ENZYME COMPOSITIONS AND METHODS OF MAKING THEM

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US Provisional Patent Application Ser. No. 62/953,361, filed December 24, 2019. The entire contents of the aforementioned patent applications are incorporated herein by reference.

BACKGROUND

[0002] Proteins are important dietary nutrients. They can serve as a fuel sources and/or as sources of amino acids, including the essential amino acids that cannot be synthesized by the body. The daily recommended intake of protein for healthy human adults is 10% to 35% of a person's total calorie needs, and currently the majority of protein intake for most humans is from animal-based sources. In addition, athletes and bodybuilders may rely upon supplemental protein consumption to build muscle mass and improve performance. Recombinantly produced proteins are free from animal-based sources and provide an alternative protein resource for consumers. With the world population growing, and the coincidental increase in global food demand, there is an unmet need for alternative sustainable, non-animal-based sources of dietary and supplemental protein.

SUMMARY

[0002a] In one aspect, provided herein is a consumable composition comprising a recombinant food preserving enzyme (rFPE), wherein the FPE is a goose-type FPE (gFPE); wherein the composition is solid, semi-solid or a gel composition.

[0002b] In another aspect, provided herein is a food composition, wherein the food composition comprises one or more consumable ingredients and the gFPE of any one of claims 1-8.

[0002c] In another aspect, provided herein is a method of preparing a consumable composition comprising steps of: a. providing an isolated foodstuff preserving enzyme (FPE) which is recombinantly produced, wherein the FPE is a goose type FPE (gFPE); b. combining the gFPE with one or more consumable ingredients.

[0003] The methods and compositions of the present disclosure provide this unmet need.

[0004] In some ~~embodiment~~examples, described herein are consumable compositions comprising a recombinant food preserving enzyme (rFPE). In some cases, the FPE may be a goose-type FPE (gFPE). In some cases, the composition may be semi-solid or a gel composition.

[0005] In some ~~embodiment~~examples, the consumable composition may be free of bacterial impurities.

[0006] In some ~~embodiment~~examples, the gFPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 1. In some ~~embodiment~~examples, the gFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 1.

[0007] In some ~~embodiment~~examples, the gFPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 2. In some ~~embodiment~~examples, the gFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 2.

[0008] In some ~~embodiment~~examples, the gFPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 3. In some ~~embodiment~~examples, the gFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 3.

[0009] In some ~~embodiment~~examples, the gFPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 4. In some ~~embodiment~~examples, the gFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 4.

[0010] In some ~~embodiment~~examples, the consumable composition may be heat treated. In some ~~embodiment~~examples, the consumable composition has a longer shelf life than a nearly identical consumable composition which does not comprise the gFPE. In some ~~embodiment~~examples, the consumable composition has a longer shelf life than the shelf life of a nearly identical consumable composition which comprises chicken egg-white muramidase rather than the gFPE. In some ~~embodiment~~examples, the gFPE may be produced in a Pichia cell.

[0011] In some ~~embodiment~~examples, described herein are compositions comprising a recombinant foodstuff preserving enzyme (FPE) wherein the FPE may have an activity of greater than 90,000 shugar U/mg.

[0012] In some cases, the FPE may have an activity in Shugar units of greater than 150,000U/mg. The FPE may have an activity in Shugar units of greater than 200,000U/mg. The FPE may have an activity in Shugar units of greater than 250,000U/mg. The FPE may have an activity in Shugar units of greater than 300,000U/mg. The FPE may have an activity in Shugar units of greater than 350,000U/mg. The FPE may have an activity in Shugar units of greater than 400,000U/mg. The FPE may have an activity in Shugar units of greater than 450,000U/mg.

[0013] The recombinant FPE may be produced in a Pichia cell.

[0014] The composition may be a food composition. The food composition may comprise one or more consumable ingredients. The food composition may have a longer shelf life than a nearly identical food composition which does not comprise the recombinant FPE. The food composition may have a longer shelf life than the shelf life of a nearly identical product which may comprise chicken egg-white muramidase rather than the recombinant FPE.

[0015] The composition may be a powder composition comprising rFPE.

[0016] The recombinant FPE may comprise an amino acid sequences with at least 85% sequence identity to SEQ ID NO: 1.

[0017] One Shugar unit may be an amount of the enzyme which will digest a suspension of *M. luteus* cells causing a decrease in absorbance of the solution at a rate of 0.001 per minute at 37°C, pH 7.0.

[0018] The composition may be hypoallergenic as compared to a composition comprising chicken egg-white muramidase.

[0019] The recombinant FPE may have a comparable activity as compared to a non-recombinant FPE and/or a FPE comprising at least 85% sequence identity to SEQ ID NO: 1 yet may be isolated from a natural source.

[0020] In some ~~embodiment~~examples, described herein is a consumable composition comprising a recombinant foodstuff preserving enzyme (FPE), wherein the FPE may comprise an amino acid sequence with at least 85% sequence identity to SEQ ID NO: 1.

[0021] The consumable composition may be a food composition. The food composition may have a gel-like texture or consistently. The food composition may be in the form of a baked product. The food composition may be in the form of an egg-white-like product. The FPE may be recombinantly produced in Pichia pastoris cells. The food composition may be in liquid form. The food composition may be in solid form.

[0022] The composition may have a longer shelf life than the shelf life of a nearly identical product which may comprise chicken egg-white muramidase rather than the recombinant FPE.

[0023] The food composition may have at least 0.1% FPE by weight. The food composition may have at most 10% FPE by weight. The FPE may be enzymatically active in the food composition. The composition may be an ingredient. The food composition may be substantially free of microorganisms or cell-debris. The food composition may be a probiotic formulation. The recombinant FPE may be at least 95% pure.

[0024] The food composition may comprise one or more recombinant proteins in addition to the recombinant FPE. The recombinant FPE may provide gel solidity or increased viscosity to the food product. The food composition may comprise more than one recombinant proteins in addition to the recombinant FPE.

[0025] In some ~~embodiment~~examples, described herein are methods of preparing a consumable composition comprising steps of: providing an isolated foodstuff preserving enzyme (FPE) which is recombinantly produced and combining the recombinantly produced FPE with one or more consumable ingredients. In some cases, the FPE is a goose-type FPE.

[0026] The recombinantly produced FPE may have an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 1.

[0027] The recombinantly produced FPE may be recombinantly produced in a yeast cell. The yeast cell may be Pichia pastoris.

[0028] The recombinantly produced FPE increases the shelf life of the consumable composition relative to a nearly identical consumable composition lacking the recombinantly produced FPE.

[0029] The recombinantly produced FPE provides a gel-like texture to the consumable composition.

[0030] The consumable composition may be a food product that may be ready for consumption by a human/animal.

[0031] In some ~~embodiment~~examples, described herein are methods of producing a xanthan gum product comprising the steps of: providing *X. campestris* cells into a fermentation medium; heat treating the cells at a temperature between 45-60℃ and a pH of between 8-10 and an alkaline protease, thereby producing a solution comprising cell debris; adding to a solution comprising cell debris a foodstuff preserving enzyme (FPE) that may be recombinantly produced and/or a recombinantly produced FPE comprising an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 1, thereby producing a xanthan gum solution. The method may also comprise adding isopropanol to the xanthan gum solution, thereby precipitating xanthan gum; isolating and drying the precipitated xanthan gum, thereby obtaining a xanthan gum product.

[0032] The amount of the FPE added may be less than the amount of chicken egg-white FPE that would be needed to produce an equivalent amount of the xanthan gum product under otherwise identical conditions.

[0033] The method of producing xanthan gum may further comprise a step of adjusting the pH of the composition after producing a solution comprising cell debris.

[0034] In some ~~embodiment~~examples, described herein are food preservatives comprising a recombinantly produced food preserving enzyme (FPE) with at least 95% sequence identity to SEQ ID NO: 1.

[0035] Additional ~~aspect~~examples and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative ~~embodiment~~examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different ~~embodiment~~examples, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also "figure" and "FIG." herein), of which:

[0037] **FIG. 1A to FIG. 1E** illustrate bactericidal activity of a recombinant foodstuff preserving enzyme (rFPE) against common food spoiling bacteria.

[0038] **FIG. 2A to FIG. 2E** illustrate gelation of an rFPE at various temperatures and concentrations.

CLAIMS

What is claimed is:

1. A consumable composition comprising a recombinant food preserving enzyme (rFPE), wherein the FPE is a goose-type FPE~~lysozyme~~ (gFPE); wherein the composition is solid, semi-solid or a gel composition.

~~2. The consumable composition of claim 1, wherein the consumable composition is free of bacterial impurities.~~

~~3.~~2. The consumable composition of claim 1, wherein the gFPE comprises an amino acid sequence with at least 95% identity to any one of SEQ ID NOs: 1-4.

~~4. The consumable composition of claim 1, wherein the gFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 1.~~

~~5. The consumable composition of claim 1, wherein the gFPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 2.~~

~~6. The consumable composition of claim 1, wherein the gFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 2.~~

~~7. The consumable composition of claim 1, wherein the gFPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 3.~~

~~8. The consumable composition of claim 1, wherein the gFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 3.~~

~~9. The consumable composition of claim 1, wherein the gFPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 4.~~

~~10. The consumable composition of claim 1, wherein the gFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 4.~~

~~11.~~3. The consumable composition of any one of ~~the previous~~ claims 1-2, wherein the consumable composition is heat treated.

~~12.~~4. The consumable composition ~~of~~ any one of ~~the previous~~ claims 1-3, wherein the consumable composition has a longer shelf life than a nearly identical consumable composition which does not comprise the gFPE.

~~13.~~5. The consumable composition of ~~any one of the previous~~ claim~~s~~ 4, wherein the consumable composition has a longer shelf life than the shelf life of a nearly identical consumable composition which comprises chicken egg-white muramidase rather than the gFPE.

~~14. The composition of any one of the previous claims, wherein the gFPE is produced in a Pichia cell.~~

15.6. TheA consumable composition of any one of claims 1-5, wherein the gFPEcomprising a recombinant foodstuff preserving enzyme (rFPE) wherein the rFPE has an activity of greater than about 90,000 Shugar U/mg.

16. The composition of claim 15, wherein the rFPE has an activity of greater than about 150,000 Shugar U/mg.

17. The composition of claim 15 or claim 16, wherein the rFPE is produced in a *Pichia pastoris* cell.

18. The composition of any one of claims 15 to 17, wherein the composition is a food composition.

19. The composition of claim 18, wherein the food composition comprises one or more consumable ingredients.

20. The composition of claim 18 or claim 19, wherein the food composition has a longer shelf life than a nearly identical food composition which does not comprise the rFPE.

21. The composition of any one of claims 18-20, wherein the food composition has a longer shelf life than the shelf life of a nearly identical product which comprises chicken egg white muramidase rather than the rFPE.

22. The composition of any one of claims 15-21, wherein the composition is a powder composition comprising rFPE.

23. The composition of any one of claims 15-22, wherein the rFPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 1.

24. The composition of any one of claims 15-22, wherein the rFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 1.

25. The composition of any one of claims 15-22, wherein the rFPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 2.

26. The composition of any one of claims 15-22, wherein the rFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 2.

27. The composition of any one of claims 15-22, wherein the rFPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 3.

28. The composition of any one of claims 15-22, wherein the rFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 3.

29. The composition of any one of claims 15-22, wherein the rFPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 4.

30. The composition of any one of claims 15-22, wherein the rFPE comprises an amino acid sequence with at least 97% identity to SEQ ID NO: 4.

~~31. The composition of any one of claims 15-22, wherein the composition is hypoallergenic as compared to a nearly identical composition comprising chicken egg white muramidase.~~

~~32. The composition of any one of claims 15-22, wherein the rFPE has higher activity as compared to a non-recombinant FPE and/or a chicken muramidase.~~

~~33. The composition of any one of claims 15-22, wherein the rFPE has an activity of greater than about 200,000 Shugar U/mg.~~

~~34. The composition of any one of claims 15-22, wherein the rFPE has an activity of greater than about 300,000 Shugar U/mg.~~

~~35. The composition of any one of claims 15-22, wherein the rFPE has an activity in Shugar units of greater than about 450,000 Shugar U/mg.~~

~~36. The composition of any one of claims 15-22,~~ wherein one Shugar unit is an amount of the enzyme which will digest a suspension of *M. luteus* cells causing a decrease in absorbance of the solution at a rate of 0.001 per minute at 37°C, pH 7.0.

~~37. A consumable composition comprising a recombinant foodstuff preserving enzyme (rFPE), wherein the FPE comprises an amino acid sequence with at least 95% identity to SEQ ID NO: 1.~~

~~38. The consumable composition of claim 37, wherein the consumable composition is a food composition.~~

~~39. The consumable composition of claim 37 or claim 38, wherein the food composition has a gel-like texture or consistently.~~

~~40. The consumable composition of claim 37 or claim 38, wherein the food composition is in the form of a baked product.~~

~~41. The consumable composition of any one of claims 37 to 40, wherein the food composition is in the form of an egg-white-like product.~~

~~42. The consumable composition of claim 37 or claim 38, wherein the food composition is in liquid form.~~

~~43. The consumable composition of claim 37 or claim 38, wherein the food composition is in solid form.~~

~~44. The composition of any one of claims 37 to 43, wherein the composition has a shelf life longer than or comparable to than the shelf life of a nearly identical composition which comprises chicken egg white muramidase rather than the rFPE.~~

~~45. The consumable composition of any one of claims 37 to 44, wherein the food composition has at least 0.05% rFPE by weight.~~

~~46. The consumable composition of any one of claim 37 to 45, wherein the food composition has at most 10% rFPE by weight.~~

~~47. The consumable composition of any one of claim 37 to 46, wherein the consumable composition is an ingredient.~~

~~48. The consumable composition of any one of claim 37 to 47, wherein the food composition is substantially free of microorganisms or bacterial cell debris.~~

~~49. The consumable composition of any one of claim 37 to 48, wherein the food composition is a probiotic formulation.~~

~~50. The consumable composition of any one of claims 37 to 49, wherein the rFPE is at least 80% pure.~~

~~51. The consumable composition of any one of claims 37 to 50, wherein the food composition comprises more than one recombinant protein in addition to the rFPE.~~

~~52. The consumable composition of any one of claims 37 to 51, wherein the rFPE provides gel solidity or increased viscosity to the food product.~~

~~53. The consumable composition of any one of claims 37 to 52, wherein the food product does not comprise a gelling agent other than the rFPE.~~

~~54. The consumable composition of any one of claims 37 to 53, wherein the rFPE is recombinantly produced in *Pichia pastoris* cells.~~

~~55. A method of preparing a consumable composition comprising steps of:~~

~~a. providing an isolated foodstuff preserving enzyme (FPE) which is recombinantly produced, wherein the FPE is a goose type FPE (gFPE);~~

~~b. combining the recombinantly produced FPE with one or more consumable ingredients.~~

~~56. The method of claim 55, wherein the recombinantly produced gFPE has an amino acid sequence with at least 95% sequence identity to one of SEQ ID NOs: 1-4.~~

~~57.~~7. ~~-~~The consumable composition of any one of claims 1-6~~method of claim 55 or claim 56~~, wherein the ~~recombinantly produced~~ gFPE is ~~recombinantly~~ produced in a yeast cell.

~~58.~~8. The consumable composition~~method~~ of claim ~~5~~7, wherein the yeast cell is a Pichia cell~~*pastoris*~~.

9. A food composition, wherein the food composition comprises one or more consumable ingredients and the gFPE of any one of claims 1-8.

~~59.~~10. The ~~method of any one of~~ food composition of claim~~s~~ ~~955 to 58~~, wherein the food composition has a longer~~recombinantly produced gFPE increases the~~ shelf life ~~of the consumable composition relative to~~than a nearly identical ~~consumable~~food composition

which does not comprise the~~lacking the recombinantly produced~~ gFPE~~ or comprising chicken egg white muramidase rather than the FPE~~.

11. The food composition of claim 10, wherein the food composition has a longer shelf life than the shelf life of a nearly identical product which comprises chicken egg-white muramidase rather than the gFPE.

12. The food composition of any one of claims 9-11, wherein the food composition has at least 0.05% gFPE by weight.

13. The food composition of any one of claims 9-12, wherein the food composition has at most 10% gFPE by weight.

14. The food composition of any one of claims 9-13, wherein the food composition is substantially free of microorganisms or bacterial cell-debris.

15. The food composition of any one of claims 9-14, wherein the food product does not comprise a gelling agent other than the gFPE.

16. The consumable composition of claim 1, wherein the consumable composition is a powder.

~~60. The method of any one of claims 55 to 59, wherein the recombinantly produced gFPE provides a gel-like texture to the consumable composition or increases the viscosity of the consumable composition.~~

~~61.~~17. The consumable composition~~method of any one~~ of claim~~s 55 to~~ 16~~0~~, wherein the consumable composition is a food ingredient~~product that is ready for consumption by a human/animal~~.

~~62. A method of producing an isolated xanthan gum product comprising the steps of:~~

~~a. providing *X. campestris* cells into a fermentation medium;~~

~~b. heat treating the cells at a temperature between 45-60°C with at a pH of between 8-10 and an alkaline protease, thereby producing a solution comprising cell debris;~~

~~c. adding to a solution comprising cell debris a foodstuff preserving enzyme (FPE) that is recombinantly produced and/or a recombinantly produced FPE comprising an amino acid sequence with at least 95% identity to SEQ ID NO: 1, thereby producing a xanthan gum solution;~~

~~d. adding an alcohol to the xanthan gum solution, thereby precipitating xanthan gum; and~~

~~e. isolating and drying the precipitated xanthan gum, thereby obtaining an isolated xanthan gum product.~~

~~63. The method of claim 62, wherein an amount of the FPE added is less than the amount of chicken egg white FPE that would be needed to produce an equivalent amount of the xanthan gum product under otherwise identical conditions.~~

~~64. The method of claim 62 or claim 63, further comprising a step of adjusting the pH of the composition after producing a solution comprising cell debris.~~

~~65. A method of preparing a consumable composition comprising combining a xanthan gum product produced according to the method of any one of claims 62 to 64 with one or more additional ingredients to form the consumable composition.~~

~~66. A food preservative comprising a recombinantly produced food preserving enzyme (FPE) with at least 95% identity to SEQ ID NO: 1.~~

18. A method of preparing a consumable composition comprising steps of:

a. providing an isolated foodstuff preserving enzyme (FPE) which is recombinantly produced, wherein the FPE is a goose type FPE (gFPE);

b. combining the gFPE with one or more consumable ingredients.

19. The method of claim 18, wherein the recombinantly produced gFPE is recombinantly produced in a yeast cell.

20. The method of claim 18, wherein the gFPE has an amino acid sequence with at least 95% sequence identity to one of SEQ ID NOs: 1-4.