

READY-TO-EAT AND READY-TO-DRINK PRODUCTS

TECHNICAL FIELD

5 The present invention relates generally to methods for making flavour modifying ingredients using dietary fibre and/or other edible cereal components and the flavour modifying ingredients made by said methods. The present invention further relates to flavour compositions and food compositions comprising said flavour modifying ingredients and the uses of said flavour modifying ingredients in food compositions, for example to improve mouthfeel of the food composition and/or mask off-notes of the food composition and/or improve sweetness of the food composition and/or enhance saltiness of the food composition. The present invention further relates to ready-to-eat and ready-to-drink products and methods of making the same using at least one edible cereal component.

15

BACKGROUND

There exists a need in the food industry to provide ingredients which can modify the flavour of various food products, for example to improve the mouthfeel, mask off-notes, improve sweetness, and/or enhance saltiness. In particular, a need exists to provide flavour modifying ingredients which are natural and/or suitable for vegans. Novel flavour modifying ingredients and methods for making said flavour modifying ingredients are therefore provided by the present invention. There also exists a need in the food industry to provide clean-label products that contain as few ingredients as possible, and which are generally recognized as natural, familiar, and simple ingredients. Typically, in order to provide ready-to-eat or ready-to-drink products with the desired organoleptic properties, the addition of other ingredients such as proteins, gums and stabilizers are required. Accordingly, such products are not considered clean-label products. Novel clean-label ready-to-eat and ready-to-drink products and methods for making said products are therefore provided by the present invention.

30

SUMMARY OF THE INVENTION

In accordance with a first aspect of the present invention there is provided a method for making a flavour modifying ingredient, the method comprising subjecting a dietary fibre to enzymatic hydrolysis and/or fermentation.

35

The method described in WO 2010/053653 A1 and the method described in US 2009/0311376 A1 are excluded from the method of the first aspect of the present invention.

- 5 For example, the method of the first aspect of the present invention may exclude a method comprising (a) contacting a fiber-digesting enzyme with a suspension comprising an amount of water and cleaned whole grain oat flour, and (b) treating the suspension for a period of time sufficient to hydrolyze fiber particles such that a modified whole grain oat flour is formed. For example, the method of the first aspect of
10 the present invention may exclude a method comprising contacting a fiber-digesting enzyme with a suspension comprising an amount of water and cleaned whole grain oat flour.

- For example, the method of the first aspect of the present invention may exclude a
15 method comprising combining a whole oat or barley flour starting mixture and a suitable enzyme to form an enzyme starting mixture, heating the enzyme starting mixture to between about 120°F and about 200°F to begin to hydrolyze the starch molecules, and extruding the resultant mixture to continue hydrolyzing the starch and further to gelatinize and cook the mixture to form the soluble oat or barley flour. For
20 example, the method of the first aspect of the present invention may exclude a method comprising combining a whole oat or barley flour starting mixture and a suitable enzyme to form an enzyme starting mixture, and heating the enzyme starting mixture to between about 120°F and about 200°F to begin to hydrolyze the starch molecules.

- 25 For example, the dietary fibre may not be cleaned whole grain oat flour. For example, the dietary fibre may not be whole oat flour and/or may not be oat flour and/or may not be barley flour. For example, the dietary fibre may not be oat flour and/or may not be barley flour. For example, the dietary fibre may not be oat fibre and/or may not be
30 barley fibre.

In certain embodiments, the dietary fibre is isolated dietary fibre. In certain embodiments, the dietary fibre is an aqueous slurry of dietary fibre.

- In certain embodiments, the dietary fibre is a cereal fibre (e.g. oat fibre), a vegetable
35 fibre (e.g. pea fibre), or a fruit fibre (e.g. citrus fruit fibre, apple fibre, blueberry fibre, cranberry fibre, grape fibre).

In certain embodiments, the enzymatic hydrolysis uses one or more enzymes selected from carbohydrases and proteolytic enzymes. In certain embodiments, the enzymatic hydrolysis uses at least one or more enzymes selected from cellulases, pectinases, and other carbohydrases. In certain embodiments, the enzymatic hydrolysis excludes
5 proteolytic enzymes.

In certain embodiments, the fermentation uses a lactic acid bacterium (e.g. *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *L. delbrueckii ssp. bulgaricus*, *Streptococcus thermophilus* and/or *Lactobacillus acidophilus*) and/or a
10 *Bifidobacterium* and/or an *Aspergillus* fungus (e.g. *Aspergillus oryzae*).

In certain embodiments, the enzymatic hydrolysis is performed at a temperature ranging from about 25°C to about 60°C.

15 In certain embodiments, the enzymatic hydrolysis takes place for a period of time ranging from about 1 hour to about 48 hours.

In certain embodiments, the fermentation is performed at a temperature ranging from about 20°C to about 45°C.

20

In certain embodiments, the fermentation takes place for a period of time ranging from about 1 day to about 10 days.

In certain embodiments, the method of the first aspect of the present invention
25 comprises subjecting the dietary fibre to enzymatic hydrolysis and fermentation. In certain embodiments, the enzymatic hydrolysis takes place before and/or simultaneously with the fermentation.

In certain embodiments, the method of the first aspect of the present invention
30 comprises subjecting a dietary fibre to enzymatic hydrolysis and does not comprise subjecting the dietary fibre to fermentation.

In certain embodiments, the method of the first aspect of the present invention
35 comprises subjecting a dietary fibre to fermentation and does not comprise subjecting the dietary fibre to enzymatic hydrolysis.

In certain embodiments, the method of the first aspect of the present invention further comprises heating the dietary fibre to a temperature equal to or greater than about 75°C prior to the enzymatic hydrolysis and fermentation.

- 5 In certain embodiments, the method of the first aspect of the present invention further comprises deactivating the enzyme and/or the fermentation microorganism following the enzymatic hydrolysis and/or fermentation.

- 10 In certain embodiments, the method of the first aspect of the present invention further comprises combining the flavour modifying ingredient with propylene glycol.

In certain embodiments, the method of the first aspect of the present invention further comprises spray-drying the flavour modifying ingredient.

- 15 In accordance with a second aspect of the present invention there is provided a flavour modifying ingredient obtainable by and/or obtained by the method of the first aspect of the present invention, including any embodiment therefore.

- 20 In accordance with a third aspect of the present invention there is provided a flavour composition comprising the flavour modifying ingredient of the second aspect of the present invention.

- 25 In accordance with a fourth aspect of the present invention there is provided a food product comprising the flavour modifying ingredient of the second aspect of the present invention.

- 30 In accordance with the fifth aspect of the present invention there is provided the use of a flavour modifying ingredient of the second aspect of the present invention to improve the mouthfeel of a food product.

- 35 In accordance with a sixth aspect of the present invention there is provided a method of providing a food product having an improved mouthfeel, the method comprising admixing the flavour modifying ingredient of the second aspect of the present invention to the food product.

In accordance with the seventh aspect of the present invention there is provided the use of a flavour modifying ingredient of the second aspect of the present invention to mask off-notes of a food product.

- 5 In accordance with an eighth aspect of the present invention there is provided a method of providing a food product having reduced off-notes, the method comprising admixing the flavour modifying ingredient of the second aspect of the present invention to the food product.

- 10 In accordance with the ninth aspect of the present invention there is provided the use of a flavour modifying ingredient of the second aspect of the present invention to improve the sweetness of a food product.

- In accordance with a tenth aspect of the present invention there is provided a method
15 of providing a food product having improved sweetness, the method comprising admixing the flavour modifying ingredient of the second aspect of the present invention to the food product.

- In accordance with the eleventh aspect of the present invention there is provided the
20 use of a flavour modifying ingredient of the second aspect of the present invention to enhance the saltiness of a food product.

- In accordance with a twelfth aspect of the present invention there is provided a method
of providing a food product having enhanced saltiness, the method comprising
25 admixing the flavour modifying ingredient of the second aspect of the present invention to the food product.

- In accordance with a thirteenth aspect of the present invention there is provided a
method for making a ready-to-eat or ready-to-drink product, the method comprising
30 subjecting at least one edible component of a cereal to fermentation, or to fermentation and enzymatic hydrolysis, wherein the fermentation uses two or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, and/or
35 *Bifidobacterium animalis lactis*, such as, for example, *Bifidobacterium animalis lactis* also known as BB-12® from Chr. Hansen or *Bifidobacterium animalis lactis* also known

as Probiotic BifidoBHN019 or DR10 or B019, wherein the cereal is selected from the group consisting of oat, maize, rice, wild rice, wheat, barley, sorghum, millet, rye, triticale, fonio or combinations thereof.

- 5 In certain embodiments of the method of the thirteenth aspect of the present invention, the at least one edible cereal component is in the form of, or derived from, cereal grain, cereal wholegrain, cereal groats, steel cut cereal, rolled cereal, cereal bran, cereal flour, cereal kernel, cereal fibre, irish oats or combinations thereof.
- 10 In certain embodiments, the method of the thirteenth aspect of the present invention comprises an aqueous slurry of the at least one edible cereal component.

In certain embodiments, the method of the thirteenth aspect of the present invention comprises using one or more enzymes selected from carbohydrases and proteolytic
15 enzymes.

In certain embodiments, the method of the thirteenth aspect of the present invention comprises using at least one or more enzymes selected from cellulases, pectinases, and other carbohydrases.
20

In certain embodiments, the method of the thirteenth aspect of the present invention comprises performing enzymatic hydrolysis at a temperature ranging from about 25°C to about 60°C.

25 In certain embodiments, the method of the thirteenth aspect of the present invention comprises performing enzymatic hydrolysis for a period of time ranging from about 1 hour to about 48 hours.

In certain embodiments, the method of the thirteenth aspect of the present invention
30 comprises performing fermentation at a temperature ranging from about 20°C to about 45°C.

In certain embodiments, the method of the thirteenth aspect of the present invention comprises performing fermentation for a period of time ranging from about 10 hours to
35 about 10 days.

In certain embodiments of the method of the thirteenth aspect of the present invention, the enzymatic hydrolysis occurs before and/or simultaneously with the fermentation.

In certain embodiments, the method of the thirteenth aspect of the present invention comprises subjecting the at least one edible cereal component to fermentation and does not comprise subjecting the at least one edible cereal component to enzymatic hydrolysis.

5

In certain embodiments, the method of the thirteenth aspect of the present invention comprises heating the at least one edible cereal component to a temperature equal to or greater than about 75°C prior to the enzymatic hydrolysis and fermentation.

10 In certain embodiments, the cereal of the thirteenth aspect of the present invention comprises oat.

In certain embodiments, the edible cereal component of the thirteenth aspect of the present invention comprises oat flour.

15

In certain embodiments, the method of the thirteenth aspect of the present invention further comprises spray-drying the ready-to-eat product.

20 In certain embodiments of the method of the thirteenth aspect of the present invention, the at least one cereal fibre comprises oat fibre, maize fibre, rice fibre, wild rice fibre, wheat fibre, barley fibre, sorghum fibre, millet fibre, rye fibre, triticale fibre, fonio fibre, or combinations thereof.

25 In certain embodiments of the method of the thirteenth aspect of the present invention, the at least one cereal fibre comprises oat fibre.

In certain embodiments of the method of the thirteenth aspect of the present invention, the edible cereal component is in the form of, or derived from, oat grain, oat wholegrain, oat groats, steel cut oats, rolled oats, oat bran, oat flour, oat kernels, oat
30 fibre, or combinations thereof.

In certain embodiments, the method of the thirteenth aspect of the present invention comprises using three or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus*
35 *bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, and/or *Bifidobacterium animalis lactis*, such as, for example, *Bifidobacterium animalis lactis* also known as BB-12® from Chr.

Hansen or *Bifidobacterium animalis lactis* also known as Probiotic BifidoBHN019 or DR10 or B019.

5 In accordance with a fourteenth aspect of the present invention there is provided a ready-to-eat or ready-to-drink product obtainable by and/or obtained by the method of the thirteenth aspect of the present invention.

10 In accordance with a fifteenth aspect of the present invention there is provided a ready-to-eat product obtained by mixing at least one edible component of a cereal in an aqueous solution, wherein the cereal is selected from the group consisting of oat, maize, rice, wild rice, wheat, barley, sorghum, millet, rye, triticale, fonio and combinations thereof, adding two or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, and/or *Bifidobacterium animalis lactis*, such as, for example, *Bifidobacterium animalis lactis* also known as BB-12® from Chr. Hansen or *Bifidobacterium animalis lactis* also known as Probiotic BifidoBHN019 or DR10 or B019 to the mixture, and incubating the mixture for a period of time sufficient
20 to ferment at least a portion of the at least one edible cereal component to form the ready-to-eat product.

In certain embodiments, the ready-to-eat product of the fifteenth aspect of the present invention is yoghurt type.

25 In certain embodiments of the ready-to-eat product of the fifteenth aspect of the present invention, the edible cereal component is in the form of, or derived from, cereal grain, cereal wholegrain, cereal groats, steel cut cereal, rolled cereal, cereal bran, cereal flour, cereal kernel, cereal fibre, or combinations thereof.

30 In certain embodiments of the ready-to-eat product of the fifteenth aspect of the present invention, the edible cereal component is in the form of, or derived from, oat grain, oat wholegrain, oat groats, steel cut oats, rolled oats, oat bran, oat flour, oat kernels, oat fibre, or combinations thereof

35 In accordance with a sixteenth aspect of the present invention there is provided a ready-to-drink product obtained by mixing at least one edible component of a cereal in

an aqueous solution, wherein the cereal is selected from the group consisting of oat, maize, rice, wild rice, wheat, barley, sorghum, millet, rye, triticale, fonio and combinations thereof, adding carbohydrase and/or proteolytic enzymes to the mixture, subsequently adding two or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*,
5 *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, and/or *Bifidobacterium animalis lactis*, such as, for example, *Bifidobacterium animalis lactis* also known as BB-12® from Chr.
10 Hansen or *Bifidobacterium animalis lactis* also known as Probiotic BifidoBHN019 or DR10 or B019 to the mixture, incubating the mixture for a period of time sufficient to ferment at least a portion of the at least one edible cereal component to form the ready-to-drink product.

15 In certain embodiments, the ready-to-drink product of the sixteenth aspect of the present invention is oat milk.

In certain embodiments of the ready-to-drink product of the sixteenth aspect of the present invention, the edible cereal component is in the form of, or derived from,
20 cereal grain, cereal wholegrain, cereal groats, steel cut cereal, rolled cereal, cereal bran, cereal flour, cereal kernel, cereal fibre, or combinations thereof.

In certain embodiments of the ready-to-drink product of the sixteenth aspect of the present invention, the edible cereal component is in the form of, or derived from,
25 oat grain, oat wholegrain oat groats, steel cut oats, rolled oats, oat bran, oat flour, oat kernels, oat fibre, or combinations thereof

In accordance with a seventeenth aspect of the present invention there is provided a consumable product obtainable by and/or obtained by the method of the thirteenth
30 aspect of the present invention.

There is a consumer demand for food products to contain wholefood ingredients and be processed to as little degree as possible. Some consumers wish to avoid wheat flour and other flours that contain gluten. Accordingly, there is consumer demand for
35 low-gluten or gluten-free products. In certain embodiments of the method of the seventeenth aspect of the present invention, the consumable product is a clean-label dairy alternative product that is considered gluten free as it has a gluten content less than 5 ppm, which is significantly lower than the U.S. Food and Drug Administration

(FDA) definition of "gluten-free" of less than 20 ppm. In certain embodiments of the method of the seventeenth aspect of the present invention, the consumable product is a clean-label dairy alternative product that is considered gluten free as it has a gluten content less than 20 ppm. Gluten free ready-to-drink oat products may be obtained
5 from the methods according to Examples 22 and 23 herein. Without wishing to be bound by theory, it is believed that aminopeptidase enzymes, in particular Flavourzyme® from Novozymes, used in an amount, for example, according to Example 22 is useful in the preparation of gluten free ready-to-drink products.

10 In certain embodiments of any aspect of the present invention, the food product is a dairy product or a dairy alternative product or a beverage or a savoury food.

In certain embodiments of any aspect of the present invention, the food product further comprises one or more sweeteners. In certain embodiments, the one or more
15 sweeteners are selected from sucrose, fructose, glucose, arabinose, rhamnose, tagatose, allulose, trehalose, isomaltulose, steviol glycosides (e.g. rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside M, stevioside), stevia, trilobatin, rebusoside, aspartame, advantame, agarve syrup, acesulfame potassium (AceK), neotame, saccharin, sucralose, high fructose corn syrup, starch syrup, Luo
20 Han Guo extract, mogrosides, neohesperidin, dihydrochalcone, naringin, and sugar alcohols (e.g. sorbitol, xylitol, inositol, mannitol, erythritol).

Certain embodiments of any aspect of the present invention may provide one or more of the following advantages:

25

- production of a natural product;
- food product with improved mouthfeel;
- food product with reduced off-notes;
- food product with improved sweetness;
- 30 • food product with enhanced saltiness;
- dairy alternative product with improved dairy-like characteristics.

The details, examples and preferences provided in relation to any particulate one or more of the stated aspects of the present invention will be further described herein and
35 apply equally to all aspects of the present invention. Any combination of the embodiments, examples and preferences described herein in all possible variations

thereof is encompassed by the present invention unless otherwise indicated herein, or otherwise clearly contradicted by context.

DETAILED DESCRIPTION

5

The present invention is based, at least in part, on the surprising finding that subjecting dietary fibre to enzymatic hydrolysis and/or fermentation produces a product that can be used as a flavour modifying ingredient, for example to improve the mouthfeel of a food product, to mask off-notes of a food product, to improve the sweetness of a food product, and/or to enhance the saltiness of a food product.

In particular, the present invention is based, at least in part, on the surprising finding that the flavour modifying ingredients described herein can be used to eliminate the unpleasant beany taste of dairy alternative products, provide a "fullness" sensation to low-fat or non-fat dairy products that is similar to the corresponding full-fat dairy product, and can enhance saltiness in savoury food products such as chips. Dietary fibre has previously been used in food products for a bulking effect so it is surprising that the flavour modifying ingredients described herein provide the advantageous taste and mouthfeel effects described herein.

20

In certain embodiments, the dietary fibre is subjected to enzymatic hydrolysis and not to fermentation. Where the dietary fibre is subjected to enzymatic hydrolysis and not to fermentation, the dietary fibre may, for example be a fruit fibre such as grape fibre. In certain embodiments, the dietary fibre is subjected to fermentation and not to enzymatic hydrolysis. Where the dietary fibre is subjected to fermentation and not to enzymatic hydrolysis, the dietary fibre may, for example, be a cereal fibre such as an oat fibre. In certain embodiments, the dietary fibre is subjected to enzymatic hydrolysis and fermentation, for example wherein enzymatic hydrolysis occurs before and/or simultaneously with fermentation.

30

The present invention is also based on the surprising finding that subjecting at least one edible cereal component to fermentation, or to fermentation and enzymatic hydrolysis, produces clean-label ready-to-eat products and ready-to-drink products having improved mouthfeel and/or flavour profiles.

35

In particular, the present invention is based, at least in part, on the surprising finding that the ready-to-eat and ready-to-drink products described herein can be used to

- provide clean-label products with the desired organoleptic properties without the addition of other ingredients such as proteins, gums, stabilizers, etc. which are typically required in ready-to-eat and ready-to-drink products. Edible cereal components have previously been used in food products for a bulking effect, so it is surprising that the
- 5 ready-to-eat and ready-to-drink products described herein provide the advantageous taste and mouthfeel effects described herein.

Dietary Fibre and Other Edible Cereal Components

- 10 The term "dietary fibre" refers to a type of carbohydrate that cannot be completely broken down by human digestive enzymes. It is found in edible plant foods such as cereals, fruits, vegetables, nuts, seeds, lentils, fungi, and grains.

- The term "dietary fibre" includes non-starch polysaccharides, resistant starch, cellulose, hemicellulose, psyllium, dextrins, inulin, lignins, lichenin, chitins, pectins, beta-glucans, and oligosaccharides. The dietary fibre may, for example, be soluble fibre or insoluble fibre.
- 15

- The dietary fibre may, for example, be a cereal fibre, a vegetable fibre, a fruit fibre, a nut fibre, a seed fibre, a lentil fibre, a fungi fibre, or a grain fibre. The dietary fibre may, for example, be a cereal fibre, a vegetable fibre, or a fruit fibre. The terms "cereal fibre", "vegetable fibre", and "fruit fibre" refer to types of fibres that are obtained and/or obtainable respectively from a cereal, a vegetable, or a fruit.
- 20

- 25 The term "cereal" refers to members of the *Gramineae* family and determines nine species: wheat (*Triticum*), rye (*Secale*), barley (*Hordeum*), oat (*Avena*), rice (*Oryza*), millet (*Pennisetum*), corn (*Zea*), sorghum (*Sorghum*), and *Triticale*, which is a hybrid of wheat and rye.

- 30 A cereal is any grass cultivated (grown) for the edible components of its grain (botanically, a type of fruit called a caryopsis), composed of the endosperm, germ, and bran. The term may also refer to the resulting grain itself (specifically "cereal grain").

- Edible cereal components may, for example, be in the form of, or be derived from, cereal grain, cereal wholegrain, cereal groats, steel cut cereal, rolled cereal, cereal bran, cereal flour, cereal kernels, cereal fibre, irish oatmeal or combinations thereof.
- 35

The methods described herein may be applied to plant-based material other than cereals, such as pea, fava, soy, lentil, chickpea, rice, quinoa, and the like, in order to produce a non-dairy consumable product (e.g., a flavour modifying ingredient, a ready-to-eat product, or a ready-to-drink product) that closely mimics the flavour and mouthfeel of dairy products.

The dietary fibre and/or other edible cereal component may, for example, be obtained and/or obtainable from one or more types of plant. The dietary fibre and/or other edible cereal component may, for example, be obtained and/or obtainable from fresh, dried, or rehydrated plant material.

The dietary fibre may, for example, be isolated dietary fibre. The term "isolated dietary fibre" refers to dietary fibre that is separate to the plant in which it is found.

The dietary fibre and/or other edible cereal component may, for example, be a side-stream from an industrial process, for example a side-stream from juice production. This may, for example, provide environmental advantages.

Cereal fibre includes, for example, oat fibre, maize fibre, rice fibre, wild rice fibre, wheat fibre, barley fibre, sorghum fibre, millet fibre, rye fibre, triticale fibre, and fonio fibre. In certain embodiments, the cereal fibre is oat fibre. The fibre may, for example, be obtained and/or obtainable from the seed of the plant. Vegetable fibres include, for example, legume fibre such as pea fibre, chickpea fibre, lentil fibre, and soybean fibre; root vegetable fibre such as potato fibre, sweet potato fibre, carrot fibre, celeriac fibre, parsnip fibre, radish fibre, and onion fibre; broccoli fibre; cabbage fibre; green bean fibre; cauliflower fibre; courgette fibre; and celery fibre. In certain embodiments, the vegetable fibre is legume fibre such as pea fibre. The fibre may, for example, be obtained and/or obtainable from the flower, fruit, stem, leaves, roots, and/or seeds of the plant.

Fruit fibres include, for example, citrus fruit fibre such as orange fibre, lemon fibre, lime fibre, clementine fibre, tangerine fibre, grapefruit fibre, kumquat fibre, yuzu fibre; apple fibre; grape fibre; tomato fibre; bell pepper fibre; cucumber fibre; berry fibre such as blueberry fibre, cranberry fibre, strawberry fibre, raspberry fibre, blackberry fibre, red currant fibre, white currant fibre, and blackcurrant fibre; avocado fibre; fig fibre; plum fibre; prune fibre; banana fibre; pear fibre; and kiwi fibre. In certain embodiments, the fruit fibre is cranberry fibre, grape fibre, or a combination of one of more thereof. The

fibre may, for example, be obtained from the fruit of the plant or from the waste stream from the processing of the fruit or from the “pomace” which is the solid remains of grapes, olives, or other fruit after pressing for juice or oil. It contains the skins, pulp, seeds, and stems of the fruit.

5

Where the dietary fibre is subjected to enzymatic hydrolysis but not fermentation, the dietary fibre may be a fruit fibre such as citrus fruit fibre, apple fibre, blueberry fibre, cranberry fibre, grape fibre.

- 10 Where the dietary fibre is subjected to fermentation but not enzymatic hydrolysis, the dietary fibre may be a cereal fibre such as oat fibre. The dietary fibre as described herein may take the form of, or be derived from, cereal groats, steel cut cereal, rolled cereal, cereal bran, cereal flour, cereal kernels, cereal fibre, or combinations thereof. In certain embodiments, the dietary fibre may take the form of, or be derived from, oat
- 15 groats, steel cut oats, rolled oats, oat bran, oat flour, oat kernels, oat fibre or combinations thereof.

- Oat groats are the hulled, whole, and unbroken inner kernels of the oat, and are the source of almost every other oat product available. They contain all three parts of the
- 20 oat, making them the most nutritious oat product, along with steel cut oats.

- Large, cylindrical steel cutters are typically used to chop groats into several pieces, creating steel cut groats (also referred to as Irish Oatmeal). With a shorter cooking time than whole groats and a chewy texture, steel cut oats retain much of their shape even
- 25 after cooking. They can be used for a hearty breakfast, as an alternative to rice, or to add texture to stuffing and other foods. Examples of steel cut oat products include steel cut groats, no-soak steel cut groats, quick-cooking steel cut groats and Scottish oatmeal.

- 30 To create rolled oats, whole grain groats are first steamed, then rolled flat into flakes. Sometimes referred to as “old-fashioned” oats, rolled oats cook faster than steel-cut oats and are a great source of soluble fibre (beta glucans) along with other phytochemicals such as avenanthramides. Rolled oats provide a rich oat flavour to a variety of common products like granola bars, cookies, muffins, cereals, and beverages
- 35 because they can provide flavour, texture, and nutrition. Examples of rolled oat products include baby rolled oats, instant rolled oats, quick rolled oats, regular rolled oats, thick rolled oats, and oat crush.

Oat Bran comes from the outermost bran or edible covering of the oat. Rich in B vitamins and antioxidants, oat bran is also a good source of soluble fibre (beta glucans) along with other phytochemicals such as avenanthramides. Examples of oat bran products include course oat bran, medium oat bran, fine oat bran, and micro-ground bran.

Examples of oat bran that can be used in the invention include SWEOAT™ brans such as SWEOAT™ Bran BG 14, SWEOAT™ Bran BG14 Bakery, and SWEOAT™ Bran BG22, and SWEOAT™ Bran BG28 (all available from Naturex SA and Swedish Oat Fiber Ab of Bua, Sweden).

Oat flour may comprise ground groats still have their bran layer intact, which makes whole oat flour a great source of soluble fibre (beta glucans) along with essential minerals. Oat flour is an ideal ingredient when looking to add flavour, nutrition, and viscosity to a wide range of end products. Examples of oat flour products include whole oat flour, colloidal oat flour, and low viscosity whole oat flour.

Examples of oat flour that can be used in the invention include SWEOAT™ flours such as SWEOAT™ Flour P12, SWEOAT™ Flour P14 Wholemeal, SWEOAT™ Flour P16, and SWEOAT™ Flour P19 (all available from Naturex SA and Swedish Oat Fiber Ab of Bua, Sweden).

SWEOAT™ Flour P12 is a fine powder (particle size less than 180 microns) having a moisture content of 7.5%, and a shelf life of 12 months. SWEOAT™ flour P12 has a very light yellowish colour and a neutral oat cereal taste.

SWEOAT™ Flour P14 Wholemeal is a fine powder (a minimum of 80% of particles have a particle size of less than 250 microns and a minimum of 90% of particles have a particle size of less than 355 microns). SWEOAT™ flour P14 has a moisture content of 7%, and a shelf life of 12 months. SWEOAT™ flour P14 has a light yellowish colour and a neutral oat cereal taste.

SWEOAT™ Flour P16 is a fine powder (particle size less than 180 microns), a moisture content of less than 12% and a very light yellowish brown colour.

SWEOAT™ Flour P19 is a fine powder (particle size less than 180 microns), a moisture content of 5%, and a shelf life of 24 months. SWEOAT™ flour P19 has a very light yellowish colour and a neutral oat cereal taste.

- 5 Oat fibre is a great source of insoluble fibre with many nutritional and functional benefits. Oat fibre also improves nutrition, yields, and functionality to foods like cereals, breads and snacks. Examples of oat fibre products include OAT FIBER BCS 20, OAT FIBER BCS 30, OAT FIBER BCS 30L, OAT FIBER BCS 30SS, OAT FIBER BCS 30SL and OAT FIBER BCS 30XS2 (all available from Grain Millers Inc. of Iowa, USA).
- 10 It has been found that enzymatic treatment of oat flour, for example SWEOAT™ Flour P12 results in a gluten free ready-to-drink product, as the final product contains less than 5 ppm gluten.

Enzymatic Hydrolysis

- 15 In certain embodiments, dietary fibre and/or other edible cereal component is subjected to enzymatic hydrolysis, wherein the dietary fibre and/or other edible cereal component is contacted with one or more enzyme(s) under conditions and for a period of time suitable for the enzyme(s) to at least partially break down the dietary fibre and/or other
- 20 edible cereal component. All enzymes should be food grade.

- The enzyme(s) used for enzymatic hydrolysis may, for example, be selected from one or more of carbohydrases and proteolytic enzymes. Where more than one enzyme is used, the enzymes may be more than one class of enzymes and/or more than one
- 25 enzymes within a single class. In certain embodiments, the enzyme(s) used for enzymatic hydrolysis include at least one or more carbohydrase(s). In certain embodiments, the enzyme(s) used for enzymatic hydrolysis include at least one or more of cellulases, pectinases, and other carbohydrases. In certain embodiments, the enzyme(s) used for enzymatic hydrolysis include at least one or more of cellulases and
- 30 pectinases.

- Carbohydrases catalyse the hydrolysis of carbohydrates. The carbohydrases may have specificity for either alpha- or beta- glycosidic bonds. Carbohydrases include, for example, cellulases, pectinases, mannanase, amylase, lactase, and beta-glucanase.

- 35 Examples of amylase enzymes include but are not limited to (i) alpha-amylase enzyme (Kleistase® SD-80, from Amano Enzyme) which is useful in breaking down amylose

and amylopectin to maltose and various dextrans and/or (ii) Glucoamylase (Gluczyme® NLP from Amano Enzyme) which is useful for example, for the breakdown of maltose and various to release glucose and/or Alpha-Amylase enzymes from Novozymes A/S and/or Endo-Amylase enzymes from Novozymes A/S which are useful for example, for the breakdown of amylose and amylopectin to maltose and various dextrans.

In certain embodiments, Alpha-Amylase enzyme (from Novozymes at a concentration of about 0.05 to about 1.0%, in particular about 0.5%) and Endo-Amylase (from Novozymes BAN at a concentration of about 0.05-2.5%, in particular about 2%) are added to a mixture of oat flour and water, and then the mixture is incubated at about 70°C for 1 hour with continuous agitation to break down the amylose and amylopectin to maltose and various dextrans.

In certain embodiments, Alpha-Amylase enzyme (from Novozymes at a concentration of about 0.05 to about 1.5%, in particular about 1.0%) and Endo-Amylase enzyme (from Novozymes BAN at a concentration of about 0.05-3.5%, in particular about 3%) are added to a mixture of oat flour and water, and then the mixture is incubated at about 70°C for 2 hours with continuous agitation to break down the amylose and amylopectin to maltose and various dextrans.

In certain embodiments, subsequent to enzymatic treatment using Alpha-Amylase enzyme and/or Endo-Amylase enzyme, proteolytic enzyme (Protana® Prime from Novozymes at a concentration of 0.05 to about 2.0%, in particular about 1.0%) and proteolytic enzymes Protana® UBoost (from Novozymes at a concentration of 0.05 to about 1.0%, in particular about 0.5%), and Alcalase® 2.4 L FG (from Novozymes at a concentration of about 0.5 to about 2.0%, in particular about 1.0%) are added to the mixture and incubated for an additional 2 to 3 hrs at 50-55°C. The mixture is then heated to 121°C for 15 minutes to inactivate the enzymes and any microbial contaminants. The mixture is then cooled down to 37°C.

Cellulases catalyse the hydrolysis of beta-1,4-glycosidic bonds found in cellulose, hemicellulose, lichenin, and cereal beta-glucans. Cellulases include, for example, hemicellulase, endo-1,4-beta-D-glucanase, xylanase, and carboxymethyl cellulase.

Pectinases catalyse the hydrolysis of alpha-1,4-glycosidic bonds between galacturonic acid residues found in pectin. An example of a pectinase is polygalacturonase (EC 3.2.1.15).

Proteolytic enzymes catalyse the hydrolysis of proteins and peptides. Proteolytic enzymes include, for example, proteinases, which hydrolyze proteins to form small peptides, and peptidases, which further hydrolyze small peptides to form amino acids. The proteolytic enzyme(s) may, for example, have endopeptidase activity (attack
5 internal peptide bonds) and/or exopeptidase activity (attack peptide bonds at the end of the protein or peptide such as amino- or carboxypeptidases).

Proteolytic enzymes include, for example, protease, peptidase, glutaminase (e.g. L-glutamine-amido-hydrolase (EC 3.5.1.2)), endoprotease, serine endopeptidase,
10 subtilisin peptidase (EC 3.4.21.62), serine protease, threonine protease, cysteine protease, aspartic acid protease, glutamic acid protease, trypsin, chymotrypsin (EC 3.4.21.1), pepsin, papain, and elastase.

Proteolytic enzymes (EC 3.4 and EC 3.5) are classified by an EC number (enzyme
15 commission number), each class comprises various known enzymes of a certain reaction type. EC 3.4 comprises enzymes acting on peptide bonds (peptidases/proteinases) and EC 3.5 comprises enzymes that act on carbon-nitrogen bonds other than peptide bonds.

Examples for EC 3.4 include, for example, the following: aminopeptidase (EC 3.4.11), dipeptidase (3.4.13), dipeptidyl-peptidase (3.4.14), peptidyl-dipeptidase (3.4.15), serine-carboxypeptidase (3.4.16), metallocarboxypeptidase (3.4.17), cysteine-carboxypeptidase (3.4.18), omegapeptidase (3.4.19), serine-endopeptidase (3.4.21), cysteine-endopeptidase (3.4.22), aspartate-endopeptidase (3.4.23),
25 metalloendopeptidase (3.4.24), threonine-endopeptidase (3.4.25).

Examples for EC 3.5 include, without limitation, proteolytic enzymes that cleave in linear amides (3.5.1), for example, without limitation, glutaminase (EC 3.5.1.2) and protein glutaminase (e.g. protein glutaminase®500 from Amano)

30 Various proteolytic enzymes, suitable for food-grade applications, are commercially available from suppliers such as Novozymes, Amano, Biocatalysts, Bio-Cat, Valey Research (now subsidiary of DSM), EDC (Enzyme Development Corporation), and others. Some examples include: Neutrase®, Alcalase®, Protamex®, Protana® Prime, 35 Protana® UBoost and Flavorzyme®, (available from Novozymes); the Promod® series: e.g. 215P, 278P, 279P, 280P, 192P, and 144P, Flavorpro® 192, Peptidase 433P, and Peptidase 436P (available from Biocatalysts); Protin PC10, Umamizyme®, Peptidase R

(or 723), Peptidase A, Peptidase M, Peptidase N, Peptidase P, Peptidase S, Acid protease II, and Thermoase GL30 (available from Amano); Peptidase 600 (available from Bio-Cat); Validase® AFP and Validase® FPII (available from Valey Research); Fungal protease, Exo-protease, Papain, Bromelain, and the Enzeco® series of
5 proteases and peptidases (available from EDC). In certain embodiments, the enzymes used for enzymatic hydrolysis comprise cellulase, beta-glucanase, and aminopeptidase. In certain embodiments, the enzymes used for enzymatic hydrolysis comprise cellulase, beta-glucanase, aminopeptidase, hemicellulose, and mannanase. In certain embodiments, the enzymes used for enzymatic hydrolysis comprise
10 carbohydrases (such as alpha-amylase and/or glucoamylase) and proteases and/or aminopeptidases (such as protein glutaminase).

The enzymes may be part of an enzyme mix. A number of enzyme preparations such as Celluclast™, Ceramix™, Alcalase™, Viscozyme™, Flavorzyme™, and
15 Umamizyme™, are commercially available and may be used in the enzymatic hydrolysis described herein.

The enzyme(s) may, for example, be obtained or obtainable from a microbial or plant source. Examples include *Aspergillus oryzae*, *Bacillus licheniformis*, pineapple, and
20 papaya.

The amount of enzyme is chosen to ensure sufficient activity and depends on the activity of the enzyme, amount of substrate, and conditions it is used in. The necessary amount of enzyme can be determined by trying out different amounts and testing the
25 effect of the resulting product in a sensory evaluation as described herein.

The ratio of enzyme: substrate may, for example, range from about 0.05:20 to about 3:20, for example from about 0.5:20 to about 3:20, for example around 1:20. The enzymes may, for example, be used in an amount ranging from about 0.1 wt% to about
30 20 wt% based on the total weight of the dietary fibre and/or other edible cereal component. For example, the enzymes may be used in an amount ranging from about 0.5 wt% to about 15 wt% or from about 1 wt% to about 10 wt% or from about 0.5wt% to about 5wt% or from about 0.5wt% to about 1.5wt% or from about 1wt% to about 1.5wt% based on the total weight of the dietary fibre and/or other edible cereal
35 component.

(Ceremix™, Novozymes, Bagsvaerd, Denmark, has an activity of 300 Beta-Glucanase Units (BGU) per gram of enzyme; Viscozyme™, Novozymes, Bagsvaerd, Denmark, has an activity of 100 Fungal Beta-Glucanase Units FBG per gram of enzyme; Alcalase™, Novozymes, Bagsvaerd, Denmark, has an activity of 2.4 Anson units (AU) per gram of enzyme; Celluclast™, Novozymes, Bagsvaerd, Denmark, has an activity of 700 Endo-Glucanase Units (EGU) per gram of enzyme; Flavourzyme™, Novozymes, Bagsvaerd, Denmark, has an activity of 1000 Leucine Aminopeptidase Units (LAPU) per gram of enzyme; Umamizyme™, Amano, Nagoya, Japan, has an activity of 70 U (Units by LGG method, LGG= L-Leucyl-Glycyl-Glycine); Flavorpro 373™, a Glutaminase, Biocatalysts, Cardiff, UK, has an activity of 30 Glutaminase Units (GU)). Useful amounts of enzyme units per gram starting material are indicated for some type of enzymes below.

Beta-Glucanase Units (BGU) per gram starting material (liquified celery slurry) 0.03 to 15 BGU, for example 0.1 to 3 BGU.

Fungal Beta-Glucanase Units FBG per gram starting material, 0.002 to 3 FBG, for example, 0.01 to 1 FBG.

Anson units (AU) per gram starting material, 0.0002 to 0.02 AU, for example 0.0005 to 0.01.

U (Units by LGG method, LGG= L-Leucyl-Glycyl-Glycine) per gram starting material 0.007 to 0.7 U, for example, 0.01 to 0.1 U are used.

Glutaminase Units (GU) per gram starting material, 0.00075 to 0.075 GU, for example, 0.001 to 0.02 GU are used.

The enzymatic hydrolysis will be performed under conditions suitable for all the enzymes involved (and all microorganisms involved if occurring simultaneously with fermentation). As will be apparent to the skilled person, the temperature and pH should be within a suitable range for hydrolysis to occur to the desired degree. The incubation length will vary accordingly, with shorter incubations when conditions are nearer to the optimum conditions. Necessary ions, if required or beneficial for the chosen enzymes may be present. Subjecting the incubated mixture to agitation, for example by stirring (e.g. at 50 to 500 rpm or 100 to 200 rpm) may improve the hydrolysis.

The enzymatic hydrolysis may, for example, be performed at a temperature less than the temperature at which the enzymes denature. The temperature may, for example, be selected to give a desired reaction rate. The enzymatic hydrolysis may, for example, be performed at a temperature ranging from about 25°C to about 60°C. For example, 5 the enzymatic hydrolysis may be performed at a temperature ranging from about 30°C to about 60°C or from about 35°C to about 55°C or from about 40°C to about 50°C or from about 50°C to about 55°C.

Where the dietary fibre and/or other edible cereal component is subjected to enzymatic 10 hydrolysis but not fermentation, the enzymatic hydrolysis may, for example, be performed at a temperature ranging from about 40°C to about 60°C.

Where the dietary fibre and/or other edible cereal component is subjected to enzymatic hydrolysis and fermentation, the enzymatic hydrolysis may, for example, be performed 15 at a temperature ranging from about 30°C to about 60°C, for example from about 30°C to about 40°C or from about 50°C to about 55°C.

The enzymatic hydrolysis may, for example, be performed at a pH at which the enzymes do not denature. The pH may, for example, be selected to give a desired 20 reaction rate. The enzymatic hydrolysis may, for example, be performed at a pH ranging from about 4 to about 8, for example from about 5 to about 8, for example from about 6 to about 8, for example from about 6.5 to about 7.5.

The enzymatic hydrolysis may, for example, take place for a period of time ranging 25 from about 1 hour to about 48 hours. For example, the enzymatic hydrolysis may take place for a period of time ranging from about 2 hours to about 48 hours or from about 4 hours to about 36 hours or from about 6 hours to about 24 hours or from about 8 hours to about 16 hours or from about 1-2 hours or up to 5 hours.

Where the dietary fibre and/or other edible cereal component is subjected to enzymatic 30 hydrolysis and not fermentation, the enzymatic hydrolysis may take place for a longer period of time compared to a method in which the dietary fibre and/or other edible cereal component is subjected to enzymatic hydrolysis and fermentation. For example, where the dietary fibre and/or other edible cereal component is subjected to enzymatic 35 hydrolysis and not fermentation, the enzymatic hydrolysis may take place for a period of time that is at least about 12 hours, for example at least about 18 hours or at least about 24 hours. For example, where the dietary fibre and/or other edible cereal

component is subjected to enzymatic hydrolysis and not fermentation, the enzymatic hydrolysis may take place for a period of time ranging from about 12 hours to about 48 hours or from about 18 hours to about 48 hours or from about 24 hours to about 48 hours.

5

Where the dietary fibre and/or other edible cereal component is subjected to enzymatic hydrolysis and fermentation, the enzymatic hydrolysis may take place for a shorter period of time compared to a method in which the dietary fibre and/or other edible cereal component is subjected to enzymatic hydrolysis only. For example, where the dietary fibre and/or other edible cereal component is subjected to enzymatic hydrolysis and fermentation, the enzymatic hydrolysis may take place for a period of time ranging from about 1 hour to about 36 hours or from about 2 hours to about 36 hours or from about 4 hours to about 24 hours or from about 1-2 hours or up to 5 hours.

15 Fermentation

In certain embodiments, dietary fibre and/or other edible cereal component is subjected to fermentation, wherein the dietary fibre and/or other edible cereal component is contacted with one or more fermenting microorganism(s) under conditions and for a period of time suitable for the microorganism(s) to at least partially break down/metabolize the dietary fibre. Where the dietary fibre and/or other edible cereal component was subjected to enzymatic hydrolysis prior to fermentation, the dietary fibre and/or other edible cereal component is the product of the enzymatic hydrolysis (a dietary fibre hydrolyzate and/or other edible cereal component hydrolyzate).

25 The dietary fibre and/or other edible cereal component that is the product of the enzymatic hydrolysis may be referred to as hydrolyzed or partly hydrolyzed dietary fibre and/or other edible cereal component.

The fermentation may, for example, use one or more species of microorganism.

30

The fermentation may, for example, use one or more lactic acid bacteria such as *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, and/or *Bifidobacterium animalis lactis*, such as, for example, *Bifidobacterium animalis lactis* also known as BB-12® from Chr. Hansen or *Bifidobacterium animalis lactis* also known as Probiotic BifidoBHN019 or

35

DR10 or B019. In certain embodiments, the fermentation uses *Lactobacillus plantarum* or *Lactiplantibacillus plantarum*. For example, the fermentation may use *Lactobacillus plantarum*, ATCC 14917.

- 5 The fermentation may, for example, use the lactic acid bacteria *Lactobacillus rhamnosus* and *Bifidobacterium animalis lactis* (LGG® and BB-12®, respectively, from Chr. Hansen A/S or *Bifidobacterium animalis lactis* also known as Probiotic BifidoBHN019 or DR10 or B019).
- 10 The fermentation may, for example, use the lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (YOFLEX® YF-L02 DA from Chr. Hansen A/S).

The fermentation may, for example, use the lactic acid bacteria *Lactobacillus*
15 *rhamnosus* and *Lactobacillus bulgaricus*.

The fermentation may, for example, use the lactic acid bacteria *Bifidobacterium animalis lactis* such as, for example, *Bifidobacterium animalis lactis* also known as BB-12® from Chr. Hansen or *Bifidobacterium animalis lactis* also known as Probiotic
20 BifidoBHN019 or DR10 or B019 and *Lactobacillus bulgaricus*.

The fermentation may, for example, use the lactic acid bacteria *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium ssp* (ABY 421 from Vivolac Cultures Corporation of Indiana, USA.)
25 The fermentation may, for example, use one or more lactic acid bacteria such as *Lactobacillus delbrueckii ssp. bulgaricus*, *Streptococcus thermophilus* and/or *Lactobacillus acidophilus*. The fermentation may, for example, use a *Bifidobacterium*.

The fermentation may, for example use an *Aspergillus* fungus such as *Aspergillus oryzae* (also known as Koji) and *Aspergillus saitoi*. In certain embodiments, the
30 *Aspergillus* fungus is *Aspergillus oryzae*.

In certain embodiments, the fermentation uses two or more lactic acid bacteria such as *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and/or *Bifidobacterium*, preferably
35 *Bifidobacterium animalis lactis* such as, for example, *Bifidobacterium animalis lactis* also known as BB-12® from Chr. Hansen or *Bifidobacterium animalis lactis* also known as Probiotic BifidoBHN019 or DR10 or B019.

In certain embodiments, the fermentation uses three or more lactic acid bacteria such as *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and *Bifidobacterium*, preferably *Bifidobacterium animalis lactis* such as, for example, *Bifidobacterium animalis lactis* also known as BB-12® from Chr. Hansen or *Bifidobacterium animalis lactis* also known as Probiotic BifidoBHN019 or DR10 or B019.

In certain embodiments, the fermentation uses a combination of the following microbial strains *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium ssp.*

Suitable microbial cultures may include the ABY Series, such as ABY 424 ND and ABY 421 ND, commercially available from Vivolac Cultures Corporation of Indiana, USA.

The microbial culture designated as ABY 421 ND has the following microbial strains: *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium ssp.* The microbial culture designated as ABY 424 ND has the following microbial strains: *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium ssp.* ABY 421 ND and ABY 424 ND were formulated with strains of the same genus species. There are several strains (bacteria) in the same genus and they are classified per their characteristics but there are differences in their plasmid profile that dictate some of their functional characteristics like viscosity production and ability to ferment lactose, phage sensitivity/resistance.

Blends of the two microbial cultures may provide different rates of fermentation depending on the ratio of strains inoculated.

In certain embodiments, the fermentation uses 100% ABY 421 ND. In other embodiments, the fermentation uses 100% and ABY 424 ND.

In certain embodiments, the fermentation uses a combination of ABY 421 ND and ABY 424 ND in about a 50/50 ratio. In certain embodiments, the fermentation uses a combination of ABY 421 ND and ABY 424 ND in about a 70/30 ratio, respectively. In certain embodiments, the fermentation uses a combination of ABY 421 ND and ABY 424 ND in about a 30/70 ratio, respectively.

The fermentation may use an overnight culture of the microorganism(s), or the dietary fibre and/or other edible cereal component (or dietary fibre and/or other edible cereal component hydrolysate or obtained from the enzymatic hydrolysis step) may be directly inoculated with a microorganism clone, and the fermentation performed for a slightly longer time accordingly.

The overnight culture (sometimes referred to as seed ferment) may be prepared by methods well-known in the art. It may be grown overnight, for example 12 hours, at the appropriate temperature for that microorganism. Approximately 37°C is an appropriate temperature for many microorganisms, including *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, *Bifidobacterium animalis lactis* such as, for example, *Bifidobacterium animalis lactis* also known as BB-12® from Chr. Hansen or *Bifidobacterium animalis lactis* also known as Probiotic BifidoBHN019 or DR10 or B019 and/or *Aspergillus oryzae*. Any suitable medium may be used, for example, MRS broth (Difco, United States of America).

The microorganism(s) may, for example, be administered on a carrier. For example, the microorganism(s) (e.g. *Aspergillus oryzae*) may be coated onto rice grains. For example, the microorganism(s) may be grown on rice grains and offered by suppliers in this form (e.g. obtainable from Rhapsody Natural Foods, Cabot VT 05647). This may, for example, induce the production of certain endogenous enzymes and/or pathways, thereby providing the microorganism(s) with desirable characteristics.

The amount of microorganism is chosen to ensure sufficient activity and depends on the activity of the microorganism, amount of substrate, and conditions it is used in. The necessary amount of microorganism can be determined by trying out different amounts and testing the effect of the resulting product in a sensory evaluation as described herein.

The amount of microorganism may, for example, range from about 0.1 % to about 1 % based on the total weight of the reaction mixture. For example, the amount of microorganism used may range from about 0.1 % to about 0.5 % or from about 0.3% to about 0.7% based on the total weight of the reaction mixture.

The fermentation will be performed under conditions suitable for all the microorganisms involved (and all enzymes involved if occurring simultaneously with enzymatic hydrolysis). As will be apparent to the skilled person, the temperature and pH should be within a suitable range for fermentation to occur to the desired degree. The incubation length will vary accordingly, with shorter incubations when conditions are nearer to the optimum conditions. Necessary nutrients if required or beneficial for the chosen microorganisms may be present. Subjecting the incubated mixture to agitation, for example by stirring (e.g. at 50 to 500 rpm or 100 to 200 rpm) may improve the fermentation. Some microorganisms such as lactic acid bacteria may grow faster under anaerobic conditions so it may be favourable to minimize stirring. In certain embodiments, aerotolerance may be manganese-dependent.

The fermentation may, for example, be performed at a temperature less than the temperature at which the microorganisms are killed and/or reduced in numbers. The temperature may, for example, be selected to give a desired reaction rate. The fermentation may, for example, be performed at a temperature ranging from about 20°C to about 45°C. For example, the fermentation may be performed at a temperature ranging from about 25°C to about 40°C or from about 30°C to about 40°C or from about 34°C to about 40°C or from about 30°C to about 37°C or from about 30°C to about 35°C.

Useful temperature ranges for *Lactobacilli*, in particular *Lactobacillus plantarum* or *Lactiplantibacillus plantarum*, include, for example, from about 20°C to about 40°C, or from about 30°C to about 40°C, or from about 35°C to about 40°C, with an optimum of about 36°C to about 38°C.

Useful temperature ranges for *Bifidobacteria* or other lactic acid bacteria, in particular, *L. delbrueckii ssp. bulgaricus*, *Streptococcus thermophilus* and/or *Lactobacillus acidophilus* include, for example, from about 20°C to about 40°C, or from about 30°C to about 40°C, or from about 35°C to about 40°C, with an optimum of about 36°C to about 38°C or from about 30°C to about 35°C or from about 30°C to about 37°C.

Where the dietary fibre and/or other edible cereal component is subjected to fermentation and not enzymatic hydrolysis, the fermentation may be performed at a temperature ranging from about 30°C to about 45°C.

The fermentation may, for example, be performed at a pH less than the temperature at which the microorganisms denature. The pH, for example, be selected to give a desired reaction rate. The fermentation may, for example, be performed at a pH ranging from about 5 to about 8, for example from about 5 to about 7 or from about 6 to about 8 or from about 6.5 to about 7.5.

The fermentation may take place for a period of time until the desired product is formed. Fermentation may, for example, take place until the fermentation medium reaches a pH of about 5.5 or lower, for example a pH of about 4.5 to about 5.5.

To produce ready-to-eat and/or ready-to-drink products, the fermentation may, for example, take place for a period of time ranging from about 5 hours to about 24 hours or longer. For example, the fermentation may take place for a period of time ranging from about 6 hours to about 23 hours, or from about 7 hours to about 22 hours, or from about 8 hours to about 21 hours, or from about 9 hours to about 20 hours, or from about 10 hours to about 19 hours, or from about 11 hours to about 18 hours, or from about 12 hours to about 17 hours, or from about 13 hours to about 16 hours, or from about 14 hours to about 16 hours, or from about 15 hours to about 16 hours. In certain embodiments, the fermentation takes place for about 16 hours.

Where the dietary fibre and/or other edible cereal components subjected to fermentation but not enzymatic hydrolysis, the fermentation may take place for a longer period of time compared to methods in which the dietary fibre and/or other edible cereal component is subjected to fermentation and enzymatic hydrolysis. For example, where the dietary fibre and/or other edible cereal component is subjected to fermentation but not enzymatic hydrolysis, the fermentation may take place for at least about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 2 days, 3 days or 4 days or longer. For example, where the dietary fibre and/or other edible cereal component is subjected to fermentation but not enzymatic hydrolysis, the fermentation may take place for a period of time ranging from about 5 hours to about 24 hours, or from about 10 hours to about 18 hours, or from about 14 hours to about 16 hours. In certain embodiments, the fermentation takes place for about 16 hours.

Where the dietary fibre and/or other edible cereal component is subjected to fermentation and enzymatic hydrolysis, the fermentation may take place for a shorter

period of time compared to methods in which the dietary fibre and/or other edible cereal component is subjected to fermentation and not enzymatic hydrolysis. For example, where the dietary fibre and/or other edible cereal component is subjected to fermentation and enzymatic hydrolysis, the fermentation may take place for a period of time ranging from about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day to about 8 days, or from about 2 days to about 6 days, or from about 2 days to about 5 days, or from about 2 days to about 4 days, or from about 1 to about 2 days. In certain embodiments, the fermentation takes place for about 16 hours.

The product of the fermentation, or the fermentation and the enzymatic hydrolysis, may be used directly as a clean-label ready-to-eat or ready-to-drink product.

15 *Further Processing Steps*

The product of the enzymatic hydrolysis and/or fermentation may, for example, be used directly as a flavour modifying ingredient. However, the methods may, for example, comprise one or more additional steps.

20

The dietary fibre and/or other edible cereal component that is subjected to the enzymatic hydrolysis and/or fermentation may, for example, be an aqueous slurry of dietary fibre and/or other edible cereal component. Thus, in certain embodiments, the method may comprise combining the dietary fibre and/or other edible cereal component with water prior to the enzymatic hydrolysis and/or fermentation. The aqueous slurry of dietary fibre and/or other edible cereal component may, for example, comprise at least about 5 wt% dietary fibre and/or other edible cereal component, for example at least about 10 wt% dietary fibre and/or other edible cereal component, for example at least about 15 wt% dietary fibre and/or other edible cereal component. The aqueous slurry of dietary fibre may and/or other edible cereal component, for example, comprise up to about 90 wt% dietary fibre and/or other edible cereal component or up to about 50 wt% dietary fibre and/or other edible cereal component or up to about 30 wt% dietary fibre and/or other edible cereal component.

35 The enzymatic hydrolysis and fermentation should be performed in a sterilized container. Thus, the container may be sterilized prior to adding the dietary fibre and/or other edible cereal component.

The dietary fibre and/or other edible cereal component (e.g. aqueous slurry of dietary fibre and/or other edible cereal component) may, for example, be heated prior to the enzymatic hydrolysis and/or fermentation. For example, the dietary fibre and/or other edible cereal component may be heated to a temperature equal to or greater than about 50°C, for example heated to a temperature in the range of 50°C to about 55°C, or heated to a temperature equal to or greater than about 75°C, for example equal to or greater than about 100°C or equal to or greater than about 110°C, prior to the enzymatic hydrolysis and/or fermentation. For example, the dietary fibre and/or other edible cereal component may be heated to a temperature equal to or less than about 140°C, for example equal to or less than about 130°C prior to the enzymatic hydrolysis and/or fermentation. For example, the dietary fibre and/or other edible cereal component may be heated to a temperature of about 121°C prior to enzymatic hydrolysis and/or fermentation. This may be to inactivate and/or kill any microbial contaminants and/or to hydrate and/or pre-heat the dietary fibre and/or other edible cereal component (e.g. aqueous slurry of dietary fibre and/or other edible cereal component) prior to enzymatic hydrolysis and/or fermentation. The dietary fibre and/or other edible cereal component is then maintained at a suitable temperature and/or cooled to a suitable temperature for the enzymatic hydrolysis and/or fermentation before the enzyme(s) and/or microorganism(s) are added.

The enzyme(s) and/or microorganism(s) may, for example, be deactivated prior to incorporation in a flavour composition or food product. This may, for example, take place by heating, for example to a temperature ranging from about 60°C to about 121°C, for example about 100°C, for a period of time sufficiently long to deactivate the enzymes and/or microorganism(s). For example, any pasteurization or sterilization methods which are well-known in the art, may be used. For example, the enzymes and/or microorganisms may be deactivated by heating to about 70°C, about 90°C or about 100°C or higher for 30 minutes or 45 minutes or 60 minutes. When heating above about 100°C, for example about 121°C, for about 30 minutes, heating may be performed under pressure, for example about 12 to about 15 psi. The microorganism deactivation step is optional if aseptic conditions are used in the preparation of the product. In particular, the disclosed lactic acid bacteria are generally regarded as safe (GRAS) for human food as defined or recognized by the United States Food and Drug Administration or the United States Department of Agriculture, and therefore are suitable for human consumption.

The product of the enzymatic hydrolysis and/or fermentation (the flavour modifying ingredient) may, for example, be filtered or centrifuged to remove large particles. The product of the enzymatic hydrolysis and/or fermentation (the flavour modifying ingredient) may, for example, be concentrated, for example by evaporation including
5 boiling at, for example, up to about 100°C. The product of the enzymatic hydrolysis and/or fermentation (the flavour modifying ingredient) may, for example, be spray-dried by methods known in the art, for example using carriers such as oat fibre, soluble corn fibre, and maltodextrin and/or anti-caking agents.

10 Filtering may be performed by any suitable filtering method, such methods are well known in the art, for example, by passing through a felt filter bag in a filter centrifuge. The filtered culture (supernatant containing the remaining smaller solids, minus the biomass that includes larger undigested proteins) can be concentrated, for example concentrated 2x by evaporation/boiling at 100°C. The resulting concentrate's solid
15 content can be determined using a moisture analyser and can be spray-dried, for example, onto a suitable carrier. Many carriers are well known in the art, for example, without limitation, a potato maltodextrin carrier (for example, a ratio of about 1:1 solids of the 2xconcentrate to carrier may be suitable). Optionally an anti-caking agent may be added, such agents are well known. A suitable anti-caking agent is, for example,
20 tricalciumphosphate (TPC); about 0.5% (wt/wt) based on total weight of the 2x concentrate would be a suitable amount.

The flavour modifying ingredient may, for example, be used in filtered and/or concentrated form.

25

The product of the enzymatic hydrolysis and/or fermentation (the flavour modifying ingredient) may, for example, be combined with one or more stabilizing agents such as propylene glycol.

30 *Products*

The ready-to-eat and ready-to-drink products made by the fermentation, alone or in combination with enzymatic hydrolysis, described herein may be used directly as a final food product without undergoing any further processing. The ready-to-eat and ready-to-
35 drink products may, for example, be considered natural, clean-label products for food labelling and/or food regulation reasons.

The final form of the ready-to-eat and ready-to-drink products may be chosen according to methods well known in the art and will depend on the particular food application. For liquid foods, for example milks, the ready-to-drink product can be used without further processing in its liquid form. For solid foods, for example non-dairy
5 yoghurt, the ready-to-eat product can be used without further processing in its solid form.

The flavour modifying ingredient made by the enzymatic hydrolysis and/or the fermentation described herein may be used directly in flavour compositions and/or food
10 compositions or may undergo further processing as described above. For example, the flavour modifying ingredient may be in filtered and/or concentrated and/or paste and/or spray-dried form. The flavour modifying ingredient may, for example, be in combination with a stabilizer such as propylene glycol, or may be in combination with one or more carriers and/or anti-caking agents used in the spray-drying process. The flavour
15 modifying ingredient may, for example, be considered to be a natural product for food labelling and/or food regulation reasons. By way of example, the flavour modifying ingredient may, for example, be considered to be a Ready to Eat (RTE) or Ready to Drink (RTD) product.

20 The final form of the flavour modifying ingredient may be chosen according to methods well known in the art and will depend on the particular food application. For liquid foods, for example soups, the flavour modifying ingredient can be used without further processing in its liquid form. For dry applications such as crackers, the spray-dried concentrated flavour modifying ingredient can be used.

25 The flavour modifying ingredient may be directly added to food products, or may be provided as part of a flavour composition for flavouring or seasoning food products.

Flavour compositions contain the flavour modifying ingredient and optionally one or
30 more food grade excipient. Suitable excipients for flavour compositions are well known in the art and include, for example, without limitation, solvents (including water, alcohol, ethanol, oils, fats, vegetable oil, and miglyol), binders, diluents, disintegrating agents, lubricants, flavouring agents, colouring agents, preservatives, antioxidants, emulsifiers, stabilisers, flavour-enhancers, sweetening agents, anti-caking agents, and the like.
35 Examples of such carriers or diluents for flavours may be found e.g. in "Perfume and Flavour Materials of Natural Origin", S. Arctander, Ed., Elizabeth, N.J., 1960; in "Perfume and Flavor Chemicals", S. Arctander, Ed., Vol. I & II, Allured Publishing

Corporation, Carol Stream, USA, 1994; in "Flavourings", E. Ziegler and H. Ziegler (ed.), Wiley-VCH Weinheim, 1998, and "CTFA Cosmetic Ingredient Handbook", J.M. Nikitakis (ed.), 1st ed., The Cosmetic, Toiletry and Fragrance Association, Inc., Washington, 1988.

5

The flavour composition may contain additional flavour ingredients including flavour compounds, flavours from natural sources including botanical sources and including ingredients made by fermentation.

- 10 The flavour composition may have any suitable form, for example liquid or solid, wet or dried, or in encapsulated form bound to or coated onto carriers/particles or as a powder.

- The flavour composition may, for example, comprise from about 0.02% to about 0.5% (wt/wt) based on the unconcentrated flavour modifying ingredient.
- 15

The term "food product" is used in a broad meaning to include any product placed into the oral cavity but not necessarily ingested, including, for example, food, beverages, nutraceuticals and dental care products including mouth wash.

20

- Food products include cereal products, rice products, pasta products, ravioli, tapioca products, sago products, baker's products, biscuit products, pastry products, bread products, confectionery products, dessert products, gums, chewing gums, chocolates, ices, honey products, treacle products, yeast products, salt and spice products, 25 savoury food products, mustard products, vinegar products, sauces (condiments), processed foods, cooked fruits and vegetable products, meat and meat products, meat analogues/substitutes/alternatives, jellies, jams, fruit sauces, egg products, dairy products (including milk), cheese products, butter and butter alternative products, milk alternative products, soy products (e.g. soy "milk"), edible oils and fat products, 30 medicaments, beverages, juices, fruit juices, vegetable juices, food extracts, plant extracts, meat extracts, condiments, nutraceuticals, gelatins, tablets, lozenges, drops, emulsions, elixirs, syrups, and combinations thereof.

- Processed foods include margarine, peanut butter, soup (clear, canned, cream, instant, 35 UHT), gravy, canned juices, canned vegetable juice, canned tomato juice, canned fruit juice, canned juice drinks, canned vegetables, pasta sauces, frozen entrees, frozen dinners, frozen hand-held entrees, dry packaged dinners (macaroni & cheese, dry

dinners-add meat, dry salad/side dish mixes, dry dinners-with meat). Soups may be in different forms including condensed wet, ready-to-serve, ramen, dry, and bouillon, processed and pre-prepared low-sodium foods.

- 5 Of particular interest are, for example, dairy products such as milk (e.g. cow's milk, goat's milk, sheep's milk, camel's milk), cream, butter, cheese, yoghurt, ice cream, and custard. The dairy products may, for example, be sweetened or unsweetened. The dairy products (e.g. milk) may, for example, be full-fat, low-fat, or non-fat.
- 10 Dairy alternative products are also of particular interest. Dairy alternative products are plant-based products that do not encompass true dairy products that have been obtained from an animal. For example, dairy alternative products include alternative "milk", "cream", and "yoghurt" products which may, for example, be derived from soy, almond, rice, pea, coconut, and nuts (e.g. cashew). The dairy alternative products may,
15 for example, be sweetened or unsweetened.

- Of further particular interest are, for example, beverages including beverage mixes and concentrates, including, for example, alcoholic and non-alcoholic ready to drink and dry powdered beverages, carbonated and non-carbonated beverages, e.g., sodas, fruit or
20 vegetable juices, alcoholic and non-alcoholic beverages. The beverages may, for example, be sweetened or unsweetened.

- Of further particular interest are, for example, food products traditionally high in sodium salt with a reduced sodium salt concentration, including condiments and sauces (cold,
25 warm, instant, preserved, sate, tomato, BBQ Sauce, Ketchup, mayonnaise and analogues, bechamel), gravy, chutney, salad dressings (shelf stable, refrigerated), batter mixes, vinegar, pizza, pasta, instant noodles, french fries, croutons, salty snacks (potato chips, crisps, nuts, tortilla-tostada, pretzels, cheese snacks, corn snacks, potato-snacks, ready-to-eat popcorn, microwaveable popcorn, caramel corn, pork
30 rinds, nuts), crackers (Saltines, 'Ritz' type), "sandwich-type" cracker snacks, breakfast cereals, cheeses and cheese products including cheese analogues (reduced sodium cheese, pasteurized processed cheese (food, snacks & spreads), savoury spreads, cold pack cheese products, cheese sauce products), meats, aspic, cured meats (ham, bacon), luncheon/breakfast meats (hotdogs, cold cuts, sausage), soya-based products,
35 tomato products, potato products, dry spice or seasoning compositions, liquid spice or seasoning compositions including pesto, marinades, and soup-type/meal-alternative

beverages, and vegetable juices including tomato juice, carrot juice, mixed vegetable juices and other vegetable juices.

5 The food product may, for example, comprise from about 0.001 % to about 0.5 % (wt/wt) based on the unconcentrated flavour modifying ingredient, for example from about 0.001 % to about 0.02 % (wt/wt) based on the unconcentrated flavour modifying ingredient.

10 If the flavour modifying ingredient is added as an unconcentrated liquid, about 0.005 to about 0.5% (wt/wt) is usually enough, for example, without limitation, in soups and topical food applications such as chips, crisps and snacks.

15 Depending on the food product more may be needed. For most topical applications, about 0.1% to about 0.5% (wt/wt) is sufficient. When using a concentrate (for example by distillation) or a spray-dried salt enhancing ingredient, the concentrations indicated need to be adjusted with an appropriate factor to take into account of the concentration change in the salt enhancing ingredient.

20 Depending on the food product, for food products that contain about 10 to 100 %, for example 25 to 50%, less sodium than a comparable food product (for example "reduced sodium" products with 25% reduction, or "light in sodium" products with a 50% reduction), the flavour modifying ingredient may be employed as follows: a useful concentration for most food applications may be, for example, about 0.001% to about 0.015% (wt/wt) based on the unconcentrated flavour modifying ingredient. Alternatively, 25 for example, 25 to 300 ppm or 0.002% to 0.03% (wt/wt) based on a spray-dried 2x concentrate may be used.

30 The flavour modifying ingredient may be used in unconcentrated or concentrated form or the concentrate may be formulated into a paste or powder by methods known in the art. In this case the amount to be used has to be adjusted accordingly. Flavour compositions such as spices are often more concentrated, for example a 10x concentrate, and the concentration will be adjusted higher accordingly (250 ppm to 3000 ppm).

35 The NaCl concentration in common food products with a regular NaCl concentration varies with most products ranging from about 0.5% to about 5% (wt/wt) NaCl. Seasoning or products used as seasoning, such as croutons, sauces or salad

dressings that are employed in a small amount (to be applied to, for example, salad or noodles), have a concentration of for example from about 2% to about 5% (wt/wt) NaCl. Soups usually contain about 0.6% to about 1.25% (wt/wt) NaCl. Salty crackers and meat products (e.g. salami, ham, and bacon) usually contain about 2% to about 4% (wt/wt) NaCl. Cereals usually contain about 0.6 to 3% (wt/wt) NaCl. Products that need to be reconstituted (dry soups) usually range in the concentration ranges indicated after reconstitution.

For low sodium products containing even less NaCl than products with reduced sodium content (e.g. 353 mg per serving), the amount of the salt enhancing ingredient may have to be increased.

For food products with added KCl, depending on the food product and said ingredients, the concentration of KCl may be from about 0.1 % or about 0.2 %, up to about 1%, up to about 1.5%, up to about 2% (wt/wt), or higher, depending on how much the sodium concentration is reduced. A KCl concentration of about 0.25 % to about 1.5% (wt/wt), for example about 0.5% to about 1.5% (wt/wt) KCl will be useful for most low sodium products. A range to which the NaCl concentration may usefully be reduced for most applications is, for example, about 0.25% (wt/wt) to about 2.5% (wt/wt), or from about 0.125% to about 1.25% (wt/wt). The amount of the flavour modifying ingredient to be added to the food product as an ingredient will depend on the concentration of KCl used, and the specific food product including the particular base and flavour. A useful concentration for most food applications may be, for example, about 0.001% to about 0.015% (wt/wt) based on the unconcentrated flavour modifying ingredient. Alternatively, for example, 25 to 300 ppm or 0.002% to 0.03% (wt/wt) based on a spray-dried 2x concentrate may be used.

The flavour modifying ingredient may be used in un-concentrated form or the concentrate may be formulated into a paste or powder or spray-dried salt enhancing ingredient by methods known in the art. In this case, the amount to be used has to be adjusted accordingly.

The appropriate concentration of the flavour modifying ingredient can be easily tested by an organoleptic titration. This technique is well known in the field of sensory analysis.

The flavour compositions and food products may, for example, comprise one or more sweeteners. Examples of sweeteners that may be used in the sweetened compositions are disclosed, for example, in WO 2016/038617, the contents of which are incorporated herein by reference.

5

The one or more sweeteners may, for example, be selected from sucrose, fructose, glucose, xylose, arabinose, rhamnose, tagatose, allulose, trehalose, isomaltulose, steviol glycosides (e.g. rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, rebaudioside G, rebaudioside H, rebaudioside I, rebaudioside J, rebaudioside K, rebaudioside L, rebaudioside M, rebaudioside N, rebaudioside O, dulcoside A, dulcoside B, rubusoside, naringin dihydrochalcone, stevioside), mogrosides (e.g. grosvenorine II, grosvenorine I, 11-O-mogroside II (I), 11-O-mogroside II (II), 11-O-mogroside II (III), mogroside II (I), mogroside II (II), mogroside II (III), 11-dehydroxy-mogroside III, 11-O-mogroside III, mogroside III (I), mogroside III (II), mogroside IIIe, mogroside IIIx, mogroside IV (I) (siamenoside), mogroside IV (II), mogroside IV (III), mogroside IV (IV), deoxymogroside V (I), deoxymogroside V (II), 11-O-mogroside V (I), mogroside V isomer, mogroside V, iso-mogroside V, 7-O-mogroside V, 11-O-mogroside VI, mogroside VI (I), mogroside VI (II), mogroside VI (III) (neomogroside) and mogroside VI (IV)), stevia, trilobatin, rebusoside, aspartame, advantame, agarve syrup, acesulfame potassium (AceK), high fructose corn syrup, neotame, saccharin, sucralose, high fructose corn syrup, starch syrup, Luo Han Guo extract, neohesperidin, dihydrochalcone, naringin, sugar alcohols (e.g. sorbitol, xylitol, inositol, mannitol, erythritol), cellobiose, psicose, and cyclamate.

25 *Uses*

The flavour modifying ingredient obtained by and/or obtainable by the methods described herein may, for example, be added to food products (e.g. as part of a flavour composition) to modify the flavour or mouthfeel of the food product.

30

The flavour modifying ingredient obtained by and/or obtainable by the methods described herein may, for example, be used to improve the mouthfeel of a food product and/or to mask off-notes of a food product and/or to improve the sweetness of a food product and/or to enhance the saltiness of a food product and/or to act as a prebiotic in a food product.

35

Thus, there is also provided herein a method of providing a food product having improved mouthfeel and/or reduced off-notes and/or improved sweetness and/or

enhanced saltiness and/or use as a prebiotic, the method comprising admixing the flavour modifying ingredient obtained by and/or obtainable by the methods described herein with the food product.

- 5 In general terms, “mouthfeel” refers to the complexity of perceptions experienced in the mouth as influenced by the aroma, taste, and texture qualities of food and beverage products. From a technical perspective, however, mouthfeel sensations are specifically associated with physical (e.g. tactile, temperature) and/or chemical (e.g. pain) characteristics perceived in the mouth via the trigeminal nerve. Accordingly, they are a
10 consequence of oral-tactile stimulations and involve mechanical, pain and temperature receptors located in the oral mucosa, lips, tongue, cheeks, palate and throat.

- Mouthfeel perceptions include, for example, one or more of texture - astringent, burning, cold, tingling, thick, biting, fatty, oily, slimy, foamy, melting, sandy, chalky,
15 watery, acidic, lactic acid type, lingering, metallic, body, body sweet, carbonation, cooling, warming, hot, juicy, mouth drying, numbing, pungent, salivating, spongy, sticky, fullness, cohesiveness, density, fracturability, graininess, grittiness, gumminess, hardness, heaviness, moisture absorption, moisture release, mouthwatering, mouthcoating, roughness, slipperiness, smoothness, creamy, creamy texture, buttery,
20 uniformity, uniformity of bite, uniformity of chew, viscosity, fast-diffusion, full body, salivation and retention.

- As stated previously, the perceived mouthfeel of a food or beverage can be broadly influenced by the presence of aroma and taste attributes in addition to textural
25 properties. Thus a number of other attributes may affect the experienced overall mouthfeel sensation of a product including, for example, one or more of taste or aroma – for example sweet, salty, umami, sour, bitter, creamy, creamy texture, creamy sour, acidic, acidic dairy, green onion, toasted onion and parsley.

- 30 By “improvement of mouthfeel” it is meant that any one or more of desired mouthfeel perceptions is/are enhanced and/or that any one or more undesirable mouthfeel perceptions is/are reduced, as compared to a non-dairy, non-fermented base. In particular, one or more of the following perceptions may be enhanced by the product and methods described herein: good texture, less gummy, creamy, creamy texture,
35 creamy sour, buttery, acidic, acidic dairy, sweet, salty, umami.

By “masking of off-notes” it is meant that the intensity and/or length of perception of undesirable attributes in a food product is reduced, as analysed by trained panellists when comparing food comprising an ingredient with off-note masking to food without an added off-note masking ingredient.

5

By “improvement in sweetness” it is meant the effect of the flavour modifying ingredient on the sweetness characteristics of a food which are found to be more favourable as analysed by trained panellists when comparing food comprising an ingredient with sweetness improving effect to food without an added sweetness improving ingredient.

10

The improvement in sweetness may, for example, provide sweetness characteristics that are more similar to the sweetness characteristics of sucrose.

The sweetness characteristics may refer to the flavour profile (taste profile), which refers to the intensity of the flavour and perceptual attributes of a given compound. Exemplary flavour attributes of sweetness are sweetness intensity, bitterness, black liquorice etc.

The sweetness characteristics may refer to the temporal profile, which refers to the changes in perception of sweetness over time. Every sweetener exhibits a characteristic appearance time (AT) and extinction time (ET). Most high-potency sweeteners, in contrast to carbohydrate sweeteners, display prolonged ET (lingering). Generally, the detected sucrose equivalence spikes to a maximal response level, then tapers off over time. The longer the taper, the greater the detected sweetness linger of a compound.

The improvement in sweetness may, for example, be particularly obtained when the flavour modifying ingredient is used in sweetened food products. The improvement of sweetness may, for example, be particularly obtained in dairy products or beverages, for example sweetened dairy products or beverages.

In certain embodiments, the flavour modifying ingredient may be used to weaken the lingering sweet taste of the food product (e.g. sweetened food product). In other words, the flavour modifying ingredient may be used to decrease the extinction time (ET) of the food product (e.g. sweetened food product). This relates to the undesirable lingering of the sweetness taste in the mouth after the food product is initially ingested or expectorated. The lingering sweet taste may, for example, refer to the length of time

that the sweetness taste remains after it is initially detected, how rapidly the intensity of the sweetness taste decreases or fades after it is initially detected and the intensity of the sweetness taste after it is initially detected. The flavour modifying ingredient may, for example, decrease the length of time that the sweetness taste remains after it is initially detected and/or increase the speed at which the sweetness taste decreases after it is initially detected and/or decrease the intensity of the sweetness taste after it is initially detected.

In certain embodiments, the flavour modifying ingredient may be used to weaken the bitter taste and/or astringent taste and/or metallic taste and/or liquorice taste of the food product (e.g. sweetened food product).

In certain embodiments, the flavour modifying ingredient may be used to strengthen the sweetness impact of the food product (e.g. sweetened food product). The sweetness impact relates to the length of time it takes before the sweetness is initially detected and the intensity at which the sweetness is initially detected. The flavour modifying ingredient may, for example, decrease the amount of time before the sweetness is initially detected and/or increase the intensity at which the sweetness is initially detected.

The degree of sweetness and other sweetness characteristics described herein may be evaluated by a tasting panel of trained experts, for example as described in the examples below.

By "salt enhancing" it is meant the effect of the flavour modifying ingredient on the salt taste of a food which is found more pronounced (stronger, enhanced) in its taste intensity and and/or longer in its duration as analysed by trained panellists sensitive to salty taste when comparing food comprising an ingredient with a salt enhancing effect to food without an added salt enhancing ingredient.

By "prebiotic" it is meant the effect of the flavour modifying ingredient, the ready-to-eat product and/or the ready-to drink product to improve the effect of gut flora, for example by increasing the activity of the gut flora and/or by increasing the population of the gut flora. By "probiotic" it is meant live bacteria, for example the microbial strains or blends of strains described herein, that ferment substances, for example plant-based products.

- The ready-to-eat and ready-to-drink products made by the methods described herein may, for example, be used directly as a final food product and may not undergo further processing. Sensory evaluation of such products may be conducted by trained panelists. The use of trained panelists is a widely recognized analytical tool for assessing sensory profiles of compounds in a statistically significant manner. See e.g. "EFFA Guidance Document on the EC Regulation on Flavourings", *European Flavor Association, 2015*. Sensory profiling is based on the concept that the overall sensory impression obtained from a sample consists of a number of identifiable sensory attributes (descriptors), each of which is present to a larger or smaller degree. Panelists are trained to recognize each descriptor by assessing typical molecules or blend of molecules that corresponds to that specific descriptor. As demonstrated by the examples, the ready-to-eat and ready-to-drink products made by the methods described herein provide a pleasant taste with good mouthfeel.
- The foregoing broadly describes certain embodiments of the present invention without limitation. Variations and modifications as will be readily apparent to those skilled in the art are intended to be within the scope of the present invention as defined in and by the appended claims.
- Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. The term "comprising" also means "including" as well as "consisting" e.g. a composition "comprising" X may consist exclusively of X or may include something additional e.g. X + Y. It must be noted also that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. By way of example, a reference to "a gene" or "an enzyme" is a reference to "one or more genes" or "one or more enzymes".
- In this document and in its claims, the verb "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, the verb "to consist" may be replaced by "to consist essentially of" meaning that a composition as described herein may comprise additional component(s) than the ones specifically identified, said additional component(s) not altering the unique characteristics of the invention. In addition, the verb "to consist" may be replaced by "to consist essentially of" meaning

that a method or use as described herein may comprise additional step(s) than the ones specifically identified, said additional step(s) not altering the unique characteristic of the invention. In addition, the verb "to consist" may be replaced by "to consist essentially of" meaning that a nucleotide or amino acid sequence as described herein
5 may comprise additional nucleotides or amino acids than the ones specifically identified, said additional nucleotides or amino acids not altering the unique characteristics of the invention.

As used herein, with "at least" a particular value means that particular value or more.
10 For example, "at least 2" is understood to be the same as "2 or more" i.e., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, ..., etc.

The word "about" or "approximately" when used in association with a numerical value (e.g. about 10) preferably means that the value may be the given value (of 10) more or
15 less 1% of the value.

As used herein, the term "and/or" is understood to mean that all members of a group connected by the term "and/or" are represented both cumulatively with respect to each other in any combination, and alternatively with respect to each other. Exemplarily, for
20 the expression "A, B and/or C", the following disclosure is to be understood thereunder: i) (A or B or C), or ii) (A and B), or iii) (A and C), or iv) (B and C), or v) (A and B and C), or vi) (A and B or C), or vii) (A or B and C), or viii) (A and C or B).

Various embodiments are described herein. Each embodiment as identified herein may
25 be combined together unless otherwise indicated. It is to be understood that this disclosure is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present disclosure which will be limited only by the
30 appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by the person skilled in the art. In accordance with the present disclosure there may be conventional molecular biology, microbiology, and recombinant DNA techniques employed which are within the skill of the art.

35 This disclosure is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the

drawings. The disclosure is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. Preferably, the terms used herein are defined as described in "A multilingual glossary of
5 biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Kolbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific
10 publications, manufacturer's specifications, instructions, GenBank Accession Number sequence submissions etc.), whether supra or infra, is hereby incorporated by reference in its entirety. The examples described herein are illustrative of the present disclosure and are not intended to be limitations thereon. Different embodiments of the present disclosure have been described according to the present disclosure. Many
15 modifications and variations may be made to the techniques described and illustrated herein without departing from the spirit and scope of the disclosure. Accordingly, it should be understood that the examples are illustrative only and are not limiting upon the scope of the disclosure.

Aspects

1. A method for making a ready-to-eat or ready-to-drink product, the method comprising subjecting at least one edible component of a cereal to fermentation, enzymatic hydrolysis, or to fermentation and enzymatic hydrolysis, wherein the
5 fermentation uses two or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, and/or *Bifidobacterium animalis lactis*,
10 wherein the cereal is selected from the group consisting of oat, maize, rice, wild rice, wheat, barley, sorghum, millet, rye, triticale, fonio and combinations thereof
2. The method of aspect 1, wherein the at least one edible cereal component is in the form of, or derived from, cereal grain, cereal wholegrain, cereal groats, steel cut
15 cereal, rolled cereal, cereal bran, cereal flour, cereal kernel, cereal fibre, or combinations thereof
3. The method of aspect 1 or aspect 2, wherein the at least one edible component of the cereal is in an aqueous slurry.
20
4. The method of aspect 1 or aspect 2 or aspect 3, wherein the enzymatic hydrolysis uses one or more enzymes selected from carbohydrases and proteolytic enzymes.
- 25 5. The method of any one of the preceding aspects, wherein the enzymatic hydrolysis uses at least one or more enzymes selected from cellulases, pectinases, and other carbohydrases.
6. The method of any one of the preceding aspects, wherein the enzymatic
30 hydrolysis uses at least one or more enzymes selected from alpha-amylases and endo-amylases.
7. The method of any one of the preceding aspects, wherein the enzymatic hydrolysis uses alpha-amylases, endo-amylases and proteolytic enzymes.
35
8. The method of any one of the preceding aspects, wherein the enzymatic hydrolysis uses alpha-amylases, endo-amylases, proteolytic enzymes and a peptidase.

9. The method of aspect 8, wherein the peptidase is a serine endo-peptidase
10. The method of any one of the preceding aspects, wherein the enzymatic
5 hydrolysis is performed at a temperature ranging from about 50°C to about 70°C for
about 1 hour using alpha-amylases and endo-amylases, and subsequently adding at
least one proteolytic enzyme and at least one bacterial enzyme and incubating for an
additional 2 to 3 hours at about 50°C to about 60°.
- 10 11. The method of any one of the preceding aspects, wherein the enzymatic
hydrolysis is performed at a temperature ranging from about 25°C to about 60°C.
12. The method of any one of the preceding aspects, wherein the enzymatic
hydrolysis takes place for a period of time ranging from about 1 hour to about 48 hours.
15
13. The method of any one of the preceding aspects, wherein the fermentation is
performed at a temperature ranging from about 20°C to about 45°C.
14. The method of any one of the preceding aspects, wherein the fermentation takes
20 place for a period of time ranging from about 1 day to about 2 days
15. The method of any one of the preceding aspects, wherein the enzymatic
hydrolysis occurs before and/or simultaneously with the fermentation.
- 25 16. The method of any one of the preceding aspects, wherein the method comprises
subjecting the at least one edible cereal component to fermentation and does not
comprise subjecting the at least one edible cereal component to enzymatic hydrolysis.
17. The method of any one of the preceding aspects, wherein the method comprises
30 heating the at least one edible cereal component to a temperature equal to or greater
than about 75°C prior to the enzymatic hydrolysis and fermentation.
18. The method of any one of the preceding aspects, wherein the cereal comprises
oat.
- 35 19. The method of aspect 18, wherein the oat comprises oat flour.

20. The method of any one of the preceding aspects, wherein the method further comprises spray-drying the ready-to-eat product.
21. The method of any one of the preceding aspects, wherein the edible cereal
5 component comprises oat fibre, maize fibre, rice fibre, wild rice fibre, wheat fibre, barley fibre, sorghum fibre, millet fibre, rye fibre, triticale fibre, fonio fibre, or combinations thereof.
22. The method of aspect 21, wherein the edible cereal component comprises oat
10 fibre.
23. The method of aspect 22, wherein the edible cereal component is in the form of, or derived from, oat grain, oat wholegrain, oat groats, steel cut oats, rolled oats, oat bran, oat flour, oat kernel, oat fibre, irish oatmeal, or combinations thereof.
- 15 24. The method of any one of the preceding aspects, wherein the fermentation uses three or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*,
20 *Bifidobacterium*, and/or *Bifidobacterium animalis lactis*.
25. A ready-to-eat product obtained by mixing at least one edible component of a cereal in an aqueous solution, wherein the cereal is selected from the group consisting
25 of oat, maize, rice, wild rice, wheat, barley, sorghum, millet, rye, triticale, fonio and combinations thereof, adding two or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, and/or *Bifidobacterium animalis lactis*
30 to the mixture, and incubating the mixture for a period of time sufficient to ferment at least a portion of the at least one edible cereal component to form the ready-to-eat product.
- 35 26. The ready-to-eat product of aspect 25, wherein the ready-to-eat product is yoghurt.

27. A ready-to-drink product obtained by mixing at least one edible component of a cereal in an aqueous solution, wherein the cereal is selected from the group consisting of oat, maize, rice, wild rice, wheat, barley, sorghum, millet, rye, triticale, fonio and combinations thereof, adding carbohydrase and/or proteolytic enzymes to the mixture, subsequently adding two or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, and/or *Bifidobacterium animalis lactis* to the mixture, incubating the mixture for a period of time sufficient to ferment at least a portion of the at least one edible cereal component to form the ready-to-drink product
28. The ready-to-drink product of aspect 27, wherein the ready-to-drink product is oat milk.
29. The ready-to-drink product of aspect 27, wherein the ready-to-drink product has a gluten content of less than 5ppm.
30. The ready-to-drink product of aspect 27, wherein the ready-to-drink product has a gluten content of less than 20 ppm.
31. A consumable product obtained by the method of any one of aspects 1-19 or comprising the product of any one of claims 25-30.
32. The consumable product of aspect 31, wherein the consumable product is a clean-label dairy alternative product.
33. The ready-to-eat product of aspect 25 or 26, wherein the edible cereal component is in the form of, or derived from, oat grain, oat wholegrain, oat fibre, oat groats, steel cut oats, rolled oats, oat bran, oat flour, oat kernel, Irish oatmeal or combinations thereof.
34. The ready-to-drink product of aspect 27 or 28, wherein the edible cereal component is in the form of, or derived from, oat grain, oat wholegrain, oat fibre, oat groats, steel cut oats, rolled oats, oat bran, oat flour, oat kernels, Irish oatmeal or combinations thereof.

35. The method of any one of aspects 1-24, wherein the at least one edible cereal component is present in an amount of about 5-20 wt.% based on the total weight of the aqueous slurry.

EXAMPLES

Example 1 – Fermented Oat Fibres

- 5 Flavour modifying ingredients were made by fermenting oat fibres by the following process.

831 g of water was added to a clean, sanitized tank. 166 g of oat fibre (AvenOLait™ oat fibre obtained commercially from Axiom Foods Inc.) was added to the water. The
10 mixture was heated to 121°C within 1 hour and with continuous mixing. The temperature of the mixture was held at 121°C for 30 minutes. The mixture was then cooled to 37°C before 3 g of seed ferment was added. The mixture was incubated at 37°C with slow agitation for four days. The mixture was then pasteurized at 100°C for 45 minutes. The product was stored at 4°C.

15

The seed ferment used was either rice coated with *Aspergillus oryzae* obtained from Rhapsody Natural Foods, Cabot VT 05647 (Flavour Modifying Ingredient (FMI)-A) or *Lactobacillus plantarum* or *Lactiplantibacillus plantarum* ATCC 14917 (FMI-B).

- 20 Organoleptic evaluation was undertaken using each flavour modifying ingredient in various food products at a concentration of about 0.1 % (pea yoghurt, non-fat yoghurt, soy yoghurt, and 2% fat milk).

In the potato chips, the flavour modifying ingredient was used in the sour cream and
25 onion base seasoning at a concentration of 0.1 % and the sour cream and onion base seasoning was added to the potato chips at a concentration of 7 %.

The various food products were as follows:

- 30 Pea Yoghurt (Ripple, Original – dairy alternative product)
Non-Fat Yoghurt (obtained from Danone – non-sweetened, non-fat dairy product)
Soy Yoghurt (Silk, plain – dairy alternative product)
2% Fat Milk (obtained from Kroger – non-sweetened, low fat dairy product)
Potato chips (Mike Sells Original Unsalted Chips) + 7% sour cream and onion base
35 flavour

Organoleptic evaluation of the pea yoghurt, non-fat yoghurt, soy yoghurt, and 2 % fat milk was carried out by flavorists (descriptive analysis).

Organoleptic evaluation of the sour cream and onion chips was carried out by a paired comparison strategy, whereby potato chips with the sour cream and onion base flavour with FMI-A were compared to potato chips with the sour cream and onion base flavour without FMI-A. Eleven panellists performed a pre-evaluation review and training with the sour cream and onion potato chips. For the organoleptic evaluation, samples were presented to the panellists in pairs as blinded samples in a randomized, fully balanced order. For each pair of products, the panellist was instructed to select the sample that was greater for each attribute (creamy sour, green onion, toasted onion, parsley, acidic dairy, sweet, salty, umami). Four repetitions of each paired evaluation were performed.

The definition of the attributes tested for the organoleptic evaluation of the sour cream and onion chips was as follows.

Creamy sour: Sour dairy aroma associated with sour cream, butter, and yoghurt

Green onion: Herbal, green, alliaceous aroma associated with green onions

Toasted onion: Sweet, brown, toasted, and alliaceous aroma associated with onion powder

Parsley: Green, leafy, and woody aroma associated with fresh parsley leaves

Acidic dairy: Basic taste on the tongue associated with lactic acid in solution, similar to fermented cow's milk

Sweet: Basic taste sensation associated with sugars and high potency sweeteners in solution

Salty: Basic taste sensation associated with table salt (NaCl) diluted in water

Umami: Basic taste sensation associated with MSG, characterized by fullness of flavour in the mouth, often found in bouillons, soy sauce, and mushrooms

The results are provided below.

Pea Yoghurt

FMI-A Taste Evaluation: Cleaned pea note, masks astringency, good cultured note profile, cleaner, sour (preferred compared to FMI-B).

FMI-B Taste Evaluation: Sweeter, less astringent, masks pea note, creamy, acid is masked, sweeter note comes through, less gritty.

Non-Fat Yoghurt

FMI-A Taste Evaluation: Very acidic, more sour, best cultured note, clean, more cultured.

5

FMI-B Taste Evaluation: Makes more balanced yogurt profile, cleaner acid note, more cultured, more dairy note, very acidic sharp, balanced, clean finish, more cultured, more sour note (Preferred compared to FMI-A).

10 ***Soy Yoghurt***

FMI-B Taste Evaluation: Good yogurt profile, less astringent, masks beany, creamy, sweet, nice upfront, acidic middle end, slight cultured, very smooth, balanced acidity.

2% Fat Milk

15

FMI-B Taste Evaluation: Fatty full, almost like full fat milk, yogurt like, creamy, cultured at the end, clean profile.

Sour Cream & Onion Chips

20

FMI-A Taste Evaluation: Creamy note enhancements, saltier than base alone ($p < 0.05$), less vegetative notes (parsley) than base alone ($p < 0.05$), increased perception of umami, acidic dairy, and toasted onion than base alone ($p < 0.1$).

25 It was surprisingly found that the flavour modifying ingredients eliminated the unpleasant beany taste of the dairy alternative products (pea yoghurt and soy yoghurt).

In addition, it was surprisingly found that the flavour modifying ingredients provided a “fullness” sensation to the low-fat or non-fat dairy products (non-fat yoghurt and 2% fat milk) that gives the impression of the corresponding full-fat dairy product.

30

It was further surprisingly found that the flavour-modifying ingredient FMI-A provided a salty taste in a savoury product (sour cream and onion chips).

35

Example 2 – Enzymatic Hydrolysis of Grape Fibre

A Flavour modifying ingredient was made by subjecting grape fibre to enzymatic hydrolysis.

5

The flavour modifying ingredient (FMI-C) was made by mixing 70 g of Concord Grape Fibre (obtained from FruitSmart) with 623.35 g of water in a clean, sanitized tank. The following enzymes were then added to the mixture: 3.5 g Celluclast® (from Novozyme), 1.4 g Viscozyme® (from Novozyme), 0.7 g Flavorzyme® (from Novozyme), 0.35 g Umamizyme® (from Amano Enzymes), and 0.7 g Ceramix® (from Novozyme). The mixture was incubated at 50°C for 24 hours with continuous agitation.

10

FMI-C was then filter-centrifuged through a felt filter bag at 1000 rpm for 10 minutes to remove large solids. The filtrate (532 g) was heated at 100°C for 1 hour to inactivate the enzymes. FMI-C was then stabilized by mixing with 228 g of propylene glycol and stored at 4°C.

15

Organoleptic evaluation was undertaken using FMI-C in various Reb A-, sucralose- and sugar-sweetened beverage bases at a concentration of 0.07 % and 0.09 %.

20

The Reb A-, sucralose- and sugar-sweetened bases were as follows:

Hybrid RebA/steviolglycoside-sugar base (a still neutral beverage sweetened with RebA/steviolglycoside sugar for 30% sugar reduction – 7.3 % sugar + 0.1 % citric acid)

25

Reduced sugar base (5% sucrose + 0.03 % citric acid in water; benchmark: 5.5 % sucrose + 0.03 % citric acid in water)

Hybrid Sucrose-Glucose-Fructose-RebA base (0.9 % sucrose, 0.45 % glucose, 0.45 % fructose and 180 ppm RebA + 0.05 % citric acid in water; benchmark: 1.4 % sucrose, 0.7 % glucose, 0.7 % fructose and 120 ppm RebA + 0.05 % citric acid in water)

30

Hybrid Sucralose-AceK base (70 ppm sucralose and 21 ppm AceK + 0.05 % citric acid in water; benchmark: 35 ppm sucralose, 21 ppm AceK and 2.5 % sucrose + 0.05 % citric acid in water)

35

Organoleptic evaluation was carried out by groups of 2-4 flavorists, comparing the samples containing FMI-C to their corresponding bases and benchmarks (paired comparison).

- 5 It was found that when FMI-C was added at a concentration of 0.09 %, there was improved upfront sweetness and decreased linger of the hybrid RebA/stevioglycoside-sugar sweetened base.

When FMI-C was added at a concentration of 0.07 %, there was increased sweetness
10 of the Reduced sugar base containing 5% sucrose by about ½ brix.

Example 3 – Enzymatic Hydrolysis and Fermentation of Cranberry Fibre

A Flavour modifying ingredient was made by subjecting cranberry fibre to enzymatic
15 hydrolysis followed by fermentation.

The flavour modifying ingredient (FMI-D) was made by mixing 105 g of the Cranberry fibre (obtained from FruitSmart) with 589.4 g of water in a clean, sanitized tank. The following enzymes were then added to the mixture: 3.5g Celluclast® (from Novozyme),
20 1.4g Viscozyme® (from Novozyme), 0.7g Flavorzyme® (from Novozyme), 0.35g Umamizyme® (from Amano Enzymes), and 0.7g Ceramix™ (from Novozyme) and the mixture was then incubated at 50°C for 24 hours with continuous agitation. The mixture was then cooled to 37°C and 3.5 g of *Aspergillus oryzae* (Koji) culture was added (obtained from Rhapsody Natural Foods). The mixture was incubated at 37°C for 96
25 hours with agitation and open ports.

The slurry was then diluted with water to bring the solids content from 15 % to 10 % and filter-centrifuged through a felt filter bag at 1000 rpm for 10 minutes to remove large solids. The filtrate (511 g) was then heated at 100°C for 1 hour to inactivate the
30 enzymes. FMI-D was then stabilized by mixing with 219 g of propylene glycol and stored at 4°C.

Organoleptic evaluation was undertaken using FMI-D at a concentration of 0.05% in a zero-calorie base containing 180 ppm Reb A and 0.05% citric acid (descriptive
35 analysis).

Organoleptic evaluation was carried out by 6 panelists (flavorists and scientists).

It was found that addition of FMI-D to the zero-calorie base resulted in reduced lingering, masked bitterness and metallic taste, and a more sugary mouthfeel.

Organoleptic evaluation was also undertaken using 0.075% FMI-D in plain pea yoghurt
5 obtained from Ripple foods sweetened with 180 ppm Reb A.
FMI-D provided sweetness, creaminess, and a more clean taste profile compared to the blind blank base.

Example 4 – Enzymatic Hydrolysis of Grape Fibre

10

A Flavour modifying ingredient was made by subjecting grape fibre to enzymatic hydrolysis.

The flavour modifying ingredient (FMI-E) was made by mixing 140g of the Concord
15 Grape fibre (obtained from FruitSmart) with 547.9g of water in a clean, sanitized tank.
The following enzymes were then added to the mixture: 5.25g Celluclast® (from Novozyme), 2.1g Viscozyme® (from Novozyme), 1.05g Flavorzyme® (from Novozyme), 0.525g Umamizyme® (from Amano Enzymes), 1.05g Ceramix™ (from Novozyme), 1.4g Hemicellulase (from Amano Enzymes), and 0.7g Mannanase (from
20 Amano Enzymes). The mixture was incubated at 50°C for 24 hours with continuous agitation.

The slurry was then filter-centrifuged through a felt filter bag at 1000 rpm for 10 minutes to remove the large solids. The filtrate (429g) was heated at 100°C for 1 hour to
25 inactivate the enzymes and the ingredient was stabilized by mixing with 184g Propylene glycol and stored at 4°C.

Organoleptic evaluation was undertaken using FMI-E in various Reb A-, sucralose- and sugar-sweetened beverage bases at concentrations between 0.02% and 0.09%

30

The Reb A-, sucralose- and sugar-sweetened bases were as follows with the same composition as the bases used in Example 2:

Reduced sugar base (5% sucrose + 0.03 % citric acid)

35

Hybrid Sucrose-Glucose-Fructose-RebA base

Hybrid Sucralose-AceK base

Organoleptic evaluations were carried out by 6 flavorists.

It was found that at 0.09 % concentration, FMI-E increased the sweetness of the reduced-sugar base (containing 5% sucrose) by more than ½ brix. At 0.045 % concentration, the sweetness of the reduced-sugar base (containing 5% sucrose) increased by about ½ brix. At 0.03 % concentration, FMI-E added body in the middle of the sweetness and modulated (reduced) metallic linger of sucralose in the hybrid sucralose-AceK-sugar base.

Example 5 – Enzymatic Hydrolysis and Fermentation of Oat Fibre

10

A Flavour modifying ingredient or probiotic drink was made by subjecting oat fibre to enzymatic hydrolysis and fermentation.

The flavour modifying ingredient or probiotic drink was made by mixing Oat flour (code name P12 or BG28 from Naturex) or Oat kernels (from Grain Millers Inc US) with water to form a slurry with 20 to 30% solids. The aqueous slurry was heated to a temperature in the range of 50°C to about 55°C prior to the enzymatic hydrolysis. Alpha-Amylase enzyme (Kleistase® SD-80, from Amano Enzyme at a concentration of 1 to 1.5%) was then added and the mixture was incubated at 50-55°C for 2 hours to break down the amylose and amylopectin to maltose and various dextrans. Glucoamylase (Gluczyme®NLP from Amano Enzyme at a concentration of 0.5 to 1.5% for another 1 to 2 hrs at 50°C to 55°C) was then added for further breakdown to release glucose. Protease/aminopeptidase enzymes (Protein Glutaminase®500 from Amano) were also added to hydrolyze the proteins at 50°C to 55°C for 1 to 2 hours. The mixture was pasteurized at 100°C for 45 minutes to inactivate all enzymes. Then it was fermented for 24 to 48 hours at 30-35°C with lactic acid bacteria (such as *L. delbrueckii ssp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*) and/or a *Bifidobacterium* at a concentration of 0.3 to 0.7%. The cultures were obtained from a commercial supplier (eg, Vivolac, US) as a frozen concentrate. The resulting flavour modifying ingredient was then either kept refrigerated or further processed by spray-drying.

Organoleptic evaluation was undertaken by adding the flavour modifying ingredient at a concentration of 0.05% to GoodBelly's® dairy-free probiotic shots. Six panellists carried out the organoleptic evaluation. All panellists found that the flavour modifying ingredient provided good body and improved mouthfeel as well as off-note masking and some sweetness improvement. The results are provided in the Table below.

Evaluation of enzymatically hydrolyzed and fermented Oat fibers at a concentration of 0.05% in GoodBelly® dairy-free probiotic shots

Sample code #	Fiber material	Comments
229-9220-00	Oat kernel	Good mouthfeel, slight sweetness.
229-9228-00	Oat flour BG28	Good mouthfeel, viscous texture, good masking, more dairy like, creamy, smooth
229-9230-00	Oat flour P12	Good mouthfeel, less off note, slight sweet, dairy like, fullness, cleaner

Example 6 – Ready-To-Eat Yoghurt-type Product Obtained from an Edible Cereal Component

A ready-to-eat yoghurt product was made by subjecting an edible cereal component to fermentation. The ready-to-eat yoghurt product was made by mixing 100 grams of oat flour (code name P12 from Naturex SA and Swedish Oat Fiber Ab of Bua, Sweden) with 900 grams of water to form a slurry with 10% solids in a glass reactor. The aqueous slurry was heated to a temperature of about 121°C for 15 minutes prior to fermentation to eliminate any initial microbial contaminants. The aqueous slurry was then cooled down to about 37°C. *Lactobacillus rhamnosus* and *Bifidobacterium animalis lactis* (LGG® and BB-12®, respectively, from Chr. Hansen A/S at a 1:1 ratio for a total inoculum level of 0.1 to 1%, in particular about 0.3%) were then added and the mixture was incubated at about 37°C with a low level of continuous mixing for about 16 hours. Initial pH of the mixture was 6.16 and as the incubation continued pH started to drop and after 16 hours of incubation pH was 4.27, and at this stage fermentation was terminated by heating at about 121°C for about 15 min. This heat treatment was optional, as low pH and refrigeration temperature of storage would be sufficient to keep the ready-to-eat product safe and prevent further microbial growth.

Organoleptic evaluation of the ready-to-eat yoghurt product was undertaken by 5-7 sensory trained expert panellists. All panellists found that the ready-to-eat yoghurt product provided a pleasant taste with a creamy and smooth texture. The organoleptic descriptors used by the panellists were: good texture, acidic, dairy lactic acid type notes, good creamy texture. The dairy lactic acid type taste is very desirable in dairy alternative consumable products.

Example 7 – Ready-To-Eat Yoghurt-type Product Obtained from an Edible Cereal Component

A ready-to-eat yoghurt product was made by the same process as Example 6, except a
5 blend of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (YOFLEX® YF-L02
DA from Chr. Hansen A/S at a concentration of about 0.1 to 1%, in particular about
0.3%) was used for the fermentation. Initial pH of the mixture was 6.21 and as the
incubation continued pH started to drop and after 16 hours of incubation pH was 4.21
10 and at this stage fermentation was terminated by heating at about 121°C for about 15
min. This heat treatment was optional as low pH and refrigeration temperature of
storage would be sufficient to keep the product safe and prevent further microbial
growth.

Organoleptic evaluation of the ready-to-eat yoghurt product was undertaken by 5-7
15 sensory trained expert panellists. All panellists found that the ready-to-eat yoghurt
product provided a pleasant taste with creamy and smooth texture. The organoleptic
descriptors used by the panellists were very nice texture with low gumminess.
Panellists determined that the microbial culture containing *Streptococcus thermophilus*
and *Lactobacillus bulgaricus* provided excellent texture.

20

Example 8 – Ready-To-Eat Yoghurt-type Product Obtained from an Edible Cereal Component

A ready-to-eat yoghurt product was made by the same process as Example 6, except a
25 combination of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (YOFLEX®
YF-L02 from Chr. Hansen A/S) and *Lactobacillus rhamnosus* (LGG® from Chr. Hansen
A/S) was used for the fermentation. Organoleptic evaluation of the ready-to-eat yoghurt
product was undertaken by 5-7 sensory trained expert panelists. All panelists found
that the ready-to-eat yoghurt product provided a pleasant taste with creamy and
30 smooth texture.

Example 9 – Ready-To-Eat Yoghurt-type Product Obtained from an Edible Cereal Component

35 A ready-to-eat yoghurt product was made by the same process as Example 6, except a
combination of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (YOFLEX®
YF-L02 from Chr. Hansen A/S) and *Bifidobacterium animalis lactis* (BB-12® from Chr.
Hansen A/S), was used for the fermentation. Organoleptic evaluation of the

ready-to-eat yoghurt product was undertaken by 5-7 sensory trained expert panellists. All panellists found that the ready-to-eat yoghurt product provided a pleasant taste with creamy and smooth texture.

5 Example 10 – Ready-To-Drink fermented Oat Product Obtained from an Edible Cereal Component

A ready-to-drink oat milk product was made by subjecting an edible cereal component to enzymatic hydrolysis and fermentation. The ready-to-drink product was made by
10 mixing 100 grams of oat flour (code name P12 from Naturex and Swedish Oat Fiber Ab of Bua, Sweden) with 900 grams of water to form a slurry. The aqueous slurry was heated to a temperature of about 55°C. Alpha-Amylase enzyme (Kleistase® SD-80, from Amano Enzyme at a concentration of about 0.05 to about 1.0%, in particular about 0.1%) was then added to the mixture and then the mixture was incubated at about
15 55°C for 1 hour with continuous agitation to break down the amylose and amylopectin to maltose and various dextrins. Glucoamylase (Gluczyme®NLP from Amano Enzyme at a concentration of 0.05 to about 1.0%, in particular about 0.1%) and proteolytic enzymes Thermoase GL-30 (from Amano Enzyme at a concentration of 0.05 to about 1.0%, in particular about 0.1%), and Umamizyme (from Amano Enzyme at a
20 concentration of about 0.005 to about 1.0%, in particular about 0.01%) were added and incubated for an additional 2 to 3 hrs at 50-55°C. The mixture was then heated to 121°C for 15 minutes to inactivate the enzymes and any microbial contaminants. The mixture was then cooled down to 37°C. *Lactobacillus rhamnosus* (LGG® from Chr. Hansen A/S at a concentration of about 0.1 to 1%, in particular about 0.3%), was then
25 added and the mixture was incubated at 37°C for 16 hours. The initial pH was 5.79 and the final pH became 3.16. The final mixture was heated to 121°C for 15 min, and then cooled down to 30°C. This heat treatment was optional, as low pH and refrigeration temperature of storage would be sufficient to keep ready-to-drink product safe and prevent further microbial growth.

30 Organoleptic evaluation of the ready-to-drink oat milk product was undertaken by 5-7 sensory trained expert panellists. All panellists found that the ready-to-drink oat milk product provided a pleasant, sweet taste with good mouthfeel. The organoleptic descriptors used by the panellists were: very liquid having a fermented sweet taste.
35 Gluten content was less than 5ppm, so the product is considered a “gluten-free” product.

Example 11 – Ready-To-Drink fermented Oat Product Obtained from Whole Oat

A ready-to-drink product was made by subjecting whole oat to enzymatic hydrolysis and fermentation. The ready-to-drink product was made by mixing 80 grams of whole
5 oat (thick rolled oats from Grain Millers Inc., Iowa, USA) with 320 grams of water to form a slurry. The aqueous slurry was heated to a temperature of about 50°C. Alpha-Amylase enzyme (Kleistase® SD-80, from Amano Enzyme at a concentration of about 0.1 to about 1.5%, in particular about 0.4%) was then added and the mixture was incubated at 50-55°C for 30 minutes. Glucoamylase (Gluczyme®NLP from Amano
10 Enzyme at a concentration of 0.1 to about 1.5%, in particular about 0.4%) was then added and incubated for 1 hour. The proteolytic enzyme Glutaminase (at a concentration of 0.05 to 1.0%, in particular about 0.1%) was added and incubated for another 3 hours at 50-55°C. The mixture was then centrifuged to remove undigested solids. The mixture was then pasteurized at about 100°C for 30 minutes to inactivate
15 the enzymes and any microbial contaminants. A microbial culture containing *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium ssp* (ABY421 from Vivolac) was then added at a total inoculum level of 0.1 to 1%, in particular about 0.3%, and the mixture was incubated at about 30°C for 16 hours. The initial pH was 5.84 and the final pH became 4.56.
20 Sensory evaluation was carried out with 5-7 trained expert panellists. All panellists found that the ready-to-drink oat milk product provided a pleasant taste with a creamy and smooth texture. The organoleptic descriptors used by the panellists were: creamy and dairy type flavour and mouthfeel. The gluten content of the ready-to-drink product was less than 5 ppm, and therefore considered a “gluten-free” product.

25

Example 12 – Ready-To-Drink fermented Oat Product Obtained from Whole Oat

A ready-to-drink product was made by subjecting whole oat to enzymatic hydrolysis and fermentation. The ready-to-drink product was made by mixing 80 grams of whole
30 oat (thick rolled oats from Grain Millers Inc., Iowa, USA) with 320 grams of water to form a slurry. The aqueous slurry was heated to a temperature of about 50°C. Alpha-Amylase enzyme (Kleistase® SD-80, from Amano Enzyme at a concentration of 0.1 to 1.5%, in particular about 0.4%) was then added and the mixture was incubated at about 50°C for 30 minutes. Glucoamylase (Gluczyme®NLP from Amano Enzyme at
35 a concentration of 0.1 to 1.5%, in particular about 0.4%) was added and incubated for 1 hour. The proteolytic enzyme Glutaminase (at a concentration of 0.05 to 1.0%, in particular about 0.1%) was added and incubated for another 3 hours at 50-55°C. The

mixture was then centrifuged to remove undigested solids. The mixture was then pasteurized at 121°C for 15 minutes to inactivate the enzymes and any microbial contaminants. A microbial culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (YOFLEX® YF-L02 DA from Chr. Hansen A/S),
5 *Bifidobacterium animalis lactis* (BB-12® from Chr. Hansen A/S), *Streptococcus thermophilus* (YOFLEX® YF-L01 DA from Chr. Hansen A/S), or *Bifidobacterium animalis lactis*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (BB-12® and YOFLEX® YF-L02 DA, both from Chr. Hansen A/S) was then added at a total inoculum level of 0.1 to 1%, in particular about 0.3, and the mixture was incubated at about
10 30-37°C for 16 hours. The initial pH was 5.24 and the final pH was between 3.47 to 4.81, depending on the microbial culture used. The mixture may optionally be pasteurized to eliminate any microbial contaminants. Sensory evaluation was carried out with 5-7 trained expert panellists. All panellists found that the ready-to-drink oat milk product provided a pleasant taste having a very distinctive dairy type flavour with a
15 creamy and smooth texture. The gluten content of the ready-to-drink product was less than 5 ppm, and therefore considered a “gluten-free” product.

Example 13 Fermentation Study

20 Fermentation trials were run on a nondairy yogurt base, namely a pea protein base, using different microbial cultures. The objective of the trials was to determine the correct pH range in a good timeline. The nondairy base contained in %weight (g) 75.74% water, 13.30% pea protein isolate (PURIS® P870), 9.00% UHT coconut cream, 1.00% cane sugar, 0.50% calcium complex, 0.050% citrus fiber (CITRI-FI
25 100M40; 200MESH), and 0.010% pea protein binder flavour. The nondairy base was prepared according to the following steps: i) adding pea protein isolate and pea protein binder flavor to water at 55-60°C; ii) hydrating protein with water for 30 minutes with high shear at 55-60°C; iii) mixing all dry ingredients and adding while hydrating protein; iv) melting and adding coconut fat and continue mixing for 15 minutes; v) heating the
30 slurry to 62°C; vi) homogenizing at 2500/500 psi; vii) heat treating at 95°C for 8 minutes; and viii) cooling to 40°C.

Two cultures were formulated with the following microbial strains: *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*,
35 and *Bifidobacterium ssp.* The strains were classified per their characteristics but there were differences in their plasmid profile that dictate some of their functional characteristics like viscosity production and ability to ferment lactose, and phage sensitivity/resistance. Culture 1 (C1- ABY421 from Vivolac) ferments very slow and

gives high viscosity with a very mild almost neutral flavour. Culture 2 (C2- ABY424 from Vivolac) is a faster acid producer with high viscosity and a slightly stronger acetaldehyde (yogurt flavour).

5 Tests performed

Base Testing – The pH of the base was tested to be 6.87 at refrigeration temperature. The base was also plated for the presence of coliform and standard plate count. The percent solids was determined to be 18.43%.

10

Overnight Fermentation Procedure:

- Ten 150 mL samples of the nondairy yogurt base were allotted in sterile jars.
- Two 150 mL samples of ultra-high-temperature (UHT) milk (shelf stable) were allotted in sterile jars.
- 15 • One set of five of the nondairy yogurt base samples and 1 UHT milk sample were tempered at 40°C and the other set at 42°C.
- Samples were inoculated at a rate of 0.4% with the ratios listed in Table 1 and agitated to blend small amounts of each of the UHT milk and nondairy yogurt base in sterile jars were added as uninoculated controls.
- 20 • Samples were incubated for 16 hours.
- pH's were measured at the end of 16 hours.

Table 1: Overnight Fermentation Culture Ratios and pH's after 16 hours.

Sample	Base	Culture Ratio	Fermentation Temp. (°C)	pH at the end of 16 hrs.
1	nondairy yogurt base	100% C1	40	4.64
2	nondairy yogurt base	100% C2	40	4.37
3	nondairy yogurt base	50%/50% C1 to C2	40	4.49
4	nondairy yogurt base	70%/30% C1 to C2	40	4.54
5	nondairy yogurt base	30%/70% C1 to C2	40	4.51
6	UHT milk	50%/50% C1 to C2	40	3.92
7	nondairy yogurt base	100% C1	42	4.58
8	nondairy yogurt base	100% C2	42	4.32
9	nondairy yogurt base	50%/50% C1 to C2	42	4.46
10	nondairy yogurt base	70%/30% C1 to C2	42	4.50

11	nondairy yogurt base	30%/70% C1 to C2	42	4.43
12	UHT milk	50%/50% C1 to C2	42	3.85

Uninoculated controls for both the UHT milk and the nondairy yogurt base did not acidify after 16 hours of incubation.

5 Daytime Fermentation Procedure:

- Two 150 mL samples of the nondairy yogurt base were allotted in sterile jars.
- One 150 mL sample of UHT milk was allotted in a sterile jar.
- Samples were tempered to 40°C.
- Samples were inoculated at a rate of 0.4% with the ratios listed in Table 2 and agitated to blend.
- Small amounts of each of the UHT milk and the nondairy yogurt base in sterile jars were added as uninoculated controls.
- Samples were incubated at 40°C for 7 ½ hours.
- pH levels were measured at hour 5 and every half hour to 7 ½ hours or until a pH of 4.4 was reached.

Table 2: Daytime Fermentation Culture Ratios

Sample	Base	Culture Ratio	Fermentation Temp. (°C)
1	nondairy yogurt base	100% C2	40
2	nondairy yogurt base	50%/50% C1 to C2	40
3	UHT Milk	50%/50% C1 to C2	40

Table 3: Daytime Fermentation pH's

Time (hr)	100% C2	50%/50% C1 to C2	50%/50% C1 to C2
	nondairy yogurt base	nondairy yogurt base	UHT Milk
5	4.89	4.90	4.37
5.5	4.77	4.80	-
6	4.69	4.74	-
6.5	4.63	4.71	-
7	4.58	4.70	-
7.5	4.55	4.66	-

The activity of the 50%/50% C1 to C2 in UHT milk reached pH 4.4 within 5 hours, however in the nondairy yogurt base the activity at the same inoculation ratio reached pH 4.66 in 7 ½ hours. The 100% C2 inoculation into the nondairy yogurt base reached

pH 4.55 in 7 ½ hours. Uninoculated controls for both the UHT milk and the nondairy yogurt base did not acidify after 7 ½ hours of incubation.

Conclusion

- 5 Uninoculated control base samples did not drop in pH in either fermentation, indicating there was not acid producing bacteria present in the base itself. The culture activity was slower when inoculated at the same rates in the nondairy yogurt base compared to UHT milk. Activities of samples fermented at 42°C were faster than samples fermented at 40°C. The 100% C2 activities were faster than the 100% C1 activities. Blends of the two cultures gave different fermentation speeds according to the ratio of microbial strains inoculated. Blends with higher ratio of C2 were faster than blends with less C2 (and more C1). The samples that reached pH 4.4 were the 16 hours fermentations of 100% C2 at both temperatures. The next closest sample to reaching pH 4.4 in 16 hours was the 30% / 70% C1 / C2 fermented at 42°C. Some difference in curd size was seen. C1 produced smooth curds with small size and good mouthfeel.

Table 4: Illustrative dairy free Microbial Cultures used to make Ready-to-Eat and Ready-to-Drink Products

General ID #	Component	Strain(s)
716593	F-DVS-BB-12	<i>Bifidobacterium animalis lactis</i>
716594	F-DVS L. Casei 431	<i>Lactobacillus paracasei</i>
720758	F-DVS-LGG	<i>Lactobacillus rhamnosus</i>
704993	YF-L01 DA	<i>Streptococcus thermophilus</i>
716628	YF-L02 DA	<i>Streptococcus thermophilus</i> and <i>Lactobacillus bulgaricus</i>
-	ABY 421 ND	<i>Lactobacillus delbrueckii subsp. bulgaricus</i> , <i>Streptococcus thermophilus</i> , <i>Lactobacillus acidophilus</i> , and <i>Bifidobacterium ssp.</i>
-	ABY 424 ND	<i>Lactobacillus delbrueckii subsp. bulgaricus</i> , <i>Streptococcus thermophilus</i> , <i>Lactobacillus acidophilus</i> , and <i>Bifidobacterium ssp.</i>
	B019	<i>Bifidobacterium animalis subsp.lactis</i> (Probiotic BifidoB HN019)

- 20 The first five microbial cultures listed in Table 4 (i.e., 716593, 716594, 720758, 704993 and 716628) were obtained from Chr. Hansen A/S of Hørsholm, Denmark. The remaining two microbial cultures listed in Table 4 (i.e., ABY 421 ND and ABY 424 ND) were obtained from Vivolac Cultures Corporation of Indiana, USA. It has been found that the cell surface structures of *Bifidobacterium animalis lactis* (BB-12®) and *Lactobacillus rhamnosus* (LGG®) provide good mouthfeel and texture in flavour applications.

Example 14 – Further Sensory Evaluation of Strain or Blend of Strains

Fermentation was continued with the strain or blend of strains of Samples 7, 8, 9 and 11 (as detailed in Table 1). Two to three samples of each strain or blend of strains were
5 taken at different pH levels and evaluated for sensory properties to find an optimum solution, which closely represents dairy alternatives. Pre-acidify the water between 5-6 pH before fermentation and review sensory properties. Increase the sugar and culture to find out how it impacts the pH and sensory properties.

10 Example 15 – Fermentation of Oat Flour

Oat flour (SWEOAT™ Flour P12) was mixed with water to prepare a slurry containing 10% solids. The slurry was mixed well and heated to about 50-55°C. Thermoase GL-30 was then added to the mixture at a level of 0.05 to 1.0% and incubated for about
15 2 hours. The mixture was then heated to about 121°C for 15 minutes and allowed to cool down to 37°C. The mixture was then inoculated with the culture LGG® (*Lactobacillus rhamnosus*) and BB-12® (*Bifidobacterium animalis lactis*) (at 0.1 to 1.0% total inoculum level) and incubated for about 16 hours at 30-37°C with very low continuous agitation. Initial pH was 6.18 and the final pH was 3.84. Alpha-amylase
20 enzyme (Kleistase® SD-80) was then added at a level in the range of 0.05 to 1.0% to the mixture and incubated at 50-55°C for 1 hour. Glucoamylase (Gluczyme® NLP) and Glutaminase SD-C-100 were then added to the mixture each at a level in the range between 0.05 and 1.0% and incubated at 55°C for about 2 more hours. The final mixture was then heated to 121°C for 15 minutes and allowed to cool to 30°C.

25 The final mixture was then subjected to a distillation process wherein a distillate (20%) was collected. Both the distillate and the pot residue (left over after distillation) were evaluated as a flavour modifier in a vegan cheese sauce base. Both samples showed good mouthfeel, creamy mouthfeel, and masking of off-notes in the vegan cheese
30 sauce base. The distillate and pot residue were used at 0.1%. These flavour modifiers can be used in any dairy alternative products as a flavour modifier. The pot residue can also be spray dried using any suitable carrier, such as, oat fibre or maltodextrin. These flavour modifiers can be formed into ready-to-eat and/or ready-to-drink products by modifying the level of solid starting material (e.g., an edible cereal component) and
35 adjusting the process ensuring the enzymes are inactivated while the inactivation of the microorganisms is optional.

Example 16 – Fermentation of Pea Protein Isolate

A slurry was prepared with a pea protein isolate at 15% in water. The pea protein in the slurry was then partially hydrolyzed with Umamizyme (Amano) added at 0.1 to 1% level for about 4 hours at 50°C. The slurry was then heated to 121°C for 45 minutes to eliminate any microbial contamination from the starting material and to inactivate the enzymes, and then fermented for about 24 hours with the cultures BB-12® (*Bifidobacterium animalis lactis*) or LGG® (*Lactobacillus rhamnosus*). The initial pH of 6.18 decreased to 5.35 with LGG® and to 4.9 with BB-12®. Final heat treatment of the samples was carried out at 121°C for 15 minutes. Sensory evaluation was carried out at 0.15% in a non-dairy yoghurt base by trained expert panellists. Both samples were deemed to provide a pleasant flavour and good mouthfeel properties. These flavour modifiers can be formed into ready-to-eat and/or ready-to-drink products by modifying the level of solid starting material and adjusting the process ensuring the enzymes are inactivated while the inactivation of the microorganisms is optional.

Example 17 – Fermentation of Chickpea Flour

A slurry was prepared with chickpea flour (Organic Chickpea Flour obtained from Cambridge Commodities Inc. of California, USA or Firebird Artisan Mills of North Dakota, USA) at 10% in water. The slurry was sterilized at 121°C for 45 minutes to eliminate any microbial contamination from the starting material and allowed to cool down to 37°C. The slurry was then inoculated with LGG® (*Lactobacillus rhamnosus*) or BB-12® (*Bifidobacterium animalis lactis*) or YOFLEX® YF-L01 DA (*Streptococcus thermophilus*) or YOFLEX® YF-L02 DA (*Lactobacillus bulgaricus*) or L. Casei 431 (*Lactobacillus paracasei*) added at 0.4% and incubated for 24 hours at 30-37°C with minimal agitation. The final slurry was then heated at 121°C for 15 minutes. The initial pH of around 6 had dropped to below 4 in all the cases except when LGG® was used, wherein the final pH was around 5. Sensory evaluation of the fermented chickpea flour at 0.15% was carried out with trained expert panellists in vegan Alfredo sauce and in a bland, non-dairy sauce. The organoleptic descriptors used by the panellists for the vegan Alfredo sauce were: adds creamy, dairy notes, as well as salty, umami, brothy; masks the beany off-notes from the base. The organoleptic descriptors used by the panellists for the bland, non-dairy sauce were: creamy and nice mouthfeel with cultured/dairy impression. These flavour modifiers can be formed into ready-to-eat and/or ready-to-drink products by modifying the level of solid starting material and adjusting the process with the final inactivation of the microorganisms being optional.

Example 18 – Ready-To-Drink Oat Product Obtained from an Edible Cereal Component

A ready-to-drink oat milk product was made by subjecting an edible cereal component to enzymatic hydrolysis. The ready-to-drink product was made by mixing 125 grams of oat flour (code name P16 from Naturex and Swedish Oat Fiber Ab of Bua, Sweden) with 875 grams of water to form a slurry. The aqueous slurry was heated to a temperature of about 70°C. Alpha-Amylase enzyme (from Novozyme at a concentration of about 0.05 to about 1.0%, in particular about 0.5%) and Endo-Amylase (from Novozyme BAN at a concentration of about 0.05-2.5%, in particular about 2%) were then added to the mixture and then the mixture was incubated at about 70°C for 1 hour with continuous agitation to break down the amylose and amylopectin to maltose and various dextrans and then cooled down to 50°C.

Proteolytic enzyme (Protana Prime from Novozymes at a concentration of 0.05 to about 2.0%, in particular about 1.0%) and proteolytic enzymes Protana U Boost (from Novozymes at a concentration of 0.05 to about 1.0%, in particular about 0.5%), and Alcalase (from Novozymes at a concentration of about 0.5 to about 2.0%, in particular about 1.0%) were added and incubated for an additional 2 to 3 hrs at 50-55°C. The mixture was then heated to 121°C for 15 minutes to inactivate the enzymes and any microbial contaminants. The mixture was then cooled down to 37°C.

The final mixture was heated to 121°C for 15 min, and then cooled down to 30°C.

Organoleptic evaluation of the ready-to-drink oat milk product was undertaken by sensory trained expert panellists. All panellists found that the ready-to-drink oat milk product provided a pleasant, sweet taste but with a slight bitterness.

Example 19 – Ready-To-Drink Oat Product Obtained from an Edible Cereal Component

A ready-to-drink oat milk product was made by subjecting an edible cereal component to enzymatic hydrolysis. The ready-to-drink product was made by mixing 125 grams of oat flour (code name P16 from Naturex and Swedish Oat Fiber Ab of Bua, Sweden) with 875 grams of water to form a slurry. The aqueous slurry was heated to a temperature of about 70°C. Alpha-Amylase enzyme (from Novozyme at a concentration of about 0.05 to about 1.5%, in particular about 1.0%) and Endo-Amylase (from Novozyme BAN at a concentration of about 0.05-3.5%, in particular about 3%) were

then added to the mixture and then the mixture was incubated at about 70°C for 2 hours with continuous agitation to break down the amylose and amylopectin to maltose and various dextrans.

- 5 The final mixture was heated to 100°C for 30 min, and then cooled down to 30°C.

Organoleptic evaluation of the ready-to-drink oat milk product was undertaken by sensory trained expert panellists. All panellists found that the ready-to-drink oat milk product provided a pleasant, sweet taste and an oat type note with a pleasant
10 mouthfeel.

Example 20 - Ready-To-Drink fermented Oat Product Obtained from an Edible Cereal Component

- 15 An additional fermentation step is added to the enzymatic treatment steps of Example 18 using one or more than one of the microbial strains or microbial cultures disclosed in Table 4 of Example 13, such as, for example, B019 and/or ABY421, typically at a concentration of about 0.1 to 1%, in particular about 0.3%), was then added and the mixture was incubated at 37°C for 16 hours. The initial pH was 5.79 and the final pH
20 became 3.16.

The final mixture was heated to 121°C for 15 min, and then cooled down to 30°C. This heat treatment was optional, as low pH and refrigeration temperature of storage would be sufficient to keep ready-to-drink product safe and prevent further microbial growth.

- 25 Organoleptic evaluation of the ready-to-drink oat milk product was undertaken by sensory trained expert panellists. The ready-to-drink oat milk product was deemed to provide a pleasant flavour and good mouthfeel properties.

30 **Example 21 - Ready-To-Drink fermented Oat Product Obtained from an Edible Cereal Component**

- An additional fermentation step is added to the enzymatic treatment steps of Example 19 using one or more than one of the microbial strains or microbial cultures disclosed in
35 Table 4 of Example 13 typically at a concentration of about 0.1 to 1%, in particular about 0.3%), was then added and the mixture was incubated at 37°C for 16 hours. The initial pH was 5.79 and the final pH became 3.16.

The final mixture was heated to 121°C for 15 min, and then cooled down to 30°C. This heat treatment was optional, as low pH and refrigeration temperature of storage would be sufficient to keep ready-to-drink product safe and prevent further microbial growth.

- 5 Organoleptic evaluation of the ready-to-drink oat milk product was undertaken by sensory trained expert panellists. The ready-to-drink oat milk product was deemed to provide a pleasant flavour and good mouthfeel properties.

Example 22 – Gluten free Ready-To-Drink Oat Product Obtained from an Edible Cereal Component

A gluten free ready-to-drink oat milk product was made by subjecting an edible cereal component to enzymatic hydrolysis. The gluten free ready-to-drink oat milk product is a clean-label dairy alternative product that is considered “gluten free” as it has a gluten content less than 5 ppm, which is significantly lower than the U.S. Food and Drug Administration (FDA) definition of “gluten-free” of less than 20 ppm.

The ready-to-drink product was made by mixing 125 grams of oat flour (code name P16 from Naturex and Swedish Oat Fiber Ab of Bua, Sweden) with 875 grams of water to form a slurry. The aqueous slurry was heated to a temperature of about 70°C. Endo alpha-amylase enzyme (BAN® from Novozymes) in an amount of about 3 grams and Amylase 300L from Novozymes in an amount of about 1 gram were then added to the mixture and then the mixture was incubated at about 70°C for 2 hours with continuous agitation to break down the amylose and amylopectin to maltose and various dextrans and then cooled to 55°C.

Aminopeptidase enzyme (Flavourzyme® from Novozymes) in an amount of 1 gram was then added and incubated for an additional 1 hour at 50-55°C. The mixture was then heated to 121°C for 15 minutes to inactivate the enzymes and any microbial contaminants. The mixture was then cooled to 37°C.

Organoleptic evaluation of the gluten free ready-to-drink oat milk product was undertaken by sensory trained expert panellists. All panellists found that the gluten free ready-to-drink oat milk product provided a pleasant, sweet taste with only slight bitterness.

Example 23 - Gluten free Ready-To-Drink fermented Oat Product Obtained from an Edible Cereal Component

An additional fermentation step is added to the enzymatic treatment steps of Example
5 22 using about 0.15 grams of a microbial culture designated as ABY 421 (*Vivolac*
Cultures Corporation of Indiana, USA) having the following microbial strains:
Lactobacillus delbrueckii subsp. bulgaricus, *Streptococcus thermophilus*, *Lactobacillus*
acidophilus, and *Bifidobacterium ssp.*, and/or about 0.15 grams of *Bifidobacterium*
animalis lactis also known as Probiotic BifidoBHN019 or DR10 or B019, and thereafter
10 the mixture was incubated at 37°C for 12 hours. The initial pH was 5.95 and the final
pH became 4.8.

The final mixture was heated to 100°C for 30 min, and then cooled to 30°C. This heat
treatment was optional, as low pH and refrigeration temperature of storage would be
15 sufficient to keep ready-to-drink product safe and prevent further microbial growth.

Organoleptic evaluation of the gluten free ready-to-drink oat milk product was
undertaken by sensory trained expert panellists. The organoleptic descriptors used by
the panellists for the gluten free ready-to-drink oat milk product were: good sweetness,
20 oat milk, nice mouthfeel.

The foregoing broadly describes certain embodiments of the present invention without
limitation. Variations and modifications as will be readily apparent to those skilled in the
art are intended to be within the scope of the present invention as defined in and by the
25 appended claims.

CLAIMS

1. A method for making a ready-to-eat or ready-to-drink product, the method comprising subjecting at least one edible component of a cereal to fermentation, enzymatic hydrolysis, or to fermentation and enzymatic hydrolysis, wherein the
5 fermentation uses two or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, and/or *Bifidobacterium animalis lactis*,
10 wherein the cereal is selected from the group consisting of oat, maize, rice, wild rice, wheat, barley, sorghum, millet, rye, triticale, fonio and combinations thereof
2. The method of claim 1, wherein the at least one edible cereal component is in the form of, or derived from, cereal grain, cereal wholegrain, cereal groats, steel cut cereal,
15 rolled cereal, cereal bran, cereal flour, cereal kernel, cereal fibre, or combinations thereof, preferably wherein the at least one edible component of the cereal is in an aqueous slurry, preferably wherein the at least one edible cereal component is present in an amount of about 5-20 wt.% based on the total weight of the aqueous slurry.
- 20 3. The method of claim 1 or claim 2, wherein the enzymatic hydrolysis uses one or more enzymes selected from carbohydrases and proteolytic enzymes, preferably wherein the enzymatic hydrolysis uses at least one or more enzymes selected from cellulases, pectinases, and other carbohydrases.
- 25 4. The method of any one of claims 1-3, wherein the enzymatic hydrolysis is performed at a temperature ranging from about 25°C to about 60°C., preferably wherein the enzymatic hydrolysis takes place for a period of time ranging from about 1 hour to about 48 hours, preferably wherein the fermentation is performed at a temperature ranging from about 20°C to about 45°C.
30
5. The method of any one of the preceding claims, wherein the fermentation takes place for a period of time ranging from about 1 day to about 2 days.
6. The method of any one of the preceding claims, wherein the enzymatic
35 hydrolysis occurs before and/or simultaneously with the fermentation.

7. The method of any one of the preceding claims, wherein the method comprises subjecting the at least one edible cereal component to fermentation and does not comprise subjecting the at least one edible cereal component to enzymatic hydrolysis.
- 5 8. The method of any one of the preceding claims, wherein the method comprises heating the at least one edible cereal component to a temperature equal to or greater than about 75°C prior to the enzymatic hydrolysis and fermentation.
9. The method of any one of the preceding claims, wherein the edible cereal
10 component comprises oat, preferably wherein the oat comprises oat flour, preferably wherein the edible cereal component comprises oat fibre, maize fibre, rice fibre, wild rice fibre, wheat fibre, barley fibre, sorghum fibre, millet fibre, rye fibre, triticale fibre, fonio fibre, or combinations thereof, preferably the edible cereal component comprises oat fibre, preferably wherein the edible cereal component is in the form of, or derived
15 from, oat grain, oat wholegrain, oat groats, steel cut oats, rolled oats, oat bran, oat flour, oat kernel, oat fibre, Irish oatmeal, or combinations thereof.
10. The method of any one of the preceding claims, wherein the method further comprises spray-drying the ready-to-eat product.
- 20 11. The method of any one of the preceding claims, wherein the fermentation uses three or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*,
25 *Bifidobacterium*, and/or *Bifidobacterium animalis lactis*.
12. A ready-to-eat product obtained by mixing at least one edible component of a cereal in an aqueous solution, wherein the cereal is selected from the group consisting
30 of oat, maize, rice, wild rice, wheat, barley, sorghum, millet, rye, triticale, fonio and combinations thereof, adding two or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, and/or *Bifidobacterium animalis lactis*
35 to the mixture, and incubating the mixture for a period of time sufficient to ferment at

least a portion of the at least one edible cereal component to form the ready-to-eat product.

13. The ready-to-eat product of claim 12, wherein the ready-to-eat product is yoghurt.

5

14. A ready-to-drink product obtained by mixing at least one edible component of a cereal in an aqueous solution, wherein the cereal is selected from the group consisting of oat, maize, rice, wild rice, wheat, barley, sorghum, millet, rye, triticale, fonio and combinations thereof, adding carbohydrase and/or proteolytic enzymes to the mixture, subsequently adding two or more lactic acid bacteria selected from the group consisting of *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Bifidobacterium*, and/or *Bifidobacterium animalis lactis* to the mixture, incubating the mixture for a period of time sufficient to ferment at least a portion of the at least one edible cereal component to form the ready-to-drink product

10

15

15. The ready-to-drink product of claim 14, wherein the ready-to-drink product is oat milk, preferably wherein the ready-to-drink product has a gluten content of less than 5ppm.

20

16. A consumable product obtained by the method of claim 1, preferably wherein the consumable product is a clean-label dairy alternative product.

25 17. The ready-to-eat product of claim 12 or 13 or the ready-to-drink product of claims 14 or 15 wherein the edible cereal component is in the form of, or derived from, oat grain, oat wholegrain, oat fibre, oat groats, steel cut oats, rolled oats, oat bran, oat flour, oat kernel, Irish oatmeal or combinations thereof.

30