

(12) **UK Patent**

(19) **GB**

(11) **2535864**

(13) **B**

(45) Date of B Publication

**20.01.2021**

(54) Title of the Invention: **Edible fungi**

(51) INT CL: **A23J 3/22** (2006.01)    **A23J 3/20** (2006.01)    **A23L 31/00** (2016.01)    **C12R 1/77** (2006.01)

(21) Application No: **1601239.5**

(22) Date of Filing: **22.01.2016**

(30) Priority Data:  
(31) **1501320**                      (32) **27.01.2015**                      (33) **GB**

(43) Date of A Publication: **31.08.2016**

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(58) Field of Search:  
As for published application 2535864 A viz:  
INT CL **A23J, A23L, C12R**  
Other: **WPI and EPODOC**  
updated as appropriate

Additional Fields  
Other: **None**

**GB 2535864 B**

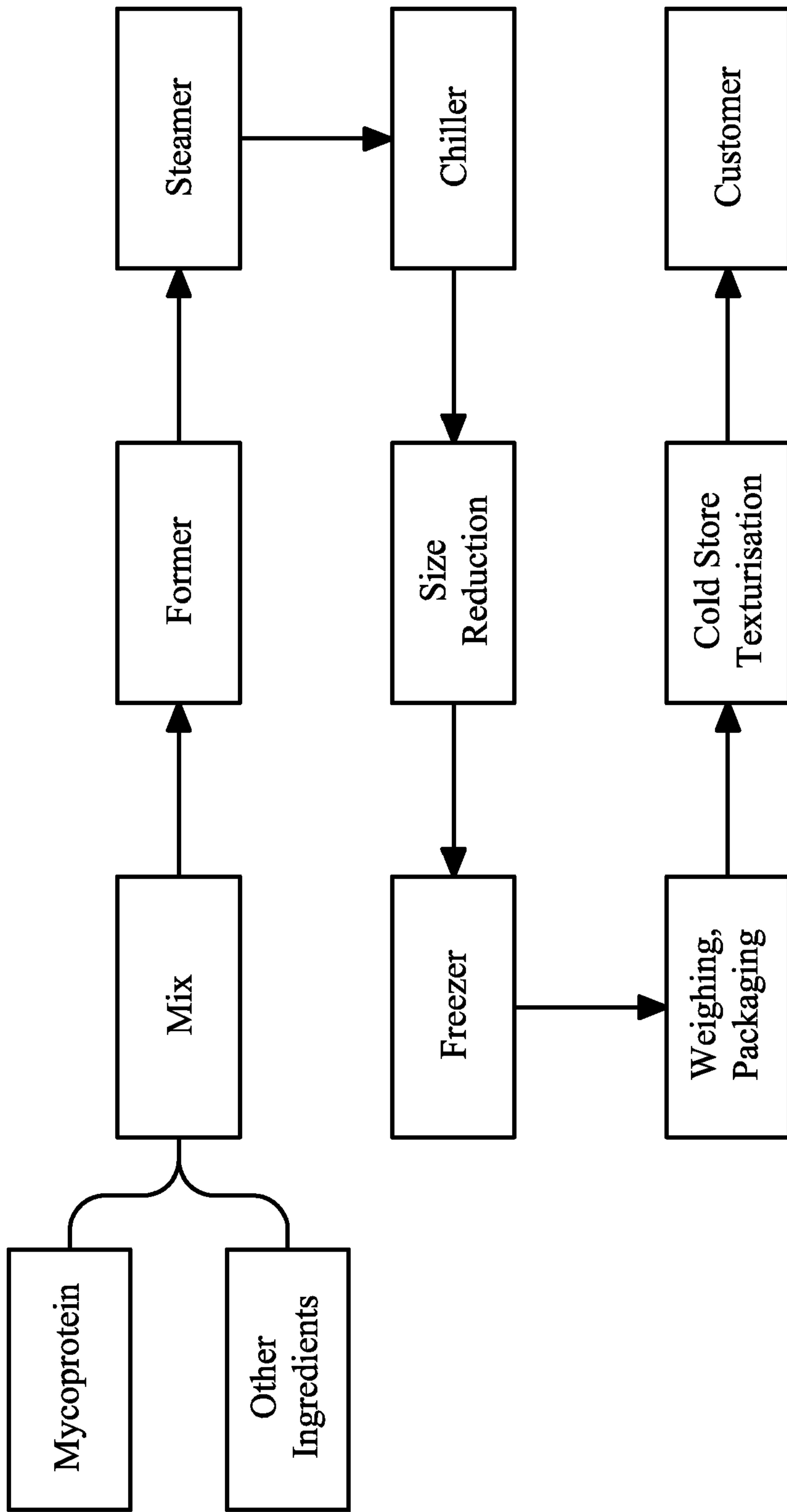


FIG. 1

## Edible Fungi

This invention relates to edible fungi and particularly, although not exclusively, relates to edible formulations comprising edible fungal particles for use as meat  
5 substitutes.

It is known, for example from WO 00/15045 (DSM), WO96/21362 (Zeneca) and  
WO95/23843 (Zeneca) to use edible filamentous fungi as meat-substitutes, for  
10 example in the preparation of burgers and sausages. In such uses, filaments of the  
fungi are bound together, for example with egg albumin, and are texturised so that the  
product resembles muscle fibres and therefore has a meat-like appearance and  
texture. Meat substitutes of the type described have been widely commercially  
available for many years under the trade mark QUORN.

15 In some circumstances, it is desirable to reduce or even eliminate the amount of  
egg albumin used with edible fungus in the manufacture of meat-substitutes for  
example on cost grounds or to produce a product suitable for vegans.

It is an object of the present invention to address the aforementioned problem.  
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According to a first object aspect of the invention, there is provided an edible  
formulation comprising edible fungal particles of a filamentous fungus and calcium  
ions.

25 One class of people may find it acceptable, generally, to avoid all animal  
products except dairy products. Thus, such a class of people may find an edible  
formulation which incorporates whey protein (e.g. up to about 10 wt% whey protein)  
as acceptable. Thus, in one embodiment, said edible formulation includes no animal  
products except, optionally, dairy products. However, in a second embodiment, said  
30 edible formulation is suitable for vegans. Consequently, in this embodiment, said  
edible formulation preferably includes 0 wt% of components derived from animals.

Thus, it follows from the aforementioned that said formulation preferably  
includes 0 wt% of a hydrocolloid derived from an animal source. Said formulation  
35 preferably includes 0 wt% of egg albumin.

Said edible formulation is preferably a meat substitute.

Said edible formulation suitably includes at least 5 wt%, preferably at least 15 wt%, more preferably at least 20 wt% of said filamentous fungus on a dry matter basis. In some cases, for example, when said edible formulation mimics a meat product (e.g. strips of meat or mince) which may be used as a food ingredient, said edible formulation may include higher levels of said filamentous fungus, for example greater than 40 wt% or greater than 60 wt% of filamentous fungus on a dry matter basis. Said edible formulation may include 5 to 80 wt%, preferably 15 to 75 wt% of filamentous fungus on a dry matter basis.

Said edible formulation suitably includes at least 2,000mg, preferably at least 4,000mg, more preferably at least 6,000mg, especially at least 8,000mg of calcium ions per Kg of filamentous fungus on a dry matter. Said edible formulation suitably includes less than 25,000mg, preferably less than 20,000mg, of said calcium ions per Kg of filamentous fungus on a dry matter basis.

Said edible formulation may include intracellular calcium ions (e.g. within the edible fungal particles) and extracellular calcium ions. Said edible formulation suitably includes the following extracellular levels of calcium ions: at least 2,000mg, preferably at least 4,000mg, more preferably at least 6,000mg, especially at least 8,000mg per Kg of filamentous fungus on a dry matter basis. The maximum extracellular level of calcium ions is suitably less than 25,000mg, preferably less than 20,000mg, per Kg of filamentous fungus on a dry matter basis.

The total amount of calcium ions in said edible formulation is suitably at least 5,000mg per Kg, preferably at least 10,000mg per Kg, of filamentous fungus on a dry matter basis. The total amount of calcium ions may be less than 40,000mg or less than 20,000mg per Kg of filamentous fungus.

Said edible formulation may include at least 0.100 wt%, preferably at least 0.200 wt%, more preferably at least 0.300 wt%, of calcium ions in total, on a dry weight basis. It may include less than 1 wt% or less than 0.8 wt% of calcium ions in total, on a dry weight basis.

Said edible formulation may include at least 50 wt%, preferably at least 60 wt%, water. Said formulation may include less than 85 wt% or less than 80 wt% water. Said edible formulation may include the aforementioned levels of water after a precursor of said edible formulation has been treated (e.g. cooked such as by steaming, chilling and/or freezing) to develop texture.

Said edible formulation may include a polysaccharide, for example a sulphonated polysaccharide, for example carrageenan. Said edible formulation, on a dry weight basis, suitably includes at least 0.1 wt%, preferably at least 0.5 wt%, more preferably at least 0.9 wt% of said polysaccharide, for example carrageenan. It may include less than 2 wt% of said polysaccharide.

Said edible formulation may include an alginate, for example a salt of alginic acid such as sodium alginate. Said edible formulation, on a dry weight basis, suitably includes 0.01 wt%, preferably at least 0.05 wt%, more preferably at least 0.12 wt% of alginate. It may include less than 1 wt%, preferably less than 0.5 wt% of alginate.

Said edible formulation may include gluten, for example wheat gluten. Said edible formulation, on a dry weight basis, suitably includes at least 0.1 wt%, preferably at least 1 wt%, more preferably at least 1.5 wt% of said gluten. It may include less than 5 wt% of said gluten.

Said edible formulation may include a protein source in addition to said edible fungal particles and which is not a wheat-based protein. Said protein source (A) may be a source of vegetable protein. Said protein source (A) may be a potato protein source. Said edible formulation, on a dry weight basis, may include at least 1 wt%, preferably at least 5 wt% of protein source (A). It may include less than 20 wt% of protein source (A).

It is found that, if calcium chloride alone is used in the formulation, even at a minimum level to achieve the effect described herein, a disagreeable taste may result. Accordingly, steps may be taken to counter the disagreeable taste. It has been found that use of calcium acetate is able to do this. Thus, said edible formulation preferably includes acetate moieties (which may have been initially incorporated into the formulation as calcium acetate).

In said edible formulation, the ratio of the wt% of acetate ions divided by the wt% of filamentous fungus on a dry matter basis is suitably at least 0.005, preferably at least 0.01. Said ratio may be less than 0.04, for example less than 0.03.

5 Said acetate ions are suitably extra-cellular ions.

Said edible formulation may include at least 0.10 wt%, preferably at least 0.40 wt% of acetate ions on the dry matter basis. It may include less than 2.00 wt%, for example less than 1.00 wt% of acetate ions on a dry matter basis.

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In a first preferred embodiment, said edible formulation may include:

- at least 2,000mg, for example at least 5,000mg, of calcium ions per Kg of filamentous fungus on a dry matter basis;
- 15 - at least 50 wt% of water.

In a second preferred embodiment, said edible formulation may include:

- 5,000mg of calcium ions per Kg of filamentous fungus on a dry matter basis;
- 20 - less than 25,000mg of calcium ions per Kg of filamentous fungus on a dry matter basis;
- at least 50 wt% of water;
- 0 wt% of egg albumin.

25 In said first and second preferred embodiments, preferably in said edible formulation, the ratio of the wt% of acetate ions divided by the wt% of filamentous fungus on a dry matter basis is at least 0.005 and is less than 0.03.

Said edible formulation preferably includes at least 0.10 wt%, preferably at least 0.50 wt%; and suitably includes less than 1.5 wt%, or less than 1.1 wt% of acetate ions, on a dry matter basis.

35 As described, fungal particles suitably comprise a filamentous fungus. Said filamentous fungus preferably comprises fungal mycelia and suitably at least 80 wt%, preferably at least 90 wt%, more preferably at least 95 wt% and, especially, at least 99 wt% of the fungal particles in said formulation comprise fungal mycelia. Some

filamentous fungi may include both fungal mycelia and fruiting bodies. Said fungal particles preferably comprise a filamentous fungus of a type which does not produce fruiting bodies. Where, however, a filamentous fungus of a type which produces fruiting bodies is used, the fungal particles in said composition suitably include at least 5 80 wt%, preferably at least 90 wt%, more preferably at least 95 wt% of fungal mycelia. Preferably, said fungal particles comprise substantially only fungal mycelia - that is, said fungal particles in said composition preferably do not include any fruiting bodies.

Preferred fungi for said fungal particles have a cell wall which includes chitin and/or chitosan. Preferred fungi have a cell wall which includes polymeric glucosamine. Preferred fungi have a cell wall which includes  $\beta$ 1-3 and 1-6 glucans. 10

Said fungal particles may include fungal cells of the order Mucorales as described in WO 00/15045 (DSM). 15

Said fungal particles preferably comprise fungus selected from fungi imperfecti.

Preferably, said fungal particles comprise, and preferably consist essentially of, cells of *Fusarium* species, especially of *Fusarium venenatum* A3/5 (formerly classified as *Fusarium graminearum*) (IMI 145425; ATCC PTA-2684 deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA.) as described for example in WO96/21361 (Zeneca) and WO95/23843 (Zeneca). 20

Preferably, said fungal particles are non-viable. Preferably, said fungal particles have been treated to lower the level of RNA which they contain. Thus, the level of RNA in the fungal particles used is preferably less than the level in an identical fungus when in a viable state. The level of RNA may be reduced as described in WO95/23843. Said fungal particles suitably have a RNA content on a dry weight basis of less than 1.9 wt%, for example 1.7 wt% or less. 25

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Fungal particles in said formulation may comprise filaments having lengths of less than 1000  $\mu\text{m}$ , preferably less than 800  $\mu\text{m}$ . Said filaments may have a length greater than 100  $\mu\text{m}$ , preferably greater than 200  $\mu\text{m}$ . Preferably, fewer than 5 wt%, preferably substantially no, fungal particles in said formulation have lengths of greater than 5000 $\mu\text{m}$ ; and preferably fewer than 5 wt %, preferably substantially no, fungal particles have lengths of greater than 2500  $\mu\text{m}$ . Preferably, values for the number 35

average of the lengths of said fungal particles in said formulation are also as stated above.

5 Fungal particles in said formulation may comprise filaments having diameters of less than 20  $\mu\text{m}$ , preferably less than 10  $\mu\text{m}$ , more preferably 5  $\mu\text{m}$  or less. Said filaments may have diameters greater than 1  $\mu\text{m}$ , preferably greater than 2  $\mu\text{m}$ . Preferably, values for the number average of said diameters of said fungal particles in said formulation are also as stated above.

10 Fungal particles in said formulation may comprise filaments having an aspect ratio (length/diameter) of less than 1000, preferably less than 750, more preferably less than 500, especially of 250 or less. The aspect ratio may be greater than 10, preferably greater than 40, more preferably greater than 70. Preferably, values for the average aspect ratio of said fungal particles (i.e. the average of the lengths of the  
15 particles divided by the average of the diameters of the fungal particles) in said formulation are also as stated above.

In said edible formulation, the ratio of the wt% of filamentous fungus (on a dry matter basis) divided by the wt% of water is at least 0.05, preferably at least 0.10.  
20 The ratio may be less than 0.5 or less than 0.4.

Said edible formulation may be provided in a package. Thus, said edible formulation is suitably surrounded by packaging material which may comprise a receptacle. Said package may include at least 50g or at least 100g of said edible  
25 formulation. Said package may include said edible formulation and include at least 10g of edible fungal particles on a dry matter basis. Said package may include an edible formulation which includes at least 20 wt%, for example at least 45 wt% water.

According to a second aspect of the invention, there is provided a method of  
30 making an edible formulation, the method comprising:

- (i) selecting a formulation comprising edible fungal particles of a filamentous fungus;
- (ii) contacting said formulation with calcium ions.



Contact may be effected so as to produce an edible formulation with calcium ions as described in the first aspect and/or at levels described in the first aspect. Thus, the edible formulation prepared may be as described in the first aspect.

5 Said edible fungal particles selected in step (i) are suitably fungal particles which have been treated after removal from a reactor in which the particles are produced in a fermentation process.

Said edible fungal particles selected in step (i) are preferably not viable.

10 Said edible fungal particles selected in step (i) preferably have been treated to lower the level of RNA they contain.

Said edible fungal particles selected in step (i) preferably have an RNA content on a dry weight basis of less than 1.9 wt%, for example 1.7 wt% or less.

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Any feature of any aspect of any invention described herein may be combined with any other invention described herein mutatis mutandis.

20 Specific embodiments of the invention will now be described, by way of example, with reference to the accompanying drawing, in which Figure 1 is a schematic representation of steps in the production of mycoprotein-containing products for human consumption.

The following materials are referred to hereinafter:

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Mycoprotein paste – Mycoprotein paste-refers to a visco-elastic material comprising a mass of edible filamentous fungus derived from *Fusarium venenatum* A3/5 (formerly classified as *Fusarium graminearum* Schwabe) (IMI 145425; ATCC PTA-2684 deposited with the American type Culture Collection, 12301 Parklawn Drive, Rockville Md. 20852) and treated to reduce its RNA content to less than 2% by weight by heat treatment. Further details on the material are provided in WO96/21362 and WO95/23843. The material may be obtained from Marlow Foods Limited of Stokesley, U.K. It comprises about 23-25 wt % solids (the balance being water) made up of non-viable RNA reduced fungal hyphae of approximately 400-750  $\mu\text{m}$  length, 3-  
35 5  $\mu\text{m}$  in diameter and a branching frequency of 2-3 tips per hyphal length.

Calcium chloride solution – refers to a 36 wt% aqueous solution of calcium chloride.

Calcium acetate – refers to calcium acetate in solid form.

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Sodium alginate refers to sodium alginate in a solid form.

In the examples which follow, Example 1 provides a general method of producing mycoprotein-containing products. Example 2 - 5 provide details of specific products which are suitable for consumption by vegans. It is found that the addition of calcium cations (via calcium chloride and calcium acetate) to the ingredients described in the examples produce a rise in firmness and produces acceptable quality of the product produced. It is believed the calcium cations interact with the mycoprotein paste to increase its firmness and strength.

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#### Example 1 – General process for preparation of products

This is summarized in Figure 1. Mycoprotein paste is mixed with other ingredients it is desired to incorporate to produce a substantially homogenous mass of a mycoprotein-containing foodstuff (for example meat-like pieces, mince, sausages and roast meats). The homogenous mass is put through a former and then a steamer (e.g. over 95°C for 35-45 minutes). The steamed product is then chilled (e.g. -5 to -10°C for about 20 minutes) which improves the texture of the product by making it slightly firmer. There follows an optional size reduction process followed by a second texturization step involving freezing. Thereafter, products are weighed and packaged prior to the final texturization step at -18°C in a cold store for at least 7 days. Thereafter product can be delivered to retail outlets for sale to customers.

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#### Example 2 – Preparation of mycoprotein-containing pieces

Following the general procedure described in Example 1, the ingredients referred to in Table 1 were combined to produce the final product.

Table 1

Vegan Pieces recipe (Table 1)		
Ingredient	Wet weight (g/kg)	Dry weight g/kg
Mycoprotein Paste	884.20	221.00
Water	12.00	<1.00
Flavour 1	10.00	9.50
Potato Protein	32.00	30.40
Vital Wheat Gluten	10.00	9.50
Calcium Acetate	4.00	3.80
Calcium Chloride Solution	12.00	4.30
Sodium Alginate	0.80	0.76
Flavour 2	3.00	2.85
Carageenan	4.00	3.80
Wheat Fibre	20.00	19.00
Pea Fibre	8.00	7.60
<b>Total</b>	<b>1000.00</b>	<b>312.60</b>

- 5 It should be appreciated that the steaming/chilling process affects the level of water in the final product which is generally in the range 70 to 77 wt% in total.

Example 3 – Preparation of mycoprotein-containing mince

- 10 Following the general procedure described in Example 1, the ingredients referred to in Table 2 were combined to produce the final product.

Table 2

<b>Vegan Mince recipe</b>		
<b>Ingredient</b>	<b>Wet weight (g/kg)</b>	<b>Dry Weight(g/kg)</b>
<b>Mycoprotein Paste</b>	<b>888.20</b>	<b>222.00</b>
<b>Water</b>	<b>0.00</b>	<b>0.00</b>
<b>Potato Protein</b>	<b>50.00</b>	<b>47.50</b>
<b>Calcium acetate</b>	<b>4.00</b>	<b>3.80</b>
<b>Calcium Chloride Sodium</b>	<b>12.00</b>	<b>4.20</b>
<b>Sodium Alginate</b>	<b>0.80</b>	<b>0.76</b>
<b>Vital Wheat Gluten</b>	<b>10.00</b>	<b>9.50</b>
<b>Malt Extract</b>	<b>6.00</b>	<b>5.70</b>
<b>Caramelized Sugar</b>	<b>9.00</b>	<b>8.55</b>
<b>Wheat Fibre</b>	<b>14.00</b>	<b>13.30</b>
<b>Flavour</b>	<b>2.00</b>	<b>1.90</b>
<b>Carageenan</b>	<b>4.00</b>	<b>3.80</b>
<b>Total</b>	<b>1000.00</b>	<b>321.00</b>

5 Example 4 – Preparation of mycoprotein-containing burger

Following the general procedure described in Example 1, the ingredients referred to in Table 3 were combined to produce the final product.

Table 3

<b>Vegan Burger Recipe</b>		
<b>Ingredient</b>	<b>Wet Weight (g/kg)</b>	<b>Dry Weight (g/kg)</b>
<b>Mycoprotein Paste</b>	<b>385.20</b>	<b>176.60</b>
<b>Water</b>	<b>171.10</b>	<b>&lt;1.00</b>
<b>Onions</b>	<b>100.00</b>	<b>15.00</b>
<b>Meatless Mince</b>	<b>83.30</b>	<b>16.70</b>
<b>Malt Extract</b>	<b>10.00</b>	<b>9.50</b>
<b>Texturized Wheat Protein</b>	<b>83.30</b>	<b>79.10</b>
<b>Oil</b>	<b>20.50</b>	<b>20.50</b>
<b>Flavour 1</b>	<b>30.00</b>	<b>28.50</b>
<b>Flaked Fat</b>	<b>38.88</b>	<b>36.90</b>
<b>Calcium Chloride Solution</b>	<b>3.90</b>	<b>1.40</b>
<b>Calcium Acetate</b>	<b>3.90</b>	<b>3.70</b>
<b>Flavour 2</b>	<b>3.00</b>	<b>2.90</b>
<b>Wheat Fibre</b>	<b>20.00</b>	<b>19.00</b>
<b>Carageenan</b>	<b>4.00</b>	<b>3.80</b>
<b>Sodium Alginate</b>	<b>0.80</b>	<b>0.76</b>
<b>Vital Wheat Gluten</b>	<b>10.00</b>	<b>9.50</b>
<b>Potato Protein</b>	<b>32.00</b>	<b>30.40</b>
<b>Total Weight</b>	<b>1000.00</b>	<b>455.00</b>

Example 5 – Preparation of mycoprotein-containing sausage

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Following the general procedure described in Example 1, the ingredients referred to in Table 4 were combined to produce the final product.

Table 4

<b>Vegan Sausage Recipe</b>		
<b>Ingredient</b>	<b>Wet Weight (g/kg)</b>	<b>Dry Weight (g/kg)</b>
<b>Mycoprotein Paste</b>	<b>431.60</b>	<b>107.90</b>
<b>Water</b>	<b>170.00</b>	<b>&lt;1.00</b>
<b>Oil</b>	<b>60.00</b>	<b>60.00</b>
<b>Onions</b>	<b>62.50</b>	<b>9.40</b>
<b>Pea Fibre</b>	<b>6.00</b>	<b>5.70</b>
<b>Textured Wheat Protein</b>	<b>37.50</b>	<b>35.60</b>
<b>Rusk</b>	<b>68.80</b>	<b>65.30</b>
<b>Meatless Mince</b>	<b>43.80</b>	<b>8.80</b>
<b>Tapioca Starch</b>	<b>10.00</b>	<b>9.50</b>
<b>Seasoning</b>	<b>31.20</b>	<b>29.70</b>
<b>Calcium Chloride Solution</b>	<b>4.40</b>	<b>1.40</b>
<b>Calcium Acetate</b>	<b>4.40</b>	<b>4.20</b>
<b>Flavour</b>	<b>3.00</b>	<b>2.80</b>
<b>Wheat Fibre</b>	<b>20.00</b>	<b>19.00</b>
<b>Carageenan</b>	<b>4.00</b>	<b>3.80</b>
<b>Sodium Alginate</b>	<b>0.80</b>	<b>0.76</b>
<b>Vital Wheat Gluten</b>	<b>10.00</b>	<b>9.50</b>
<b>Potato Protein</b>	<b>32.00</b>	<b>30.40</b>
<b>Total</b>	<b>1000.00</b>	<b>405.00</b>

The invention is not restricted to the details of the foregoing embodiment(s). The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

## Claims

1. An edible formulation comprising edible fungal particles of a filamentous fungus and calcium ions;
- 5 wherein said edible formulation includes 0 wt% of components derived from animals;
- wherein said edible formulation includes at least 20 wt% of said filamentous fungus on a dry matter basis;
- 10 wherein said edible formulation includes at least 0.100 wt% and less than 1 wt% of calcium ions in total, on a dry weight basis and wherein said edible formulation includes at least 50 wt% of water;
- wherein said edible formulation includes acetate moieties; and
- 15 wherein said edible fungal particles comprise filaments with an aspect ratio of greater than 40 and wherein said edible fungal particles have a number average length of greater than 200  $\mu\text{m}$ ;
- wherein said edible formulation is provided in a package which includes at least 50g of said edible formulation.
2. A formulation according to claim 1, wherein said edible formulation includes at least 8,000mg, of calcium ions per Kg of filamentous fungus on a dry matter.
- 20
- 3., A formulation according to claim 1 or claim 2, wherein said edible formulation includes at least 2,000mg, preferably at least 8,000mg, per Kg of filamentous fungus on a dry matter basis, of extracellular calcium ions.
- 25
4. A formulation according to any preceding claim, wherein the total amount of calcium ions in said edible formulation is at least 5,000mg per Kg; and may be less than 40,000mg per Kg of filamentous fungus.
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- 5.. A formulation according to any preceding claim, wherein said edible formulation includes a polysaccharide, for example a sulphonated polysaccharide.
- 6 A formulation according to any preceding claim, wherein said edible formulation includes an alginate.
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7. A formulation according to any preceding claim, wherein said edible formulation includes gluten.

8. A formulation according to any preceding claim, wherein said edible formulation includes a protein source (A) in addition to said edible fungal particles.

9. A formulation according to claim 8, wherein said protein source (A) is a source of vegetable protein but is not a wheat-based protein.

10. A formulation according to claim 8 or claim 9, wherein said edible formulation, on a dry weight basis, includes at least 1 wt%, preferably at least 5 wt%, of protein source (A); and it may include less than 20 wt% of protein source (A).

11. A formulation according to any preceding claim, wherein in said edible formulation, the ratio of the wt% of acetate ions divided by the wt% of filamentous fungus on a dry matter basis is at least 0.005 and may be less than 0.04.

12. A formulation according to any preceding claim, wherein said edible formulation includes at least 0.10 wt% and may include less than 2.00 wt% of acetate ions on a dry matter basis.

13. A formulation according to any preceding claim, wherein said edible formulation includes:

- 5,000mg of calcium ions per Kg of filamentous fungus on a dry matter basis;
- less than 25,000mg of calcium ions per Kg of filamentous fungus on a dry matter basis.

14. A formulation according to any preceding claim, wherein in said edible formulation, the ratio of the wt% of acetate ions divided by the wt% of filamentous fungus on a dry matter basis is at least 0.005.

15. A formulation according to any preceding claim, wherein said edible formulation includes at least 0.10 wt% and suitably includes less than 1.5 wt% of acetate ions, on a dry matter basis.



16. A formulation according to any preceding claim, wherein said fungal particles have a RNA content on a dry weight basis of less than 1.9 wt%.

5 17. A formulation according to any preceding claim, wherein in said edible formulation, the ratio of the wt% of filamentous fungus (on a dry matter basis) divided by the wt% of water is at least 0.05 and said ratio may be less than 0.5.

18 A formulation according to any preceding claim, wherein said package includes  
10 at least 100g of said edible formulation.

19 A formulation according to any preceding claim, wherein said edible formulation is surrounded by packaging material which comprises a receptacle.

15 20 A formulation according to any preceding claim, wherein said package includes said edible formulation and includes at least 10g of edible fungal particles on a dry matter basis.

20 21. A formulation according to any preceding claim, wherein said fungal particles comprise fungus selected from fungi imperfecti.

22. A formulation according to any preceding claim, wherein said fungal particles comprise cells of *Fusarium* species.

25 23. A formulation according to any preceding claim, wherein said fungal particles comprise cells of *Fusarium venenatum* A3/5.

24. A method of making an edible formulation as described in any preceding claim, the method comprising:

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(i) selecting a formulation comprising edible fungal particles of a filamentous fungus;

(ii) contacting said formulation with calcium ions.