

ANIMAL-FREE SUBSTITUTE FOOD PRODUCTS

FIELD OF THE INVENTION

The present invention relates to a method for producing a substitute food product, such as a dairy product (such as cheese or yoghurt), or an egg replacement composition. The invention further extends to a substitute food product, such as a substitute cheese or egg product, made using the method of the invention and to compositions used to produce the substitute food products.

BACKGROUND OF THE INVENTION

Protein is a key nutritional requirement and forms a key component of the human and animal diet. Proteins can be animal derived (for example, meat, fish, milk or eggs) or can also be sourced from plants, for example legumes, or microbes.

Cheese is typically made out of milk or milk-derived ingredients. Milk production is a resource intensive method and has a negative effect on the environment, leading to a questionable sustainability of cheese made from milk or milk derived ingredients. In fact, cheese is the third most unsustainable animal product globally in terms of greenhouse gas emissions per kg of product.

Hence, great efforts are undertaken to produce substitute dairy products, such as cheese-like and other substitute dairy products of vegan source (including milk and yogurt-substitute products), i.e., without the use of milk and other animal-derived ingredients. However, to date, the use of vegan ingredients, like plant proteins, results in products that lack the organoleptic properties of cheese. Further, the nutritional quality of these products is also low due to the low protein content and/or low nutritional value of the vegetable proteins compared to animal-derived proteins. The plant-based alternatives not resembling the organoleptic properties of the dairy original and/or having low nutritional quality is one of the major reasons why the consumption of dairy cheese has not been slowed down by these plant-based products.

Eggs, particularly from avian species such as chickens, while high in protein, include components that are allergenic or are considered unhealthy for certain groups of people. However, eggs have several desirable functional attributes that permit the use of egg in preparing a wide variety of egg-based food products of pleasurable taste/flavour and texture, including emulsions, batters, doughs, baked/cooked goods, and the like. Eggs are useful in these products because they contribute to processes such as gelling/thickening/coagulating, foaming/leavening, emulsifying, and water binding. Therefore, eggs are considered essential ingredients of many commonly consumed food products, imparting or contributing to volume, texture, emulsification, shape, taste/flavour, cooking stability, processing tolerance, and shelf life.

There is an interest in vegan egg replacers. Known prior art egg replacers are, for example, based on mung bean protein isolate, chickpea flour, pea protein or lupin protein isolate. Companies producing such egg replacers are include Greenforce, Just Egg, Rewe Bio, Plant-B. Aquafaba from commercially canned chickpeas can also be used as a potential egg replacer, see Institute of Food Science and Technology, 2019-10-24 which discusses the recipe optimisation and storage stability of Aquafaba in vegan mayonnaise. Likewise, WO2022124988 discloses an egg yolk replacer.

There is a need for an egg replacer that provides similar nutritive content, similar functional, taste, flavour, and organoleptic attributes, and fewer or none of the undesired attributes. It is an object of the current invention to provide an improved egg replacer.

Hence, there is a need for the provision of animal-free substitute food products, including dairy and egg substitutes, in particular substitute products resembling the organoleptic properties of the corresponding animal-based food.

SUMMARY OF THE INVENTION

The inventors have found that a denatured amylase can be used in the product as an ingredient, for example, at a minimum level of 3% (w/w of protein) within a

substitute food product. Optionally, the amylase can be present as the main protein constituent of the substitute food product. The amylase allows formation of a product with acceptable organoleptic properties as well as having suitable physical characteristics. In an egg replacer product (which is intended for combination with other ingredients before cooking), the enzyme may be denatured as part of the cooking process itself.

Optionally, the amylase is present in an amount of about 0.01 g/L to about 1000 g/L inclusive, typically between 10 g/L to 450 g/L, inclusive, more specifically between 60 g/L and 350 g/L, inclusive.

The inventors have found that a substitute food product can be formed by using an amylase as an ingredient within the substitute food product. Where we refer to the enzyme being present as an “ingredient”, we mean that the enzyme is not present as a biocatalyst to break down other biomolecules or as dietary supplements but is rather used for its structural or nutritional properties. Thus, the amylase is subjected to a process step which process step converts the amylase into at least one converted protein having a structural function. Since the enzyme is not being used as a biocatalyst, it can conveniently be denatured, optionally during cooking of the food product itself. Optionally, the amylase is heat treated prior to or during such that it is suitable for human consumption. The term “amylase” is used herein to refer to a wild type amylase enzyme or to variants thereof, which have an amino acid sequence with at least 80% sequence identity relative to the amino acid sequence of a wild type amylase enzyme. Optionally any variant has more than 80% sequence identity to a wild type amylase enzyme, for example has 85% or more, 90% or, 95% or more or 98% or more sequence identity to the amino acid sequence of a wild type amylase enzyme.

More specifically, the inventors have found that it is possible to obtain a curd or emulsified composition by using at least one amylase to produce an animal-free substitute food product resembling the organoleptic or other desirable properties of equivalent food products, for example traditional dairy products such as cheese. Optionally, the amylase is denatured using heat-treatment, which further ensures that it is suitable for human consumption.

The at least one amylase may be treated to render it partially or substantially devoid of its catalytic function prior to or during the process of forming the food product. Such treatment may be by way of hydrolysis (pH-based hydrolysis or enzymatic hydrolysis), heat-treatment, or by mechanical shearing to partially or fully denature the catalytic activity of the amylase. The at least partially denatured amylase is then suitable for use as a structural ingredient for producing a substitute food product. The treatment may be by way of enzymatic hydrolysis, for example using a protease and/or by means of heat treatment.

The invention thus extends to a substitute dairy product wherein the main protein constituent is at least one partially or fully denatured amylase.

According to another aspect of the invention, there is provided an amylase which, when converted in accordance with an aspect of the invention, is rendered as a protein component having a desirable attribute. By "desirable attribute" is meant an attribute that may be useful in making a food-like or substitute food product, such as an egg replacer.

In a further aspect, the present invention provides a process of forming a substitute egg product, wherein amylase is mixed with water to form a mixture and said mixture is homogenised with a fat, wherein said amylase provides at least 5g / 100g protein of the product. Optionally, the amylase is mixed with a carbohydrate source together with the water, for example a seed mucilage or a flour. Instead of a seed mucilage, fibres, such as flaxseed fibre, hydrocolloids, modified starches or soluble fibres such as β -glucan, xanthan gum, pectin and the like can also be used. Optionally a fat can be added to the mixture and homogenized to form the substitute egg product. Optionally other flavourings or colorants can be added, as described below with regard to the composition.

The invention thus also extends to a substitute egg product wherein the main protein constituent is at least one partially or fully denatured amylase.

The inventors have found that the amylase (which may be partially or fully denatured) can be used to provide improved structural or nutritional value within the animal-free substitute food products of the invention.

FIGURES

Figure 1 shows a substitute cheese product made as described in the Example.

Figure 2: shows a substitute cream cheese product made as described in the Example;

Figure 3 shows a substitute feta cheese product made as described in the Example;

Figure 4 shows a substitute yoghurt product made as described in the Example;

Figure 5 Amplitude-sweeps of Alpha-Amylase (top), Glucoamylase (middle); yoghurt (bottom);

Figure 6 shows a substitute fresh cheese product made as described in the Example: Fresh cheeses made of alpha-Amylase (left) and Glucoamylase (right);

Figure 7 shows a substitute egg product made as described in the Example: Egg substitute based on alpha-Amylase (left) and Glucoamylase (right);

Figure 8 shows the gelation of glucoamylase (product A) at different pH values and salt concentrations;

Figure 9 shows the gelation of glucoamylase (product B) at different pH values and salt concentrations. Top row: no salt, bottom row: 1% NaCl (w/w). From left to right: pH 5.0, 5.5, 6.6

Figure 10 shows the evolution of storage and loss modulus of heat-induced gels based on purified Lactase (β -APC) and Alpha-Amylase (α -APC) gels as a function of temperature at pH 6 (a and b); at pH 4.8 (c and d).

DETAILED DESCRIPTION OF THE INVENTION

Dairy milk products and eggs form a significant part of a western diet. Typically, most animal-free food products rely heavily on the inclusion of plant proteins. The process of separating plant proteins from associated carbohydrates and other molecules is inefficient and expensive. Therefore, known animal-free egg replacement compositions produced with plant proteins suffer from intrinsically

poor performance due to co-extracted compounds that negatively affect taste, smell, and texture. The proteins used in these products are structural in nature.

Typically, cheese products are made using either animal milk or using protein and fat extracts from plants. To date, most animal-free substitute dairy products rely heavily on the inclusion of plant proteins. The process of separating plant proteins from associated carbohydrates and other molecules is inefficient and expensive. Therefore, an animal-free substitute cheese (for example) produced with plant proteins suffers from intrinsically poor performance due to co-extracted compounds that negatively affect taste, smell, and texture. Surprisingly, the Applicant has found that it is possible to use amylase which is generally used in the animal and human food industries (but which has previously been employed only as a biocatalyst to break down carbohydrate or as dietary supplements and has not been used for their structural or nutritional properties), as a major ingredient to create substitute food products such as substitute dairy products. It was surprising that the amylase could serve the same structural purpose as animal or plant based globular proteins. Optionally, the amylase can be treated to render it suitable for human consumption, for example can by heat treatment. Specifically, heat treatment of the amylase produces a cheese-like gel, emulsion, or curd, which was further treated to render a substitute cheese or cheese-like product, specifically a vegan or animal-free substitute cheese.

It was then found that the gel, emulsion or curd formed could also be used to form other substitute dairy products, such as yoghurt, and also to form other substitute food products, such as substitute egg products.

Surprisingly, the Applicant has found that it is possible to treat or manipulate globular enzymes, specifically amylase, that are generally used in the animal and human food industries (but which have previously been employed only as biocatalysts to break down other biomolecules or as dietary supplements and have not been used for their structural properties) to create substitute-egg food products, such as an animal-free egg replacement composition, egg-based food product or egg-white substitute. It was, therefore, surprising that amylase could provide the required structure as natural egg proteins to form egg-like

food products, egg-white substitute or an egg replacement composition for use in egg-based food products or compositions for such products. Specifically, heat treatment of the amylase produces an egg-like gel or emulsion product which could be used to form an (optionally vegan or animal-free) egg replacement composition, or an (optionally vegan or animal-free) egg-like product, such as scrambled eggs.

To successfully deliver a substitute-egg food product, the technical properties of the protein are important for providing the characteristics of the final product, in conjunction with the ability for the protein to be produced in sufficient quantity in a sustainable way. At the same time, the protein should deliver health (nutrition) benefits similar to egg proteins.

Egg white contains many functionally important proteins; Ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%), and lysozyme (3.5%). However, production of all of these proteins using genetic engineering and precision fermentation in order to simulate an animal-free egg replacement product is very challenging and costly. Accordingly, finding a single protein which can deliver one or more of the technical, nutritional and/or organoleptic properties of egg proteins as well as egg experience to consumers is important.

The amylase has the ability to form a gel formation (coagulate) upon heating.

Enzymes for biocatalysis, such as amylase, are used almost exclusively for a precisely specified catalytic function. In contrast, in the present invention, the amylase is used to provide the main protein constituent (i.e., main protein nutrition) and an improved structure to a substitute food product, such as a substitute cheese or egg product. Advantageously, the amylase used herein is already generally regarded as safe ("GRAS") due to its extensive use in the food industry.

According to a first aspect of the invention there is provided a method for the production of a substitute food product, the method comprising the steps of:

- i. providing at least one amylase;
- ii. subjecting the at least one amylase to one or more process steps to at least partially denature the amylase or providing the amylase in the product in an amount of at least 3g/100g protein; and
- iii. incorporating said amylase into a composition to form a substitute food product.

The term “amylase” is used herein to refer to a wild type amylase enzyme or to variants thereof, which have an amino acid sequence with at least 80% sequence identity relative to the amino acid sequence of a wild type amylase enzyme. Optionally any variant has more than 80% sequence identity to a wild type amylase enzyme, for example has 85% or more, 90% or, 95% or more, or 98% or more sequence identity to the amino acid sequence of a wild type amylase enzyme.

Optionally, the amylase used within the present invention is recombinantly produced, that is expressed under controlled conditions using a heterologous host cell, for example in a genetically modified organism (GMO), such as a micro-organism (GMO). Optionally, the amylase is expressed naturally from a host cell. The host cell can be a fungus, for example can be *Aspergillus oryzae* or *Aspergillus niger*. Optionally, the amylase is naturally produced by plants. Optionally the amylase is naturally produced by micro-organisms. Optionally, the amylase can be provided in the form of a crude or purified ferment.

The amylase used in the present invention can be neutrally flavoured and can be manipulated to form gels, emulsions, or curds with a texture and mouthfeel that can be optimised through modification of process conditions known to those skilled in the field of the invention. The amylase may be commercially available in the marketplace, including any alpha or beta amylase, for example alpha amylase and glucoamylase (amyloglucosidase). The availability of the amylase presents an opportunity for ameliorating the sub-optimal economics involved in making substitute food products, for example making substitute dairy products from recombinantly produced dairy proteins.

alpha Amylase (1,4- α -D-glucan glucohydrolase) is an *endo*-acting glucanase that belongs to the glycoside hydrolase family 13 (GH13). It catalyzes the cleavage of α -1,4-glycosidic bonds of starch and other related polysaccharides to generate oligosaccharides of varying lengths with α -configuration and α -limit dextrins, which constitute the branched oligosaccharides. The β -Amylases (EC 3.2.1.2) are exoenzymes that cleave the penultimate α -(1 $\rightarrow$ 4) linkage from the nonreducing end of the polymeric chains and release the disaccharide maltose.

The invention is based on the realisation that the amylase, can be used to produce compositions that exhibit a similar taste, aroma, and mouth feel as equivalent food products, for example dairy products such as cheese made using animal-produced milk ("traditional milk" or "traditional dairy products" when animal milk is used to make products such as cheese). The present invention therefore provides compositions, methods of making the compositions, and kits including amylase, compositions, curds, and mixtures useful for making these substitute food products.

The present invention includes the use of an amylase enzyme produced through precision fermentation which is converted from a protein having a catalytic activity (i.e., an enzyme) into a protein providing a structural function. The invention also provides a protein with a structural function produced using the method of the invention. As such, the invention extends to, an amylase enzyme treated in accordance with the method of the invention to render it suitable for inclusion in a substitute food product as a structural component of the food product, i.e., to denature it at least partially to render it suitable for inclusion in a food product as a structural component of the food product.

The substitute food products or compositions of the invention have a similar taste, mouth feel, aroma, and nutritional value as compared to equivalent products or compositions made using animal products.

For example, the substitute dairy products or compositions of the invention have a similar taste, mouth feel, aroma, and nutritional value as compared to equivalent products or compositions made using animal-produced milks.

Additionally, the inventors have found that it is possible to obtain an egg replacement composition ("egg replacer") which can be used with other ingredients to form an egg-based food product or egg-based food product composition. The egg replacement composition can be formed by using at least one amylase enzyme. The egg replacement composition formed is preferably an animal-free product. The egg replacement composition formed preferably exhibits the organoleptic or other desirable properties of avian eggs.

The invention thus extends to an egg-based food product, that is a food product in a form which is ready to eat comprising the egg replacement composition of the invention.

The invention also extends to an egg-based food product composition comprising the egg replacement composition of the invention, that is a composition for a food product which requires cooking to be in a form which is ready to eat. Thus, the present invention provides a food product or food product composition which comprises the egg replacement composition of the present invention together with at least one other ingredient to form an egg-based food product or an egg-based food product composition.

In one embodiment of the invention an alpha amylase glucosidase has been used. In one embodiment of the invention a glucosidase, specifically an amyloglucosidase, commonly known as glucoamylase, has been used. In one embodiment of the invention an alpha amylase and a glucoamylase have been used,

Optionally, the substitute food product, for example egg replacer composition/product comprises an amylase enzyme, with the amylase providing a minimum level of protein of 3% (w/w of protein), optionally with at least 5% (w/w protein), optionally with at least 10% (w/w protein), for example at least 20% (w/w

protein) of the food product. Optionally the amylase can be the main protein constituent of the food product.

The term “main protein constituent” refers to the ingredient which provides the majority protein content within the product of the invention compared to any other ingredient or sum of other ingredients. For example, the “main protein constituent” can provide more than 50% (w/w) of the protein content within the product, for example at least 80% (w/w), for example at least 90% (w/w) of the protein content within the product.

The term “substitute dairy product” refers to a composition that resembles or is equivalent to a product made using a dairy milk or a dairy milk component. In particular, the substitute dairy product will resemble or be equivalent in terms of one or more of: taste, aroma, flavour, mouthfeel, organoleptic qualities, colour, and the like to a product made using dairy milk or a dairy milk component. Thus, the term “substitute dairy product” as used herein means a product resembling a traditional dairy product, but made with proteins produced by processes not involving mammals. For example, the proteins used can be plant-based proteins or can be proteins expressed by a micro-organism or, more preferably, are recombinantly expressed proteins, for example are proteins which are expressed using heterologous microbial host cells. The terms “synthetic dairy product” or “synthetic cheese product” can be used interchangeably with the term “substitute dairy product”.

The term “animal-produced milk” refers to a milk which has been obtained from a female mammal such as a cow, goat, sheep, camel, human etc. by lactation.

The term “isolated” protein or “isolated” enzyme refers to a protein or enzyme which is substantially separated from other cellular components that naturally accompany the native protein or enzyme in its natural host cell.

The term “animal-free” refers to a component which has not been obtained or produced from an animal, but excludes a recombinantly-produced protein, even

where the recombinantly produced protein has the same amino acid sequence as a wild type equivalent.

“Heat treated” means that the amylase has been treated with heat to a temperature sufficient to reduce the likelihood of bacterial contamination, thereby rendering the amylase suitable for use within a foodstuff. A “heat treated” amylase enzyme may also be at least “partially denatured”.

“Partially denatured” means that the protein structure of the amylase has been irreversibly changed so as to impair its original catalytic activity by at least 50%, preferably by at least 75% relative to the activity of the untreated amylase.

“Totally denatured” or “fully denatured” means that the protein structure of the amylase has been irreversibly changed so as to reduce its catalytic activity to 10% or less, for example to 5% or less. Optionally the catalytic activity of the amylase has been completely lost.

The term “lipids” means one or more molecules that include a fatty acyl group (e.g., saturated or unsaturated acyl chains). For example, the term “lipids” includes fats, oils, phospholipids, free fatty acids, monoglycerides, diglycerides, and triglycerides. Non-limiting examples of lipids are described herein and include sunflower oil, coconut oil, rapeseed oil, palm fat, tributyrin, mono- and diglycerides, free fatty acids, and phospholipids, cultured oils or fats, or microbially-produced or microbially-derived oils or fats. Non-animal fats or oils are an option and refer to plant or microbial, fungal or recombinantly produced fats or oils. Additional examples of lipids are known in the art.

The term “sweetening agent” means a saccharide (e.g., a monosaccharide, a disaccharide, or a polysaccharide) or an artificial sweetener (e.g., a small molecule artificial sweetener or a protein artificial sweetener) that, when added to a composition, makes the composition taste sweet when ingested by a mammal, such as a human. Non-limiting examples of sweetening agents are described herein. Additional examples of sweetening agents are known in the art.

The term “ash” is a term known in dairy science and refers to one or more ions, elements, minerals, and/or compounds that can be found in an animal-produced milk. Non-limiting examples of the ions, elements, minerals, and compounds that are found in an animal-produced milk and which are covered by the term “ash” are described herein. Additional ions, elements, minerals, and compounds that are found in an animal-produced milk are also known in the art. The term “ash” as used herein refers to residue remaining after the product has been burned for analysis includes minerals, such as salts. In some embodiments, the minerals can be salts or ions or one or more of the following: sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, chloride, manganese, selenium, iodine. The term “ash” also includes, retinol, carotene, and other vitamins (such as vitamin D, vitamin E, vitamin B12, thiamine, and riboflavin). As used herein, the term “ash” also includes anions. Examples of such anions include one or more of the following: phosphate, citrate, sulphate, carbonate, and chloride.

The term “colour balancing agent” or “colouring agent” means an agent added to a composition to modulate the colour of the composition, e.g., to make the colour of the composition appear more similar to an equivalent product made using animal-produced milk or avian egg. Non-limiting examples of colour balancing agents or colouring agents include 3-carotene and annatto. Other examples of colouring balancing agents are known in the art. Optionally, the colour balancing agent or a colouring agent can be produced by or obtained from a plant.

Desirably, the substitute food products and compositions of the present invention exhibit functional characteristics and organoleptic properties of an equivalent food-based product. In some embodiments, the substitute food products and compositions of the present invention have a nutritional profile similar to an equivalent conventional food product, and replicates one or more, if not all, of the core functionalities thereof.

The term “core functionalities” refers to the sensory, chemical, biochemical, or mechanical characteristics of a traditional food product. The term “core functionalities”, includes but is not limited to: flavour, taste, appearance,

nutritional value, handling and mouthfeel, desired density, structure, texture, elasticity, springiness, coagulation, binding, leavening, aeration, foaming, creaminess, and emulsification.

The term “flavour” refers to the taste and/or the aroma of a food or drink.

As used herein, the term “predominantly” or variations thereof will be understood to mean, for instance, a) in the context of fats the amount of a particular fatty acid composition relative to the total amount of fatty acid composition; b) in the context of protein the amount of a particular protein composition (e.g., amylase relative to the total amount of protein composition (e.g., α -, β -, and κ -casein).

The term “about,” “approximately,” or “similar to” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which can depend in part on how the value is measured or determined, or on the limitations of the measurement system. It should be understood that all ranges and quantities described below are approximations and are not intended to limit the invention. Where ranges and numbers are used these can be approximate to include statistical ranges or measurement errors or variation. In some embodiments, for instance, measurements could be plus or minus 10%.

According to one aspect of the invention there is provided a method for the production of a substitute food product, the method comprising the steps of:

providing at least one amylase;

and incorporating said at least one amylase into a composition to form a substitute food product, and wherein said amylase is subjected to one or more process steps to at least partially denature the amylase during formation of the substitute food product.

In one embodiment, the amylase is denatured. The amylase is therefore caused to gel or coagulate during denaturation. Optionally, the amylase will be in a solution or suspension having a pH of about 5.0 to about 7.2 (e.g., about 6.2 to about 6.8) prior to gelling / coagulation.

The step of at least partially denaturing the amylase may take place before or after the amylase is added to other ingredients to form the composition.

Optionally, the at least partially denatured enzyme amylase comprises at least 3% by weight of protein in the substitute food product, for example at least 5% by weight, for example at least 7% (w/w protein), for example up to 10% (w/w protein) such as 7 to 10% (w/w protein) of the substitute food product.

Optionally, the amylase is the main protein constituent of the substitute food product (i.e., can comprise at least 10% (w/w protein), for example 20% (w/w protein) or more within the substitute food product, for example from 20 to 50% (w/w protein), for example from 30 to 50% (w/w protein). Specifically, the amylase may comprise 3 to 15% (w/w protein), for example 5 to 15% (w/w protein), including 5 to 12% (w/w protein), 5 to 10% (w/w protein) or 5 to 12% (w/w protein) within the substitute dairy food product.

Optionally the amylase is totally denatured during production of the substitute food product.

The present invention thus further provides a substitute food composition comprising at least one amylase and one or more of: lipids, proteins, sweetening agents, and/or ash, wherein said amylase comprises at least 3% by weight of protein within the substitute food composition. Optionally, the content of the amylase can be more than 3%, for example at least 10% w/w protein, for example at least 20% w/w protein.

Optionally, the amylase is at least partially denatured in the substitute food product.

Optionally, the substitute food product is a substitute dairy product, such as a substitute cheese or yoghurt.

Optionally, the substitute food product is a substitute egg product.

Optionally the composition includes one or more of the following components: lipids, proteins, sweetening agents, colour balancing agent and/or ash. Optionally the composition includes a fat.

The composition used to form the substitute food product forms a further aspect of the present invention.

In one aspect the present invention provides an egg-like food product comprising an amylase enzyme providing a minimum level of protein of 3% (w/w of protein), optionally with at least 5% (w/w protein), optionally with at least 10% (w/w protein), for example at least 20% (w/w protein). Optionally the enzyme can be the main protein constituent of the food product. The egg-like food product will have one or more properties selected from: foaming, emulsification, or binding properties, which are similar to that of whole eggs obtained from natural (avian) eggs. The egg-like food product can, for example, be used instead of avian eggs, for example can be fried directly to produce, a scrambled egg-type product or an omelette.

In one aspect the present invention provides an egg-white substitute with properties suitable for use in baked or cooked goods, the egg-white substitute comprising an amylase enzyme, with the enzyme providing a minimum level of protein of 3% (w/w of protein), optionally with at least 5% (w/w protein), optionally with at least 10% (w/w protein), for example at least 20% (w/w protein). Optionally the amylase enzyme can be the main protein constituent of the egg-white substitute. The egg white-like substitute will have one or more properties selected from: foaming, emulsification, or binding properties, which are similar to that of egg whites obtained from natural (avian) eggs. The egg-white substitute can, for example, be added to a dough instead of avian eggs and contribute to a foamy texture.

The egg replacement composition of the present invention may also be prepared as a spray-dried or desiccated composition and may be in the form of a powder that can be reconstituted with water and/or other liquids.

The egg replacement composition product and egg-based food product may have a natural egg-like texture, which is obtained by combining the protein with

other modifiers, such as texture modifiers (such as a mucilage, e.g., a flaxseed extract or carbohydrate compounds, such as dextrose) and taste modifiers (for example yeast extract or salts). Salts added as taste modifiers may include conventional culinary salts such as sodium chloride and potassium chloride, but also salts such as kala namak, also known as Indian black salt, or Hawaiian black lava salt.

The egg white-like emulsion product may have a natural egg white-like texture, which is obtained by combining the protein with other modifiers, such as texture and taste modifiers, for example by the addition of a mucilage, e.g., flaxseed extract or flaxseed flour, carbohydrates (e.g., dextrose), yeast extract or salts. These salts may include conventional culinary salts such as sodium chloride and potassium chloride, but also salts such as kala namak, also known as Indian black salt, or Hawaiian black lava salt.

Another taste modifier can be yeast flocks. This results in a food product, specifically an egg replacer or egg white-like emulsion product, with a high versatility suitable for culinary applications in which eggs, and specifically egg whites, would typically find application.

The amylase enzyme of the invention may be commercially produced, either by microbial fermentation, purified from non-animal or non-mammalian sources or produced by recombinant expression in a microbial host cell (typically a microorganism or plant cell).

Optionally, the amylase may be commixed or admixed with one or more plant proteins to form the composition of the invention. Inclusion of one or more plant proteins can be desirable to improve the economics of manufacturing a substitute food product using a purified or recombinant protein, since a purified or recombinant protein is generally more expensive to produce than traditional food products.

Substitute Dairy Products

In one embodiment of the invention there is provided a method for the production of a substitute dairy product, the method comprising the steps of:

providing at least one amylase;

incorporating said at least one amylase into a composition to form a substitute dairy product, for example a cheese, yoghurt or other dairy based product, and wherein said amylase is subjected to one or more process steps to at least partially denature the amylase during formation of the substitute dairy product.

Optionally, the amylase comprises at least 3% by weight of protein in the substitute dairy product, for example at least 5% by weight, for example at least 7% (w/w protein) of the substitute dairy product or at least 10% (w/w protein) of the substitute dairy product.

Optionally, the amylase is the main protein constituent of the substitute dairy product.

Optionally the amylase is totally denatured.

The substitute dairy composition (intermediate to the substitute dairy product) may be a curd. The curd may be used to create further substitute dairy products. The substitute dairy product may be a substitute butter, cheese, yoghurt, custard, cream cheese, medium-hard cheese, hard cheese, pasta filata-type cheese, soft-ripened cheeses, feta-style cheese and the like.

When the substitute dairy product is in the form of a cheese or a yoghurt, the method may include a step of ripening the substitute dairy product. The substitute dairy product may thus be a ripened substitute dairy cheese or a ripened substitute dairy yoghurt.

For some cheese varieties, one or more bacteria or other microbial species can be employed in the cheese-making process for ripening or fermentation, where

fermentative products and by-products such as lactic acid, carbon dioxide, alcohols, aldehydes and ketones are produced.

Optionally, the method of producing the substitute dairy product in accordance with the invention may include the step of adding one or more lipids, fats, or oils to form a substitute dairy composition including the amylase.

Optionally, the method of producing the substitute dairy product in accordance with the invention may include the step of adding one or more of: carbohydrates, sweetening agents, and ash to form a substitute dairy composition including the amylase.

The substitute dairy composition may include the amylase in a concentration of about 0.01 g/L to about 1000 g/L inclusive, for example of from 10 g/L to 450 g/L, inclusive, more specifically of from 60 g/L and 350 g/L, inclusive.

The amylase may be added in a purified form (for example at least 70%, 80%, 90%, 95%, 99%, or higher purity). Alternatively, the amylase may be produced by microbial fermentation and may be provided in the form of a crude extract having a purity of 70% or less.

The substitute dairy product, such as a cheese or yogurt (joghurt) product, may include one or more different amylases, for example an alpha amylase and a glucoamylase.

The substitute dairy product of the invention may include one or more other proteins to form a substitute dairy composition including the amylase. The one or more proteins to be included in the composition may include one or more non-dairy or dairy proteins. Soy protein or faba bean can be included, for example. Dairy proteins may be included, typically as purified or recombinant dairy proteins. For the avoidance of doubt, a recombinant dairy protein is considered to be "animal free". The dairy proteins may include one or more casein proteins. Alternatively, or additionally, the dairy proteins may include one or more whey proteins. Optionally, the casein and/or whey proteins can be recombinant

proteins, for example can be produced by microbial fermentation. In some embodiments of these methods, the protein mixture can include one or more proteins selected from the group of: α -lactalbumin, β -lactoglobulin, α -S1-casein, α -S2-casein, lactoferrin, transferrin, and serum albumin.

The substitute dairy product or composition may include a total concentration of one or more lipids of about 0 weight % to about 45 weight %; a total concentration of one or more flavour compounds of about 0.01 weight % to about 6 weight %; a total concentration of about 0.1 weight % to about 6 weight % of one or more carbohydrates or sweetening agents; and a total concentration of ash of about 0.15 weight % to about 1.5 weight %, wherein the total of all ingredients sums to 100 weight%, with the balance being the amylase and water. The lipids may be cultured lipids or microbially-produced lipids.

Optionally, the one or more lipids are selected from the group consisting of: sunflower oil, coconut oil, tributyrin, mono- and di-glycerides, free fatty acids, and phospholipids. Optionally, the substitute dairy product includes one or more of: a final concentration of sunflower oil of about 1 weight % to about 28 weight %; a final concentration of coconut oil of about 0.5 weight % to about 14 weight %; a final concentration of tributyrin of about 0.05 weight to about 1.0 weight %; a final total concentration of monoglycerides and diglycerides of about 0.08 weight % to about 1.2 weight %; a final total concentration of free fatty acids of about 0.02 weight % to about 0.28 weight %; and a final total concentration of phospholipids of about 0.02 weight % to about 0.3 weight percent.

Optionally, the free fatty acids comprise at least one fatty acid selected from the group of: butyric acid, caproic acid, caprylic acid, myristic acid and capric acid. In some embodiments of any of the compositions described herein, the phospholipids are soy lecithin phospholipids, sunflower lecithin phospholipids, cotton lecithin phospholipids, or rapeseed lecithin phospholipids. In some embodiments of any of the compositions described herein, the monoglycerides and diglycerides are plant-derived monoglycerides and diglycerides, or are bacteria-derived monoglycerides and diglycerides.

Optionally, the flavour compounds include at least one flavour compound selected from the group consisting of: δ -decalactone, ethyl butyrate, 2-furyl methyl ketone, 2,3-pentanedione, γ -undecalactone, and δ -undecalactone.

Optionally, the one or more sweetening agents is a saccharide. The saccharide can be selected from the group consisting of: glucose, mannose, maltose, fructose, galactose, lactose, sucrose, monatin, and tagatose. Alternatively, the one or more sweetening agents is an artificial sweetener. For example, the artificial sweetener can be selected from the group of: *stevia*, aspartame, cyclamate, saccharin, sucralose, mogrosides, brazzein, curculin, erythritol, glycyrrhizin, inulin, isomalt, lacticitol, mabinlin, malititol, mannitol, miraculin, monatin, monelin, osladin, pentadin, sorbitol, thaumatin, xylitol, acesulfame potassium, advantame, alitame, aspartame-acesulfame, sodium cyclamate, dulcin, glucin, neohesperidin dihydrochalcone, neotame, and P-4000.

Optionally, the ash includes one or more of: calcium, phosphorus, potassium, sodium, citrate, and chloride. In some embodiments of any of the compositions described herein, the ash comprises one or more (e.g., one, two, or three) of CaCl_2 , KH_2PO_4 , and Na_3 citrate. For example, the CaCl_2 can have a final concentration of about 0.05 g/L to about 0.2 g/L; the KH_2PO_4 can have a final concentration of about 0.2 g/L to about 0.4 g/L; and/or the Na_3 citrate can have a final concentration of about 0.1 g/L to about 0.3 g/L.

Optionally, the substitute dairy product includes one or more colour balancing agents, for example β -carotene or annatto.

Substitute Egg Products

According to a further aspect of the invention there is provided substitute-egg food product comprising:

at least one amylase enzyme; wherein said at least one amylase enzyme provides at least 3% (w/w) of the protein content of the product (optionally is the main protein constituent of the substitute-egg food product).

In an alternative aspect, there is provided a method for the production of a substitute egg food product, the method comprising the steps of:

- providing at least one amylase;
- subjecting the at least one amylase to one or more process steps to at least partially denature the amylase; and
- incorporating said at least partially denatured amylase into a composition to form a substitute egg food product.

The substitute-egg food product can be an egg replacement composition (egg replacer), an egg-like food product or an egg-white substitute.

Optionally, the amylase enzyme is partially or totally denatured. Alternatively, the amylase is denatured during cooking of the food product itself or a food composition incorporating the food product (for example where an egg replacement composition or egg-white substitute is added to other ingredient to form a food product).

Optionally, the substitute-egg food product includes one or more lipids. Optionally, the substitute-egg food product further comprises water, carbohydrate (such as dextrose, flaxseed powder or extract), a fat (such as coconut fat), and a salt, and optionally may also comprise a colorant (e.g., β -carotene). The substitute-egg food product can be produced by hydration of the amylase and carbohydrate in water followed by homogenization to incorporate the fat and any colorant. The emulsion can then be pasteurized. If the product is to be used in liquid form, the pasteurized liquid can be filled into bottles and can then be used for the different applications. Alternatively, the liquid can be formed into a powder, for example by freeze-drying.

Optionally the substitute-egg food product includes one or more additional proteins (preferably non-egg proteins, more preferably plant proteins). A small amount of additional proteins can be provided by a seed mucilage, for example a flaxseed mucilage. However, a seed mucilage will mainly comprise carbohydrate. Optionally the substitute-egg food product includes one or more carbohydrates. Optionally the substitute-egg food product includes one or more sweetening agents. Optionally the substitute-egg food product includes one or

more colouring agents. Optionally the substitute-egg food product includes one or more vitamins, such as a yeast extract. Optionally the substitute-egg food product includes one or more minerals or salts.

Optionally, the amylase is denatured by a heat treatment, for example by heating to a temperature of 40°C to 100°C for a suitable period of time, for example from 10 to 90 minutes. The amylase enzyme can be denatured during cooking of the final food composition to provide a cooked foodstuff.

The amylase may be an isolated (purified) enzyme.

The amylases may be commercially produced enzymes, optionally produced either by recombinant (for example recombinant microbial) processes or purified from non-animal sources.

Optionally, the amylase used within the product of the present invention is recombinantly produced, that is a gene encoding the protein sequence of the enzyme(s) are expressed under controlled conditions. Any suitable host cell can be used, for example a micro-organism such as a yeast or bacteria. Optionally the gene encoding the enzyme is expressed using a heterologous host cell. Alternatively, the gene encoding the amylase can be expressed within a homologous host cell, which can optionally be engineered to exhibit above-normal levels of expression, for example by manipulation of the native promoter and/or enhancer sequences.

The amylases used in the present invention can be neutrally flavoured and can be manipulated to form gels, emulsions, or egg replacers with a texture and mouthfeel that can be optimised through modification of process conditions known to those skilled in the field of the invention. Many of these amylases are commercially available in the marketplace: the availability of these enzymes presents an opportunity for ameliorating the costly economics involved in making substitute food product from recombinantly produced food proteins.

Optionally, the amylase can then be combined with other ingredients to form the substitute-egg food product. Thus, the invention provides a method of producing a substitute-egg food product, which method comprises providing an

amylase and adding one or more lipids, proteins, carbohydrates, sweetening agents, colouring agents, vitamins, minerals and/or salts to the amylase enzyme to form a substitute-egg food product. Optionally the amylase comprises at least 3% of the protein content of the substitute egg food product (optionally with at least 5 % w/w protein, for example at least 10% w/w protein, for example at least 20% w/w protein). Optionally the amylase enzyme can be the main protein constituent of the food product.

Where the substitute-egg food product is an egg replacement composition, this composition can then be formed into an egg-based food product, such as a pancake, baked goods (biscuits, cakes, breads and the like), or mixtures therefore.

The invention is based on the realisation that amylases, when denatured or hydrolysed (partially or fully) can be used to produce egg-based food product compositions that exhibit a similar taste, aroma, and mouth feel as such products made using eggs ("traditional eggs" or "traditional egg-based food products", the latter being products which would normally include eggs as an ingredient, typically as a binder, for example baked products, pancakes, deserts). The present invention therefore provides compositions, methods of making the compositions, and kits including amylase, compositions, emulsions, and mixtures useful for making these egg-based food product compositions.

The substitute-egg food products, (egg replacement compositions, egg-like food products and egg-white substitutes) provided herein have a similar taste, mouth feel, aroma, and nutritional value as compared to equivalent products made using an avian egg.

The substitute-egg food products may include the at least one amylase enzyme in a concentration of about 30 to 98 g enzyme / 100 g (total protein content), typically of from 55 to 98 g enzyme / 100 g (total protein content), more specifically of from 70 to 98 g enzyme / 100 g (total protein content).

The amylase enzyme used may be in a purified form containing at least 40% by weight protein (enzyme) per enzyme composition (for example at least 50%,

at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or higher purity). This reflects the content of commercially available enzyme compositions, which can include fillers such as maltodextrin. Alternatively, the amylase may be produced by microbial fermentation and may be provided in the form of a crude extract having a protein content of 70% or less (w/w). These percentages are by reference to the total content of the enzyme composition.

The substitute-egg food product of the invention may include one or more other additional proteins to produce a protein mixture. The one or more additional proteins to be included in the protein mixture may include one or more enzymes or food proteins. The term "food proteins" means any non-animal derived protein which can be added to food. Optionally, the additional proteins can be purified or recombinant proteins obtained from non-animal sources. The additional proteins may include one or more avian egg proteins, but could also include other enzymes. Alternatively, or additionally, the additional proteins may include one or more recombinant dairy proteins, such as casein or whey proteins, optionally produced by microbial fermentation. Optionally, the protein mixture can include one or more additional proteins selected from the group of: α -lactalbumin, β -lactoglobulin, α -S1-casein, α -S2-casein, lactoferrin, transferrin, and serum albumin.

Optionally, the substitute-egg food product (for example the egg replacement composition) can comprise one or more lipids. The lipids can be in the form of oils or fats. The lipids may be selected from the group consisting of: sunflower oil, coconut oil, rapeseed oil, palm fat, tributyrin, mono- and di-glycerides, free fatty acids, and phospholipids. The lipids may be cultured oils or fats, or microbially-produced or microbially-derived oils or fats. Optionally, the substitute-egg food product (for example the egg replacement composition) can comprise a carbohydrate. The carbohydrate may be obtained from a plant. The carbohydrate may be a monosaccharide, a disaccharide, a polysaccharide or a mixture thereof. The carbohydrate may be a starch, a gum, an edible fibre, a flour, or a mixture thereof. When the carbohydrate is in the form of a starch, the starch may be selected from the group consisting of tapioca starch, corn starch, potato starch, wheat starch, waxy maize starch, modified starch and

mixtures thereof. Starch may comprise of from 0.1% and 50% by mass of the egg replacer. When the carbohydrate is in the form of a gum, the gum may be selected from the group consisting of arrowroot flour, xanthan gum, tara gum, guar gum, agar gum, locust bean gum, and gum arabic, and mixtures thereof. Gum may comprise between 0.1% and 50% by mass of the egg replacer. The carbohydrate may be in the form of exopolysaccharides, typically originated from microbial cultures, such as dextran, fructan, galactan, xanthan and their derivatives with a molecular weight of 1 to 10,000 kDa.

In one embodiment of the invention, the substitute-egg food product (for example the egg replacement composition) can comprise a mucilage containing plant seeds, or its flour, its fractionated flour, or its extracted mucilage to which the amylase enzyme is added. Optionally, the mucilage may be a flaxseed mucilage or aloe vera extract. Optionally, flaxseed flour or fractionated flour can be used. Optionally, the mucilage is based on chia seeds, psyllium husk, aqua faba extract, chickpea water, hemp seeds, wheat germ, and the like. Instead of flaxseed mucilage, fibers, such as flaxseed fiber, hydrocolloids, modified starches or soluble fibres such as β -glucan, xanthan gum, pectin and the like can be used.

Optionally β -carotene is added to the substitute-egg food product (for example the egg replacement composition) as a natural colorant. However, other yellow, orange, red colourants, natural extracts, or artificial colourants may be used e.g., turmeric, pepper extract, capsicum annum L, tomato extract, lycopene, carrot extract, annatto, safflower and the like. Optionally, one or more flavour compounds may be added to the substitute-egg food product (for example the egg replacement composition). The flavour can be selected from the group consisting of: fully or partially degraded beta-lactoglobulin, extracts containing fully or partially degraded beta-lactoglobulin (specifically biomass extracts containing such compounds created using precision fermentation), δ -decalactone, ethyl butyrate, 2-furyl methyl ketone, 2,3-pentanedione, γ -undecalactone, and δ -undecalactone, sulphur containing flavour compounds (e.g. 2-acetyl thiazoline, dimethyl sulphide, allyl isothiocyanate, diallyl thiosulfinate, diallyl disulphide, and the like), hydrogen sulphide. The flavour compounds may be natural or artificial. Optionally, one or more sweetening

agents may be added to the substitute-egg food product (for example the egg replacement composition). The one or more sweetening agents may be a saccharide(s). Optionally, the saccharide is selected from the group consisting of: glucose, mannose, maltose, fructose, galactose, lactose, sucrose, monatin, and tagatose. The sweetening agent may also have a neutral taste, such as a maltodextrin. Optionally, the one or more sweetening agents may be an artificial sweetener. Optionally, one or more salts may be included in the substitute-egg food product (for example the egg replacement composition). Exemplary salts include one or more of: calcium, phosphorus, potassium, sodium, citrate, and chloride salts.

Optionally, the egg replacement composition has a pH of about 4.6 to 8.0, for example about 6.2 to about 7.2 (e.g., about 6.2 to about 6.8).

The egg replacement composition or egg-white substitute of the invention may be used in baking or cooking, as binding agent, such as a replacer for eggs in frozen desserts, bakery mixes, meringues, coatings, batters, cakes, pancakes, tarts, confections, panade for use in, for example, croquettes, in alcoholic beverages such as whisky sour, pisco sour, and the like. The invention extends to a baked or cooked product (i.e., an "egg-based food product") produced using the egg replacer or egg-white substitute of the invention.

In one embodiment of the invention, an alpha amylase and/or a glucoamylase is used to form the substitute-egg food product (for example the egg replacement composition) of the invention.

The term "egg replacement composition" (or "egg replacer") means a product which resembles a composition formed using an avian egg as its main component, for example a beaten egg. Thus, the term "egg replacement composition" as used herein means a product resembling a traditional egg product, but made with proteins produced by processes not involving mammals. For example, the proteins used can be plant-based proteins or, more preferably, are recombinantly expressed proteins, for example are proteins which are expressed using microbial host cells. Optionally, the "egg replacement composition" of the invention is similar to, equivalent to, or is

nearly identical to the corresponding product formed using an avian egg especially as regards one or more of taste, aroma, flavour, mouthfeel, organoleptic qualities, colour, and the like. Optionally, the “egg replacement composition” of the invention is made with proteins produced by processes not involving animals.

The “egg replacement composition” of the present invention refers to a composition which comprises an at least partially denatured amylase in addition to other ingredients. The egg replacement composition is an intermediate composition for addition to an egg-based food product or composition.

The term “egg-white substitute” (or “egg-white replacer”) means a product which resembles a composition formed using the white (non-yolk) of an avian egg without any other components. Thus, the term “egg-white substitute” as used herein means a product resembling an avian egg-white, but made with proteins produced by processes not involving mammals. For example, the proteins used can be plant-based proteins or, more preferably, are recombinantly expressed proteins, for example are proteins which are expressed using microbial host cells. Optionally, the “egg-white substitute” of the invention is similar to, equivalent to, or is nearly identical to the corresponding product formed using an avian egg white especially as regards one or more of taste, aroma, flavour, mouthfeel, organoleptic qualities, colour, and the like. Optionally, the “egg-white substitute” of the invention is made with proteins produced by processes not involving animals.

The term “egg-based food product” refers to a food product which is in a form suitable for eating or for sale to consumers and which has been made using the egg replacement composition or the egg-white substitute of the invention. Optionally, these products can be referenced as “synthetic food products”. Optionally, these goods can be cooked or baked at home by the consumer or can be in kit form, for example a cake mix which requires other ingredients to be added (for example fat, milk or water). The term “egg-based food product composition” refers to a composition for production of a food product which composition includes the egg replacement composition or the egg-white substitute of the invention. The composition can include all ingredients for the

end food product, which can optionally be in a “ready-to-cook” or part-baked format or may be in the form of a kit to which other ingredients are intended to be added to form the final food product.

The term “avian egg” refers to an egg which has been laid by a bird such as chicken, duck, quail or the like.

Egg Replacement Composition (Egg Replacer)

Optionally, the amylase enzyme may be commixed or admixed with one or more plant products, such as plant proteins or fats, to form the egg replacement composition of the invention. Inclusion of more or more plant products can be desirable to improve the economics of making an egg-replacement composition and any food product containing the same from a purified or recombinant protein, since a recombinant protein is generally more expensive to produce than traditional food products.

The egg replacement composition may include a final total concentration of one or more lipids of about 0 weight % to about 30 weight %; a final total concentration of one or more flavour compounds of about 0.01 weight % to about 4 weight %; a final total concentration of about 0.1 weight % to about 60 weight % of one or more carbohydrates or sweetening agents; and a final total concentration of salts of about 0.15 weight % to about 1.5 weight %.

Optionally, the egg replacement composition may contain one or more lipids, in the form of oils or fats. Optionally, the lipids may be selected from the group consisting of: sunflower oil, coconut oil, rapeseed oil, palm fat, tributyrin, mono- and di-glycerides, free fatty acids, and phospholipids. Additionally, or alternatively, the lipids may be cultured lipids or microbially-produced lipids. Such lipids, including phospholipids, may be produced by precision fermentation techniques. Optionally, lipid is a free fatty acid selected from the group of: butyric acid, caproic acid, caprylic acid, and capric acid. Optionally, the lipid is a phospholipid, such as soy lecithin phospholipids, sunflower lecithin phospholipids, cotton lecithin phospholipids, or rapeseed lecithin phospholipids. Optionally, the lipid can be monoglycerides and diglycerides

which are plant-derived monoglycerides and diglycerides, or are bacteria-derived monoglycerides and diglycerides.

As noted above, the egg replacement composition may comprise a carbohydrate. The carbohydrate may be obtained from a plant. The carbohydrate may be a monosaccharide, a disaccharide, a polysaccharide or a mixture thereof. The carbohydrate may be a starch, a gum, an exopolysaccharide, an edible fibre, a flour, or a mixture thereof. When in the form of a starch, the starch may be selected from the group consisting of tapioca starch, corn starch, potato starch, wheat starch, waxy maize starch, modified starch and mixtures thereof. The starch may comprise from 0.1% to 50% by mass of the egg replacer. When the carbohydrate is in the form of a gum or exopolysaccharide, the gum may be selected from the group consisting of arrowroot flour, tara gum, guar gum, agar gum, locust bean gum, and gum arabic, and mixtures thereof. Exopolysaccharides may be selected from the group consisting of dextran, fructan, galactan, xanthan, and mixtures thereof. The gum may comprise from 0.1% to 50% by mass of the egg replacer.

Optionally, one or more sweetening agents may be added to the egg replacement composition. The one or more sweetening agents may be a saccharide. Optionally, the saccharide is selected from the group consisting of: glucose, mannose, maltose, fructose, galactose, lactose, sucrose, monatin, and tagatose. The sweetening agent may also have a neutral taste, such as a maltodextrin. Optionally, the one or more sweetening agents is an artificial sweetener. Optionally, the artificial sweetener is selected from the group of: *stevia*, aspartame, cyclamate, saccharin, sucralose, mogrosides, brazzein, curculin, erythritol, glycyrrhizin, inulin, isomalt, lactitol, mabinlin, maltitol, mannitol, miraculin, monatin, monelin, osladin, pentadin, sorbitol, thaumatin, xylitol, acesulfame potassium, advantame, alitame, aspartame-acesulfame, sodium cyclamate, dulcin, glucin, neohesperidin dihydrochalcone, neotame, and P-4000. Optionally, the composition will include one or more colourants, such as β -carotene.

As noted above, the egg replacement composition can include one or more salts. Optionally, the egg replacement composition can include one or more

salts selected from: calcium, phosphorus, potassium, sodium, citrate, and chloride salts. Optionally, the salts used include one or more (e.g., one, two, or three) of CaCl_2 , KH_2PO_4 , and $\text{Na}_3\text{citrate}$, K_2CO_3 , $\text{Mg}_3(\text{C}_6\text{H}_5\text{O}_7)_2$. Optionally, the egg replacer includes CaCl_2 at a final concentration of about 0.05 g/L to about 0.2 g/L; and/or KH_2PO_4 at a final concentration of about 0.2 g/L to about 0.4 g/L; and/or $\text{Na}_3\text{citrate}$ at a final concentration of about 0.1 g/L to about 0.3 g/L; and/or K_2CO_3 at a final concentration of about 0.1 g/L to about 0.5 g/L; and/or $\text{Mg}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ at a final concentration of about 0.01 g/L to about 0.3 g/L.

Optionally, depending on the required end use of the egg replacement composition, one or more bacteria or other microbial species is employed in the process of forming the egg replacement composition for ripening or fermentation where fermentative products and by-products such as lactic acid, carbon dioxide, alcohols, aldehydes and ketones are produced.

The amylase enzyme(s) used in the present invention may be commercially produced enzymes, produced either by recombinant microbial processes, or purified from non-animal or non-mammalian sources.

In one embodiment the egg replacer combines amylase with potato protein, flaxseed fiber, sunflower oil, sunflower lecithin, salt, colorant and water.

Egg-White Substitute

The egg-white substitute is made as described above for the egg replacement composition, but will typically not include a colourant such as β -carotene. An example composition would include the amylase, water, flaxseed mucilage or flaxseed fibre, and fat (such as coconut oil or sunflower oil, for example).

The egg replacement composition and/or egg-white substitute can each be combined with other food ingredient to form a foodstuff which would typically comprise avian eggs.

Egg-Like Food Product

The egg-like food product is made as described above for the egg replacement composition, and will typically include a colourant such as β -carotene. An example composition would include the amylase, water, flaxseed mucilage or flaxseed fibre, fat (such as coconut oil or sunflower oil, for example), a colourant such as β -carotene and optionally a dextrose.

In a further aspect, the present invention provides a process of forming a substitute egg product, wherein an amylase enzyme is mixed with water to form a mixture and said mixture is homogenised with a fat, wherein said amylase enzyme provides at least 5g / 100g protein of the product. Optionally, the amylase enzyme is mixed with a carbohydrate source together with the water, for example a seed mucilage or a flour. One suitable seed mucilage is a flax seed mucilage. Optionally a fat can be added to the mixture and homogenized to form the substitute egg product. Suitable fats are described above and include, for example vegetable oils, sunflower oils, coconut fat. Optionally other flavourings, sweetening agents, ash, salts and/or colorants can be added, as described above with regard to the composition. These additional components can be added before or after homogenisation, as convenient.

The present invention may be further defined by reference to the following clauses:

1. According to one aspect of the invention there is provided a method for the production of a substitute dairy product, the method comprising the steps of:

providing an enzyme having a catalytic function;

subjecting the enzyme to one or more treatment steps to convert said enzyme into a converted protein having a structural function; and

incorporating said converted protein into a substitute dairy product.

2. The method of clause 1, wherein the enzyme is treated to render it substantially devoid of its catalytic function.

3. The method of clause 1 or clause 2, wherein the at least one enzyme is a globular enzyme.

4. The method of any one of clause 1 to 3, wherein the enzyme is selected from the group consisting of: a lactase, a glucosidase, a cellulase, an amylase, an invertase, a protease, xylanase, glutenase, phytase, lipase, gelatinase, glucose oxidase, transglutaminase, pectinase, beta amylase, pullulanase, naringinase,

limoninase, aminopeptidase, laccase, tyrosinase, cutinase, superoxide dismutase, endoglycosidase, glycosyl transferase, glucose isomerase, amidase, lignin peroxidase, invertase, and a kinase.

5. The method of clause 4, wherein the enzyme is selected from the group consisting of: beta-galactosidase, alpha glucosidase, a cellulase, alpha amylase, nattokinase, glucoamylase, and invertase.

6. The method of any one of clauses 1 to 5, wherein a curd or emulsified composition from such globular enzymes is obtained, such curd or emulsified composition being suitable for production of an animal-free product resembling one or more desired properties of a dairy product.

7. The method of any one of clauses 1 to 6, wherein the enzyme is treated by way of hydrolysis (pH-based hydrolysis or enzymatic hydrolysis), heat-treatment, or mechanical shearing to at least partially, or fully, denature the enzyme, rendering a structural protein component or structural protein constituent.

8. The method of any one of clauses 1 to 5, wherein the enzyme is heat-treated to between 40 and 100 °C.

9. The method of clause 8, wherein the heat treatment is performed for a period of between 1 min and 500 min, typically between 5 min and 90 min, most typically between 10 to 30 minutes

10. A non-milk protein component having a desirable attribute.

11. The non-milk protein component of clause 10, which is a non-dairy protein treated to convert it into a protein having a structural function suitable for use in the production of a substitute dairy product, such as, for example, a substitute cheese product.

12. A substitute dairy product produced by treating at least one enzyme with a catalytic function to render such enzyme suitable for structural purposes in a substitute dairy product.

13. The substitute dairy product of clause 12, which is a substitute curd, substitute milk, butter, cheese, yoghurt, custard, cream cheese, medium-hard cheese, hard cheese, pasta filata-type cheese, soft-ripened cheeses and other substitute dairy products.

14. The substitute dairy product of clause 13 which, when in the form of a substitute cheese product or substitute yoghurt product, is a ripened substitute cheese product or ripened substitute yoghurt product.

15. The substitute dairy product of any one of clauses 12 to 14, wherein the protein treated in accordance with the method of the invention is present in a concentration of about 0.01 g/L to about 1000 g/L inclusive, typically between 10 g/L to 450 g/L, inclusive, more specifically between 60 g/L and 350 g/L, inclusive.

The present invention may be further defined by reference to the following clauses:

1A. A method for the production of an egg replacer, the method comprising the steps of:

providing an enzyme having a catalytic function;

subjecting the enzyme to one or more treatment steps to convert said enzyme into a converted protein having a structural function; and

incorporating said converted protein into an egg replacer.

2A. The method of clause 1, wherein the enzyme is at least partially denatured to render it substantially devoid of its catalytic function.

3A. The method of clause 1 or clause 2, wherein the at least one enzyme is an isolated enzyme, specifically a globular enzyme.

4A. The method of any one of clauses 1 to 3, wherein the enzyme is selected from the group consisting of: a lactase, a glucosidase, a cellulase, an amylase, an invertase, a protease, xylanase, glutenase, phytase, lipase, gelatinase, glucose oxidase, transglutaminase, pectinase, beta amylase, pullulanase, naringinase, limoninase, aminopeptidase, laccase, tyrosinase, cutinase, superoxide dismutase, endoglycosidase, glycosyl transferase, glucose isomerase, amidase, lignin peroxidase, invertase, and a kinase.

5A. The method of clause 4, wherein the enzyme is selected from the group consisting of: beta-galactosidase, alpha glucosidase, a cellulase, alpha amylase, nattokinase, glucoamylase, and invertase.

6A. The method of any one of clauses 1 to 5, wherein an egg white-like emulsified composition from such globular enzymes is obtained, such egg white-like emulsified composition being suitable for production of an animal-free food product including one or more desired properties of a food product.

7A. The method of any one of clauses 1 to 6, wherein the enzyme is treated by way of hydrolysis (pH-based hydrolysis or enzymatic hydrolysis), heat-treatment,

or mechanical shearing to at least partially, or fully, denature the enzyme, rendering a structural protein component or structural protein constituent.

8A. The method of any one of clauses 1 to 5, wherein the enzyme is heat-treated to between 40 and 100 °C.

9A. The method of clause 8, wherein the heat treatment is performed for a period of between 1 min and 500 min, typically between 5 min and 90 min, most typically between 10 to 30 minutes

10A. An enzyme treated to convert it into a protein having a structural function suitable for use in the production of a substitute food product, such as, for example, an egg replacer.

11A. A substitute food product produced by treating at least one enzyme with a catalytic function to render such enzyme suitable for structural purposes in a substitute food product.

12A. The substitute food product of clause 11, wherein the protein treated in accordance with the method of the invention is present in a concentration of about 0.01 g/L to about 1000 g/L inclusive, typically between 10 g/L to 450 g/L, inclusive, more specifically between 60 g/L and 350 g/L, inclusive.

13A. An egg replacer comprising a non-egg protein as the main structural protein.

14A. The egg replacer of clause 13, wherein the non-egg protein comprises an at least partially denatured isolated enzyme.

15A. The egg replacer of clause 14, wherein the enzyme is a deactivated isolated enzyme or fully denatured isolated enzyme.

16A. An egg white-like emulsion product comprising a non-egg protein as the main protein source.

17A. The egg white-like emulsion product of clause 16, wherein the non-egg protein is an at least partially denatured isolated enzyme.

18A. The egg white-like emulsion product of clause 16 or clause 17, wherein the non-egg protein component is a deactivated isolated enzyme, or fully denatured isolated enzyme

Further features of the invention will become apparent from the following description, by way of example only, with reference to the accompanying figures and examples.

EXAMPLES

EXAMPLE 1

Substitute Cheese Product using Alpha-amylase

Alpha-amylase was sourced from Rajvi Biotech Pvt. Ltd.

Ingredients:

Details of the enzyme used: supplier Enzyme Bioscience, Alpha-Amylase [Aspergillus oryzae], containing a protein content of 71.4%. (referred to “Alpha-Amylase”)

INGREDIENTS			
Supplier	Name	%	g
	Tap Water	65,3	65,3
Vandemoortele L0404	Coconut fat	18,00	18,00
Enzyme Bioscience	Alpha-amylase	13,6	13,59
Rewe LXX20294801	Salt (NaCl)	0,7	0,70
AZELIS / B. E489R	Nutriose FM10	2,0	2,00
Ja (Rewe) L116008000	Sugar	0,3	0,30
Ch. Hansen	Lactic acid bacteria	0,3	0,3
Total weight (g)		100%	100

Process steps:

- Dissolve alpha-amylase in water with all other dry ingredients, in a beaker in a stirring plate, and hydrate for 15 minutes at room temperature.
- Add melted coconut fat and emulsify (Ultraturrax, shear level 10000rpm, room temperature).
- Adjust pH to 6.2 with 1M NaOH.

- Ferment the mix with lactic acid bacteria until pH 5.5, followed by acidification with lactic acid (30%w/w) to pH 4.5.
- Heat treatment of solution in a waterbath (79°C, 20 minutes).
- Cool down to below 40°C and gently mix in salt and cultures for ripening
- Manually press the curd for 1 minute, flip the cheese and press for another minute.
- Ripen for 3 days at 12°C and then store in the fridge.

Results and Discussion

The formulation yielded a Feta/salad-cheese like structure, slightly crumbly. Smell and taste of the product was neutral. Color of the product was off-white. Overall taste and appearance resemble a mild Feta/salad-cheese when cut into square cubes. The substitute dairy product is shown in Figure 1.

Example 2: Protocol for production of cream cheese alternative

Ingredients:

Details of the enzyme used: supplier Enzyme Bioscience, Alpha-Amylase [Aspergillus oryzae], containing a protein content of 71.4%. (referred to "Alpha-Amylase")

INGREDIENTS									
		1 - Standard		2 - Flaxseed		3 - Pectin		4 - Protease	
Supplier	Name	%	g	%	g	%	g	%	g
	Tap Water	73.3	73.3	72.8	72.8	72.3	72.3	73.3	73.3
Vandemoortele / L0404	Coconut fat	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0
Enzyme Bioscience	Alpha-amylase	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Rewe / LXX202 94801	Salt (NaCl)	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
AZELIS / B. E489R	Nutriose FM10	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
HI-FOOD	Flaxseedfiber Hi-Smooth			0.5	0.5				
CPKelco	Genu Explorer Pectin ND-200					1.0	1.0		
Amano	Protease 2FG							0.01	0.01
Total		100	100	100	100	100	100	100	100

Process steps:

1. production of milk
 1. weighing dry ingredients (see recipe)
 2. hydrating 30 min
 3. adding fat (liquid)
 4. protease: time frame until coagulation ~2h
2. homogenisation
 1. pre-homogenisation with UT: 1min, 12000 rpm
 2. homogenisation with Panda
 1. batch 1: 130bar total pressure (2nd stage 50 bar)
 2. batch 2-8: standard (250 bar, 2nd stage 50 bar)
3. adjusting pH to 4.5 with 30 % w/w lactic acid
4. coagulation in foils in water bath

1. 1a: 74°C 15 min
2. 1b: 74°C 20 min
3. 1c & 2-8: 70°C 25 min
5. cooling curd in foils, storage at 6°C until next day
6. analysis of curd
 1. fill curd into beaker and on plate
 2. take pictures
 3. sensory: texture, mouthfeel, appearance
 4. measurement of pH

Results and Discussion: The formulation yielded a cream cheese like curd within a range of temperatures and times. The substitute dairy product is shown in Figure 2.

Example 3 - Feta/Salad-cheese alternative

Ingredients:

Details of the enzyme used: supplier Enzyme Bioscience, Alpha-Amylase [Aspergillus oryzae], containing a protein content of 71.4%. (referred to "Alpha-Amylase")

INGREDIENTS									
		1 - Standard		2 - Protease		3 - Protease-Flaxseed		4 - Protease-Pectin	
Supplier	Name	%	g	%	g	%	g	%	g
	Tap Water	70.8	70.8	70.8	70.8	70.4	70.4		
Vandemoortele / L0404	Coconut fat	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0
Enzyme Bioscience	Alpha-amylase	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Rewe / LXX202 94801	Salt (NaCl)	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
AZELIS / B. E489R	Nutriose FM10	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
HI-FOOD	Flaxseedfiber Hi-Smooth					0.4	0.4		
CPKelco	Genu Explorer Pectin ND-200							0.5	0.5
Amano	Protease 2FG			0.01	0.01	0.01	0.01	0.01	0.01
Total		100	100	100	100	100	100	100	100

Process steps:**1. production of milk**

1. weighing dry ingredients (see recipe)
2. hydrating 30 min on stirring plate
3. adding protease
4. adding fat (liquid)

2. Homogenization

1. pre-homogenization with UT: 1min, 12000 rpm

- 2. Homogenization with Panda
 - 1. 250/50 bar
- 3. coagulation
 - 1. Fill milk into foils
 - 2. Place them in a water bath at 74 °C for 25 min
- 4. Smoothening
 - 1. Cool curd in foils to RT
 - 2. Fill curd into Kenwood mixer
 - 3. shear 30 s at level 1.5
- 5. Pressing
 - 1. Weight cheese into mold with cheesecloth
 - 2. Press with hydraulic press 2 min at 1.5 bar
 - 3. Flip cheese and press again
- 6. analysis of curd when cooled down
 - 1. filling curd into beaker and on plate
 - 2. taking pictures
 - 3. analyzation of sensory: texture, mouthfeel, appearance

Results and discussions: The substitute dairy product is shown in Figure 3. Protease additions result in creamier texture, as well as reduced crumbliness (see Figure 3, 2 vs 1)

Example 4 - Substitute Joghurt product

Hydrated alpha-amylase and gluco-amylase paste can be converted into a substitute yoghurt.

Ingredients:

Details of the enzyme used: supplier Breatec, Glucoamylase [Aspergillus niger], Bakemyl AGSX containing a protein content of 43%. (referred to “Glucoamylase”)

Details of the enzyme used: supplier Enzyme Bioscience, Alpha-Amylase [Aspergillus oryzae], containing a protein content of 71.4%. (referred to “Alpha-Amylase”)

		%
	Tap Water	71.8

Protein	alpha Amylase or Glucoamylase	9
Fibre	flaxseed fibre	0.5
Salt	Salt	0.7
Fat	Coconut fat	18

Protocol:- If required, grind using mortar and pestle or other appropriate method

- Hydrate dry ingredients (alpha Amylase or Glucoamylase, Salt, Nutriose, flaxseed fibre) for 30 min and add the fat
- Homogenise the mix
- Adjust pH to 4.5
- Heat to 75 °C for 20 minutes in a waterbath
- smoothening
- Let the curd cool down and store the curd under refrigerated conditions for 12-48 h until analysis

Results and discussions: Visual examination: yoghurt-like texture. The substitute dairy product is shown in Figure 4.

Rheological measurement:

Amplitude-sweeps of Yoghurt, Alpha-Amylase, Glucoamylase. The cow-milk based yoghurt contained fat: 3.5 g, carbohydrates: 4.6 g, protein 3.7 g and salt: 0.12 g and was produced for Netto by Marken Discount AG & Co Kg.

The amplitude sweeps aim to describe the deformation behavior of samples in the non-destructive deformation range and at determining the upper limit of this range. The measuring results of amplitude sweeps are usually presented as a diagram with strain (or shear stress) plotted on the x-axis and storage modulus

G' and loss modulus G'' plotted on the y-axis; both axes on a logarithmic scale. Our amplitude sweep results (see Figure 5) showed that the height and the course of the storage modulus of alpha Amylase and Gluco-Amylase is very similar to the Joghurt sample.

Example 5 - Substitute cheese product – fresh cheese

Hydrated alpha-amylase and gluco-amylase paste converted into a substitute fresh cheese.

Ingredients:

Details of the enzyme used: supplier Breatec, Glucoamylase [Aspergillus niger], Bakemyl AGSX containing a protein content of 43%. (referred to “Glucoamylase”)

Details of the enzyme used: supplier Enzyme Bioscience, Alpha-Amylase [Aspergillus oryzae], containing a protein content of 71.4%. (referred to “Alpha-Amylase”)

		%
	Tap Water	69.3
Protein	alpha Amylase or Glucoamylase	12
Salt	Salt	0.7
Fat	Coconut fat	18

Protocol fresh cheese

- If required, grind using mortar and pestle or other appropriate method
- Hydrate dry ingredients (alpha Amylase or Glucoamylase, Salt) for 30 min and add the fat

- Homogenise the mix
- Adjust pH to 4.5
- Heat to 80 °C for 20 minutes in a waterbath
- Pressing (3 bar, 10 min)
- Let the curd cool down and store the curd under refrigerated conditions for 12-48 h until analysis

Results and discussions: The substitute dairy product is shown in Figure 6. Fresh cheeses made of alpha Amylase (left) and Glucoamylase (right)

Rheological measurement:

Amplitudesweeps of commercial Almette fresh cheese and fresh cheese made of Alpha-Amylase, Glucoamylase. The cow milk based fresh cheese Almette contained (in 100 g) 25 g fat, 3 g carbohydrates, 5.6 g protein and 0.68 g salt. It was produced by Hochland Deutschland GmbH. Ingredient list: fresh cheese, salt and nitrogen.

Our amplitude sweep data showed that the height and the course of the storage modulus of Amylase and Gluco-Amylase fresh cheeses is very similar to the Almette fresh cheese sample.

Example 6 - Egg substitute

Hydrated alpha-amylase and gluco-amylase paste converted into a substitute egg.

Ingredients:

Details of the enzyme used: supplier Breatec, Glucoamylase [*Aspergillus niger*], Bakemyl AGSX containing a protein content of 43%. (referred to "Glucoamylase")

Details of the enzyme used: supplier Enzyme Bioscience, Alpha-Amylase [*Aspergillus oryzae*], containing a protein content of 71.4%. (referred to "Alpha-Amylase")

Protocol

- If required, grind using mortar and pestle or other appropriate method
- Hydrate dry ingredients (Alpha-Amylase or Glucoamylase, Salt, Nutriose, flaxseed fibre, β -carotene, flavour) for 30 min and adjust pH to 6.3
- Add the oil/lecithin mix
- Homogenise the mix
- Frying in the pan

Results and discussions: The substitute egg product is shown in Figure 7. Egg substitute based on alpha Amylase (left) and Glucoamylase (right)

Example 7

Functionality/gelation of glucoamylase: Tap water was mixed with an enzyme product to obtain a solution containing 10 % (w/w) protein. Enzyme ingredients tested:

- A) Glucoamylase [*Aspergillus niger*], powder, 43 % protein (w/w, measured by Dumas method), Bretec
- B) Glucoamylase [*Aspergillus niger*], liquid solution, 25 % protein (w/w, measured by Dumas method), WeissBio Tech

Each solution was split in 3 tubes and pH was adjusted by Lactic acid 80% (w/w) or 1 M NaOH (w/w) to pH 6.5, 5.5 and 5.0 respectively. Each of the samples was split in 2 microtubes, and in one of those 1 % (w/w) NaCl was added.

Process steps:

- samples were mixed
- samples were centrifuged (10 seconds, 13300 rpm)
- incubation at room temperature for 30 min
- heat treatment (80 °C, 30min)
- cooling down in cold water

- sample removal from microtubes for visual analysis

Results and Discussion: All samples containing 10 % (w/w) protein (glucoamylase product A or B) formed a strong gel after incubation at 80 °C. The gel strength was higher for pH 6.5 than for pH 5.0 and 5.5. The crumbly texture suggests suitability for use in a grateable cheese substitute, like a parmesan substitute. (See Figure 8 and Figure 9)

Example 8 - Heat-induced gelation – analysis of gel structures

A heat-induced gel produced from alpha-Amylase enzyme powder in water and compared to a gel that was produced based on lactase (beta-galactosidase) enzyme. Resulting properties can be influenced by processing conditions as well as composition. The gel properties are measured by rheological methods using small deformation oscillation tests.

Standard protocol:

- Use Alpha-Amylase or purified Lactase (beta-galactosidase) enzymes.

Details of the enzymes used:

1. Supplier Vita actives, Lactase [Beta-D galactosidase] [Aspergillus oryzae] 100,000 ALU/g powder, containing a protein content of 44% , a lactase activity of 100,000 ALU/g powder and 18 to 22% maltodextrin powder according to supplier. The protein content of this preparation was increased to 60-70% by applying common filtration procedures followed by subsequent spray drying (referred to "purified Lactase").
 2. Supplier Angel Yeast Co. Ltd, Alpha-Amylase [Aspergillus oryzae] 55000 U/g powder. The protein content of this preparation was 52.7% (referred to "Alpha-Amylase").
- If required, grind using mortar and pestle or other appropriate method
 - The standard procedure and recipe used involves addition of powder to liquid at a rate of 6% protein weight/volume (6 grams protein in 100 ml total volume)

- Add coconut fat at a rate of 6% weight/volume (6 grams fat in 100 ml total volume)
- Add salts (0.1% weight/volume Sodium chloride and 1.39% weight/volume Calcium chloride)
- Adjust pH (to pH 4.8 or pH 6)
- Heat at a rate of 4 K/min to a final temperature of 80°C to denature the enzyme and induce coagulation and measure the process of coagulation in the rheometer
- Inside the rheometer, cool the gel to room temperature at a rate of 10 K/min
- Analysis of rheological properties (gel properties) within rheometer

One commonly applied test procedure to study the rheological properties of protein gels are oscillation tests using a rheometer. The basic principle of these small deformation oscillation tests can be described using a two-plate model.

In a rheometer, applying oscillation tests the viscoelastic behavior of a sample can be described using the storage modulus, G' (given in [Pa]). The G' value is a measure of the deformation energy stored in the material during the shear process. G' represents the elastic behavior of the sample. A high gel elasticity corresponds to a high elastic component (high G').

The data (see Figure 10) showed the temperature dependent evolution of G' and G'' of alpha Amylase and lactase preparations upon heating until 80 °C. For both proteins the G' curve runs above the G'' curve, indicating that the solid behavior dominates over the liquid behavior. The temperature at which G' increases reflect thermally induced changes that lead to the formation of a gel. For alpha-Amylase, G' increases in an almost linear manner. The temperature at which changes in G' occurred was ~ 47 to 50 °C, while for lactase gels G' increased at 66 °C. This may indicate that under the selected conditions the gel structure formation starts earlier but is also much slower in the alpha amylase gels than in the Lactase gels.

CLAIMS

1. A method for the production of a substitute food product, the method comprising the steps of.
 - i. providing at least one amylase enzyme;
 - ii. subjecting the at least one amylase to one or more process steps to at least partially denature the amylase or providing the amylase in the product in an amount of at least 3g/100g protein; and
 - iii. incorporating said amylase into a composition to form a substitute food product.
2. The method of claim 1, wherein the amylase is the main protein constituent of the substitute food product.
3. The method of claim 1 or claim 2 wherein the amylase is at least partially denatured.
4. The method of any one of claims 1 to 3, wherein the amylase is totally denatured within the substitute food product.
5. The method of any one of claims 1 to 4, wherein the substitute food product is a curd.
6. The method of any one of claims 1 to 5, wherein the amylase is denatured by hydrolysis, heat-treatment, or mechanical shearing.
7. The method of any one of claims 1 to 6, wherein the amylase is heat-treated by heating to a temperature of from 40 to 100 °C
8. The method of claim 7, wherein the heat treatment is performed for 10 to 30 minutes.
9. A dairy substitute food composition comprising at least one amylase and one or more of: lipids, proteins, sweetening agents, and/or ash, wherein

said amylase comprises at least 3% by weight of protein in the substitute food composition.

10. An egg substitute food composition comprising an amylase enzyme which is present in the product in an amount of at least 3g / 100g protein.
11. The composition of claim 9 or 10 which comprises lipids.
12. The composition of claim 11 wherein the lipids are coconut fat or vegetable oil.
13. The composition of any one of claims 9 to 12 which is a liquid composition and comprises the amylase in a concentration of from 0.01 g/L to 1000 g/L.
14. The composition of claim 13 which comprises the amylase in a concentration of from 60 g/L to 350 g/L.
15. The composition as claimed in any one of claims 9 to 14 wherein said amylase is the main protein constituent of said composition.
16. The composition as claimed in any one of claims 9 to 15 wherein said amylase comprises at least 5% by weight of said composition.
17. The composition as claimed in any one of claims 9 to 17 which further comprises a mucilage formed from soaking flaxseeds.
18. A substitute dairy product formed using the composition of any one of claims 9 or 11 to 16, which is a substitute curd, substitute milk, butter, cheese, yoghurt, custard, cream cheese, medium-hard cheese, hard cheese, pasta filata-type cheese, soft-ripened cheeses and other substitute dairy products.

19. The substitute dairy product of claim 18 which is a ripened substitute cheese product or ripened substitute yoghurt product.
20. The substitute-egg food product of any one of claims 10 to 17, in the form of an egg-white substitute.
21. A substitute-egg-based food product which comprises the egg replacement composition of any one of claims 10 to 17, together with one or more ingredients for a food product.
22. The substitute-egg-based food product as claimed in claim 21, which comprises the at least partially denatured amylase in a concentration of from 6 g/ 100g to 35 g/ 100g.
23. The substitute-egg-based food product as claimed in claim 22 which is selected from baked products, deserts, coatings, batters, pancakes, tarts, confections, or panade.
24. A substitute-egg-based food product composition which comprises the egg replacement composition of any one of claims 10 to 17 together with one or more ingredients and in the form of a ready to bake or part-baked food product.
25. A substitute-egg-based food product composition which comprises the egg replacement composition of any one of claims 10 to 17 together with one or more ingredients and in the form of a kit for a food product.
26. A process of forming a substitute egg product, wherein an amylase enzyme is mixed with water to form a mixture and said mixture is homogenised with a fat, wherein said enzyme provides at least 3g / 100g protein of the product.
27. The process as claimed in claim 26 wherein said enzyme is combined with water and a carbohydrate to form the mixture.
28. The process as claimed in claim 27 wherein said carbohydrate is a seed mucilage.
29. The process as claimed in claim 28, wherein said seed mucilage is a flaxseed mucilage.

30. The process as claimed in any of claims 26 to 29 wherein said fat is a vegetable oil, sunflower or coconut fat.
31. The process as claimed in any one of claims 26 to 30 wherein one or more flavour modifiers, sweeteners, ash, salt or colourants are added.