

COMPOSITIONS COMPRISING TEXTURIZED PROTEIN PRODUCTS AND METHODS FOR OBTAINING SAME

FIELD OF TECHNOLOGY

[001] The present technology generally relates to compositions comprising texturized protein products and relates to methods for obtaining the same.

BACKGROUND

[002] Traditional plant-based proteins suffer from textural and flavor shortcomings, thereby limiting their market reach. The present technology addresses these gaps by synergistically combining a texturized protein product and a fungi. Plant proteins are limited in specific amino acid or vitamin and mineral bioavailability. Primarily in the sulfur containing aminos such as methionine or amino acids required for muscle synthesis such as leucine (ref. 1), or in minerals such as iron. Extruding plant and mushroom proteins creates novel texturized proteins with distinct physicochemical properties and use cases.

SUMMARY OF TECHNOLOGY

[003] According to one aspect, the present technology relates to a composition comprising a vegetable protein extruded with a fungi protein containing material, creating a texturized protein product (TPP) with applications as meat analogues wherein the composition is suitable as a foodstuff. In some instances, the TPP is a vegetable protein. In one aspect the TPP comes from a blend of vegetable proteins and mushroom material.

[004] In one embodiment the TPP is from a blend of vegetable proteins, vegetable-derived starches and additives, and fungi material.

[005] In one embodiment the TPP from vegetable proteins, fungi material, and other additives for either flavour and/or texture enhancement. These additives may be chemically or naturally derived.

[006] According to one aspect, the present technology relates to a method obtaining the composition as defined herein. The method comprises the steps of: i) milling a vegetable source; ii) performing air or liquid classification for fractionation of the milled vegetable source into protein-rich and starch-rich fractions; iii) mixing the mushroom or mushroom mycelium powder or flour with obtained protein-rich and starch-rich fractions with; iv) extrude the mixture to create a texturized protein product; v) dry the TPP to < 5% moisture content.

BRIEF DESCRIPTION OF DRAWINGS

[007] All features of embodiments which are described in this disclosure are not mutually exclusive and can be combined with one another. For example, elements of one embodiment can be utilized in the other embodiments without further mention. A detailed description of specific embodiments is provided herein below with reference to the accompanying drawings in which:

[008] Figures 1A-K are photographs showing a composition in solid form according to embodiments of the present technology. FIG. 1A: Pleurotus - Pea Protein Blend for Larger sized extrudates; FIG. 1B: Pleurotus - Pea Proteins Extrudate; FIG. 1C: Trametes versicolor - Pea Proteins Extrudate; FIG. 1D: Ganoderma lucidum - Pea Proteins Extrudate; FIG. 1E: Cordyceps militaris - Pea Proteins Extrudate; FIG. 1F: Pleurotus - Pea - Faba Proteins Extrudate; FIG. 1G: Pleurotus - Pea - Chickpea Proteins Extrudate; FIG. 1H: Pleurotus - Soy Proteins Extrudate; FIG. 1I: Pleurotus - Rice Proteins Extrudate; FIG. 1J: Pleurotus - Wheat Proteins Extrudate; and FIG. 1K: Pleurotus - Hemp Proteins Extrudate;

[009] Figure 2 is a graph showing the hydration rate over time of compositions according to some embodiments of the present technology;

[010] Figure 3 is a graph showing the protein content of the identified compositions according to some embodiments of the present technology;

[011] Figure 4 is a graph showing the metabolomic analysis of amino acids of the compositions according to some embodiments of the present technology;

[012] Figure 5 is a graph showing other protein components of interest according to some embodiments of the present technology;

[013] Figure 6 is a graph showing the polyphenols breakdown of the compositions according to some embodiments of the present technology;

[014] Figure 7 is a graph showing the breakdown of amines in the compositions according to some embodiments of the present technology;

[015] Figure 8 is a graph showing the acylcarnitines breakdown of the compositions according to some embodiments of the present technology;

[016] Figure 9 is a graph showing the texturization index of certain foods and predicted TI for CanPro products according to Mohamad Mazlan et al., 2020 (Physical and Microstructure Properties of Oyster Mushroom-Soy Protein Meat Analog via Single-Screw Extrusion. *Foods*, 9(8) and Physical and Microstructure Properties of Oyster Mushroom-Soy Protein Meat Analog via Single-Screw Extrusion. *Foods*, 9(8); and Samard and Ryu, 2019 (*Journal of the Science of Food and Agriculture*, 99(6), 2708–2715), the content of which are incorporated herein by reference; and

[017] Figure 10 is a principle component analysis of the flavour profile of the mushroom compared to other plant based protein powders. The mushroom component was described as mushroom, seaweed, earthy aroma. Acidic, bitter, salty and strong umami (savoury) taste. Astringent, thin, and gritty mouthfeel when dissolved in water.

DETAILED DESCRIPTION OF TECHNOLOGY

[018] The present technology is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the technology may be implemented, or all the features that may be added to the instant technology. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure which variations and additions do not depart from the present technology. Hence, the following description is intended to illustrate some particular embodiments of the technology, and not to exhaustively specify all permutations, combinations and variations thereof.

[019] As used herein, the singular form “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

[020] The recitation herein of numerical ranges by endpoints is intended to include all numbers subsumed within that range (e.g., a recitation of 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 4.32, and 5).

[021] The term “about” is used herein explicitly or not, every quantity given herein is meant to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including equivalents and approximations due to the experimental and/or measurement conditions for such given value. For example, the term “about” in the context of a given value or range refers to a value or range that is within 20%, preferably within 15%, more preferably within 10%, more preferably within 9%, more preferably within 8%, more preferably within 7%, more preferably within 6%, and more preferably within 5% of the given value or range.

[022] The expression “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example, “A and/or B” is

to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

[023] As used herein, the term “comprise” is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded.

[024] One of the problems identified in the field of the present technology is the paucity of texturized vegetable protein ingredients that meet the standards of food manufactures. To meet their standards, the product must achieve specific standards both in quality and operational compatibility. Standards relating to texture such as chew that mimics already on the market meat products such as chicken and pork, a subtle or umami flavour, a neutral colour, and made from natural ingredients. Operational standards include product loss of less than 10% passing through processing equipment, hydration rates less than 24 hours, shear strength to withstand processing, and thermal stability sufficient to withstand pasteurization at 65°C to 80°C. All while being food safe and with a minimum 2-year shelf life.

[025] The present investigators have identified that extruding vegetable protein with a mushroom source to create a TPP solve some of these identified problems. The present investigators provide herein a composition exhibiting a high-quality protein ingredient with increased firmness and chewiness and a distinct or unique flavour profile.

[026] In one embodiment, the present technology relates to a composition comprising a texturized protein product and a fungi. In some instances, the texturized protein product is a vegetable protein.

[027] In one embodiment, the present technology relates to a composition comprising a vegetable protein and a fungi.

[028] The fungi protein and material allows to alter the product texture and taste, creating a novel meat analog compared to one using vegetable proteins alone. The texture is altered due to the specific protein, fibre, and carbohydrate profile of the mushroom. When extruded with plant

proteins, the microstructure is changed, creating unique textures and properties (ref. 2). Due to the addition of specific amino acids from the mushroom itself, glutamic acid and glutamates, along with methionine and leucine, enhances the flavour (ref. 3), creating a unique flavour profile compared to other proteins.

[029] In some embodiments, the vegetable protein source is selected from a group consisting of vegetable protein isolates, protein concentrates, and flours, wherein said source is derived from one or more of the following: peas (*Fabaceae* e.g. *Pisum sativum* var *sativum* or *Pisum sativum* var. *arvense*), faba beans (*Fabaceae*), soybeans (*Fabaceae*), wheat (*Poaceae*), oats (*Poaceae*), cotton seeds (*Malvaceae*), broad beans (*Fabaceae*), chickpeas (*Fabaceae*), barley (*Poaceae*), and corn (*Poaceae*), Mung bean (*Vigna*).

[030] In one embodiment, the vegetable protein source is a vegetable selected from: peas, faba, corn and soy.

[031] In one embodiment, the vegetable protein source is a vegetable selected from: peas, corn and soy.

[032] In one embodiment, the vegetable protein source is a vegetable selected from: peas, and soy.

[033] In one embodiment, the fungi protein source is a mushroom or a mushroom mycelia in a powder or flour form.

[034] In one embodiment, the fungi protein source is Generally Regarded as Safe (GRAS) gourmet mushrooms.

[035] In one embodiment, the fungus is selected from the genus *Pleurotus*, including but not limited to Oyster (*P. ostreatus*) Phoenix Oyster (*P. pulminarius*), Black King Oyster (*P. eryngii*), Pink Oyster (*P. dejamour*), *P. citrinopileatus*, *P. tuber-regium*, and *P. salmoneostramineus*. The fungus may also be selected from the genus *Agaricus* (e.g., *Agaricus bisporus*, *Agaricus blazei*),

Coprinus (e.g., *Coprinus comatus*), Lentinula (e.g., *Lentinula edodes*, also known as Shiitake), Trametes (e.g., *Trametes versicolor*), Ganoderma (e.g., *Ganoderma lucidum*, known as Reishi). The fungi types mentioned are exemplary and should not limit the scope of the present technology. The fungi can be utilized at various lifecycle stages, including spores, mycelium, and mature fruiting bodies.

[036] Non-limiting examples of previously isolated forms of *Pleurotus* potentially suitable for this process include: Common Oyster Mushroom (*Pleurotus ostreatus*), King Oyster Mushroom (*Pleurotus eryngii*), Golden Oyster Mushroom (*Pleurotus citrinopileatus*), Pink Oyster Mushroom (*Pleurotus djamor*), Phoenix Oyster Mushroom (*Pleurotus pulmonarius*), Branched Oyster Mushroom (*Pleurotus cornucopiae*), Veiled Oyster Mushroom (*Pleurotus dryinus*), Abalone Mushroom (*Pleurotus cystidiosus*), Ferula Oyster Mushroom (*Pleurotus ferulae*), Blue Oyster Mushroom (*Pleurotus columbinus*), Salmon Oyster Mushroom (*Pleurotus salmoneostramineus*), Indian Oyster Mushroom (*Pleurotus sajor-caju*), Cinnamon Cap Mushroom (*Pleurotus calypttratus*), Florida Oyster Mushroom (*Pleurotus florida*), Tasty Oyster Mushroom (*Pleurotus sapidus*), Aspen Oyster Mushroom (*Pleurotus populinus*), Fragrant Oyster Mushroom (*Pleurotus euosmus*), Golden Hue Branched Oyster Mushroom (*Pleurotus cornucopiae* var. *citrinopileatus*), and King Tuber Mushroom (*Pleurotus tuber-regium*).

[037] Non-limiting examples of previously isolated *Hericium* species include: Lion's Mane Mushroom (*Hericium erinaceus*), Bear's Head Tooth Mushroom (*Hericium americanum*), Bearded Tooth Mushroom (*Hericium coralloides*), Comb Tooth Mushroom (*Hericium ramosum*), Monkey's Head Mushroom (*Hericium alpestre*), Pom Pom Mushroom (*Hericium abietis*), Eastern Coral Tooth Mushroom (*Hericium flagellum*), Pine-Spike Mushroom (*Hericium abietis*), Snow Coral Mushroom (*Hericium clathroides*), and Hazel Bracket (*Hericium circinatum*).

[038] Non-limiting examples of previously isolated *Trametes* species include: Turkey Tail Mushroom (*Trametes versicolor*), Violet Toothed Polypore (*Trametes pubescens*), Cinnamon Bracket (*Trametes cinnabarina*), Multicolor Gill Polypore (*Trametes hirsuta*), *Trametes suaveolens*, *Trametes ochracea*, *Trametes gibbosa*, *Trametes trogii*, *Trametes villosa*, *Trametes meyenii*, *Trametes lactinea*, *Trametes elegans*, *Trametes ljubarskyi*, and *Trametes menziesii*.

[039] A non-limiting examples of previously isolated *Agaricus* species include: Button Mushroom (*Agaricus bisporus*), Horse Mushroom (*Agaricus arvensis*), Field Mushroom (*Agaricus campestris*), Prince Mushroom (*Agaricus augustus*), Almond Mushroom (*Agaricus subrufescens*), Yellow-staining Mushroom (*Agaricus xanthodermus*), Wood Mushroom (*Agaricus silvicola*), Scaly Wood Mushroom (*Agaricus langei*), Garden Mushroom (*Agaricus bitorquis*), Blewit-Like *Agaricus* (*Agaricus placomyces*), Blushing Wood Mushroom (*Agaricus silvaticus*), *Agaricus devoniensis*, Snowball Mushroom (*Agaricus niveus*), Sweet Almond Mushroom (*Agaricus dulcidulus*), *Agaricus bernardii*, *Agaricus impudicus*, *Agaricus moelleri*, *Agaricus phaeolepidotus*, *Agaricus cupreobrunneus*, and *Agaricus pseudopratensis*.

[040] In one embodiment, the present technology relates to a composition comprising a vegetable protein and a fungi, wherein the composition has an enhanced taste.

[041] Enhanced taste is defined as an improved point hedonic scale rating in which the protein with mushrooms added scores higher than protein without mushrooms added. Enhanced taste could also be defined as elevated levels of presence of glutamic, aspartic acids and the 5 nucleotides which give mushrooms the umami flavour. The characteristic aroma of mushrooms is produced by a mixture of compounds including alcohols, aldehydes, ketones, esters, alkanes, and heterocyclic compounds. Enhanced taste could also describe the profile of the flavour as distinguished from principal component analysis in which different levels of flavour can be added (see Figure 10).

[042] Enhanced taste also refers to kokumi properties such as taste development (mouthfulness, continuity, duration and depth); punch (the initial taste and impact); and roundness and balance (richness, thickness, spread, and unity). The kokumi sensation is a distinct flavour from the five basic tastes (ref. 4).

[043] In one embodiment the taste profile of the composition in question was assessed During a sensory profiling in which panelists rated the intensity of 24 attributes of aroma, taste and mouthfeel from the lexicon developed in the training phase of the project for the following sensory

data: **Aroma** (13): cereal/grain, doughy, green pea, green pepper, nutty, beany, seaweed, cardboard, earthy, mushroom, sulfur, onion, burnt. **Taste** (5): sweet, acidic, salty, bitter, umami. **Mouthfeel** (6): astringency, chalky, gritty, viscosity, mouth-coating, residual particles.

[044] The trained panelists (N=14) are all experienced with descriptive techniques and a wide variety of horticultural products. They were aware that they were evaluating plant-based protein powders, but had no other knowledge of the products, to reduce the impact of potential bias. In order to negate the influence of other ingredients or backgrounds, products were profiled in water. Samples were prepared as 10%w/v dispersions in filtered water 24-hr before evaluation and kept in the fridge until 1-hr before tasting. Products were tasted in ISO tasting glasses, labeled with 3-digit codes, in duplicate according to a balanced design. During tasting, the sensory booths were illuminated with red lighting to minimize visual differences. To minimize carry-over effects and sensory fatigue, sparkling water was used as a rinse aid and a forced 2-min. break between samples was implemented.

[045] Sensory profiling data were analyzed to determine significant differences by attribute through Analysis of Variance (ANOVA), sensory similarities and differences through Principal Component Analysis (PCA) and Agglomerative Hierarchical Cluster Analysis (AHC) using XLStat[®] software. An alpha limit of 0.05 was used for all analyses unless otherwise specified.

[046] Figure 10 outlines the Sensory map (PCA biplot) with protein powder products (coded for confidentiality and coloured according to cluster (AHC analysis)).

[047] In some embodiments, the composition of the present technology comprises a vegetable protein in an amount between about 10% wt and about 90% wt of the total weight of the composition. In some embodiments, the composition of the present technology comprises a vegetable protein in an amount between about 20% wt and about 80% wt of the total weight of the composition. In some embodiments, the composition of the present technology comprises a vegetable protein in an amount between about 25% wt and about 75% wt of the total weight of the composition. In some embodiments, the composition of the present technology comprises a vegetable protein in an amount between about 50% wt and about 90% wt of the total weight of the

composition. In some embodiments, the composition of the present technology comprises a vegetable protein in an amount of about 85% wt of the total weight of the composition.

[048] In some embodiments, the isolated fungal strain and/or progeny thereof may grow at low pH conditions of up to about 7.0, about 6.5, about 6.0, about 5.5, about 5.0, about 4.5, about 4.0, about 3.5, about 2.0, For example, the fungal strain may be grown under low pH conditions from 9.0 to 2.0.

[049] In some embodiments, the composition of the present technology comprises a fungus in an amount between 10% wt and 90% wt of the total weight of the composition. In some embodiments, the composition of the present technology comprises a fungus in an amount between 20% wt and 90% wt of the total weight of the composition. In some embodiments, the composition of the present technology comprises a fungus in an amount between 25% wt and 75% wt of the total weight of the composition. In some embodiments, the composition of the present technology comprises a fungus in an amount between 10% wt and 50% wt of the total weight of the composition. In some embodiments, the composition of the present technology comprises a fungus in an amount between 10% wt and 25% wt of the total weight of the composition. In some embodiments, the composition of the present technology comprises a fungus in an amount of about 15% wt of the total weight of the composition.

[050] In some embodiments the extrusion system is a Clextral dual screw extrusion apparatus. An extrusion process is then performed to form a texturized protein product. A Clextral EV32 twin-screw extruder (Firminy, France) equipped with a volumetric feeder (Clextral VF/40/25-2) and a die face cutter was used. A 4.8mm conical-size die was used. A large air gap configuration and a 2-blade, 2-piece cutter were used for the trial. The extruder barrel length: diameter was 24:1. The temperatures, moisture content, screw speed, and feed rate were altered to gain the most desirable product. During the trials, the extrusion operating conditions were monitored, and their effect on the final product quality was used to determine the next steps. The preconditioner, a 75L pre-hydrating vessel containing 2 counter-rotating shafts with 136 paddles and 6 ports for steam or water injection was placed on top of the extruder for use. Oven temperatures were set to 120° C for a residence time of 3-5 minutes.

[051] Mushroom cultures were obtained from certified mushroom growers. The mushrooms are grown on a proprietary mix of grain, wood, starch, or other lignocellulosic material, along with supplemental vitamins and minerals and nitrogen sources. The material is hydrated and sterilized before the mushroom is inoculated.

[052] In some embodiments the mushroom substrate has a carbon content between 50% to 90% and with nitrogen or protein being between 10% to 50%.

[053] In some embodiments the mushroom substrate was sterilized at 15 psi for 30, 60, 90, 120, or 180 minutes.

[054] In some embodiments the mushroom substrate pH is adjusted using sodium bicarbonate or acetic acid.

[055] In some embodiments the mushroom substrate is agitated to aerate at 0, 5, 10, 30, 60, 120, hour intervals.

[056] In some embodiments the mushroom and mushroom mycelium is harvested from the substrate, dried to < 5%, and powderized. The mushroom is dried between 30, 40, 60, 80 degrees celsius. In some embodiments the mushrooms are dried using an infrared, microwave vacuum dryer.

[057] In some embodiments, the compositions of the present technology have a total protein content of between about 20% wt and about 80% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of between about 20% wt and about 60% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 20% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 25% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have

a total protein content of at least about 30% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 35% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 40% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 45% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 50% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 55% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 60% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 65% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 70% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 75% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 80% wt of the total weight of the composition. In some other embodiments, the compositions of the present technology have a total protein content of at least about 85% wt of the total weight of the composition.

[058] Starch, salts and dry spices and flavorings, high moisture flavourings, or additional mushroom powders and flours of different combinations, or crushed grape pulp and by products of wine fermentation, such as a polyphenol extract.

[059] In one embodiment, the present technology relates to a method for obtaining the composition material of the present technology. The method comprises milling a vegetable source using dry milling techniques, such as hammer milling, roller milling, or pin milling, and wet milling techniques, such as stone milling, colloid milling, or disc milling.

[060] In one embodiment, the present technology relates to integrating the method for producing the material described with a process in which the method is obtaining protein-rich and starch-rich fractions from a vegetable source. The method could comprise both wet and dry milling or fractionation techniques. In the wet milling process, the vegetable source is first steeped in water or an aqueous solution to soften the material and facilitate separation, before being ground and separated into different components using various separation techniques, such as centrifugation or filtration. The protein-rich fraction and a starch-rich fraction can be further processed or refined as needed.

[061] In one embodiment the present technology co-locates with a dry milling or fractionation process in which the method is where the vegetable source is ground without the addition of water. The dry ground material is then subjected to air classification or sieving to separate the protein-rich fraction from the starch-rich fraction. Both wet and dry milling processes can be tailored to optimize the yield and purity of the protein and starch fractions, depending on the specific requirements of the application.

[062] The vegetable source is milled, an air classification is performed for fractionation of the milled vegetable source into protein-rich and starch-rich fractions. Seeds are ground down to grits - visible and coarse fragments or flours - fine powders. Flours can be further fractionated by air classification. Light - fine fraction rich in protein, Heavy - heavy, coarse fraction rich in starch. During air classification, as the flour is dispersed into the classifier, a rising air column causes smaller particles to become airborne while larger particles sink.

[063] In some embodiments the composition of the present technology has a colour profile (L^* , a^* , and b^*) of 61.77, 8.06, 20.94, and 55.42, 7.59, 17.95, and 67.7, 5.65, 22.74. The L^* , a^* , and b^* values were captured using a Minolta Spectrophotometer CM2500d.

[064] In some embodiments the composition of the present technology has a moisture content of between about 2.5% and about 5.5%. In some embodiments the composition of the present technology has a moisture content of between about 2.5%, 2.8%, and about 5.5%. In some

embodiments, the moisture content of the composition of the present technology is measured using a Mettler Toledo HE53 IR Moisture Analyzer.

[065] In some embodiments the composition of the present technology has a bulk density in g / L of between about 105 and about 225. In some embodiments the composition of the present technology has a bulk density in g / L of between about 107, 142, and about 225. In some embodiments, the bulk density of the composition of the present technology is measured using volume measurements.

[066] In some embodiments the composition of the present technology has a protein content (%) between about 40, 60, 70, 75, 80, 85 and 90. In some embodiments, the protein content of the present technology is measured using the combustion method, utilizing an Elementar protein analyzer to accurately analyze protein content in the samples.

[067] In some embodiments the composition of the present technology has a particle shape that is sliced irregularly, or sliced round and uniform, or irregularly shaped and bite sized. In some embodiments, the particle shape of the composition of the present technology is measured using Carbon Fiber Composites Electron Caliper.

[068] In some embodiments the composition of the present technology has an average particle size (LxWxH mm) of 7x7x7, 20x15x7, and about 80x30x7. In some embodiments, the particle size of the composition of the present technology is measured using Carbon Fiber Composites Electron Caliper.

[069] In some embodiments the composition of the present technology has a Protein Digestibility (PDCAAs) of between about 0.80 and about 0.92. In some embodiments, the PDCAAs of the composition of the present technology is measured using High performance liquid chromatography to assay the amino acid composition. Then the amino acids are compared against standard known amino acid profiles of egg or milk protein.

[070] In some embodiments the composition of the present technology has an oil holding capacity (g of oil / g of sample) of between about 0.70 and about 1.1. In some embodiments the composition of the present technology has an oil holding capacity (g of oil / g of sample) of between about 0.72, 0.88, and about 1.09. In some embodiments, the oil holding capacity of the composition of the present technology is measured using IEC Micromax centrifuge and Sartorius CP124S analytical balance.

[071] In some embodiments the composition of the present technology has a hydration rate of 2.94 g of water retained per g of sample over 60 minutes (see Figure 2). The hydration rate of the composition of the present technology is measured using a weight differential method and determined using a Heraeus Megafuge 16R centrifuge and Sartorius CP124S analytical balance.

[072] In some embodiments the composition of the present technology has a chewiness (m.J) of between about 60 and about 395. In some embodiments the composition of the present technology has a chewiness (m.J) of between about 61 and about 393. In some embodiments the composition of the present technology has a chewiness (m.J) of between about 61.05, 166.58, and about 392.55. In some embodiments, the chewiness of the composition of the present technology is measured using a Food Technology Corporation TMS-Pro texture analyzer with a modified standard program. Probe: 10 mm diameter Steel Cylinder Probe Test Speed: 60 mm/min, % Deformation: 50%, Interval time between compressions: 8s. As used herein the term “chewiness” refers to the texture of the property of food describing the effort required to masticate the product until it is ready to swallow.

[073] In some embodiments the composition of the present technology has a cohesiveness (ratio) of between about 0.3 and about 0.7. In some embodiments the composition of the present technology has a cohesiveness (ratio) of between about 0.31, 0.65, and about 0.68. In some embodiments, the cohesiveness of the composition of the present technology is measured using a Food Technology Corporation TMS-Pro texture analyzer with a modified standard program. As used herein the term “cohesiveness” means how well a food product retains its form and structure when subjected to mechanical forces such as biting, cutting or chewing, measuring how much a substance can be deformed before it breaks apart.

[074] In some embodiments the composition of the present technology has a springiness of between about 5 and about 15. In some embodiments the composition of the present technology has a springiness of between about 6 and about 12. In some embodiments the composition of the present technology has a springiness of between about 6.04, 8.34, and about 11.3. In some embodiments, the springiness of the composition of the present technology is measured using a Food Technology Corporation TMS-Pro texture analyzer with a modified standard program. As used herein the term “springiness” means the ability of a food product to return to its original shape after being compressed. Springiness is a measure of elasticity.

[075] The waste starch is then utilized as a growth medium for filamentous fungi.

EXAMPLES

Example 1 – Preparation of a composition comprising Pleurotus spp. and pea protein

Fungus

[076] *Pleurotus spp.* were used to produce the mycelia used for the mushroom-pea textured protein product developed. All species used are Generally Regarded as Safe (GRAS) mushrooms. Species included Oyster (*P. ostreatus*) Phoenix Oyster (*P. pulmonarius*), Black King Oyster (*P. eryngii*), and Pink Oyster (*P. dejamour*). Mushrooms were maintained on a complex proprietary medium at 4°C for long-term storage and 25°C or 27°C or 30°C for growth and short-term use.

Bulk Substrate

[077] A combination of grains included 2 parts oats, 4 parts barley, and 3 rye. In other embodiments, the grain could be 1 part oats, 5 parts barley, and 3 parts rye; or, 0 parts oats, 5 parts barley, and 5 rye; or, any combination thereof. Grains were stored in a cool, dry place until needed. Grains were then soaked for 18 to 24 hours such as 18 or 20 or 24 hours in water with 0.2 mg/L of chlorine while stirring between 2 and 18 hours such as at 2 or 4 or 8 or 16 or 18 hours. Excess water was drained, and substrate was weighed into the vessel used. The substrate was sterilized in a steam sterilizer (Yamato SK101C Autoclave) using distilled water at 15 psi for 160 to 180

minutes, depending on vessel size at 121°C. The substrate was allowed to cool in front of flow hoods overnight.

Vessel

[078] The vessels were polypropylene Unicorn SM-4T Filter Patch Bags. The vessel was heat-sealed following inoculation.

Growing time and conditions

[079] Mycelia was allowed to grow in a 10 x 10 x 6'6'' incubation chamber at temperature chamber was recorded between 20°C to 25°C and between 25% to 30% humidity for 120 to 135 days such as at 120 or 125 or 130 or 135 days. Vessels were routinely agitated between 18 to 48 hours such as every 18 or 24 or 48 hours.

RNA reduction, drying, separating, grinding and packaging

[080] To reduce RNA levels and kill the fungus, the mycelium was dried on food dehydrators at 65°C - 75°C for 1 hour followed by drying between 45°C to 55°C such as at 45°C or 50°C or 55°C between 4 and 10 hours such as for 4 or 6 or 8 or 10 hours. Dried mycelium was separated from the dried grains as much as possible. The mycelium product was ground into a fine powder and vacuum seal packaged.

Regrowth assessment

[081] Samples of dried mycelium were collected, rehydrated, and allowed to sit at room temperature to determine if mycelium would regrow. The tests were conducted using distilled water or distilled water and dextrose. The following microbiological testing was performed at Intertek:

- *E. coli* – method MFHPB-34
- *Salmonella* – method MFHPB-20 (presence/absence)
- *S. aureus* – method MFLP-21
- *Listeria monocytogenes* – method MFHPB-30 (presence/absence)
- *Clostridium perfringens* – method MFHPB-23

Extrusion

[082] A Clextral EV32 twin-screw extruder (Firminy, France) equipped with a volumetric feeder (Clextral VF/40/25-2) and a die face cutter was used. A 4.8mm conical-size die was used. A large air gap configuration and a 2-blade, 2-piece cutter were used for the trial. The extruder barrel length: diameter was 24:1. The temperatures, moisture content, screw speed, and feed rate were altered to gain the most desirable product. During the trials, the extrusion operating conditions were monitored, and their effect on the final product quality was used to determine the next steps. The preconditioner, a 75L pre-hydrating vessel containing 2 counter-rotating shafts with 136 paddles and 6 ports for steam or water injection was placed on top of the extruder for use. Oven temperatures were set to 120° C for a residence time of 3-5 minutes.

Extrusion Samples Analyzed.

- E15723-5 (70/30 Pea Protein 870 (Puris), Mycoprotein (Maia Farms))
- E15723-7 (90/10 Pea Protein 870 (Puris), Mycoprotein (Maia Farms))
- E15723-8 (100/0 Mycoprotein (Maia Farms))

Moisture content

[083] Moisture content was measured using a Mettler Toledo HE53 IR Moisture Analyzer. The L*, a*, and b* values were captured using a Minolta Spectrophotometer CM2500d. Length and height measurements were done using a Carbon Fiber Composites Electron Caliper. Water holding capacity was determined using a Heraeus Megafuge 16R centrifuge and Sartorius CP124S analytical balance. Approximately 2.0±0.1g of flour was added to a 50 mL centrifuge tube. The tube was filled with excess water, vortexed and allowed to sit for 30 min. The tubes were centrifuged for 30min at 3000 RPM, and excess water was decanted. Samples were allowed to air out for 10 minutes; then a final weight was taken. $WHC = \frac{(\text{Moisture Content} * \text{Dry Sample Weight}) + (\text{Wet Sample weight} - \text{Dry Sample Weight})}{\text{Dry Sample Weight} - (\text{Moisture Content} * \text{Dry Sample Weight})}$.

Oil holding capacity

[084] Determined using an IEC Micromax centrifuge and Sartorius CP124S analytical balance. Approximately 0.05 ± 0.01 g of flour was added to a 2mL microcentrifuge tube. The tube was filled with canola oil, vortexed and allowed to sit for 30 minutes. The tubes were centrifuged for 30 min at 10000rpm, excess oil was decanted, and samples were allowed to air out for 10 min. $OHC = \frac{\text{Wet Sample Weight} - \text{Dry Sample Weight}}{\text{Dry Sample Weight}}$.

Hydration rate

[085] Hydration rate was determined using a weight differential method. Samples were weighed, given an excess of water, drained, and weighed again. The time and weight were closely monitored and the rate at which the sample incorporated the water was calculated. The results of the measurements are shown in Figure 2.

Double compression

[086] was run using a Food Technology Corporation TMS-Pro texture analyzer with a modified standard program to calculate cohesiveness, chewiness, and springiness. Parameters:

- Probe: 10 mm diameter Steel Cylinder Probe
- Test Speed: 60 mm/min
- % Deformation: 50%
- Interval time between compressions: 8s

[087] Total plate count, coliform count, and rapid yeast & mould were performed using 3M plates. Samples were plated and incubated for 48 hours at their appropriate temperatures. Results within 25 CFU/g – 250 CFU/g are considered statistically significant, and the highest dilution within these constraints was reported. Final product quality characteristics included: bulk density and moisture content with ingredients provided by the client. To grow the mycelium required cultures were purified and transferred to the sterilized grain using aseptic techniques. Transferring half an agar plate of healthy mycelium to the grains in front of a flow hood, the vessel was sealed, tested for leaks, shaken, and placed in the growing chamber. The vessels were then kept in a controlled environment growth chamber until they needed to be harvested. Vessels were cut open, substrate was broken apart, dried, separated, ground up, and packaged before being extruded. The

ingredients used for each trial, a combination of pea protein and mushroom protein, were weighed and placed into a 750g high speed electric stainless steel grain mill grinder for two minutes to blend the ingredients. Pea protein, pea fibre, corn starch, faba bean concentrate, and soy protein concentrate were used as the primary ingredients to run the trials with mycelia included in increments to determine its effects. The pre-conditioner was started, along with the feeder and water and/or steam was added as needed. The product reached the end of the preconditioner and fed directly into the extruder. Water was added to the barrel while the extruder ran based on the operating conditions needed. The temperatures were increased or decreased as needed. Variations were given in the percent of the ingredients to run different trials at different moisture and temperature conditions. The product was dropped onto a belt conveyor drying oven. The dried extrudate left the oven and was transferred to a bulk bag via an uplift conveyor. Each sample was scored on the floor through sensory and hydration tests.

[088] As shown in Figure 1, the combination of mycelium with pea protein results in a better-textured product compared to pea protein alone or pea protein with starches. When cooked and flavoured, the product is chewy and delicious.

Microbial testing results

[089] The regrowth tests showed that the RNA reduction steps of air drying the mycelium at 65°C for 1 hour was sufficient to prevent the regrowth of the mycelia. The mycelia and most other potential food-borne illness agents were eliminated during the extrusion and drying step as temperatures reached much greater than required to reduce RNA in the mycelium samples. Microbial tests show that the product was within safe limits by the end of production. Table 1 shows the microbial tests for two of the products. According to our limits of 25 to 250 CFU/g being significant, the total plate count is 670 and 20 CFU/g for E1573-5 and E15723-7, respectively. All of the other tests were either <5 CFU/g or not detectable.

Table 1. Microbiology testing results

	E15723-5	E15723-7
Table Plate Count (CFU/g)	67 x 10 ¹	2 x 10 ¹
Coliforms (CFU/g)	ND	ND
Rapid Yeast & Mould (CFU/g)	ND	ND
<i>E. coli</i> (CFU/g)	<5	<5
<i>Salmonella</i>	-ve	-ve
<i>S. aureus</i> (CFU/g)	<5	<5
<i>Listeria monocytogenes</i>	-ve	-ve
<i>Clostridium perfringens</i> (CFU/g)	<5	<5

ND = not detectable

Functionality Testing

Table 2. Functionality results

Tests	E15723-5	E15723-7	E15723-8
Moisture content (%)	2.50	4.03	5.95
Colour- L*	61.77	67.70	55.42
Colour- a*	8.06	5.65	7.59
Colour- b*	20.94	22.74	17.95
Length (mm)	19.4	13.82	20.18
Height (mm)	7.75	10.21	8.17
Water Holding Capacity (g of water / g of sample)	2.25	3.05	2.79
Oil Holding Capacity (g of oil / g of sample)	0.88	1.09	0.72
Chewiness (mJ)	166.58	392.55	61.05
Cohesiveness (ratio)	0.65	0.68	0.31
Springiness (mm)	8.34	11.30	6.04

Protein content and amino acids

Dehulling

[090] Seed coat or hull is removed by machines using abrasive force to break apart seed and hull

Milling

[091] Seeds are ground down to grits - visible and coarse fragments or flours - fine powders. Flours can be further fractionated by air classification. Light - fine fraction rich in protein, Heavy - heavy, coarse fraction rich in starch. During air classification, as the flour is dispersed into the

classifier, a rising air column causes smaller particles to become airborne while larger particles sink.

Extrusion

[092] Flour is fed into a barrel with rotating screws that knead the material as it passes. Combinations of pressure, temperature, moisture and sharp edges are applied. The material begins to turn doughy and cook.

[093] Figures 3, 4, 5, 6, 7, 8, 9 and 10 show the content of the compositions obtained with the method of the present technology. The methods of the present technology employ in some instances Liquid Chromatography-Mass Spectrometry (LC-MS). LC-MS method provides a comprehensive solution for the precise identification and quantification of small molecules in complex samples for the qualitative and quantitative analyses of food assays and fatty acids. The method comprises two primary stages, Liquid Chromatography (LC) and Mass Spectrometry (MS). The sample is first subjected to LC, where compounds are separated based on their affinity. Each compound's retention time is recorded for identification purposes. Post-separation, the compounds are ionized, and their mass-to-charge ratios (m/z) are measured. Isotopic patterns may also be obtained for further identification and differentiation. For quantitative analysis, traditional multipoint calibration curves are generated using known concentrations of the analyte for accurate quantification. Alternatively, when isotopically labeled standards are available, internal calibration is performed to directly determine the concentration of the unlabeled analog, eliminating the need for separate calibration curves. The analytical method adheres to international guidelines for validation, ensuring robustness, reliability, and reproducibility. The values presented represent the amount of each material in the sample in $\mu\text{Mol/g}$ of the sample.

[094] The present technology also employs the combustion method, utilizing an Elemental protein analyzer to accurately analyze protein content in the samples. The sample is prepared to ensure it is free from any contaminants that could interfere with the analysis. The prepared sample is introduced into the Elemental protein analyzer. Combustion is initiated to convert all organic nitrogen present in the sample to elemental nitrogen gas. The elemental nitrogen gas produced is quantified using the analyzer's detection system. A nitrogen-to-protein conversion factor of 6.25

is applied to the measured nitrogen content to accurately determine the protein content of the sample. The combustion method demonstrates a precision rate within an interval of 2-4%, making it both reliable and efficient. Variance analysis is employed to identify any statistically significant differences in protein content when compared with other methodologies. This combustion method provides a rapid, precise, and reliable technique for protein content analysis, suitable for a wide range of applications. The method is particularly beneficial for samples with variable non-protein nitrogen content, offering a robust alternative to traditional methods. The values presented here are the percent of protein in the given sample. For figure 10 specifically, the texturization index was determined using background research to find the values that are not the present technology. The TI of the present technology was approximated based on cohesiveness and subjective chew. TI is the ratio of cutting strength along the sample's longitudinal and orthogonal axis relative to the extrusion direction.

Fungus

[095] Dry *Agaricus campestris* fruiting body was harvested, dried, and powderized. The content of Powderized *Agaricus Campestris* is presented in Table 3.

Table 3: Certificate of Analysis for Batch No. MGF230610 of Powderized *Agaricus Campestris*

Item of Analysis	Specification	Result
Organoleptic		
Appearance	Light grey brown fine powder	Conforms
Odor	Characteristic	Conforms
Taste	Characteristic	Conforms
Physical Characteristics		
Particle Size	100% Through 80 mesh	Conforms
Loss on Drying	≤7.0%	3.95%
Ash	≤7.0%	4.10%
Heavy Metals		

Total Heavy Metals	≤10ppm	Conforms
Arsenic	≤2ppm	Conforms
Lead	≤2ppm	Conforms
Cd	≤1ppm	Conforms
Hg	≤0.1ppm	Conforms
Microbiological Tests		
Total Plate Count	≤10,000cfu/g	448cfu/g
Total Yeast & Mold	≤100cfu/g	7cfu/g
E.Coli	Negative	Negative
Salmonella	Negative	Negative
Staphylococcus	Negative	Negative

Extrusion

[096] Extrusion took place using the same machine and practices as outlined in the extrusion of the mycelium product. Extrusion Results are presented in Table 4.

Table 4: Extrusion Results

Ingredient	Minimum Inclusion	Maximum Inclusion
Pea Protein Isolate	60	90
Pea Protein Concentrate	0	25
Premix Conditioner	0	100
Mushroom Powder	5	30

[097] All references cited in this specification, and their references, are incorporated by reference herein in their entirety where appropriate for teachings of additional or alternative details, features, and/or technical background.

[098] While the disclosure has been particularly shown and described with reference to particular embodiments, it will be appreciated that variations of the above-disclosed and other features and functions, or alternatives thereof, may be desirably combined into many other different systems or applications. Also, that various presently unforeseen or unanticipated alternatives, modifications, variations or improvements therein may be subsequently made by those skilled in the art which are also intended to be encompassed by the following claims.

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CLAIMS:

1. A composition comprising a blend of co-extruded plant protein and fungi protein, wherein the composition is a texturized protein product (TPP).
2. The composition of claim 1, wherein the plant protein is a vegetable protein.
3. The composition of claim 2, wherein the vegetable protein is obtained from a vegetable selected from peas, faba beans, soybeans, wheat, oats, cotton seeds, broad beans, chickpeas, barley, and corn.
4. The composition of claim 2, wherein the vegetable protein is obtained from (Fabaceae), faba beans (Fabaceae), soybeans (Fabaceae), wheat (Poaceae), oats (Poaceae), cotton seeds (Malvaceae), broad beans (Fabaceae), chickpeas (Fabaceae), barley (Poaceae), and corn (Poaceae).
5. The composition of any one of claims 1 to 4, wherein the mushroom protein is obtained from a fungi selected from the genus *Pleurotus*, *Agaricus*, *Coprinus*, *Lentinula*, *Trametes*, and *Ganoderma*.
6. The composition of any one of claims 1 to 5, wherein the vegetable protein is present in the composition in an amount of between about 10% wt and 90% wt of the total weight of the composition.
7. The composition of any one of claims 1 to 6, wherein the vegetable protein is present in the composition in an amount of between about 50% wt and about 90% wt of the total weight of the composition.
8. The composition of any one of claims 1 to 7, wherein the vegetable protein is present in the composition in an amount of about 85% wt of the total weight of the composition.
9. The composition of any one of claims 1 to 8, wherein the fungi protein is present in the composition in an amount of between about 10% wt and 90% wt of the total weight of the composition.

10. The composition of any one of claims 1 to 9, wherein the fungi protein is present in the composition in an amount of between about 10% wt and 50% wt of the total weight of the composition.
11. The composition of any one of claims 1 to 10, wherein the fungi protein is present in the composition in an amount of about 15% wt of the total weight of the composition.
12. The composition of any one of claims 1 to 11, wherein the composition has a moisture content of between about 2.5% and about 5.5%.
13. The composition of any one of claims 1 to 12, wherein the composition has a bulk density in g / L of between about 105 and about 225.
14. The composition of any one of claims 1 to 13, wherein the composition has a Protein Digestibility (PDCAAs) of between about 0.80 and about 0.92.
15. The composition of any one of claims 1 to 14, wherein the composition has an oil holding capacity (g of oil / g of sample) of between about 0.70 and about 1.1.
16. The composition of any one of claims 1 to 15, wherein the composition has a hydration rate of 2.94 g of water retained per g of sample over 60 minutes.
17. The composition of any one of claims 1 to 16, wherein the composition has a chewiness (m.J) of between about 60 and about 395.
18. The composition of any one of claims 1 to 17, wherein the composition has a cohesiveness (ratio) of between about 0.3 and about 0.7.
19. The composition of any one of claims 1 to 17, wherein the composition has In some embodiments the composition of the present technology has a springiness of between about 5 and about 15.
20. A composition comprising a blend of co-extruded vegetable protein and mushroom protein, wherein the composition is a texturized protein product (TPP), wherein the vegetable protein is present in the composition in an amount of between about 50% wt and about 90% wt of the total

weight of the composition and the mushroom protein is present in the composition in an amount of about 10% and 50% of the total weight of the composition.

21. The composition of claim 20, wherein the vegetable protein is obtained from a vegetable selected from peas, faba beans, soybeans, wheat, oats, cotton seeds, broad beans, chickpeas, barley, and corn.

22. The composition of claim 21, wherein the vegetable protein is obtained from (Fabaceae), faba beans (Fabaceae), soybeans (Fabaceae), wheat (Poaceae), oats (Poaceae), cotton seeds (Malvaceae), broad beans (Fabaceae), chickpeas (Fabaceae), barley (Poaceae), and corn (Poaceae).

23. The composition of any one of claims 20 to 22, wherein the mushroom protein is obtained from a fungi selected from the genus *Pleurotus*, *Agaricus*, *Coprinus*, *Lentinula*, *Trametes*, and *Ganoderma*.

24. The composition of any one of claims 20 to 23, wherein the vegetable protein is present in the composition in an amount of between about 10% wt and 90% wt of the total weight of the composition.

25. The composition of any one of claims 20 to 23, wherein the vegetable protein is present in the composition in an amount of between about 50% wt and about 90% wt of the total weight of the composition.

26. The composition of any one of claims 20 to 25, wherein the vegetable protein is present in the composition in an amount of about 85% wt of the total weight of the composition.

27. The composition of any one of claims 20 to 26, wherein the fungi protein is present in the composition in an amount of between about 10% wt and 90% wt of the total weight of the composition.

28. The composition of any one of claims 20 to 27, wherein the fungi protein is present in the composition in an amount of between about 10% wt and 50% wt of the total weight of the composition.

29. The composition of any one of claims 20 to 28, wherein the fungi protein is present in the composition in an amount of about 15% wt of the total weight of the composition.
30. The composition of any one of claims 20 to 29, wherein the composition has a moisture content of between about 2.5% and about 5.5%.
31. The composition of any one of claims 20 to 30, wherein the composition has a bulk density in g / L of between about 105 and about 225.
32. The composition of any one of claims 20 to 31, wherein the composition has a Protein Digestibility (PDCAAs) of between about 0.80 and about 0.92.
33. The composition of any one of claims 20 to 32, wherein the composition has an oil holding capacity (g of oil / g of sample) of between about 0.70 and about 1.1.
34. The composition of any one of claims 20 to 33, wherein the composition has a hydration rate of 2.94 g of water retained per g of sample over 60 minutes.
35. The composition of any one of claims 20 to 34, wherein the composition has a chewiness (m.J) of between about 60 and about 395.
36. The composition of any one of claims 20 to 35, wherein the composition has a cohesiveness (ratio) of between about 0.3 and about 0.7.
37. The composition of any one of claims 20 to 36, wherein the composition has In some embodiments the composition of the present technology has a springiness of between about 5 and about 15.
38. A method for obtaining a composition comprising a blend of co-extruded plant protein and fungi protein, wherein the composition is a texturized protein product (TPP), the method comprising the steps of:
- i) milling a vegetable source;
 - ii) performing air or liquid classification for fractionation of the milled vegetable source into protein-rich and starch-rich fractions;

iii) mixing the mushroom or mushroom mycelium powder or flour with obtained protein-rich and starch-rich fractions with;

iv) extrude the mixture to create a texturized protein product; and

v) dry the TPP to a < 5% moisture content.

39. The method of claim 38, wherein prior to step iv), the mushroom is grown using a waste starch growth medium.