including but not limited to by blind tasting performed by human testers or human sensory
15 experts.

In some embodiments, the second edible composition (e.g., the dairy-like product) may also contain other ingredients corresponding to the "other ingredients" contained in the first edible composition (e.g., in the dairy-substitute product). Therefore, the second edible composition (e.g., the dairy-like product) may also contain the "other ingredients" detailed in
20 the section "First edible composition (e.g., Dairy-substitute product)" above.

The second edible composition (e.g., the dairy-like product) provided herein may be used as a base for production of other edible compositions (e.g., other dairy product analogs) with certain nutritional profiles. For example, such dairy product analogs may be milk analogs, yogurt analogs, cheese analogs or cream analogs.

25 In some embodiments, the second edible composition (e.g., the dairy-like product) has a pH below pH 7, such as from pH 3 to pH 6.9.

In some embodiments, the second edible composition (e.g., the dairy-like product) has a pH from pH 3 to pH 5, such as from pH 4 to pH 5, such as pH 4.5.

In some embodiments, the second edible composition (e.g., the dairy-like product) has a pH
30 from pH 5 to pH 6, such as pH 4.8.

In some embodiments, the second edible composition (e.g., the dairy-like product) has a pH from pH 6 to pH 6.9, such as pH 6.8.

In some specific embodiments, the casein of the casein composition is sodium or calcium caseinate and the second edible composition (e.g., the dairy-like product) has a pH from pH 6 to pH 6.9, such as from pH 6.5 to pH 6.9, such as pH 6.8.

In a third object of the present invention relates to a second edible composition comprising:

5 a) a first edible composition, and

 b) a casein composition comprising casein in the sole form of non-animal origin,

wherein the second edible composition comprises proteins in the sole form of non-animal origin,

wherein the second edible composition comprises from 0.1 % to 10 % of casein of non-animal origin, and

wherein second edible composition has preferably a pH below pH 7, such as from pH 3 to pH 6.9, such as such as from pH 3 to pH 5, such as from pH 4 to pH 5, such as pH 4.5, such as from pH 5 to pH 6, such as pH 5.8, such as from pH 5 to pH 6.9, such as pH 6.8.

In some embodiments, the present invention relates to a dairy-like product comprising:

15 a) a dairy-substitute product, and

 b) a casein composition comprising casein in the sole form of non-animal origin,

wherein the dairy like product comprises proteins in the sole form of non-animal origin,

wherein the dairy-like product comprises from 0.1 % to 10 % of casein of non-animal origin, and

20 wherein the dairy-like product has preferably a pH below pH 7, such as from pH 3 to pH 6.9, such as from pH 3 to pH 6.9, such as such as from pH 3 to pH 5, such as from pH 4 to pH 5, such pH 4.5, such as from pH 5 to pH 6, such as pH 5.8, such as from pH 5 to pH 6.9, such as pH 6.8.

In some embodiments, the second edible composition (e.g., the dairy-like product) comprises

25 from 0.1 % to 9 % of casein of non-animal origin, such as from 0.1 % to 8 %, such as from 0.1 % to 7 %, such as from 0.1 % to 6 %, such as from 0.1 % to 5 %, such as from 0.1 % to 4 %, such as from 0.5 % to 10 %, such as from 0.5 % to 9 %, such as from 0.5 % to 8 %, such as from 0.5 % to 7 %, such as from 0.5 % to 6 %, such as from 0.5 % to 5 %, such as from 1 % to 10 %, such as from 1 % to 9 %, such as from 1 % to 8 %, such as

30 from 1 % to 7 %, such as from 1 % to 6 %, such as from 1 % to 5 %, such as from 1 % to 4 %, such as from 2 % to 10 %, such as from 2 % to 9 %, such as from 2 % to 8 %, such

as from 2 % to 7 %, such as from 2 % to 6 %, such as from 2 % to 5 %, such as from 2 % to 4 %, such as from 3 % to 10 %, such as from 3 % to 9 %, such as from 3 % to 8 %, such as from 3 % to 7 %, such as from 3 % to 6 %, such as from 3 % to 5 %, such as from 3 % to 4 % of casein of non-animal origin, such as about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10% of casein of non-animal origin.

The specific embodiments detailed in the section "Second edible composition (e.g., Dairy-like product)" above apply to the third object of the invention. For example:

In some embodiments, the second edible composition (e.g., the dairy-like product) has a pH below pH 7, such as from pH 3 to pH 6.9.

In some embodiments, the second edible composition (e.g., the dairy-like product) has a pH from pH 3 to pH 5, such as from pH 4 to pH 5, such as pH 4.5.

In some embodiments, the second edible composition (e.g., the dairy-like product) has a pH from pH 5 to pH 6, such as pH 4.8.

In some embodiments, the second edible composition (e.g., the dairy-like product) has a pH from pH 6 to pH 6.9, such as from pH 6.5 to pH 6.9, such as pH 6.8.

In some specific embodiments, the casein of the casein composition is sodium or calcium caseinate and the second edible composition (e.g., the dairy-like product) has a pH from pH 5 to pH 6.9, such as from pH 5.5 to pH 6.9, such as from pH 6 to pH 6.9, such as from pH 6.5 to pH 6.9, such as pH 6.8.

Specific and non-limiting embodiments of the invention (Objects 1, 2 and 3), wherein the first edible composition is a dairy-substitute product and the second edible composition is a dairy-like product, are disclosed below:

Specific embodiment 1: (i) the casein of non-animal origin is alpha-S1 casein, beta casein, a mixture of alpha-S1 casein and beta casein, a mixture of alpha-S1 casein and alpha-S2 casein, or a mixture of beta casein, alpha-S1 casein and alpha-S2 casein; (ii) the dairy-like product contains from 1 % to 10 % of casein of non-animal origin, such as from 1% to 10%, such as from 4% to 10%, such as 4% or 8%; and (iii) at least 50% of the casein of the casein composition is sodium caseinate, calcium caseinate, or a mixture thereof, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at

least 99%, such as 100% of the casein of the casein composition is sodium caseinate, calcium caseinate, or a mixture thereof.

Specific embodiment 2: (i) the casein of non-animal origin is alpha-S1 casein, beta casein, a mixture of alpha-S1 casein and beta casein, a mixture of alpha-S1 casein and alpha-S2 casein, or a mixture of beta casein, alpha-S1 casein and alpha-S2 casein; (ii) the dairy-like product contains from 5 % to 10 % of casein of non-animal origin, such as from 5% to 10%, such as 8%; and (iii) at least 50% of the casein of the casein composition is sodium caseinate and/or calcium caseinate, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is sodium caseinate, calcium caseinate, or a mixture thereof.

Specific embodiment 3: (i) the casein of non-animal origin is alpha-S1 casein, beta casein, a mixture of alpha-S1 casein and beta casein, a mixture of alpha-S1 casein and alpha-S2 casein, or a mixture of beta casein, alpha-S1 casein and alpha-S2 casein; (ii) the dairy-like product contains from 1 % to 10 % of casein of non-animal origin, such as from 1% to 10%, such as from 1.5% to 10%, such as 4% or 8%; (iii) at least 50% of the casein of the casein composition is sodium caseinate, calcium caseinate, or a mixture thereof, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is sodium caseinate, calcium caseinate, or a mixture thereof; and (iv) the dairy-like product has a pH below pH 5, such as from pH 3 to pH 5, such as pH 4.5. In this specific embodiment in which the pH is below pH 5, the Applicant has shown that the viscosity is particularly improved compared to a pH above pH 5.

Specific embodiment 4: (i) the casein of non-animal origin is alpha-S1 casein, beta casein, a mixture of alpha-S1 casein and beta casein, a mixture of alpha-S1 casein and alpha-S2 casein, or a mixture of beta casein, alpha-S1 casein and alpha-S2 casein; (ii) the dairy-like product contains from 1 % to 10 % of casein of non-animal origin, such as from 3% to 10%; (iii) at least 50% of the casein of the casein composition is sodium caseinate, calcium caseinate, or a mixture thereof, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least

97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is sodium caseinate, calcium caseinate, or a mixture thereof; and (iv) the dairy-like product has a pH below pH 7, such as from pH 5 to pH 6, such as pH 5.8.

Specific embodiment 5: (i) the casein of non-animal origin is alpha-S1 casein, beta casein, a mixture of alpha-S1 casein and beta casein, a mixture of alpha-S1 casein and alpha-S2 casein, or a mixture of beta casein, alpha-S1 casein and alpha-S2 casein; (ii) the dairy-like product contains from 5 % to 10 % of casein of non-animal origin, such as from 5% to 10%, such as 8%; (iii) at least 50% of the casein of the casein composition is sodium caseinate, calcium caseinate, or a mixture thereof, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is sodium caseinate, calcium caseinate, or a mixture thereof; and (iv) the dairy-like product has a pH below pH 7, such as from pH 5 to pH 7, such as from pH 6 to pH 6.9, such as pH 6.8. In this specific embodiment in which the dairy-like product contains from 5 % to 10 % of casein, the Applicant has shown that the viscosity is surprisingly improved even if the dairy-like product has a pH above pH 5, such as from pH 6 to pH 6.9.

Specific embodiment 6: (i) the casein of non-animal origin is alpha-S1 casein, beta casein, a mixture of alpha-S1 casein and beta casein, a mixture of alpha-S1 casein and alpha-S2 casein, or a mixture of beta casein, alpha-S1 casein and alpha-S2 casein; (ii) the dairy-like product contains from 1 % to 10 % of casein of non-animal origin, such as from 3% to 10%; (iii) at least 50% of the casein of the casein composition is sodium caseinate, calcium caseinate, or a mixture thereof, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is sodium caseinate, calcium caseinate, or a mixture thereof; and (iv) the dairy-like product has a pH below pH 7, such as from pH 5 to pH 6, such as pH 5.8.

Specific embodiment 7: (i) the casein of non-animal origin is a mixture of alpha-S1 casein and beta casein; (ii) the casein composition is in a powder form, such as a spray-dried casein composition, and the casein of non-animal origin represents at least 50 % w/w of the casein composition, such as at least 75% w/w of the casein composition; (iii) wherein the viscosity of the dairy-like product is increased by at least 100% relatively to the viscosity of the dairy-substitute product; (iv) the casein of non-animal origin is substantially free of

- phosphorylation (v) the dairy-substitute product is a beverage having a viscosity of less than 3 cP at 20°C; (vi) the dairy-like product contains from 2.5 % to 10 % of casein of non-animal origin, such as from 3% to 10%, such as from 5% to 10%; (vii) at least 50% of the casein of the casein composition is sodium caseinate, such as at least 60%, such as at least 70%,
5 such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is sodium caseinate; and (viii) the dairy-like product has a pH below pH 7, such as from pH 4.5 to pH 6.9.
- 10 Specific embodiment 8: (i) the casein of non-animal origin is a mixture of alpha-S1 casein and beta casein; (ii) the casein composition is in a powder form, such as a spray-dried casein composition, and the casein of non-animal origin represents at least 50 % w/w of the casein composition, such as at least 75% w/w of the casein composition; (iii) wherein the viscosity of the dairy-like product is increased by at least 300% relatively to the viscosity of the dairy-
15 substitute product; (iv) the casein of non-animal origin is substantially free of phosphorylation (v) the dairy-substitute product has a viscosity from 40 cP to 250 cp at 4°C, such as from 40 cp to 100 cP at 4°C; (vi) the dairy-like product (e.g., yogurt-like) contains from 2.5 % to 10 % of casein of non-animal origin, such as from 2.5% to 5% of casein of non-animal origin; (vii) at least 50% of the casein of the casein composition is sodium
20 caseinate, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% of the casein of the casein composition is sodium caseinate; and (viii) the dairy-like product has a pH from pH 4 to pH 7, such as from pH 4.5
25 to pH 7.

DESCRIPTION OF THE FIGURES

Figure 1: Rheological characterization of a vegan yoghourt plant-based substitute, at pH=4.5 and 6.8. A plant-based composition was supplemented with 4% of a sodium caseinate compositions made with caseins from precision fermentation, at pH 4.5 or about 6.8, as described in Example 4, and viscosity was tested in function of shear rate, as described in Example 4. The base composition without casein addition, is featured as control.

Figure 2: Comparison of vegan yoghourt plant-based substitutes and commercial products. A plant-based yoghourt substitute (pH=4.5) as described in Example 4 is compared with conventional milk-based products, i.e. drinkable yoghourt (Yop, Yoplait, France), spoonable yoghourt (Danone velouté, Danone, France), and skyr (Skyr nature, Danone, France). Viscosity was tested in function of shear rate, as described in Example 4. The base composition without casein addition (pH=4.5), is also featured.

Figure 3: Impact of sodium caseinate made by precision fermentation on the rheology of a plant-based composition in different pH conditions. The impact of the addition of 4% of a sodium caseinate composition on a same plant-based composition was tested in various pH conditions: pH=4.5, or 5.8, or 6.8, as described in Example 4. Curves at pH=4.5 and pH 6.8 are also featured in Figure 1.

Figure 4: Rheological characterization of a drinkable yoghourt supplemented with a caseinate composition made with caseins from precision fermentation. A drinkable yoghourt was supplemented with 4% of a sodium caseinate composition made with precision fermentation, as described in example 5.

Figure 5: Rheological characterization of a spoonable yoghourt plant-based substitute supplemented with a caseinate composition made with caseins from precision fermentation. A spoonable yoghourt was supplemented with 2% or 4% of a sodium caseinate composition made with precision fermentation, as described in example 6.

EXAMPLES

Example 1: Synthesis of recombinant casein

Strain

Synthetic genes coding for alpha-S1 and beta casein (related to natural genes P02662, and
5 P02666, respectively), were modified, in order to remove the signal peptide, and the
sequence of the new synthetic open reading frames (SEQ ID NO: 2 and SEQ ID NO: 4) are
shown in Table 2, last column. They were cloned together into pET25b+, in a same operon
under the control of the T7 promoter of pET25b+, with (i) both synthetic genes being under
the control of a T7 ribosome binding site and (ii) the gene beta casein gene being proximal
10 to the promoter and the alpha-S1 distal to the promoter. The resulting plasmid, capable of
co-expressing the two proteins, was transformed into BL21(DE3) strains (Novagene).
Individual transformed clones were isolated, and for each synthetic gene, one clone was
used to inoculate LB medium.

Name and Uniprot reference of protein precursor	sequence of casein precursor	sequence of recombinant casein
Alpha-S1 casein P02662	MKLLILTCLVAVALAR PKHPIKHQGLP QEVLNENLLRFFVAPFPEVFGKEKVNELS KDIGSESTEDQAMEDIKQMEAESISSSE EIVPNSVEQKHIQKEDVPSERYLGYLEQ LLRLKKYKVPQLEIVPNSAEERLHSMKE GIHAQQKEPMIGVNQELAYFYPELFRQF YQLDAYPSGAWYYVPLGTQYTDAPSF SDIPNPIGSENSEKTTMPLW (SEQ ID NO: 1)	MR PKHPIKHQGLPQEVLNENLLRFFVAP FPEVFGKEKVNELSKDIGSESTEDQAME DIKQMEAESISSSEIIVPNSVEQKHIQKE DVPSEYRLGYLEQLRLKKYKVPQLEIVP NSAEERLHSMKEGIHAQQKEPMIGVNQ ELAYFYPELFRQFYQLDAYPSGAWYYVP LGTQYTDAPSFSDIPNPIGSENSEKTTM PLW (SEQ ID NO: 2)
Beta casein P02666	MKVLILACLVALALARE LEELNVPGEI VESLSSSEESITRINKKIEKFQSEEQQQT EDELQDKIHFAQTQSLVYFPFGPIPNLS PQNIPPLTQTPVVVPPFLQPEVMGVSKV KEAMAPKHKEMPFKYPVEPFTEQSLSLT LTDVENLHPLPLLSWMLHQP HQPLPPT VMFPPQSVLSLSQSKVLPVPQKAVPYPQ RDMPIQAFLLYQEPVLGPVRGPFPIIV (SEQ ID NO: 3)	MRE LEELNVPGEIVESLSSSEESITRINK KIEKFQSEEQQQTEDELQDKIHFAQTQ SLVYFPFGPIPNLSLPQNIPPLTQTPVVV PFLQPEVMGVSKVKEAMAPKHKEMPF KYPVEPFTEQSLSLTLDVENLHPLPLLS SWMLHQP HQPLPPTVMFPPQSVLSLSQS KVLPVPQKAVPYPQRDMPIQAFLLYQEP VLGPVRGPFPIIV (SEQ ID NO: 4)

Table 2: Sequences of natural casein (precursors) and of the related recombinant proteins. In natural casein, signal peptide is indicated in bold. In recombinant casein, the methionine resulting from cloning into the pET expression system is indicated in bold.

- 5 A typical casein production and purification experiment is described below.

Cell cultivation

In order to produce a batch of alpha-S1 and beta caseins, the resulting strain was cultivated as follow (3500 L fermentation scale).

Five concentrated stocks of the strain of each 1 mL, stored in LB with 10 % DMSO at
10 -80°C, were thawed and used to inoculate four or five precultures of a volume of 1 L each, respectively (0.1 % v/v inoculation rate), in a revivification medium Y15 composed of yeast extract (15 g/L) and NaCl (5 g/L), and supplemented with 100 µg/mL of ampicillin. The five precultures were grown in 4 L or 5 L Erlenmeyer at 30°C, for 9 hours, at 170 rpm (orbital diameter: 25 mm). After 9 hours, optical densities (600 nm) in the range of 2.5-3 were
15 achieved.

5 L of the first preculture were used to inoculate a volume of 250 L (2 % v/v inoculation rate), in an optimized culture medium, supplemented with 100 µg/mL of ampicillin and with 15 g/L of glucose. The culture was stirred in a 350 L bioreactor at 37°C for 10 hours. Stirring, aeration and pressure were adjusted to maintain pO₂ at 15%. The pH
20 was regulated to 7.2, with the automatic addition of a solution of ammonia (20.5 % v/v) and a solution of phosphoric acid (5 mol/L). After 10 hours, an optical density in the range of 15-17 was obtained.

160 L of this culture were used to inoculate a fermenter with an initial volume of 2700 L (6 % v/v inoculation rate), in an optimized culture medium, supplemented with 100
25 µg/mL of ampicillin and 1 g/L of glucose. The culture was stirred in a 3500 L bioreactor at 37°C, until optical density (600nm) reached 20, then at 35.4°C until the end of the run. After 30 minutes, glucose was added regularly in the bioreactor, using a concentrated 400 g/L glucose solution, and the feed was adjusted to sustain growth and production, without glucose accumulation in the medium. Ampicillin was added in the bioreactor each 9 hours, to
30 maintain a concentration of 100 µg/mL, and antifoam (5% stock solution, Struktol J673A) was added when necessary. Stirring, aeration and pressure were adjusted to maintain pO₂ at 15%. pH was regulated to 7.2, with the automatic addition of a solution of ammonia (20.5 % v/v) and a solution of phosphoric acid (5 mol/L). IPTG was added at a final concentration of

0.2 mM when optical density reached 40 ± 5 , and the culture was stopped after about 24 hours, optical densities (600 nm) in the range of 80 could be achieved. This resulted in around 3.5 tons of culture broth with 4.4 % of dry biomass.

5 ***Downstream processing***

The culture medium was concentrated using a self-cleaning disk-stack centrifuge (GEA, Easy-scale, SSE20 or SC15 model, centrifugal force: 17000-20000 G, back pressure : 5-5.5 bars, temperature < 20 °C).

10 The concentrated biomass was then washed two times with osmosis water, in order to remove all compounds from the fermentation medium (salts, sugars, nutrients...). The concentrated biomass was mixed with water, until obtaining a homogeneous suspension. The suspension was then separated by centrifugation, using a self-cleaning disk-stack centrifuge (GEA, Easy-scale or SC15 model, centrifugal force: 17000-20000 G, back pressure: 4-5 bars, temperature < 20 °C). Concentrated cells were obtained after separation, and washing water
15 was eliminated. This washing procedure was repeated twice, according to a similar procedure, to finally obtain a pellet of concentrated washed cells.

Washed concentrated bacteria were then resuspended in osmosis water, until having a homogeneous suspension, and the pH was adjusted to 8.5 with NaOH 30 % w/v (Brenntag, 144562). The resulting suspension was heated during 1 hour at 95°C, in a 2 m³ double-
20 jacket tank, under N₂ atmosphere, in which water vapor was directly injected, in order to reach and maintain such a temperature during all the thermal treatment, allowing efficient bacterial lysis and thus, *E. coli* strain deactivation, *E. coli* proteins precipitation and caseins solubilization. After 1 hour, the pH of the suspension was 7.82; it was re-adjusted to 8.5 with NaOH 30 % w/v. After cooling, a dense suspension was obtained.

25 The suspension was then separated by centrifugation, using a self-cleaning disk-stack centrifuge (GEA, Easy-scale model, centrifugal force: 17000 G, bowl rotation speed, back pressure : 5 bars, temperature < 20 °C), with the aim of eliminating all the insoluble particles (cellular debris, *E.coli* precipitated proteins), and recovering purified caseins in the soluble fraction. After separation, the supernatant containing caseins and a heavy phase
30 were obtained. The heavy phase was washed with osmosis water and resulting suspension was separated by centrifugation in the same experimental conditions than those described for the first separation. The supernatant was collected.

To improve their quality and reduce the quantity of insoluble particles inside, both supernatants were filtered on a 0.8 μm ceramic mineral membrane (Pall).

The pH of the permeate was then adjusted to 4.6 with a 20 % v/v solution of lactic acid (Corbion, 1001013890), at room temperature, in an agitated double-jacket tank from VMI, in order to reach the isoelectric point of caseins, and consequently, lead to their precipitation. The resulting suspension was concentrated by centrifugation using a self-cleaning disk-stack centrifuge (GEA, Easy-scale model, centrifugal force: 17000 G, bowl rotation speed: 12000 rpm, bowl filling: 80%-90% of the capacity, back pressure: 5 bars, temperature < 20 °C). After separation, concentrated acid caseins were collected, while the light phase was discarded. Acid caseins were then washed with osmosis water until obtaining a homogeneous suspension, which was then concentrated by centrifugation, using a self-cleaning disk-stack centrifuge (GEA, Easy-scale model, centrifugal force : 17000 G, back pressure : 5 bars, temperature < 20 °C). The pH of the suspension before separation was measured and adjusted to 4.6 with lactic acid if it was necessary, to avoid caseins loss in the washing water.

Acid casein

Concentrated acid washed caseins were obtained after separation, and washing water was eliminated. This washing procedure was repeated twice, according to a similar procedure, to finally obtain a pellet of concentrated white and odorless washed acid casein.

20 *Calcium caseinate production*

A suspension of calcium hydroxide (1 mol.L^{-1}) was added slowly to the suspension of acid casein to adjust the pH to 7, at room temperature, in order to obtain a suspension of calcium caseinate.

Sodium caseinate production

25 A solution of sodium hydroxide (1 mol.L^{-1}) was added slowly to the suspension of acid casein to adjust the pH to 7, at room temperature, in order to obtain a suspension of sodium caseinate.

The suspensions of acid casein, calcium caseinate or sodium caseinate were dried using a multi-stage spray-dryer (Sicca Dania, direct configuration, inlet temperature: 80-85°C, outlet temperature: 180-185°C, dual-fluid buses : 1-3.5 bars), to obtain a powder, with a dry matter from 94 % to 97 %.

Caseins content could be estimated by the analysis of SDS PAGE gels, and the comparison of samples with protein standards (Purified alpha and beta caseins from milk

(Sigma)). Sodium and calcium caseinate powders contained about 80% of recombinant casein (and about 90% of total proteins), with a ratio of about 50% (48% to 52%, depending on experiment), similar to the initial composition in the cell before treatment, showing that in the condition we used, there was no bias regarding the purification process of α s1 casein vs. β casein.

Further analysis was conducted for other compounds by various methods. Analysis of a calcium caseinate sample is described in Table 3.

Compound	% (w/w)
Fat	4.26
Including saturated fat	1.35
Carbohydrates	ND
Proteins (total)	87.38
Calcium	1.07
Sodium Chloride	0.59
Polyphenols	0.96
Moisture (water)	4.57

Table 3 : Composition of a calcium caseinate sample. Only detected compounds accounting for more than 0.1 % are accounted for

Example 2: Texturing impact of recombinant casein on a composition for making an animal-free yoghurt substitute, using 3.7% of a recombinant caseinate composition (spray-dried; casein purity: about 80% w/w)

We designed a recipe for making an animal-free yoghurt substitute using a sodium or calcium caseinate composition made by precision fermentation, as described in Example 1.

We also used coconut milk (Jardin Bio Etic, Lea Nature, France), cornstarch (*Fleur de Maïs*, Maïzena, France), and lactic ferments (lactic ferments for yoghurt, Alsa, France). The coconut milk contained 17.0% fat (including 16% saturated fatty acids), 2.6% carbohydrate (including 2.2% sugars) and 1.7% proteins. The corn starch contained 86% of carbohydrates (1% of sugars), 0.5% of fat (including 0.1% of saturated fatty acids), 0.5% proteins and 1% of fibers. Calcium caseinate composition is indicated in Table 3, and sodium caseinate composition is considered to be the same, but for salts, since sodium hydroxide instead of

calcium hydroxide, was used to adjust pH to 7. The list of ingredients and corresponding used amounts are described in Table 4. The casein content was 3% (80% of 3.7%).

Ingredients	Weight (g)
Coconut milk	30.0
Water	63.1
Corn Starch	3.0
Sodium caseinate composition (spray-dried; casein purity : 80% w/w)	3.7
Lactic ferments	0.2
Total	100.0

Table 4: Ingredients for the making of yoghourt substitute

- 5
- Half of the water, coconut milk and sodium caseinate composition were gently mixed for 3 minutes at room temperature. In parallel, corn starch was mixed in hot (99°C) water. The two compositions were then mixed for 20 minutes at 50°C, and cooled down to 40°C. Ferments were then added and gently mixed. At this stage, the composition is liquid.
- The composition was then incubated at 40°C for about 5 hours, until pH reached 4.5. It was
- 10
- then stored at 4°C for 16 hours, and a firm, bona fide spoonable yoghourt texture. Final composition is described in Table 5.

Compound	% (w/w)
Fat	5.3
Including saturated fat	4.9
Carbohydrates	3.4
Including sugars	0.7
Proteins	3.8
Including casein from precision fermentation	3.0
Others (including salts)	0.2
Water	87.4
Total	100.0

Table 5: Composition of yoghourt substitute

When the same experiment was conducted exactly in the same conditions, but without the caseinate composition (replaced with the same weight of water), pH reached 4.4, but texture remained loose, showing the key role of the casein composition in texturing the product.

In both cases, texture remained loose in the absence of lactic ferment, with observed pH 6.4 with casein composition, and pH 6.1 without casein composition (In the absence of casein, texture was slightly firmer at pH 4.4 than at pH 6.1, but in both cases, texture remained loose).

Results are summarized in Table 6, and show that in this experiment, achieving a firm yoghurt-like texture was essentially linked with the presence of the casein composition at pH around 4.5. We can infer that casein curdling at acidic pH is involved in this texture change.

Casein	Texture	pH
No casein	-	4.4
	-	6.1
Sodium caseinate	+	4.5
	-	6.4

Table 6: Summary of results. “+” indicates that a firm yoghurt-like texture is achieved.

Similar results, with a less firm texture have been achieved with a calcium caseinate composition (as described in Example 1) instead of sodium caseinate.

Example 3: Texturing impact of recombinant casein on a composition for making an animal-free yoghurt substitute, using 2.0% of a recombinant caseinate composition (spray-dried; casein purity: 80% w/w).

We designed a recipe for making an animal-free yoghurt substitute using a sodium caseinate composition made by precision fermentation, as described in Example 1 (spray-dried; casein purity: 80% w/w).

We also used coconut milk (Jardin Bio Etic, Lea Nature, France), cornstarch (*Fleur de Maïs*, Maïzena, France), and lactic ferments (lactic ferments for yoghurt, Alsa, France). The coconut milk contains 17.0% fat (including 16% saturated fatty acids), 2.6% carbohydrate (including 2.2% sugars) and 1.7% proteins. The corn starch contains 86% of carbohydrates (1% of sugars), 0.5% of fat (including 0.1% of saturated fatty acids), 0.5% proteins and 1%

of fibers. The list of ingredients and corresponding used amounts are described in Table 7. The casein content is 1.6%.

Ingredients	Weight (g)
Coconut milk	30.0
Water	64.8
Corn Starch	3.0
Sodium caseinate composition (spray-dried; casein purity: 80% w/w)	2.0
Lactic ferments	0.2
Total	100.0

Table 7: Ingredients for the making of yoghourt substitute

Half of the water, coconut milk and sodium caseinate was gently mixed for 3 minutes at room temperature. In parallel, corn starch was mixed in hot (99°C) water. The two compositions were then mixed for 20 minutes at 50°C and cooled down to 40°C. Ferments were then added and gently mixed. At this stage, the composition is liquid.

The composition was then incubated at 40°C for 5 hours, until pH reached 4.5. It was then stored at 4°C for 16 hours, resulting in an increase of the viscosity, and a bona fide stirred yoghourt texture. Final composition is described in Table 8.

Compound	% (w/w)
Fat	5.2
Including saturated fat	4.8
Carbohydrates	3.4
Including sugars	0.7
Proteins	2.3
Including casein from precision fermentation	1.6
Others (including salts)	0.1
Water	89.1
Total	100.0

Table 8: Composition of yoghourt substitute

When the same experiment was conducted exactly in the same conditions, but without the caseinate composition (replaced with the same weight of water), pH reached 4.5, but texture remained loose, showing the key role of the casein composition in texturing the product.

In both cases, texture remained loose in the absence of lactic ferment, with observed pH 6.4 with casein composition, and pH 6.7 without casein composition (In the absence of casein, texture was slightly firmer at pH 4.5 than at pH 6.7, but in both cases, texture remained loose).

Results are summarized in Table 9, and show that in this experiment, achieving a stirred yoghurt-like texture was essentially linked with the presence of the casein composition at pH around 4.5. We can infer that casein curdling at acidic pH is involved in this texture change.

Casein	Texture	pH
No casein	-	4.5
	-	6.7
Sodium caseinate	+	4.5
	-	6.4

Table 9: Composition of yoghurt substitute. “+” indicates that a stirred yoghurt-like texture is achieved.

Example 4: Texturing impact of recombinant casein on a composition for making an animal-free yoghurt substitute, using 4.0% of a recombinant caseinate composition (spray-dried; casein purity: 80% w/w)

We compared the impact on viscosity of the sodium caseinate composition on a base for the making of a vegan yoghurt, at nearly neutral and acidic pH, with rapid pH adjustment with lactic acid.

We used as a base composition containing 30% coconut milk (Jardin Bio Etic, Lea Nature, France), 2.25% cornstarch (*Fleur de Maïs*, Maïzena, France), and 67.75% water. The coconut milk contains 17.0% fat (including 16% saturated fatty acids), 2.6% carbohydrate (including 2.2% sugars) and 1.7% proteins. The corn starch contains 86% of carbohydrates (1% of sugars), 0.5% of fat (including 0.1% of saturated fatty acids), 0.5% proteins and 1% of fibers.

Water and corn starch were added in a pot and mix until a homogeneous mix is obtained. The mix was then heated on an induction plate until ebullition. This mix was added to a multifunction robot Mambo 8590 (Cecotec) with the coconut milk and was mixed at speed 3 for 15 min at 45°C. The mixture was put in the refrigerator for around 2 hours until it reached 21°C and then mixed at speed 3 for 2 min at room temperature. The pH of the composition was pH=6.8 and the protein content was 0.5%. This texture was not modified when the pH of this composition was adjusted to pH=4.5 as illustrated by viscosity analysis (Figure 1 – Table 17), and as shown on Figure 2 (Table 18), the texture was very like the texture of a drinkable yoghourt.

We tested the impact of the addition of the sodium caseinate on this composition, at neutral and acidic pH, i.e. around pH=6.8 and pH=4.5. For this, we used a recombinant casein composition containing alpha-S1 casein and beta casein and produced as described in example 1 (spray-dried; casein purity: 80% w/w). The caseinate composition (4% w/w final), to obtain samples of 30g. Composition is described in Table 10. The casein content is 3.2%.

Compound	% (w/w)
Fat	5.1
Including saturated fat	4.7
Carbohydrates	2.6
Including sugars	0.7
Proteins	4.0
Including casein from precision fermentation	3.2
Others (including salts)	0.2
Water	88.1
Total	100.0

Table 10: Composition of basis for a yoghourt substitute

Samples were homogenized with a magnetic stirrer for 7 minutes at 1500 RPM; samples were made in duplicates and pH was adjusted at pH=4.5 with lactic acid in one sample and not adjusted in the other one. In samples wherein pH had not been adjusted, marginal pH variations could be observed upon caseinate admixture (pH=6.8 ± 0.2). The final composition was kept 24 hours at 4°C before testing. We also tested the addition of caseinate after pH adjustment of the base at pH=4.5. In this case, pH after casein addition was about 5.8.

The flow properties of prepared samples were measured by using MCR 92 Rheometer (Anton Paar, GmbH, Germany); with geometric cone and plate (diameter = 50 mm and angle = 4 °). The sample was applied on the surface of the plate with a 0.499 mm gap between plates. The sample was homogenized at a shear rate of 5 s⁻¹ for 30 s at 4°C before the data
5 acquirement. The instrument was operated at shear rates of 1 - 100 s⁻¹ and temperature of 4 °C. The data points were set as 50 points, logarithmic ramp duration, 10 s initial time and 1 s final time. Flow curves were then analyzed to determine the properties of the different samples in terms of viscosity.

As shown on Figure 1, the addition of 4% of recombinant sodium caseinate composition
10 increased viscosity at pH=4.5. As shown on Figure 2, viscosity was intermediate between a drinkable and a spoonable yoghurt (Danone velouté, Danone, France). Upon addition of casein after adjustment of the base composition at pH=4.5 (and thus at a final pH of 5.8), intermediate results were achieved (Figure 3 – Table 19).

In these conditions, a pH-independent effect could also be observed with sodium caseinate
15 at nearly neutral pH at higher concentrations (8%). In such condition, pH was barely impacted by casein additions and kept in the range of pH=6.8, but a strong increase in viscosity was observed (not shown).

**Example 5: Texturing impact of recombinant casein on a commercial drinkable
20 yoghurt, using 4.0% of a recombinant caseinate composition (spray-dried;
casein purity: 80% w/w)**

We tested the impact of the addition of recombinant sodium caseinate made by precision fermentation on a commercial composition, i.e. a drinkable yoghurt from Yoplait (*Yop vanille*, Yoplait, France). This product is made from cow's milk, and protein content is 3.0%,
25 for 10.4% of carbohydrates and 0.8% of fat and pH=4.5.

We used a sodium caseinate composition made by precision fermentation as described in Example 1 (spray-dried; casein purity: 80% w/w). Caseinate composition was added (4% w/w), to obtain samples of 30g. Samples were homogenized with a magnetic stirrer for 7 minutes at 1500 RPM; and the final composition was kept 24 hours at 4°C before testing.
30 After admixing casein, pH was pH=5.2. This protocol is very similar to one of the protocols used in example 4 (pH adjustment before admixing casein). Casein content was 3.2% (w/w). Samples were analyzed as described in Example 4.

As shown on Figure 4 (Table 20), with this product having a relatively low viscosity (about 1Pa.s with low shearing), 4% of recombinant sodium caseinate composition were sufficient to significantly increase viscosity. In such compositions, texture change was concomitant with a very significant increase in protein composition.

5

Example 6: Texturing impact of recombinant casein on a commercial animal-free spoonable yoghurt substitute, using 2.0% or 4.0% of a recombinant caseinate composition (spray-dried; casein purity: 80% w/w)

We tested the impact of the addition of recombinant caseinate made by precision fermentation on a commercial composition, i.e. a spoonable plant-based yoghurt substitute from Andros (*Brassé nature au lait de coco*, France). This product contains low-fat coconut milk 89%, starch and modified starch, pea proteins, calcium citrate, sodium chloride, and ferments. In this type of yoghurt, protein content is 0.6%, for 7% of carbohydrates and 4.9% of fat (w/w), and pH=4.5.

We used a sodium caseinate compositions made with recombinant casein made by precision fermentation, produced as described in Example 1 (spray-dried; casein purity: 80% w/w). The caseinate composition was added (2% or 4%, w/w) and mixed gently, to obtain samples of 30 g, with casein contents of 1.6% or 3.2% (w/w). Samples were gently homogenized with a spoon; and the final composition was kept 24 hours at 4°C before testing. With addition of 2% caseinate composition, pH was pH=4.9, and with 4% of casein, pH=5.3. Regarding pH adjustment, this protocol is very similar to one of the protocols used in example 4 (pH adjustment before admixing casein). Samples were analyzed as described in Example 4.

As shown on Figure 5, with this type of product, having a relatively high viscosity (about 60Pa.s with low shearing), higher than dairy yoghurt displayed on Figure 2), recombinant sodium caseinate had an impact on viscosity that was detectable by 2% of caseinate composition addition, and very significant by 4%. In such compositions, texture change was concomitant with a very significant increase in protein composition, from 0.6% to about 2.4% or 3.6%.

30

Example 7: the texturizing property of casein of non-animal origin is superior to the texturizing property of casein of animal origin at pH 7

Sodium caseinates from cow milk (Armor Protein - Spray dried - Sodium caseinates) and sodium caseinates of non-animal origin prepared as described in Example 1 (alphaS1 (45 to 55% of the casein), Beta (45 to 55%of the casein)) were added to water at casein concentrations of 0%, 2.5%, 5% and 10% (w/w), considering the casein purity of each powder. Caseinate powders were slowly added to water under continuous stirring until fully dissolved. The pH was then adjusted to 7 using a 1M NaOH solution, followed by 1 hour of stirring to allow pH equilibration.

10 The concentrations of the solutions were reverified by doing a dry matter measure with a Smart 6 moisture analyzer.

Solutions were analyzed on a MCR 92 rheometer (AntonPaar, GmbH, Germany) with geometric cone and plate (diameter = 50 mm and angle = 1°). The sample was applied on the surface of the plate with a 0.1 mm gap between plates. The temperature was adjusted to 20°C. The instrument was operated at shear rates of 100 s⁻¹ to 10 s⁻¹, the data points were set as 10 points, with a constant measurement duration by point of 10 s. Flow curves were then analyzed and viscosity values on shear rate of 50 s⁻¹ and 100s⁻¹ were used to compare the different samples in terms of viscosity.

The results are detailed in Table 11 (pH 7 & Shear Rate = 50s⁻¹) and Table 12 (pH 7 & Shear Rate = 100s⁻¹).

20

Casein concentration	Viscosity of animal CasNa (cP)	Viscosity of CasNa SO (cP)
0%	1	1
2.5%	2.1	3.6
5%	5.1	13.5
10%	106.2	247.1

Table 11

Casein concentration	Viscosity of animal CasNa (cP)	Viscosity of CasNa SO (cP)
0%	1	1
2.5%	2.0	3.7
5%	5.1	13.1
10%	105.9	214.4

Table 12

Tables 11 & 12: Viscosity values (cP: centipoise) on shear rate of 50 s^{-1} and 100 s^{-1} for different protein concentrations at pH 7.0 (animal CasNa: sodium caseinate of animal origin, CasNa SO: sodium caseinate of non-animal origin).

For all the concentrations tested, sodium caseinate of non-animal origin (CasNa SO) was correlated to a much higher viscosity compared to sodium caseinate of animal origin (animal CasNa). In particular, at a casein concentration of 5% and 10%, the viscosity obtained with CasNa SO was more than 100% higher than the viscosity of animal CasNa.

Example 8: the texturizing property of casein of non-animal origin is superior to the texturizing property of casein of animal origin at pH 5.5

Sodium caseinates from milk (Armor Protein - Spray dried - Sodium caseinates) or sodium caseinates of non-animal origin prepared as described in Example 1 (alphaS1 (45 to 55% of the casein), Beta (45 to 55% of the casein)) were added to water at casein concentrations of 0%, 2.5%, 5% and 10% (w/w), considering the casein purity of each powder.

Caseinate powders were slowly added to water under continuous stirring until fully dissolved. The pH was then adjusted to 5.5 using a 1M HCl solution, followed by 1 hour of stirring to allow pH equilibration.

The concentrations of the solutions were reverified by doing a dry matter measure with a Smart 6 moisture analyzer.

Solutions were analyzed on a MCR 92 rheometer (AntonPaar, GmbH, Germany) with geometric cone and plate (diameter = 50 mm and angle = 1°). The sample was applied on the surface of the plate with a 0.1 mm gap between plates. The temperature was adjusted to 20°C . The instrument was operated at shear rates of 100 s^{-1} to 10 s^{-1} , the data points were set as 10 points, with a constant measurement duration by point of 10 s. Flow curves were then analyzed and viscosity values on shear rate of 50 s^{-1} and 100 s^{-1} were used to compare the different samples in terms of viscosity.

The results are detailed in Table 13 (pH 5.5 & Shear Rate = 50 s^{-1}) and Table 14 (pH 5.5 & Shear Rate = 100 s^{-1}).

Casein concentration	Viscosity of animal CasNa (cP)	Viscosity of CasNa SO (cP)
0%	1	1
2.5%	1.8	1.8
5%	2.9	3.9
10%	41.9	225.9

Table 13

Casein concentration	Viscosity of animal CasNa (cP)	Viscosity of CasNa SO (cP)
0%	1	1
2.5%	1.7	1.8
5%	2.9	3.7
10%	41.3	177.0

Table 14

Tables 13 & 14: Viscosity values (cP: centipoise) on shear rate of 50s⁻¹ and 100s⁻¹ for different protein concentrations at pH 5.5 (animal CasNa: sodium caseinate of animal origin, CasNa SO: sodium caseinate of non-animal origin).

For all the concentrations tested, sodium caseinate of non-animal origin (CasNa SO) was correlated to a higher viscosity compared to sodium caseinate of animal origin (animal CasNa). In particular, at a casein concentration of 10%, the viscosity obtained with CasNa SO was more than 300% higher than the viscosity of animal CasNa.

Example 9: the texturizing property of casein of non-animal origin is superior to the texturizing property of casein of animal origin on oat milk

Sodium caseinates from milk (Armor Protein - Spray dried - Sodium caseinates), or sodium caseinates of non-animal origin prepared as described in Example 1 (alphaS1 (45 to 55% of the casein), Beta (45 to 55% of the casein)) were added to oat milk (Alpro, Danone) at casein concentrations of 0, 2.5% and 5% (w/w), considering the protein purity of each powder.

Caseinate powders were slowly added to oat milk under continuous stirring until fully dissolved.

Solutions were analyzed on a MCR 92 rheometer (AntonPaar, GmbH, Germany) with geometric cone and plate (diameter = 50 mm and angle = 1°). The sample was applied on the surface of the plate with a 0.1 mm gap between plates. The temperature was adjusted to 20°C. The instrument was operated at shear rates of 100 s⁻¹ to 10 s⁻¹, the data points were set as 10 points, with a constant measurement duration by point of 10 s. Flow curves

were then analyzed and viscosity values on shear rate of 100 s⁻¹ were used to compare the different samples in terms of viscosity.

The results are detailed in Table 15.

Casein concentration	Viscosity of animal CasNa (cP)	Viscosity of CasNa SO (cP)
0%	1.8	1.8
2.5%	3.3	4.1
5%	6.6	13.9

5 Table 15: Viscosity values (cP: centipoise) on shear rate of 100 s⁻¹ for different protein concentrations (animal CasNa: sodium caseinate of animal origin, CasNa SO: sodium caseinate of non-animal origin).

For all the concentrations tested, sodium caseinate of non-animal origin (CasNa SO) was correlated to higher viscosity compared to sodium caseinate of animal origin (animal CasNa) and calcium caseinate of animal origin (animal CasCa). In particular, at a casein
10 concentration of 5%, the viscosity obtained with CasNa SO was increased by more than 100% in comparison with the viscosity of animal CasNa.

Example 10: The texturizing property of casein of non-animal origin in animal-free yoghurt substitute.

15 Sodium caseinates of non-animal origin prepared as described in Example 1 (alphaS1 (45 to 55% of the casein), Beta (45 to 55% of the casein)) were added to a base (at casein concentrations of 0 and 3.2 %) for the making of a vegan yoghurt, at nearly neutral (pH7.0) and acidic pH (pH4.5).

20 We used as a base composition containing 30% coconut milk (Jardin Bio Etic, Lea Nature, France), 2.25% cornstarch (*Fleur de Maïs*, Maïzena, France), and 67.75% water. The coconut milk contains 17.0% fat (including 16% saturated fatty acids), 2.6% carbohydrate (including 2.2% sugars) and 1.7% proteins. The corn starch contains 86% of carbohydrates (1% of sugars), 0.5% of fat (including 0.1% of saturated fatty acids), 0.5% proteins and 1% of
25 fibers.

The vegan yoghurt preparation was done in two steps:

- Half of the water was mixed with coconut milk and sodium caseinate of non-animal origin for 30 minutes.

- Half of the water was mixed with corn starch until homogenous mix is obtained. The mix was then heated on an induction plate until ebullition.

Both solutions were mixed together for 15 minutes. pH was adjusted to 7.0 or 4.0 with HCl (1M). The mixture was put in the refrigerator for around 2 hours.

- 5 The flow properties of prepared samples were measured by using MCR 92 Rheometer (Anton Paar, GmbH, Germany); with geometric cone and plate (diameter = 50 mm and angle = 4 °). The sample was applied on the surface of the plate with a 1 mm gap between plates. The sample was homogenized at a shear rate of 5 s⁻¹ for 30 s at 4°C before the data acquirement. The instrument was operated at shear rates of 1 s⁻¹ to 100 s⁻¹ and
- 10 temperature of 4 °C. The data points were set as 50 points, logarithmic ramp duration, 10 s initial time and 1 s final time. Flow curves were then analyzed to determine the properties of the different samples in terms of viscosity.

The results are detailed in table 16:

	Viscosity at 100 s ⁻¹ (cP)	Viscosity at 50 s ⁻¹ (cP)
3.2% NaCas SO - pH 4.5	328.3	495.13
0% NaCas SO - pH 4.5	59.771	82.336
3.2% NaCas SO - pH 7	97.164	121.87
0% NaCas SO - pH 7	54.027	71.887

- 15 Table 16: Viscosity values (cP: centipoise) on shear rate of 100 s⁻¹, 50 s⁻¹, 10 s⁻¹ for different protein concentrations (CasNa SO: sodium caseinate of non-animal origin) in an animal free yoghourt substitute

Supplementary tables

- 20 Table 17 (corresponding to Figure 1)

Sodium caseinate composition concentration	Viscosity at 100 s ⁻¹		Viscosity at 50 s ⁻¹	
	pH 4.5	pH 6.8	pH 4.5	pH 6.8
0%	135.0	138.2	201.4	200.9
4%	227.1	170.0	403.6	226.7

Table 18 (corresponding to Figure 2)

	Viscosity at 100 s-1	Viscosity at 50 s-1
Drinkable Yogourt	63.8	89.7
Spoonable Yogourt	1001.1	1433.1
Skyr	3172.2	4075.7
No added casein pH4.5	135	201.4
Sodium caseinate pH4.5	277.1	403.6

Table 19 (corresponding to Figure 3)

pH	Viscosity at 100 s-1	Viscosity at 50 s-1
4,50	277.1	403.6
5,80	233.3	315.3
6,80	170.0	226.7

Table 20 (corresponding to Figure 4)

Sodium Caseinate composition concentration	Viscosity at 100 s-1	Viscosity at 50 s-1
0%	63.8	89.7
4%	139.1	221.3

CLAIMS

1. A use of a casein composition for obtaining a second edible composition from a first edible composition, said second edible composition having an improved texture compared to the first edible composition, wherein said casein composition comprises casein in the sole form of non-animal origin.
2. A method for improving the texture of a first edible composition, said method comprising a step of incorporating a casein composition comprising casein in the sole form of non-animal origin to a first edible composition thereby obtaining a second edible composition, said second edible composition having an improved texture compared to the first edible composition.
3. A method for preparing a second edible composition having an improved texture, comprising the step of incorporating a casein composition comprising casein in the sole form of non-animal origin into a first edible composition, thereby obtaining a second edible composition having an improved texture compared to the first edible composition.
4. The method or use according to any of the preceding claims, wherein the second edible composition comprises from 0.1 % to 10 % of casein of non-animal origin, such as from 0.1 % to 2.5 % of casein of non-animal origin.
5. The method or use according to any of the preceding claims, wherein the second edible composition comprises from 0.1 % to 10 % of casein of non-animal origin, such as from 2.5 % to 5 % of casein of non-animal origin or from 5 % to 10 % of casein of non-animal origin.
6. The method or use according to any of the preceding claims, wherein the viscosity of the second edible composition is increased by at least 25% relatively to the viscosity of the first edible composition.
7. The method or use according to any of the preceding claims, wherein the viscosity of the second edible composition is increased by at least 100% relatively to the viscosity of the first edible composition.

- 5 **8.** The method or use according to any of the preceding claims, wherein the second edible composition comprises from 0.1 % to 10 % of casein of non-animal origin and the viscosity of the second edible composition is increased by at least 50% relatively to the viscosity of the first edible composition, such as at least 100% relatively to the viscosity of the first edible composition.
- 9.** The method or use according to any of the preceding claims, wherein the first edible composition is a beverage having a viscosity of less than 50 cP at 20°C.
- 10 **10.** The method or use according to claims 1 to 8, wherein the first edible composition is a mixture of ingredients suitable for preparing a yogurt, said mixture having a viscosity from 40 cP to 250 cP at 4°C, such as from 40 cP to 100 cP at 4°C.
- 11.** The method or use according to any of the preceding claims, wherein the first edible composition is a beverage having a viscosity of less than 3 cP at 20°C.
- 15 **12.** The method or use according to any of the preceding claims, wherein the casein of non-animal origin represents at least 25 % w/w of the casein composition.
- 13.** The method or use according to any of the preceding claims, wherein the casein composition is in a powder form, such as a spray-dried casein composition.
- 20 **14.** The method or use according to any of the preceding claims, wherein the casein composition is in a powder form, such as a spray-dried casein composition, and the casein of non-animal origin represents at least 50 % w/w of the casein composition, such as at least 75% w/w of the casein composition.
- 25 **15.** The method or use according to any of the preceding claims, wherein the casein of non-animal origin is substantially free of phosphorylation.
- 30 **16.** The method or use according to any of the preceding claims, wherein the casein of non-animal origin is produced in bacteria, such as *E. Coli*.
- 17.** The method or use according to any of any of the preceding claims, wherein at least 90% of the casein of the casein composition is non-micellar casein.
- 35

18. The method or use according to any of the preceding claims, wherein the second edible composition has a pH below or equal to pH 7.

19. The method or use according to any of the preceding claims, wherein the second edible composition has a pH from pH 4 to pH 5.5.

20. The method or use according to claims 1 to 18, wherein the second edible composition has a pH from pH 5.5 to pH 6.9.

21. The method or use according to any of the preceding claims, wherein the first edible composition is a plant-based edible composition, preferably the first edible composition is a dairy-substitute product made from plant milk.

22. The method or use according to any of the preceding claims, wherein the first edible composition is a dairy-substitute product and the second edible composition is a dairy-like product.

23. The method or use according to claim 4, wherein the dairy-substitute product is a milk-substitute or a dairy-substitute product made from a milk-substitute, such as a yogurt-substitute, a cheese-substitute, or a cream-substitute.

24. The method or use according to any of the preceding claims, wherein the first edible composition is a plant-based edible composition, preferably the first edible composition is a dairy-substitute product made from plant milk.

25. The method or use according to any of the preceding claims, wherein the improved texture is selected from increased creaminess, increased density, increased thickness, increased viscosity, increased pulpiness, increased smoothness, or combinations thereof.

26. The method or use according to any of the preceding claims, wherein the casein of non-animal origin is one, two or three casein(s) selected from the group consisting of alpha-S1 casein, alpha-S2 casein, beta casein and kappa casein.

27. The method or use according to any of the preceding claims, wherein the casein of non-animal origin is alpha-S1 casein, beta casein, a mixture of alpha-S1 casein and beta

casein, a mixture of alpha-S1 casein and alpha-S2 casein, or a mixture of beta casein, alpha-S1 casein and alpha-S2 casein.

28. The method or use according to any of the preceding claims, wherein the casein composition does not comprise at least one casein protein selected from the group consisting of alpha-S1 casein, alpha-S2 casein, beta casein and kappa casein.

29. The method or use according to any of the preceding claims, wherein the casein composition does not comprise kappa casein.

10

30. The method or use according to any of the preceding claims, wherein the casein of non-animal origin is mixture of alpha-S1 casein and beta casein.

31. The method or use according to any of the preceding claims, wherein at least 50% of the casein of the casein composition is caseinate, such as 1, 2, 3 or 4 caseinates selected from the group consisting of sodium caseinate, calcium caseinate, potassium caseinate and magnesium caseinate.

32. The method or use according to any of the preceding claims, wherein at least 50 % of the casein of the casein composition is sodium caseinate.

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33. The method or use according to any of the preceding claims, wherein the second edible composition (i) has protein content from 0.5 % to 40 %, and/or (ii) has lipid content from 0 % to 50 %.

25

34. A second edible composition having an improved texture comprising:

- a) a first edible composition, and
 - b) a casein composition comprising casein in the sole form of non-animal origin,
- wherein the second edible composition comprises proteins in the sole form of non-animal origin,
- 30 wherein the second edible composition comprises from 0.1 % to 10 % of casein of non-animal origin, and
- wherein the second edible composition has preferably a pH below pH 7.

35. The edible composition according to claim 34, wherein the casein of non-animal origin is as defined in any of the preceding claims.

36. The edible composition according to any of claims 34-35, wherein casein composition is
5 as defined in any of the preceding claims.

37. The edible composition according to any of claims 34-36, wherein the first and second edible compositions is free of texturing agents selected from starches, modified starches, gums [e.g., xanthan gum, bean gum, gear gum, gum arabic, gum ghatti, gum karaya, gum
10 tragacanth, gellan gum], hydrocolloids [e.g., guar, acacia, locust bean gum, xanthan, gellan, carrageenan, cellulose, carboxymethyl cellulose, microcrystalline cellulose, methylcellulose hydroxypropyl methyl cellulose, hydroxypropyl cellulose, pectin, low methoxyl pectin, gelatin, agar, furcellaran, dextran, or combinations thereof.