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(54) Title: PUMPKIN SEED PROTEIN

(57) Abstract: The invention provides a food product comprising or prepared with a pumpkin seed protein isolate, said isolate comprising at least 60 wt.% of pumpkin seed protein, in which food product the pumpkin seed protein is present as an emulsifier and/or as a gelling agent. The invention furthermore provides methods to obtain such food products, as well as use of pumpkin seed protein as an emulsifier and/or gelling agent.

Title: pumpkin seed protein

BACKGROUND

A major trend in the food industry nowadays is the development of clean-label ("natural"), plant-based food products. This development requires clean label, plant-based substitute products for a range of functional additives for food products which traditionally were animal-derived and/or non-natural.

For this purpose, many common food ingredients may be considered to require substitution. Examples are gelatin as an animal-derived gelling agent, egg as an animal derived emulsifier and/or gelling agent, and OSA-modified starch as a "non-natural" emulsifier.

Potato protein, in particular patatin, has been identified as a key plant-based, clean label functional ingredient for food purposes. Potato protein provides for good emulsification of lipids in water, as well as for gelation (PCT/NL2007/050513). However, potato protein, in particular patatin, displays lipolytic activity, which activity, though advantageous in some applications (e.g. cheese flavor formation; PCT/NL2013/050488), may cause formation of off-flavors, in particular when patatin is used in combination with particular types of lipid (PCT/NL2021/050481). In addition, potato protein may display oxidative activity, which may be a further source of off-flavor formation in many food products, in particular food products comprising lipids.

Although various ways to circumvent this problem have been identified (European application 21160302.2; European application 21206223.6), a further solution to this problem would expand the options for the food industry.

Alternative protein types providing the same functionality as patatin have proven difficult to identify. Emulsification and (heat) gelling are properties which are rare in plant-based protein, while lipolytic and

oxidative activity is widespread. It is well-known at least for protein derived from oil seed, that seeds are roasted prior to oil extraction in order to avoid free fatty acid release, implying lipolytic activity of (native, non-roasted) seed oil protein. The presence of lipolytic protein ("lipase") in various seed types has been confirmed by among others Avramiuc (Food & Environment Safety **2016**, Vol. 15 Issue 1, pages 21-28) and Amid (Molecules **2015**, *20*, pages 11184-11201).

In addition, off-flavor formation may also be caused by lipid oxidation. (Native, unroasted) protein from oil seed is also known to comprise lipoxygenase.

Commercial seed oil proteins, among which pumpkin seed protein, are generally isolated after roasting, and as such commercial seed oil proteins, among which pumpkin seed protein, are generally not enzymatically active. Absent activity, such protein sources cannot provide for emulsification or gelation, nor provoke off-taste.

There thus remains a need for a plant-based protein which can be used as an emulsifier and/or a gelling agent in the presence of plant-based lipids, without causing the formation of off-flavors. It has been found that pumpkin seed protein allows for such use.

FIGURES

Figure 1: gelation properties of pumpkin seed protein

Figure 2: gel strength of pumpkin seed protein after thermal gelation.

Figure 3: meat substitutes prepared using pumpkin seed protein and/or patatin as an emulsifier and a gelling agent.

Figure 4: texture of meat substitutes using pumpkin seed protein and/or patatin as an emulsifier and a gelling agent. 2% S200 = B1; 5% S200 = B2; 10 % pumpkin seed protein = B3; 15 % pumpkin seed protein = B4; 8 % pumpkin seed protein + 2 % S200 = B5.

Figure 5: vegan scrambled eggs using pumpkin seed protein and/or patatin as an emulsifier and gelling agent.

Figure 6 (from left to right): cake with 0 %, 0.5 %, 1.0 % and 1.5 % pumpkin seed protein.

DETAILED DESCRIPTION

The invention provides a food product comprising a pumpkin seed protein isolate, said isolate comprising at least 60 wt.% of pumpkin seed protein, in which food product the pumpkin seed protein is present as an emulsifier and/or as a gelling agent.

Pumpkin seed protein, in the present context, is preferably active pumpkin seed protein. Active pumpkin seed protein is functionally active protein, so that active pumpkin seed protein is preferably not a coagulated protein, and not a hydrolyzed protein. Pumpkin seed protein is further described below.

It has been found that pumpkin seed protein is capable of providing emulsification and/or gelation similar to patatin. Contrary to patatin however, presence of pumpkin seed protein does not cause off-flavors. Pumpkin seed protein does neither cause hydrolytic off-flavors when used in combination with a lipid, nor cause oxidative degradation of either lipids or non-lipid materials.

Pumpkin seed protein can generally be present in a food product in a quantity of 1 – 50 wt.%, preferably 2 – 30 wt.%, more preferably 3 – 27.5 wt.%, more preferably 4 – 25 wt.%, even more preferably 6 – 22 wt.%.

In some embodiments, the food product comprises pumpkin seed protein as defined elsewhere as a gelling agent and/or as an emulsifier. In preferred embodiments, the food product comprises pumpkin seed protein as defined elsewhere as a gelling agent. In further preferred embodiments, the food product comprises pumpkin seed protein as defined elsewhere as an

emulsifier. In such embodiments, the food products further comprises a lipid as defined elsewhere and water.

Pumpkin seed protein being present as an emulsifier and/or as a gelling agent is understood to mean that the pumpkin seed protein provides the functionality of gelling and/or emulsification. Pumpkin seed protein thus acts as a gelling agent and/or as an emulsifier, in the form of a functional ingredient.

Pumpkin seed protein can be present in the food product as an emulsifier. Emulsifiers are generally known in the art. An emulsifier is defined as a substance which stabilizes an emulsion: a biphasic system comprising a lipid and water.

The biphasic system is preferably a system in which droplets of 0.2 – 250 μm, preferably 0.2 – 150 μm of a dispersed phase (for example a lipid phase) are present in a continuous phase (for example an aqueous phase), preferably homogeneously distributed. Stabilizing the biphasic system (the emulsion), in this regard, means that the biphasic system retains its appearance for at least one day under standard room conditions. A stabilized biphasic system does not collapse, shift, breakdown or otherwise phase-separate.

An emulsifier comprises polar and apolar molecular substructures, which renders the molecule preferentially present on a lipid-water interface. This presence on a lipid-water interface allows to stabilize lipid droplets in an aqueous phase (lipid-in-water emulsion), or to stabilize water droplets in a lipid phase (water-in-lipid emulsion). In an emulsion, the dispersed phase is generally present in the continuous phase in droplets of about 0.2 – 250 μm, preferably 0.2 – 150 μm. The droplet size of an emulsion can be determined by laser light scattering, as is generally known in the art.

In food products where pumpkin seed protein is present as an emulsifier, the pumpkin seed protein is present on a lipid-water interface.

Protein used as an emulsifier preferably comprises active protein, meaning that it is at least to some extent soluble in water and at least to some extent surface active such that it is capable of stabilizing the interface between water and oil. Active protein encompasses native, undenatured protein.

In a food product where pumpkin seed protein is present as an emulsifier, the pumpkin seed protein is present in a quantity of 1 – 50 wt.%, preferably 2 – 20 wt.%, more preferably 4 – 15 wt.%, even more preferably 5 – 10 wt.%, based on the weight of the food product.

Alternatively or additionally, pumpkin seed protein can be present in the food product as a gelling agent. Gelling agents are generally known in the art. In the present context, presence of pumpkin seed protein as a gelling agent in a food product means that the pumpkin seed protein is either present as a (thermally induced) gel in the food product, or is present in a quantity which allows for gelling after an appropriate heat treatment step.

It has been found that pumpkin seed protein displays heat gelling behavior similar to native potato protein. Heat gelling refers to the property that upon heating the protein in the presence of water (e.g. to 70 °C - 95 °C for at least 1 minute, preferably at least 5 minutes), the protein denatures and forms a gel, which gel further solidifies upon cooling. This property can be used in food products in order to increase viscosity of a food product or to provide for a solid food product, capable of retaining its shape.

In food products where pumpkin seed protein is present as a gelling agent, the food product may comprise pumpkin seed protein in the form of a proteinaceous gel network. Alternatively, the pumpkin seed protein can be present as a quantity of active pumpkin seed protein sufficient to allow for gelling after an appropriate heat treatment, such as heating to 70 °C - 95 °C for at least 1 minute, preferably at least 5 minutes.

An appropriate quantity of active pumpkin seed protein in a food product for use as a gelling agent is 5 – 25 wt.%, preferably 6 – 22 wt.%, more preferably 8 – 20 wt.%, based on the weight of the food product.

The advantage of not causing off-flavor when using pumpkin seed protein as a gelling agent materializes in the period up until gelation, as the protein after gelation is no longer active.

The invention thus also provides use of pumpkin seed protein as a gelling agent and/or as an emulsifier in a food product. Pumpkin seed protein may preferably be used as a clean label proteinaceous emulsifier or as a clean label proteinaceous gelling agent. Pumpkin seed protein has the unexpected benefit relative to other proteinaceous emulsifiers and/or gelling agents of not causing off-flavors. Off-flavors in the present context are defined as a lingering bitter sensation upon ingestion, which is accompanied by a stingy smell that can be described as “paint” or “vomit”.

Off-flavor can preferably be determined by sensory evaluation.

Off flavor can also be determined by measuring the release of free fatty acids as described below and/or by measuring the para-anisidine value (pAV; AOCS, 2004, Official method Cd. 18-90 in: Official methods and recommended practices of the American Oil Chemists Society).

In such cases, off-flavor can be defined as not present provided that the pAV of the lipid is maintained at 2 or less, preferably 1.5 or less, even more preferably 1 or less, and/or provided that the release of free fatty acids from the lipid is less than 40 mmol/kg oil, preferably less than 25, more preferably less than 15 mmol/kg oil.

Pumpkin seed protein has the further unexpected benefit that it has high stability against swift temperature changes, irrespective whether it concerns use as a gelling agent or as an emulsifier. This means that food products in which pumpkin seed protein is present as an emulsifier and/or as a gelling agent have high stability towards temperature changes, such as

cooling or heating. When present as a gelling agent, pumpkin seed protein provides high stability against fast temperature changes, and when used as an emulsifier, food products with pumpkin seed protein allow for quick freezing and cooling without breaking the emulsion.

The pumpkin seed protein

Pumpkin seed protein, in the present context, is protein derived from pumpkin seed. Pumpkin seeds are widely available as pumpkin seed is an important source of pumpkin seed oil. Protein derived from pumpkin seed is also known. The favorable properties of pumpkin seed protein when used as a gelling agent or emulsifier in a food product have not been reported.

Pumpkin in this regard refers to a plant family which provides pumpkin fruit. The word "pumpkin" in the present context refers to the fruit of plants in the family of Cucurbitaceae which are referred to as pumpkin, but which may also be called squash or calabash.

Preferably, pumpkin is a fruit which stems from the plant genera of Cucurbita, Lagenaria or Telfairia. Among Cucurbita, the species Cucurbita argyrosperma, Cucurbita ficifolia, Cucurbita maxima, Cucurbita moschata, and Cucurbita pepo are preferred, preferably Cucurbita pepo. Other preferred species of pumpkin are Lagenaria siceraria (Calebash) and Telfairia occidentalis (fluted pumpkin).

Pumpkin seed protein is protein which has been derived from the seeds of the pumpkin, that is, from the seeds present in the pumpkin fruit. Pumpkin seeds are generally known as a source of seed oil, in particular pumpkin seed oil. However, pumpkin seeds can also be a good source of protein. In order to obtain protein from pumpkin seeds, pumpkin seeds can be used as such, ground and subjected to protein extraction, as is known in the art. Alternatively, the remainder of pumpkin seed after oil extraction

may be used to obtain pumpkin seed protein, by methods well known in the art.

It will be appreciated that pumpkin seed protein in the present context is preferably active protein. Active, in this regard, means that the protein is preferably enzymatically active at least to some extent, and/or is preferably soluble in water at least to some extent; active protein is not denatured or coagulated, nor hydrolyzed. Active protein encompasses native protein, which is protein which exists in its natural three-dimensional structure, without significant denaturation or degradation.

Active protein is the protein as can be obtained from (potentially defatted) pumpkin seed, or (potentially defatted) pumpkin seed flour, without it being roasted or otherwise heat-treated prior to protein extraction.

Protein is considered "active" when in a water solubility test, at least 20 wt.%, preferably at least 25 wt.%, more preferably at least 30 wt.%, more preferably at least 35 wt.%, more preferably at least 40 wt.%, more preferably at least 45 wt.%, more preferably at least 50 wt.%, more preferably at least 55 wt.%, more preferably at least 60 wt.%, more preferably at least 65 wt.%, more preferably at least 70 wt.%, more preferably at least 75 wt.%, more preferably at least 80 wt.%, more preferably at least 85 wt.%, more preferably at least 90 wt.%, of all protein can be dissolved in water. A water solubility test can be executed by suspending a material, such as a pumpkin seed protein isolate, in a large excess of water buffered at pH 8.5 under thorough mixing, and determining the proportion of protein from the material which dissolves in the water. Protein content can be determined by Kjeldahl analysis, using a correction factor of 6.25.

Active pumpkin seed protein is protein which is isolated from pumpkin seeds without these seeds having been subjected to heat treatment (wherein heat treatment is defined as heating to a temperature of at least

35 °C, preferably at least 41 °C, more preferably at least 46 °C, even more preferably at least 51 °C, for a period of at least 10 minutes), and without other processing steps which generally cause protein denaturation. Thus, pumpkin seed protein in the present context can be provided to the food product of the invention in the form of a pumpkin seed protein isolate comprising active pumpkin seed protein.

The pumpkin seed protein isolate preferably comprises at least 60 wt.%, preferably at least 70 wt.%, more preferably at least 75 wt.%, more preferably at least 80 wt.%, more preferably at least 85 wt.%, even more preferably at least 90 wt.% pumpkin seed protein, which pumpkin seed protein is preferably active pumpkin seed protein. The protein content of a pumpkin seed protein isolate is expressed as Kjeldahl nitrogen times 6.25, expressed relative to the dry weight of the isolate.

The pumpkin seed protein contained in the pumpkin seed protein isolate (and thus also in the food product) may comprise any pumpkin seed protein fraction alone or in combination. Pumpkin seed protein contained in the pumpkin seed protein isolate may comprise albumin, globulin, prolamin and/or glutelin fractions. Preferably, the pumpkin seed protein contained in the pumpkin seed protein isolate comprises the majority of all protein present in the pumpkin seed prior to protein isolation, such as at least 60 wt.%, preferably at least 70 wt.%, more preferably at least 80 wt.%, even more preferably at least 90 wt.% of all protein contained in the pumpkin seed prior to protein isolation.

The quantity of pumpkin seed protein which may be used in the food product depends on the type of food product in question; the skilled person can vary the quantity of pumpkin seed protein based on the intended purpose.

Generally, pumpkin seed protein can be present in the food product in a quantity of 1 – 50 wt.%, preferably 2 – 30 wt.%, more preferably 4 – 25 wt.%, even more preferably 6 – 22 wt.%.

If the function of pumpkin seed protein in the food product is primarily emulsification, then the quantity which can be added is in the range of 1 – 50 wt.%, preferably 2 – 20 wt.%, more preferably 4 – 15 wt.%, even more preferably 5 – 10 wt.%.

If the function of the pumpkin seed protein is primarily gelation, then the quantity which can be added is in the range of 5 – 25 wt.%, preferably 6 – 22 wt.%, more preferably 8 – 20 wt.%. The skilled person appreciates that in many food products, pumpkin seed protein may induce both gelation and emulsification, and that quantities may be optimized based on routine experiments for any particular type of food product.

Food products comprising pumpkin seed protein as a gelling agent

In some embodiments, the food product comprises pumpkin seed protein as defined elsewhere as a gelling agent. In such embodiments, the food product further comprises water, and may or may not comprise a lipid, as further defined below. Preferred food products in this context include meat substitutes, egg substitutes and bakery products (preferably egg-free bakery products) such as cake and muffins.

It is noted that some food products in which pumpkin seed protein is present as a gelling agent can be lipid-free.

Meat substitutes, egg substitutes and bakery products in which pumpkin seed protein is present as a gelling agent normally further comprise water and a lipid.

It is an advantage of any food product comprising pumpkin seed protein present as a gelling agent that a gel obtained using pumpkin seed protein is particularly stable to temperature changes. This is an advantage for food products in which the gelled state is subjected to large temperature changes. Such food products include preferably bakery products (cakes, muffins and the like), but also meat substitutes and egg substitutes. For

example, a cake obtained using pumpkin seed protein as a gelling agent is particularly stable against cake collapse during cooling.

It is a further advantage of food products comprising pumpkin seed protein that using pumpkin seed protein as a gelling agent instead of other proteinaceous gelling agents such as patatin, off-flavor formation is suppressed. This is true in lipid-free food products due to the lower oxidative action of pumpkin seed protein on non-lipid materials. This is even more so in food products further comprising a lipid and water, due to the lower oxidative and hydrolytic action of pumpkin seed protein on lipids, as compared to other proteinaceous gelling agents such as patatin, as further detailed elsewhere.

Food products comprising pumpkin seed protein as an emulsifier

In some embodiments, the food product comprises pumpkin seed protein as defined elsewhere as an emulsifier. In such embodiments, the food product further comprises a lipid and water.

A lipid, in the present context, is a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol, preferably at least 94 wt.%, more preferably at least 96 wt.%. The wt.% fatty acid tri-esters of glycerol is expressed relative to the total weight of the substance.

In preferred embodiments, the lipid is a plant-based lipid, preferably a plant oil, fruit oil or seed oil.

The lipid may be referred to as a fat, an oil or a butter. A fat is solid at ambient temperature (20 °C) and pressure, solid defined as being capable of holding shape for at least one hour. An oil is liquid or viscous at ambient temperature and pressure, liquid or viscous being defined as displaying flow under the influence of gravity. A butter is highly viscous at ambient temperature and pressure; the term “butter” may be considered to overlap the lower viscosity range of fats, and the higher viscosity range of oils.

The words “fat”, “oil” and “butter” can be used interchangeably, in the present context. For example, coconut fat can also be referred to as coconut oil, and sunflower oil can also be referred to as sunflower fat. Regardless of the colloquial distinction between fat, oil and butter, in the present context, fat, oil and butter are all encompassed by the term “lipid”.

The plant-based lipid is preferably coconut fat, palm oil, corn oil, soybean oil, rapeseed oil, sunflower oil, grape seed oil, pumpkin seed oil, peanut oil, sesame oil, olive oil, shea butter, cocoa butter, and rice bran oil. The plant-based lipid may optionally have been partially or fully hydrogenated. Preferred types of lipid are lipids well-known in the food industry, such as sunflower oil, olive oil, coconut fat or palm oil.

The off-flavor reduction in food products of the present invention comprising lipid and water relative to food products comprising lipid and water in which another proteinaceous emulsifier and/or gelling agent such as patatin is present occurs for any type of lipid, as pumpkin seed protein has been found to be considerably less lipolytic, and less oxidative toward both lipids and non-lipids.

Off-flavor reduction relative to patatin-based (lipid-containing) food products is particularly strong in food products where the lipid is prone to off-flavor development by patatin. Such lipids include in particular coconut fat and palm oil, but also include, to a lesser extent, for example corn oil and soybean oil.

The fatty acids present in the lipid are not particularly limited. It is an advantage of pumpkin seed protein that it displays little or no lipolytic or oxidative activity toward lipids, thereby allowing for the presence of many types of lipid while providing for emulsification and gelation similar to patatin. Fatty acids may thus be C2 – C26 fatty acids, preferably C4 – C22 fatty acids. Fatty acids may be saturated or unsaturated. In plant-based lipids, the fatty acids are generally a mixture of fatty acids with varying chain length, and with varying degrees of unsaturation. The fatty

acid composition of a particular plant-based lipid is fairly characteristic for the origin of the lipid, as is generally known.

The lipid to be used in the food product is preferably as pure as possible. That is, the quantity of free fatty acids ("FFA") in the lipid is preferably less than 18 mmol per kg lipid, more preferably less than 9 mmol per kg lipid, even more preferably less than 3 mmol per kg lipid. The quantity of free fatty acids in the lipid can be determined by a chemical titration method, as described below. The quantity of free fatty acids can also be determined by HPLC, as is generally known in the art.

Additionally or alternatively, the total quantity of diacylglycerols ("DAG") and monoacylglycerols ("MAG") in the lipid to be used in the food product is preferably less than 10 wt.%, more preferably less than 6 wt.%, even more preferably less than 4 wt.%, relative to the total lipid. The quantity of DAG and MAG in the lipid can be determined by column chromatography or capillary gas chromatography as described in "Standard Methods for the Analysis of Oils, Fats and Derivatives", 1st supplement to the 7th edition (IUPAC, 1987).

The lipid may be present in the food product in any quantity which is conventional for the type of food product in question. Preferably, the food product comprises, as wt.% of the food product, 2 – 50 wt.% lipid, more preferably 4 – 30 wt.%, even more preferably 6 – 20 wt.%.

Food products of the invention

A food product of the invention comprises pumpkin seed protein as defined elsewhere as a gelling agent and/or as an emulsifier. Said food product furthermore may preferably comprise water and/or a lipid as detailed elsewhere. Water in this context is water suitable for human consumption. Mains water is preferred. Water may be present in a quantity of 5 – 95 wt.%, preferably 10 – 90 wt.%, more preferably 15 – 85 wt.%. The

quantity of water varies with the type of food product being prepared, as is well known in the art.

Throughout the present disclosure, the food product "comprising" pumpkin seed protein can be interpreted as said food product being "based on" pumpkin seed protein. Alternatively, the term "comprising" may be replaced by the term "prepared from". Quantities of ingredients by which a food product is defined refer to the quantities as used for preparing the food product, expressed as wt.% of the total of ingredients used.

In an aqueous mixture comprising lipid and another proteinaceous gelling agent such as patatin, enzymatic action of the protein on the lipid can be a cause for the formation of off-flavors. This occurs in particular when lipid, protein and water are in intimate contact, such as in a homogenous mixture, or an emulsion. However, also when not mixed, diffusion may cause formation of off-flavors, as long as the protein is active. Such off-flavor formation can be avoided by using pumpkin seed protein as a gelling agent or emulsifier in food products.

Food products of the invention can be made by methods generally known for the making of the type of food product in question. Reference is made to common general knowledge on the making of any individual food product which may benefit from the presence of pumpkin seed protein as a proteinaceous emulsifier and/or gelling agent.

In preferred embodiments, the food product is vegan food product, defined as a food product not comprising animal-derived ingredients. Alternatively, the food product may be a vegetarian food product, defined as a food product not comprising animal-derived meat. Further alternatively, the food product may be a food product comprising meat or fish, such as a burger or sausage which is bound by pumpkin seed protein. Also, the food product can be an extended meat product, which is a food product comprising animal-derived meat, but in which part of the animal-derived meat has been substituted for a plant-based protein.

Food products which particularly may benefit from addition of pumpkin seed protein are food products in which the protein is active for at least some time prior to deactivation, and where said protein is used as a gelling agent or emulsifier. Deactivation in this case requires heating, so as to denature the protein which may also induce gelling.

Such food products can be food products which are heated prior to consumption, but which prior to said heating have not been heated so as to inactivate the protein, and have been kept cold (-35 °C - +20 °C), such as during storage. Cold storage in such cases may be preferably for at least 2 days, or at least 2 weeks, or at least 2 months, depending on temperature and the further ingredients of the food product.

Thus, a particularly preferred type of food product in the present context is a "cold storage" type food product, defined as a food product which is kept in cold storage for a period of at least 1 day, preferably at least 2 days, and which food product during said cold storage comprises active pumpkin seed protein. Examples are a meat product, vegetarian or vegan meat substitute, cheese, yoghurt, cream, sauce (such as mayonnaise or dressing), batter, or dough.

Alternative preferred food products are food products which are heated while comprising active pumpkin seed protein. Enzymatic activity is generally a temperature-dependent process, and an increase in temperature may increase the enzymatic activity responsible for the formation of off-flavors, prior to deactivation of the protein. Such food products ("heat shock" type food products) include for example bakery products (such as cake and muffin), vegetarian or vegan meat substitutes, or cheese products intended for heating (for example, a vegetarian or vegan cheese sauce or fondue, a vegetarian or vegan croque monsieur, or a cream cheese filling).

The two groups of preferred food product ("heat shock" type and "cold storage" type) are not mutually exclusive. In many food products, both the "cold storage" mechanism and the "heat shock" mechanism can be

responsible for off-flavor formation. Many cold storage type food products are heated prior to consumption: a dough or a batter, which may be cold-stored, is baked prior to obtaining a bakery product; a raw type meat substitute such as a burger can be distributed cold only to be fried at home. Such food products particularly benefit from pumpkin seed protein as a functional, clean label, emulsifier or gelling agent.

In preferred embodiments, the food product is a meat product, meat substitute, vegan egg product, vegan fish product, vegan sea food product, batter, dough, bakery product, butter, cheese, cream cheese, yoghurt, sauce, dressing, fondue or cream, preferably a meat substitute, a vegan egg product, a sauce or a bakery product, more preferably a meat substitute, a vegan egg product, or a bakery product.

A meat substitute, in this context, encompasses extended meat products as well as vegetarian or vegan meat substitutes. Preferably, the meat substitute is a vegetarian or vegan meat substitute, most preferably a vegan meat substitute. Preferred types of meat substitutes are (an extended, vegetarian or vegan version of) a burger, meatball, sausage, minced meat, schnitzel, skewer, nugget, rib, filet, fish ball or meat chunk.

A vegan egg product is a food product which has the appearance of egg, but which is fully plant based. A preferred type of vegan egg product is vegan scrambled eggs, or vegan egg powder.

A bakery product, in this context, is a food product prepared from dough or batter, said dough or batter comprising water, a lipid, and pumpkin seed protein, which dough or batter is subsequently baked, for example at a temperature of at least 100 °C, preferably at least 125 °C, more preferably at least 150 °C, even more preferably at least 175 °C, such as 100 – 400 °C, preferably 125 – 350 °C, more preferably 150 – 300 °C, most preferably 175 – 250 °C, for a period of at least 15 minutes, preferably at least 30 minutes, more preferably at least 45 minutes, such as 15 – 3 hrs, preferably 30 min – 2 hrs.

Preferred types of bakery products include cake (among which cupcake), muffin, cheese cake, and the like.

In some embodiments, the food product may comprise, in addition to the pumpkin seed protein, a native potato protein, preferably patatin. As the functionality of pumpkin seed protein in food products has been found to largely overlap the functionality of patatin while allowing for decreased off-flavor formation, food products comprising potato protein, in particular patatin, as well as pumpkin seed protein, benefit from the possibility of further tailoring the nutritional profile by varying the relative quantities of potato protein and pumpkin seed protein, while still displaying decreased off-flavor formation due to substitution of patatin by pumpkin seed protein.

If pumpkin seed protein is used as well as native potato protein, for example patatin, then the weight ratio between pumpkin seed protein and patatin is preferably 10 : 1 to 1 : 1 (pumpkin seed protein : native potato protein). In such embodiments, native potato protein may be present in the food product in quantities ranging from 1 – 10 wt.%, preferably 2 – 8 wt.%, more preferably 3 – 5 wt.%.

In addition to the ingredients mentioned so far, the food product may comprise further ingredients, appropriate for the type of food product in question. For example, the food product may comprise flavors, colorants and further ingredients, such as for example listed below.

Preferably, the food product may comprise one or more salts, such as a salt selected from the group consisting of sodium, potassium or calcium chloride, sodium or potassium glutamate and calcium sulfate.

Alternatively or additionally, the food product may comprise one or more pigments, such as a pigment selected from the group consisting of heme-like pigment, red beet pigment, carotene, caramel, beet juice extract, tomato pigment, radish pigment, paprika pigment and amaranth.

Alternatively or additionally, the food product may comprise one or more fibers. Fiber may be provided in the form of a fiber isolate derived

from plants, preferably comprising at least 50 wt.%, preferably at least 60 wt.%, of plant fiber. Plant fiber, in this regard, refers to a material which is commercially available, generally in the form of a free flowing powder. Many types of plant fiber are generally known in the art. Examples include soy fiber, pea fiber, potato fiber, and grain fiber, among which wheat fiber, barley fiber or oat fiber. Plant fibers, as referred to herein, generally include various types of indigestible carbohydrates among which pectins, hemi-cellulose, and/or gums, but furthermore comprises non-soluble cell wall components remaining after acid and alkaline hydrolysis.

Suitable plant fibers may be selected from the group consisting of potato fiber, sweet potato fiber, carrot fiber, psyllium fiber, bamboo fiber, soy fiber, pea fiber, mungbean fiber, tapioca fiber, coconut fiber, banana fiber, grain fiber, among which wheat fiber, barley fiber or oat fiber, cellulose, resistant starch, resistant dextrins, inulin, lignin, chitin, pectin, beta-glucan, and oligosaccharide.

Alternatively or additionally, the food product may comprise one or more texturisers such as a texturizer selected from the group consisting of native starch, modified starch, cellulose derivatives, carrageenan, alginate, agar, konjac, xanthan, and pectin. In further preferred embodiments, the food product does not comprise a hydrocolloid or a modified starch.

Alternatively or additionally, the food product may comprise one or more flavor development aids selected from the group consisting of dextrose, ribose and maltodextrin, and/or one or more flavorings, such as a sweetener selected from the group consisting of sucrose, glucose, fructose, syrup, and artificial sweeteners.

In much preferred embodiments, the food product comprises 0.3 – 3 wt.%, preferably 0.5 – 2 wt.% of salt, preferably sodium chloride or potassium chloride, or a mixture of sodium and potassium chloride. This

provides for an increased gel strength when the quantity of pumpkin seed protein in the food product is lower than 23 wt.%.

A meat substitute of the invention preferably comprises, as wt.% of the mixture,

- 45 – 75 wt.%, preferably 50 – 70 wt.% of water; and
- 3 – 15 wt.%, preferably 5 – 15 wt.% of lipid, said lipid preferably being as defined elsewhere, more preferably olive oil, sunflower oil, palm oil or coconut fat, most preferably sunflower oil; and
- 3 – 25 wt.% pumpkin seed protein, preferably 5 – 23, more preferably 7 – 20 wt.%.

In preferred embodiments, said meat substitute further comprises, as wt.% of the mixture,

- 12 – 30 wt.% texturized vegetable protein, preferably 15 – 27 wt.%, said texturized vegetable protein preferably comprising texturized pea protein, texturized soy protein, and/or texturized gluten, most preferably a 3 : 1 to 10 : 1 weight ratio of texturized pea or soy protein to texturized gluten; and/or
- 0.5 – 5 wt.%, preferably 1 – 2.5 wt.% of plant fiber, defined as a fiber isolate derived from plants, such as potato fiber, sweet potato fiber, carrot fiber, psyllium fiber, bamboo fiber, soybean fiber, pea fiber, mungbean fiber, tapioca fiber, coconut fiber, banana fiber, citrus fiber, cellulose, resistant starch, resistant dextrins, inulin, lignin, chitin, pectin, beta-glucan, and oligosaccharide; and/or
- 0.3 – 3 wt.%, preferably 0.5 – 2 wt.% of sodium or potassium chloride, preferably sodium chloride; and/or

- 1 – 10 wt.%, preferably 2 – 8 wt.% of a native potato protein isolate, preferably a native patatin isolate; and/or
- Optionally one or more further flavorants, colorants or other ingredients as defined elsewhere, in a quantity of at most 5 wt.% per ingredient, preferably at most 2 wt.% per ingredient.

In much preferred embodiments, the meat substitute at least also comprises 0.3 – 3 wt.%, preferably 0.5 – 2 wt.% of salt, preferably sodium chloride or potassium chloride, or a mixture of sodium and potassium chloride. This provides for an increased gel strength when the quantity of pumpkin seed protein in the meat substitute is 3 – 23 wt.%.

Texturized vegetable protein for preparing a meat substitute is well known in the art. A coagulated protein may be used, or a texturized protein, or a coagulated and texturized protein. Well known examples for use in meat substitutes include texturized pea protein and texturized soy protein.

In other embodiments, the food product can be a vegan egg product, preferably vegan scrambled eggs. In this case, the food product comprises, as wt.% of the mixture,

- 55 – 85 wt.%, preferably 60 – 75 wt.% of water; and
- 3 – 15 wt.%, preferably 5 – 15 wt.% of lipid, said lipid preferably being as defined elsewhere, more preferably olive oil, sunflower oil, palm oil or coconut fat, most preferably sunflower oil; and
- 5 – 30 wt.% pumpkin seed protein, preferably 8 – 23 wt.%.

In preferred embodiments, said vegan egg product further comprises, as wt.% of the mixture,

- 1 – 10 wt.%, preferably 2 – 8 wt.%, of starch, preferably corn starch, potato starch, wheat starch or rice starch; said starch most preferably being a granular starch or a pregelatinized starch; and/or
- 0.2 – 5 wt.%, preferably 0.5 – 2 wt.% of sodium or potassium chloride, preferably sodium chloride; and/or
- 1 – 10 wt.%, preferably 2 – 8 wt.% of a native potato protein isolate, preferably a native patatin isolate; and/or
- Optionally one or more further flavorants, colorants or other ingredients as defined elsewhere, in a quantity of at most 5 wt.% per ingredient, preferably at most 2 wt.% per ingredient. Egg flavor is preferred.

In much preferred embodiments, the vegan egg product at least also comprises 0.2 – 5 wt.%, preferably 0.5 – 2 wt.% of salt, preferably sodium chloride or potassium chloride, or a mixture of sodium and potassium chloride. This provides for an increased gel strength when the quantity of pumpkin seed protein is 5 – 23 wt.%.

A vegan egg powder may be prepared from the above ingredients, without the water. A vegan egg powder thus may comprise

- 10- 80 wt.%, preferably 20 – 60 wt.%, even more preferably 35 – 50 wt.% of lipid, said lipid preferably being as defined elsewhere, more preferably olive oil, sunflower oil, palm oil or coconut fat, most preferably sunflower oil; and
- 20 – 90 wt.%, preferably 30 – 80 wt.%, even more preferably 45 – 75 wt.% pumpkin seed protein.

In preferred embodiments, said vegan egg powder further comprises, as wt.% of the mixture,

- 1 – 30 wt.%, preferably 4 – 20 wt.%, of starch, preferably corn starch, potato starch, wheat starch or rice starch; said

starch most preferably being a granular starch or a pregelatinized starch; and/or

- 1 – 10 wt.%, preferably 0.5 – 5 wt.% of sodium or potassium chloride, preferably sodium chloride; and/or
- 1 – 15 wt.%, preferably 4 – 15 wt.% of a native potato protein isolate, preferably a native patatin isolate; and/or
- Optionally one or more further flavorants, colorants or other ingredients as defined elsewhere, in a quantity of at most 8 wt.% per ingredient, preferably at most 4 wt.% per ingredient. Egg flavor is preferred.

In much preferred embodiments, the vegan egg powder at least also comprises 1 – 10 wt.%, preferably 0.5 – 5 wt.% of salt, preferably sodium chloride or potassium chloride, or a mixture of sodium and potassium chloride. This provides for an increased gel strength. Said vegan egg powder is suitable for use in recipes for preparing a food product as an egg substitute.

In other embodiments, the food product can be a bakery product, preferably a cake or a muffin, or a dough or batter for preparing a bakery product. In such embodiments, the bakery product is prepared by baking as defined elsewhere a dough or batter comprising

- 10 – 35 wt.%, preferably 15 – 30 wt.% of water; and
- 10 – 40 wt.%, preferably 15 – 30 wt.% of lipid, said lipid preferably being as defined elsewhere, more preferably a plant-based butter, fat or margarine; and
- 0.5 – 10 wt.% pumpkin seed protein, preferably 1 – 5 wt.%.

In preferred embodiments, said dough or batter for preparing the bakery product further comprises, as wt.% of the mixture,

- 10 – 45 wt.%, preferably 20 – 35 wt.% of sugar; and/or
- 10 – 40 wt.%, preferably 15 – 30 wt.% of flour, preferably wheat flour, for example low gluten wheat flour; and/or
- 0.1 – 2.5 wt.% of salt, preferably sodium chloride or potassium chloride, in a quantity of 0.25 – 1.5 wt.%; and/or
- 0.5 – 10 wt.%, preferably 0.75 – 5 wt.% of a native potato protein isolate, preferably a native protease inhibitor isolate; and/or
- Optionally one or more further flavorants, colorants, texturisers and/or other ingredients as defined elsewhere, in a quantity of at most 5 wt.% per ingredient, preferably at most 2 wt.% per ingredient. Preferred optional ingredients include starch, preferably native starch, such as native potato starch, as well as one or more of a further emulsifier, an aroma, a texturizer or a aerating compound such as sodium or potassium bicarbonate.

In further preferred embodiments, said bakery product may further comprise separate ingredients, which can be applied to the bakery product after baking, or which may be baked together with the dough or batter. Preferably, all separate ingredients are vegetarian ingredients, most preferably vegan ingredients. The separate ingredients may include

- A filling, such as a jam, cheese, custard or pudding filling; and/or
- A coating, such as a sugar coating, a chocolate coating, or a fruit-based coating; and/or
- A cream, such as a whipped cream or a coffee cream.

In other embodiments, the food product can be a sauce, preferably a vegetarian or vegan sauce. A sauce in the present context is preferably a sauce in the form of an emulsion, preferably an oil-in-water emulsion. Sauces in the form of an emulsion are preferably mayonnaise, Hollandaise sauce, cocktail sauce, garlic sauce, or ravigotte sauce. A much preferred sauce of the invention is mayonnaise. A sauce may comprise, as wt.% of the sauce in question:

- 15 – 70 wt.% water, preferably (for regular, "full fat" variants) 15 – 30 wt.%, or (for "light" variants) 30 – 65, preferably 50 – 65 wt.% water; and
- 0.25 – 5 wt.% pumpkin seed protein, preferably 0.5 – 2.5 wt.%.
- 15 – 85 wt.% lipid, preferably (for regular, "full fat" variants) 50 – 85 wt.%, or (for "light" variants), 15 – 35 wt.%.

In preferred embodiments, the sauce may further comprise, as wt.% of the sauce in question,

- maltodextrin in quantity of 0.01 – 1 wt.%; and/or
- taste active agents, such as vinegar, lemon juice, mustard, sugar and/or salt, each in a quantity of 0.1 – 5 wt.%; and/or
- a stabilizer (e.g. potassium sorbate) in a quantity of 0.01 – 1 wt.%
- In "light" versions, part of the lipid in a full-fat version (50 – 75 wt.% of the lipid in the full fat version) may be replaced by e.g. a cold-water soluble starch and (further) water. Suitable starch in this context can be modified starch, such as a crosslinked potato starch, acetylated potato starch, or preferably a crosslinked acetylated potato starch. In much preferred embodiments, the starch is a waxy starch, defined as a starch having an amylopectin content of 90 – 100 wt.%

Methods of the invention

The invention further provides a method for preparing a food product from one or more ingredients, said preparing comprising one or more of the steps of shaping, mixing, cooling, heating, fermentation, and/or a period of storage of said ingredients, preferably cold storage at a temperature of less than 10 °C, wherein said preparing further comprises a step of combining a pumpkin seed protein isolate with at least one of the said ingredients to obtain a mixture, said isolate comprising at least 60 wt.% of pumpkin seed protein. In these food products, pumpkin seed protein is present as an emulsifier and/or as a gelling agent.

In some embodiments, the food product may or may not comprise a lipid. In such embodiments, the pumpkin seed protein is generally present as a gelling agent. Food products in which pumpkin seed protein is present as a gelling agent, are preferably subjected to a heat treatment of the mixture comprising the pumpkin seed protein as well as one or more further ingredients to 70 °C - 95 °C for at least 1 minute, preferably at least 5 minutes. This heat treatment, and preferably the subsequent cooling, provokes gelation of the pumpkin seed protein.

In further preferred embodiments, the mixture comprising one or more ingredients and the pumpkin seed protein isolate further comprises water and a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol. In such embodiments, the pumpkin seed protein can be present as a gelling agent and/or as an emulsifier in the food product. Preferred food products of this type include a batter, dough, bakery product, meat substitute, cheese, cream cheese, yoghurt, sauce, dressing, fondue or cream, preferably a batter, dough, bakery product, meat substitute or sauce, most preferably a batter, dough, bakery product or meat substitute, which may be obtained by a further step of emulsification of said mixture comprising water, pumpkin seed protein and the lipid.

Methods of the invention for obtaining food products can be in particular:

- the food product is a meat substitute, preferably a burger, meatball, sausage, minced meat, schnitzel, skewer, nugget, rib, filet or meat chunk, said method comprising
    - providing a mixture comprising water, said pumpkin seed protein isolate, and a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol; and
    - enriching said mixture with a denatured protein, preferably a texturized vegetable protein; and
    - shaping the meat substitute; and
    - optionally cooling the meat substitute to a temperature of from -35 °C to 20 °C for a period of at least one day; or
- the food product is a vegan egg product, said method comprising
    - providing a mixture comprising water, said pumpkin seed protein isolate, and a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol;
    - heating the mixture of water, pumpkin seed protein, and lipid to a temperature of at least 75 °C, preferably at least 85 °C; or
- the food product is a vegan egg powder, said method comprising
    - providing a mixture comprising said pumpkin seed protein isolate, and a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol; and
    - homogenizing said mixture to provide a powder; or
- the food product is a bakery product, preferably a cake or a muffin, said method comprising
    - providing a mixture comprising water, said pumpkin seed protein isolate, and a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol; and

- mixing and/or kneading the said mixture to obtain a batter or dough; and
- baking the batter or dough to obtain the said bakery product; or

- the food product is a sauce, preferably a mayonnaise, Hollandaise sauce, cocktail sauce, garlic sauce, or ravigotte sauce, said method comprising
  - providing a mixture comprising water, said pumpkin seed protein isolate, and further optional ingredients,
  - providing a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol; and
  - adding said lipid to the mixture while mixing so as to obtain a stable emulsion;
  - optionally mixing in further optional ingredients to the obtained emulsion.

For all methods for obtaining a food product listed above, the quantity of pumpkin seed protein present in the food product and the quantities of all other ingredients, including the types and quantities of further optional ingredients, are as described further above; the execution of method steps to obtain the said food products is in line with common general knowledge on these types of food products. Optional ingredients for preparing any food product may be added at the same time or consecutively, in a single batch or in portions, in line with the common general knowledge for the preparation of the said food product. In this context, reference is made to Plant Protein Foods, ISBN: 978-3-030-91205-5.

As regards meat substitutes, in preferred embodiments, the meat substitute is cooked prior to consumption. Cooking, in this regards, comprises heating the mixture to a core temperature of at least 75 °C, preferably at least 85 °C.

A meat substitute is preferably vegetarian or vegan, most preferably vegan. A meat substitute, in this context, can be a non-meat (vegetarian or vegan) analogue of a burger, meatball, sausage, minced meat, schnitzel, skewer, nugget, rib, filet or meat chunk.

The texturized vegetable protein is preferably hydrated prior to including it in the mixture comprising water, pumpkin seed protein, and the lipid. The obtained mixture is subsequently homogenized, such as by further mixing or extrusion. Also, flavors, colorants and further ingredients as defined elsewhere may be added to the mixture, in line with common general knowledge.

The meat substitute is subsequently shaped to a desired shape. The shape is not particularly limited, but is preferably customary for the type of meat substitute in question. For example, a burger may be "patty" shaped (a generally round, disc-like shape), and a sausage may be provided with an elongated cylindrical and optionally bent shape.

In some embodiments, the meat substitute can subsequently be subjected to a step of cooling, such as to a temperature of from -35 °C to 20 °C for a period of at least one day. This is particularly preferred for "raw-type" meat substitutes (meat substitutes which have an appearance similar to raw (uncooked) meat, and which can be heated at home by a consumer, and during heating provide a cooking experience similar to that of the baking of raw meat). For raw-type meat substitutes, the cooling preferably comprises a period of cooling to a temperature of 0 – 15 °C, for at least 1 day, in order to portray the meat substitute as "fresh" raw meat.

For both raw-type and ready-made meat substitutes (a ready-made meat substitute being a meat substitute which is sold to a consumer in the form in which it will be consumed, and/or which does not come with a cooking experience similar to that of raw meat. Cooking of a ready-made meat substitute does not significantly affect the appearance of the product, apart from some browning during cooking), the step of cooling may comprise

a step of cooling to a temperature of – 35 °C to 0 °C, preferably -25 to -5 °C, for a period of at least one day. This period, the "freezing period", can be applied to increase the life time of the product, so that it may be kept for at least one week, or at least one month, preferably at least six months.

Prior to consumption, the meat substitute is preferably cooked. Cooking in this regard refers to a step of heating of the meat substitute to a core temperature of at least 70 °C, preferably at least 75 °C, more preferably at least 85 °C. In preferred embodiments, the meat substitute is a raw-type meat substitute, and the step of cooking of the meat substitute provokes, among others, gelling of the pumpkin seed protein, giving the meat substitute a hard and solid shape similar to the form of a baked raw meat product. For ready-made meat substitutes, the cooking and concomitant gelation have already been performed in the factory, where after generally, the meat substitute is subjected to a step of cooling, as described, only to be followed by a step of reheating by the end-consumer.

As regards vegan egg products, the method preferably provides a vegan version of "scrambled eggs". In further preferred embodiments, a mixture comprising the pumpkin seed protein isolate and the lipid can be provided in the form of a powder by providing the mixture of lipid and pumpkin seed protein and any further optional ingredients defined above, and homogenizing said mixture to provide a powder. This powder may be used as an egg substitute in a recipe for obtaining a food product.

As regards bakery products, the method preferably comprises a step of mixing and/or kneading, to obtain the dough or batter. For batter, mixing is preferred, whereas for a dough, kneading is preferred. Mixing or kneading may be performed in line with common general knowledge, by hand or by applying known cooking equipment.

The method for preparing a bakery product further preferably comprises a step of baking the dough or batter. Baking preferably refers to a step of heating the dough or batter to a temperature of for example at least

100 °C, preferably at least 125 °C, more preferably at least 150 °C, even more preferably at least 175 °C, such as 100 – 400 °C, preferably 125 – 350 °C, more preferably 150 – 300 °C, most preferably 175 – 250 °C, for a period of at least 15 minutes, preferably at least 30 minutes, more preferably at least 45 minutes, such as 15 – 3 hrs, preferably 30 min – 2 hrs.

The method for preparing a sauce preferably comprises a step of careful addition of the lipid in order to obtain a stable emulsion. Stable, in this regard, means that the emulsion does not collapse, shift, breakdown or otherwise phase-separate. Techniques to obtain a stable emulsion from a mixture comprising water and lipid in the presence of an emulsifier are commonly known, and include slow addition of the lipid to the mixture comprising water and pumpkin seed protein. Slow addition, in this regard, my refer to a rate of at most 10 vol% per minute, preferably at most 5 vol.% per minute. The quantity vol.% per minute refers to the quantity of lipid, expressed as a volumetric percentage of the quantity of mixture to which the lipid is added, per minute. In further preferred embodiments, the mixture is subjected to a step of homogenization, during or after the addition of the lipid to the mixture.

It is an advantage of the present method that in food products comprising a proteinaceous emulsifier and/or gelling agent (e.g. patatin), the emulsifier and/or gelling agent can be substituted for pumpkin seed protein, thereby reducing the formation of off-flavors, and enhancing stability against temperature changes. Food products which particularly may benefit, and detailed descriptions of all parameters underlying the invention have been described above, and also apply to the methods of the invention.

In other embodiments, the invention provides use of a pumpkin seed protein isolate comprising at least 60 wt.% of pumpkin seed protein (as defined elsewhere) as a gelling agent and/or an emulsifier in a food product.

For use as an emulsifier, a mixture of water, lipid and pumpkin seed protein provided by the pumpkin seed protein isolate must be subjected

to a step of emulsification. The food product preferably comprises a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol, as defined elsewhere. In this embodiment, the mixture can be an emulsion comprising mainly liquid ingredients, such as in a sauce or a cheese. In some embodiments, an emulsion-based food product may be heated later, so as to provide additional use of the pumpkin seed protein as a gelling agent.

For use as a gelling agent, the mixture comprising the pumpkin sed protein can be a mixture comprising solid ingredients, such as for example ground meat or texturized vegetable protein, so as to prepare an extended, vegetarian or vegan meat product. The mixture can also be a mixture comprising liquid ingredients, such as in a batter for preparing a bakery product. In this embodiment, the step of providing a mixture may be a step of stirring, kneading, extruding or shaking, as is known in the art, prior to be heated to provoke gelation.

In embodiments where pumpkin seed protein is used as a gelling agent, the food product is heated at some point in order to provoke gelation. Heating, in this context, refers to heating the mixture to a temperature of at least 70 °C for at least 1 minute, preferably heating to a temperature of 70 – 110 °C, more preferably 80 – 100 °C, for a period of 1 – 45 minutes, preferably 5 – 30 minutes. Heating may also be to a core temperature of at least 70 °C, preferably at least 75 °C , more preferably at least 85 °C. The core temperature can be determined by inserting a thermometer into a food product while cooking or baking, as is generally known. Preferred food products are vegetarian or vegan meat substitutes, a vegan egg product or a bakery product.

The mixture comprising pumpkin seed protein is further treated in conventional ways to obtain the food product. Thus, said mixture may be subjected to a step of heating as defined above to provoke gelation. Furthermore, said mixture may be subjected to a step of fermentation,

ripening or baking (which may coincide with the step of heating to provoke gelation, but which may also be a an individual step, such as a step of baking a batter or dough to obtain a bakery product).

For the purpose of clarity and a concise description features are described herein as part of the same or separate embodiments, however, it will be appreciated that the scope of the invention may include embodiments having combinations of all or some of the features described.

EXAMPLES

Materials

Pumpkin seed protein

Pumpkin seed protein was obtained from commercial pumpkin seeds, obtained from a local supermarket. The seeds were soaked in potable water for 2 h at ambient temperature. After peeling off the skins, the seeds were subjected to milling in a blender. Skins were discarded. After milling the material was pressed through a cheesecloth (150 μm mesh) to obtain a soluble protein solution.

The solution was then subjected to fat extraction using pentane (5:1 (v/v) ratio, respectively) for 2 h at ambient temperature while mixing. After centrifugation of the mixture at 4,000 g, the organic phase (holding the fats) was removed by decantation. The aqueous phase was combined with the obtained pellet and homogenized.

The pH of the aqueous protein mixture was adjusted to pH 8.5 using 1 M NaOH. The proteins were homogenized using short mixing and freeze-dried to reach a moisture content less than 10%.

The isolated pumpkin seed protein had the following characteristics:

Protein content (active protein)	85 ± 5 wt.%, relative to dry matter
Insoluble fiber	15 ± 5 wt.%, relative to dry matter

Chemicals

The patatin used is commercially available (Solanic 200®, Avebe, abbreviated "S200"). Potato fiber was Paselli FP from Avebe.

Lipids used were 100 % pure coconut oil (KTC), sunflower oil (Reddy); and soybean oil (Levo).

Texturized vegetable protein in the experiments was texturized soy protein (Soy TVP) "Tradcon T" from Serbia and/or gluten TVP "Unitex S2030" from Vitablend Nederland.

Egg flavour was obtained from TRS, and corn starch was native, granular corn starch obtained from Cargill.

Water was mains water, and sodium chloride ("salt") was general table salt obtained from a local supermarket (Jozo).

Methodology

Equipment for emulsification

When the experiments denote "emulsification", a T18 Ultraturrax with T18N (10 or 19 g) disperging tool or a T25 Ultraturrax with T25N (8g) disperging tool from IKA were used. Results with the two types of equipment are identical. In addition, an Analog vortex mixer from VWR was used, and a Multifuge 1S-R or X3R benchtop centrifuge from Thermo Scientific. For weighing, a BP3100 S balance from Satorius was used.

Incubation of protein with lipids and extraction of the lipid

A protein solution was prepared of 3.3 % in demineralized water. Solid lipids were melted at 50 or 60 °C. The lipid was added in a 1:1 (w/w) ratio to the protein solution or to demineralized water, which served as a control. The solutions were mixed by turrax for 1 minute at about 10.000 rpm. Then, the solutions were left at room temperature overnight under gently shaking so that release of fatty acids could occur.

Subsequently hexane was added in a quantity of about 5 ml per 2-gram solution, and the sample was vortexed several times in a time frame of 30 minutes to extract the lipids from the aqueous phase. Subsequently, the layers were separated by centrifugation (5 minutes, 4700 rpm, swing-out). The hexane layer (top layer) was used for determination of free fatty acids. The protocol above was followed unless indicated otherwise.

Determination of free fatty acid formation

Lipolytic protein may cleave the ester linkage between a fatty acid and the glycerol core, producing free fatty acids. Titrimetry was used to determine the free fatty acid content of mixtures of patatin and a lipid after hexane extraction. The method is based on chemical titration method published by the Cyberlipid Center (Leray).

A solvent mixture (ethanol / tert-Butyl methyl ether, 1/1, v/v) was prepared and 10 ml phenolphthalein solution was added. As titrant a 10 mM KOH in ethanol solution was prepared. The hexane layer of the oil phase was transferred by a glass pipet to a 100 ml Erlenmeyer with cap. Solvent mixture was added to obtain approximately 30 – 50 ml solution. Titrant was added while stirring the solution on a magnetic stirrer to the end point of the indicator (light purple colour persisting for few seconds). The amount of titrant added was determined by weighting the Erlenmeyer before and after titrant addition. The weight was used to calculate the mmol alkaline / kg of oil was used. The value was corrected for the blank.

Equation = $\frac{m_{titrant} * M_{titrant}}{m_{oil}} * 1000$ = mmol KOH / kg oil

in which $m_{titrant}$ is mass of titrant added to sample in *g*, $M_{titrant}$ is the molar mass in mmol KOH / g titrant and m_{oil} is the mass of oil in the sample in g.

Example 1: pumpkin seed protein emulsification

In order to assess the stability of different lipids in the presence of pumpkin seed protein, a series of emulsions was prepared from 33 gram per liter demiwater solutions of pumpkin seed protein and an equal amount by weight of lipid. Fats were melted before use, oils were used as is.

Comparative experiments featuring emulsions prepared using the same quantities and types of lipid, but based on patatin as emulsifier, were similarly prepared.

The lipid and water were emulsified by means of an ultraturrax (T18 Ultraturrax with T18N dispersing tool) operating at 10 krpm for 1 minute and these emulsions were incubated at ambient temperature (20 °C ± 0.2 °C) for one day under mild agitation. Blanks were measured at room temperature.

Stable emulsions were obtained, which remained stable for at least a day, thereby indicating that pumpkin seed protein can be used as an emulsifier.

The free fatty acid content was then determined by titration as described. The results are shown in table 1.

Table 1: off-flavor development in pumpkin seed protein as compared to patatin in emulsions with different lipids.

Protein	Oil	mmol FFA/kg
Patatin	Sunflower Oil	7
Patatin	Soybean Oil	38
Patatin	Coconut fat	51
Pumpkin Seed Protein	Sunflower Oil	1.8
Pumpkin Seed Protein	Soybean Oil	0.4
Pumpkin Seed Protein	Coconut fat	0.6

The results show that pumpkin seed protein is a functional proteinaceous emulsifier similar to patatin, which provides for less off-flavor formation than patatin in the presence of lipids. In many lipids, off-flavor

formation of patatin is acceptable, but in some types of lipid, off-flavor formation caused by patatin is rather high. For pumpkin seed protein, off-flavor formation in the presence of lipid is lower than for patatin, for all types of lipid.

Example 2: pumpkin seed protein gelation

Pumpkin seed protein gels were prepared in demiwater at a pH of 8.5, at 15, 20, 25 and 30 wt.% protein concentration, with and without the presence of 1 wt.% salt. Gelation was induced by heating to a temperature of 95 °C for ten minutes, where after the result was evaluated visually (Figures 1 and 2).

The results show that pumpkin seed protein is functional gelling agent, which would be clean label in food products. The presence of salt increases the gel strength at lower concentrations of pumpkin seed protein. At 12 – 23 wt.% pumpkin seed protein, addition of 0.3 – 3 wt.%, preferably 0.5 – 2 wt.% of salt, preferably sodium chloride, provides for increased gel strength after heating, an effect not apparent at higher protein concentration. With or without salt, gels of increasing gel strength are obtained at 15 – 35 wt.% protein concentration in demiwater. Solid gels can be obtained in demiwater at 20 – 35 wt.% protein concentration, preferably 20 – 30 wt.%.

Example 3: application in a meat substitute

Method for preparing meat substitutes

Meat substitutes were prepared by hydrating texturized plant protein (Soy TVP "Tradcon T", Soy protein a.d., Serbia) and gluten TVP ("Unitex S2030", Vitablend Nederland) for a period of one hour in the quantity of water listed as "Water for hydrating TVP". The hydrated texturized plant protein was combined with the rest of the ingredients listed in table 2. Combining and mixing was performed in a Hobart mixer.

Subsequently, the meat substitutes were shaped into a burger patty, which was cooled to a temperature of 4°C for a period of 24 hours to obtain a raw, uncooked meat substitute.

Thus obtained raw meat substitutes were submitted to adhesion tests. Subsequently, the burgers were cooked in a steam oven at 150 °C until a core temperature of 95 °C was reached, thereby inducing gelation of the protein. Proper gelation is required in order to obtained a cooked burger of appropriate hardness and mouthfeel.

Table 2: burger recipes

Ingredients (wt.%)	B1	B2	B3	B4	B5
Soy TVP	18	18	18	18	18
Gluten TVP	3	3	3	3	3
Water for hydrating TVP	53	53	53	53	53
Water (additional)	13	10	5	-	5
Sunflower oil	8	8	8	8	8
Solanic 200	2	5	-	-	2
Pumpkin seed protein	-	-	10	15	8
PaselliFP	2	2	2	2	2
Sodium salt	1	1	1	1	1
TOTAL	**100**	**100**	**100**	**100**	**100**

Analyses

Adhesion and hardness of the meat substitute were determined using a Shimatzu EZ-SX Food Texture Analyzer (Schimatzu Corporation, Kyoto, Japan). Mechanical compression tests for both determinations applied a cylindrical probe of 75 mm diameter (SMS P/75). The meat substitutes were compressed to 40% at a constant rate of 10 mm/s, after the start at 1 N pressure.

Adhesion (J) is measured on raw, uncooked meat substitutes. Adhesion is defined as the negative area below the curve for the first peak (after the first compression).

Hardness (N) is measured in cooked meat. Hardness is defined as the highest peak force measured during the first compression.

Results

Table 3

Texture measurement	B1	B2	B3	B4	B5
Firmness (N)	67.5909	117.984	44.8136	47.8392	96.1189
Adhesiveness (J)	0.0003	0.0029	0.0185	0.0239	0.0091

The results in table 3 show that burgers of good firmness and adhesiveness can be obtained using pumpkin seed protein as an emulsifier and gelling agent. Although the gel strength of pumpkin seed protein is lower than the gel strength of patatin, the reduced off-flavor formation renders pumpkin seed protein a viable alternative to patatin for preparing meat substitutes.

Burgers could be kept at 4 °C for several days without development of off-flavors.

Example 4: vegan egg products

Vegan egg products were prepared on the basis of the recipes shown in table 4. Pumpkin seed protein, corn starch, egg flavor and salt were dry mixed to a homogenous powder mixture. Subsequently, sunflower oil and water were added to the powder mixture, and the total mixture was well mixed in a Hobart mixer to obtain vegan raw egg batter in the form of an emulsion. Both vegan raw egg batters could be stored cold until use (2 days) without off-flavor development.

The batter was fried in a cooking pan until egg-like gelation occurred, and until golden brown. No off-flavor development could be detected, and the product was very similar to scrambled eggs in both structure and taste.

When the recipes were repeated using coconut oil to replace sunflower oil, off-flavor development was noticeable after cold storage in recipe B, but not in recipe A, indicating pumpkin seed protein can be used to substitute patatin in order to reduce off-flavor formation.

Table 4: vegan egg recipes

Ingredient	Recipe A (%)	Recipe B (%)
Pumpkin seed protein	20	10
Solanic 200	0	4
Corn starch	4	4
Sunflower oil	10	10
Salt	1	1
Egg flavor	1	1
Water	64	70

Example 5: off-flavor formation in protein emulsions.

Emulsions were prepared from a 10 wt.% solution of protein in water, by emulsification of the lipid in a water solution : lipid wt. ratio of 1 : 2. The emulsions are tested for off-flavor formation by sensoric testing by a panel of trained sensoric testers. The tests were performed immediately after preparation, and after two days of storage at room temperature, mimicking an accelerated cool storage period. The results are shown in table 5.

The results show that pumpkin seed protein emulsions do not result in off-flavor immediately after preparation, and are stable to storage, regardless of the type of oil used.

Table 5: results of sensory tests on protein-lipid emulsions

Lipid	Patatin		Pumpkin	
	Off flavor after preparation*	Of flavor after storage*	Off flavor after preparation*	Of flavor after storage*
Sunflower oil	-	-	-	-
Olive oil	-	-	-	-
Rapeseed oil	-	-	-	-
Corn oil	-	-	-	-
Soybean oil	-	-	-	-
Sesame oil	-	-	-	-
Peanut oil	-	-	-	-
Grapeseed oil	-	-	-	-
Coconut oil	++	+++	-	-
Palm kernel oil	+	+	-	-
Red palm oil	+	+	-	-

*- not detected; + detected; ++ medium off flavor; +++ very strong off flavor

Example 6: cake based on pumpkin seed protein

Cake was prepared in cups following a regular (cup) cake preparation procedure, based on the recipes shown in Table 6, using 0 % PSP, 0.5 % PSP, 1.0 % PSP and 1.5 % PSP. A similar cake without PSP but with added patatin could also be prepared.

Preparation:

1. Mix “Part 1” (margarine, sugar and inverted sugar) for 2 minutes at medium speed using a Hobart mixer.
2. Hydrate sugar, Solanic®300 and pumpkin seed protein/patatin (“Part 2”) in water.
3. Combine Part 1, Part 2 and the ingredients from “Part 3” and mix in the Hobart mixer for 30 seconds at slow speed, then aerate for 5 minutes at medium speed to a density of ~800g/l.
4. Fill in cups (45g/cup) and bake at 170 ˚C for 30-38 minutes
5. Cool by immediately after baking, taking the cakes out of the oven into a room temperature environment.

Table 6: cake ingredients

Ingredients	0 % PSP, 0.5 % pat	0% PSP	0.5% PSP	1.0% PSP	1.5% PSP
Part 1					
Margarine (Akomarba 107-27/SG: AAK)	21.4	21.4	21.4	21.4	21.4
Sugar	17.8	17.8	17.8	17.8	17.8
Inverted sugar	1.61	1.61	1.61	1.61	1.61
Part 2					
Water	20.06	20.06	20.06	20.06	20.06
Sugar	8.92	8.92	8.92	8.92	8.92
native potato protease inhibitor (Solanic 300)	1.70	1.70	1.70	1.70	1.70
Pumpkin seed protein	-	-	0.5	1.0	1.5
Patatin (Solanic 200)	0.5	-	-	-	-
Part 3					
Wheat flour (Koopmans)	25.89	26.39	25.89	25.39	24.89
Potato starch	0.89	0.89	0.89	0.89	0.89
Cake emulsifier (Emul Pastry 16: Breatec)	0.27	0.27	0.27	0.27	0.27
Sodium stearoyl lactlate (SSL 70 SG: Breatec)	0.27	0.27	0.27	0.27	0.27
Salt	0.27	0.27	0.27	0.27	0.27
Sodium acid pyrophosphate (SAPP 28)	0.16	0.16	0.16	0.16	0.16
Sodium bicarbonate	0.12	0.12	0.12	0.12	0.12
Aroma (caramel flavor)	0.09	0.09	0.09	0.09	0.09
Xanthan gum	0.03	0.03	0.03	0.03	0.03

PSP = pumpkin seed protein; pat = patatin

Portions of the batter prepared following steps 1 – 3 were stored at 4 °C for up to three days, simulating a "cold-storage" type food product. No off-flavor formation could be detected by sensory evaluation for any of the batters not comprising patatin. Some off-flavor formation for the patatin reference is unavoidable.

Baked cakes were evaluated for off-flavor formation (as a "heat-shock" type food product), as described above. Baked cakes were further evaluated for stability against temperature change. Stability against temperature change was evaluated by determining numerically the "cake collapse" after cooling.

Cake collapse was determined by cutting each cake in the middle, and measuring the height of the cake from the lowest top point to the

bottom. The distance was expressed in mm. The lower the value, the more cake collapse took place. Cake collapse was expressed numerically as a shape maintenance factor, by expressing the height of the cakes with added pumpkin seed protein relative to the height of the cake prepared without pumpkin seed protein. The results can be seen in Figure 6, and in Table 7.

Table 7: Result of baked cake sensory evaluation and cake collapse

Cake	0 % PSP, 0.5 % pat	0% PSP	0.5% PSP	1.0% PSP	1.5% PSP
height	NR	22.7	26.8	28.8	29.5
Shape maintenance	NR	1	1.18	1.27	1.3
Off-flavor	+	-	-	-	-

PSP = pumpkin seed protein; pat = patatin; NR: not relevant.

Example 7: mayonnaise comprising pumpkin seed protein

As a model sauce, a plant -based (vegan) mayonnaise could be prepared on the basis of the ingredients displayed in Table 8.

The mayonnaise can be prepared by introducing the cold water and the mustard into a kitchen blender. The pumpkin seed protein, maltodextrin, salt, sugar, preservative (potassium sorbate) are added, and all is mixed with the water and mustard to obtain a homogenous dispersion. Lipid is added slowly while mixing at high intensity, in order to obtain a stable emulsion, without “breaking” the emulsion. When about 2/3 of the lipid has been added, vinegar and lemon juice are mixed in, prior to slowly adding the remaining oil and homogenizing to obtain a smooth and creamy white vegan mayonnaise. The mayonnaise can be filled into sealable containers and stored cool (4 °C) for several days.

Table 8: ingredients for "light" and "full-fat" mayonnaise

	"light" version		"full-fat" version	
	%	g	%	g
Water	57.8	433.5	21.5	161.3
Eliane SC160	4.5	33.8	0	0
Pumpkin seed protein	1	7.5	1	7.5
Avebe MD20	0.1	0.75	0.1	0.75
Dijon mustard	2	15	2	15
sugar	3.5	26.3	3.5	26.3
salt	1	7.5	1	7.5
potassium sorbate	0.1	0.75	0.1	0.75
lipid	25	187.5	65	487.5
vinegar 5%	5	37.5	3	22.5
lemon juice			2.8	21
Total	100	750	100	750

Mayonnaise types prepared using a lipid which is sensible to off-flavor formation (coconut oil), a lipid which is intermediately stable against off-flavor formation (corn oil), and a lipid which is more or less stable against off-flavor formation (sunflower oil), can be prepared in a full-fat (65 % lipid) and a light (25 % lipid) version. None of these types of mayonnaise would display off-flavor formation after three days of storage.

Claims

1. A food product comprising or prepared with a pumpkin seed protein isolate, said isolate comprising at least 60 wt.% of pumpkin seed protein, in which food product the pumpkin seed protein is present as an emulsifier and/or as a gelling agent.

2. A food product according to claim 1, said food product further comprising water and a lipid, which lipid is defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol.

3. A food product according to claim 2, wherein the lipid comprises one or more of the lipids in the group of coconut oil, palm oil, corn oil, soybean oil, rapeseed oil, sunflower oil, grape seed oil, pumpkin seed oil, peanut oil, sesame oil, olive oil, shea butter, cocoa butter, and rice bran oil, which lipids may optionally have been hydrogenated.

4. A food product according to any of claims 1 - 3, wherein the pumpkin seed protein is present as a gelling agent.

5. A food product according to any of claims 2 - 4, wherein the pumpkin seed protein is present as an emulsifier.

6. A food product according to any of claims 1 - 5, wherein the food product is a vegan food product, defined as a food product not comprising animal-derived ingredients.

7. A food product according to any of claims 1 – 6, wherein the food product is a meat substitute, vegan egg product, vegan fish product, vegan sea food product, batter, dough, bakery product, butter, cheese, cream cheese, yoghurt, sauce, dressing or cream.

8. A food product according to any of claims 1 – 7, further comprising native potato protein, preferably patatin.

9. A food product according to any of claims 1 – 8, which food product is

- a meat substitute, preferably a burger, meatball, sausage, minced meat, schnitzel, skewer, nugget, rib, filet or meat chunk, comprising 45 – 75 wt.%, preferably 50 – 70 wt.% of water, 3 – 15 wt.%, preferably 5 – 15 wt.% of lipid, and 3 – 25 wt.%, preferably 5 – 23 wt.%, more preferably 7 – 20 wt.% pumpkin seed protein; or
- a vegan egg product comprising 55 – 85 wt.%, preferably 60 – 75 wt.% of water, and 3 – 15 wt.%, preferably 5 – 15 wt.% of lipid, and 5 – 30 wt.%, preferably 8 – 23 wt.% pumpkin seed protein; or
- a vegan egg powder comprising 10- 80 wt.%, preferably 20 – 60 wt.%, even more preferably 35 – 50 wt.% of lipid, and 20 – 90 wt.%, preferably 30 – 80 wt.%, even more preferably 45 – 75 wt.% pumpkin seed protein; or
- a bakery product, prepared by baking a dough or batter comprising 10 – 35 wt.%, preferably 15 – 30 wt.% of water, 10 – 40 wt.%, preferably 15 – 30 wt.% of lipid, and 0.5 – 10 wt.%, preferably 1 – 5 wt.% pumpkin seed protein; or
- a sauce, preferably mayonnaise, Hollandaise sauce, cocktail sauce, garlic sauce, or ravigotte sauce, most preferably mayonnaise, comprising 15 – 70 wt.% water, 0.25 – 5 wt.% pumpkin seed protein, and 15 – 85 wt.% lipid, preferably 50 – 85 wt.% or 15 – 35 wt.% of lipid.

10. A method for preparing a food product from one or more ingredients, said preparing comprising one or more of the steps of shaping, mixing, cooling, heating, fermentation, and/or a period of storage of said ingredients, wherein said preparing further comprises a step of combining a pumpkin seed protein isolate with at least one of the said ingredients to obtain a mixture, said isolate comprising at least 60 wt.% of pumpkin seed protein.

11. A method according to claim 10, said method further comprising a

heat treatment of the mixture to a temperature of 70 – 110 °C, more preferably 80 – 100 °C, for a period of 1 – 45 minutes, preferably 5 – 30 minutes.

12. A method according to claim 10 or 11, wherein said mixture comprises water and a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol.

13. A method according to claim 12, wherein the food product preferably is a batter, dough, bakery product, cheese, cream cheese, yoghurt, sauce, dressing, fondue or cream, said method further comprising a step of emulsification of said mixture.

14. A method for preparing a food product according to any of claims 10 - 13, wherein

- the food product is a meat substitute, preferably a burger, meatball, sausage, minced meat, schnitzel, skewer, nugget, rib, filet or meat chunk, said method comprising
    - providing a mixture comprising water, said pumpkin seed protein isolate, and a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol; and
    - enriching said mixture with a denatured protein, preferably a texturized vegetable protein; and
    - shaping the meat substitute; and
    - optionally cooling the meat substitute to a temperature of from -35 °C to 20 °C for a period of at least one day; or
- the food product is a vegan egg product, said method comprising
    - providing a mixture comprising water, said pumpkin seed protein isolate, and a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol;
    - heating the mixture of water, pumpkin seed protein, and lipid to a temperature of at least 75 °C, preferably at least 85 °C; or
- the food product is a vegan egg powder, said method comprising providing a mixture comprising said pumpkin seed protein isolate,

and a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol, and homogenizing said mixture to provide a powder; or

- the food product is a bakery product, preferably a cake or a muffin, said method comprising
    - providing a mixture comprising water, said pumpkin seed protein isolate, and a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol; and
    - mixing and/or kneading the said mixture to obtain a batter or dough; and
    - baking the batter or dough to obtain the said bakery product; or
- the food product is a sauce, preferably a mayonnaise, Hollandaise sauce, cocktail sauce, garlic sauce, or ravigotte sauce, said method comprising
    - providing a mixture comprising water, said pumpkin seed protein isolate, and further optional ingredients,
    - providing a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol; and
    - adding said lipid to the mixture while mixing so as to obtain a stable emulsion;
    - optionally mixing in further optional ingredients to the obtained emulsion.

15. Use of a pumpkin seed protein isolate comprising at least 60 wt.% of pumpkin seed protein as a gelling agent and/or an emulsifier in a food product.

16. Use according to claim 15, wherein said food product further comprises a lipid, defined as a substance comprising at least 90 wt.% fatty acid tri-esters of glycerol.

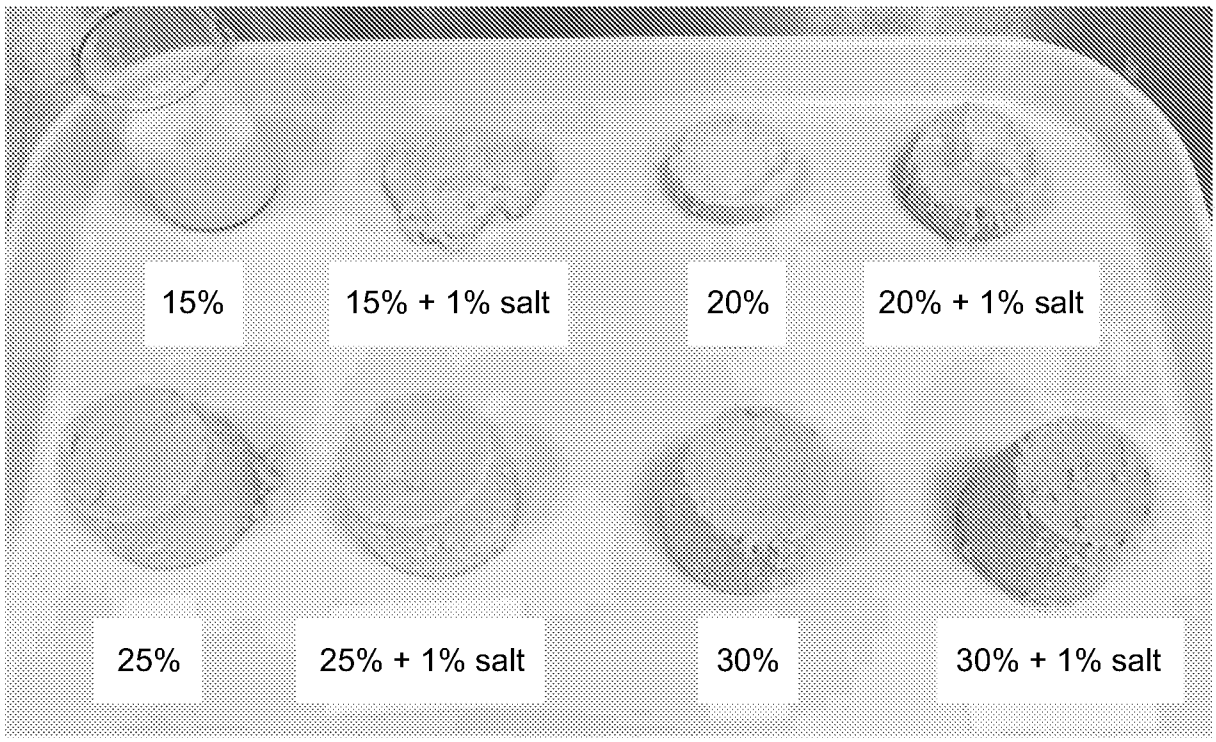
15%
15% + 1% salt
20%
20% + 1% salt
25%
25% + 1% salt
30%
30% + 1% salt

Fig. 1

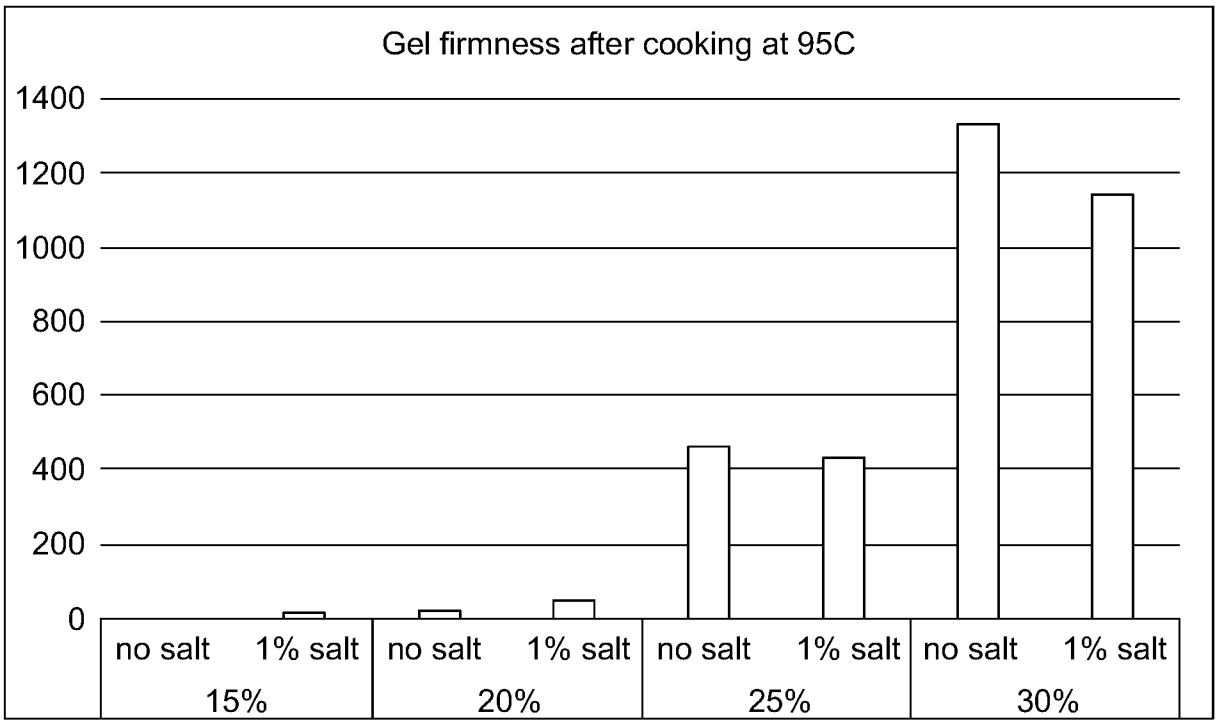
Gel firmness after cooking at 95C
1400
1200
1000
800
600
400
200
0
no salt
1% salt
15%
no salt
1% salt
20%
no salt
1% salt
25%
no salt
1% salt
30%

Fig. 2

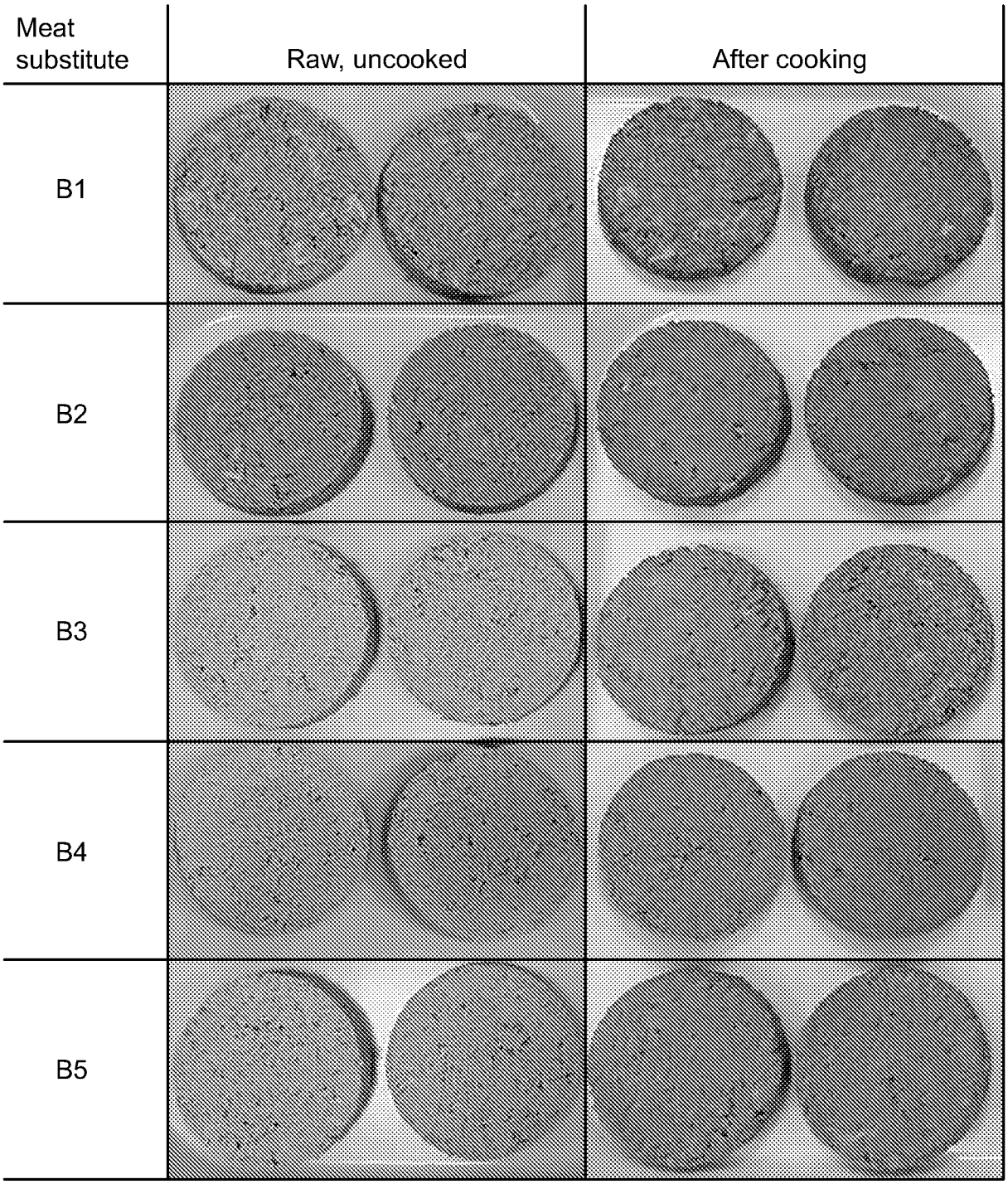

Fig. 3

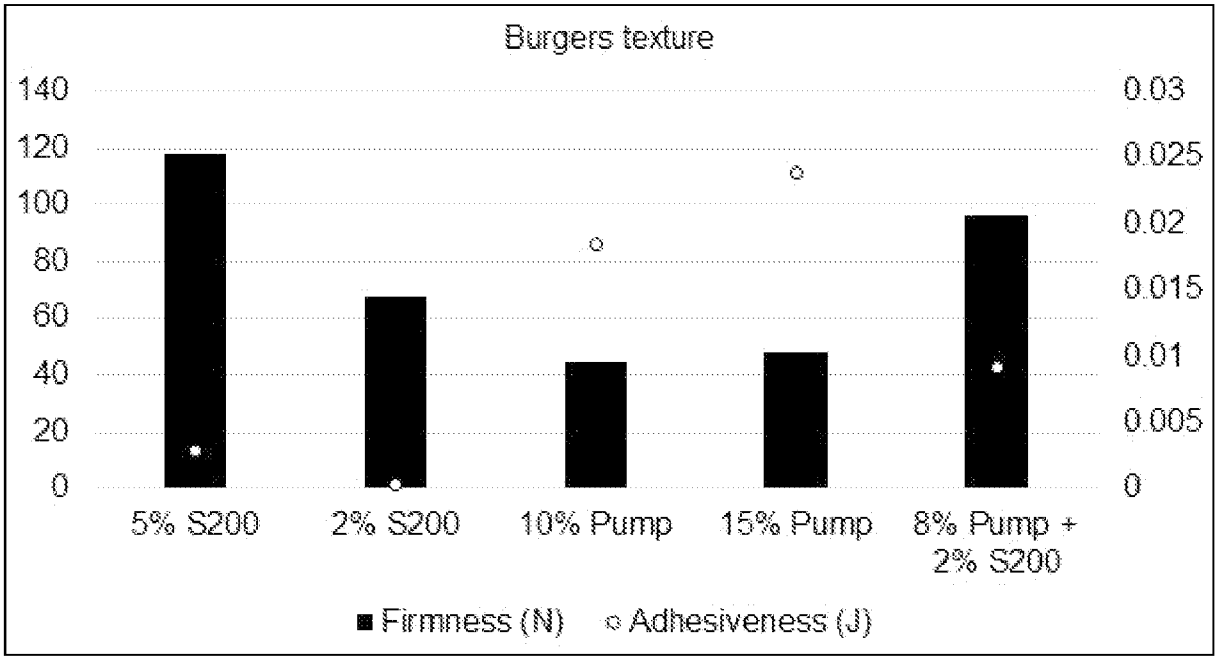

Fig. 4

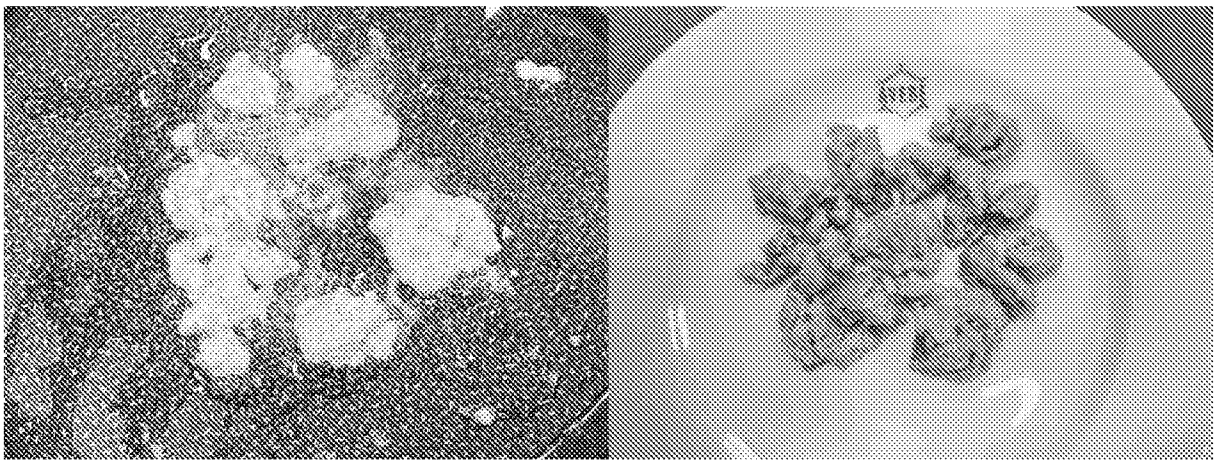

Fig. 5

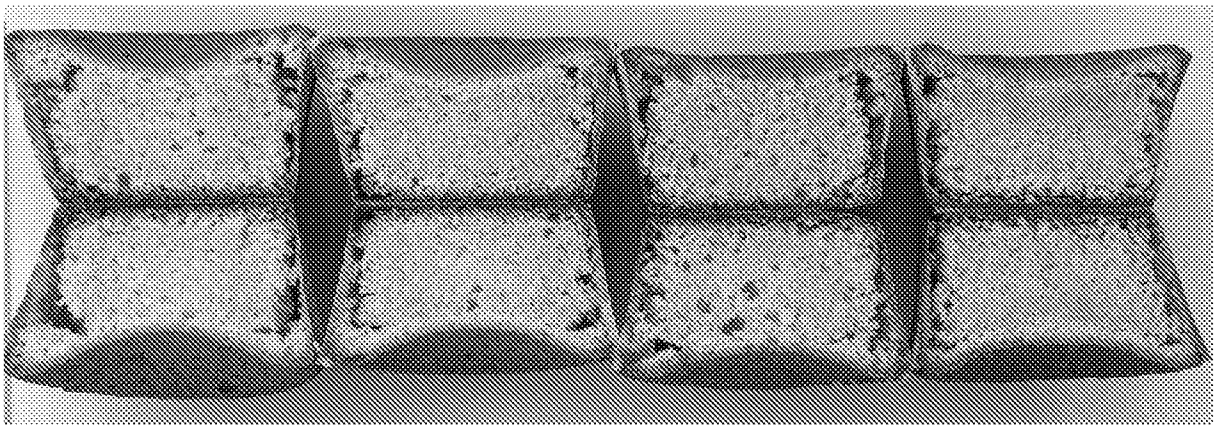

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2022/050756

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23J1/14 A23D7/005 A23J3/22 A23J3/26 A23L15/00
A23L33/185
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23J A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUCKO SANDRA ET AL: "Investigation on solubility, interfacial and emulsifying properties of pumpkin (Cucurbita pepo) seed protein isolate", LWT- FOOD SCIENCE AND TECHNOLOGY, vol. 64, no. 2, 1 December 2015 (2015-12-01), pages 609-615, XP093030361, United Kingdom ISSN: 0023-6438, DOI: 10.1016/j.lwt.2015.06.054 Abstract; page 609 2.6. Preparation and characterization of emulsions; page 611	1-3,5-7, 10,12-16

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 March 2023	30/03/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Steiner Ribeiro G.

1

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INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2022/050756

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ATUONWU A. C. ET AL: "FUNCTIONAL AND PASTING PROPERTIES OF PUMPKIN (Cucurbita pepo) SEED PRODUCTS", , [Online] 30 September 2010 (2010-09-30), pages 1-14, XP093030631, Cenresin Publications Retrieved from the Internet: URL:https://www.cenresinjournals.com/wp-content/uploads/2020/03/Page-36-49531.pdf> [retrieved on 2023-03-10] Abstract; page 1 Effect of pH and sample concentration on emulsion capacity and stability; page 37 Effect of sodium chloride concentration on emulsion capacity and stability; Pasting properties; page 38	1,2,4-7, 9-16
X	GB 2 596 121 A (MARLOW FOODS LTD [GB]) 22 December 2021 (2021-12-22) examples 1,16	1,4,6-8, 10,13,15

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2022/050756

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2596121 A	22-12-2021	GB 2596121 A	22-12-2021
		WO 2021255435 A1	23-12-2021