PROBIOTIC COMPOSITIONS AND METHODS OF FERMENTATION THEREOF

Technical Field

The present invention relates, in general terms, to probiotic compositions and their methods of fermentation thereof.

Background

Probiotics can impart a range of beneficial effects including improving digestion, strengthening the immune system and modifying the gut microbiome. It is recognized that the probiotic needs to be viable for maximum benefit, and it is generally accepted that the finished product should contain at least 10^6–10^7 viable cells per mL (CFU per mL). Consequently, there has been increasing interest in improving the survival of probiotics in the finished product, during storage and when consumed and exposed to digestive tract conditions. Traditional fermented dairy foods are frequently used for probiotic delivery, because probiotic strains have been shown to survive well under these conditions. However, there is an increasing demand for non-dairy probiotic foods because of the rise in lactose intolerance, milk allergy and an interest in low cholesterol content products. Probiotic fermented non-dairy products have the advantage of being lactose free and having a low cholesterol content. Cereals, fruits and vegetable based probiotic fermented products have received increasing attention in recent years because they can be alternatives to dairy based products and also because such products often contain complex carbohydrates that can be preferentially utilized by the probiotics and also by the beneficial gut microbes when ingested. Such carbohydrates, referred to as prebiotics, can promote the growth of probiotics and beneficial gut microbes, and thereby enhance the performance of the probiotic, for example by inhibiting the growth of potentially pathogenic gut microbes as well as promoting the growth of the indigenous beneficial microbes.

In recent years, oats consumption has been linked to numerous health benefits, such as anti-inflammatory and antioxidant activity, and shown to have the potential to reduce the risk of cardiovascular diseases (CVD), type 2 diabetes, gastrointestinal disorders and cancer. Oats contains bioactive compounds, especially natural antioxidant phenolic compounds and β-glucan. Thus, oats is becoming a popular matrix of choice for innovative functional probiotic containing foods. It has been shown that oats can promote the growth of lactic acid bacteria. In addition, there are reports of the optimization of the total phenolic content and antioxidant capacity in oats by

fermentation using yeast or bacteria. Unfortunately, most studies did not achieve improvements in both probiotic and bioactive properties, or only focused on one aspect.

Fermentation is the breakdown of carbohydrates, such as starch and sugar, by bacteria and yeast. It is an ancient technique for preserving food. Common fermented foods include kimchi, sauerkraut, kefir, tempeh, kombucha and yogurt. More recently, many health benefits have been proposed for fermented foods and these included reducing heart disease risk, aiding digestion, enhancing immunity and weight loss.

It would be desirable to overcome or ameliorate at least one of the above-described problems.

Summary

The present disclosure relates to a process that improves the viability of a probiotic, for example *L. fermentum* PC1, during exposure to simulated digestive tract conditions and storage through the use of a delivery matrix which is fermented in situ with the probiotic. This benefit is also maintained when the probiotic composition is stored for 14 days. The process can also enhances and maintains the bioactive ingredients including antioxidant potential, total phenolic acid content, phenolic composition and β-glucan. The delivery matrix can be a combination of oats and honey or other sugar rich ingredients. The process can be used to produce a probiotic composition with prebiotic properties which contributes to a beneficial impact on the gut microbes and their metabolites. The probiotic composition exerts the beneficial impact on the gut microbes when fresh and when freeze dried.

The present invention provides a fermentation process, comprising:

a) mixing a grain flour and a sugar in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the grain flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the sugar is about 1 % w/v to about 20% w/v of the mixture.

Disclosed herein is a fermentation process, comprising:

a) mixing a grain flour and a sugar in an aqueous medium to form a mixture;

b) inoculating a probiotic microorganism selected from probiotic bacterium and/or probiotic yeast in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the grain flour is 5 % w/v to 30 % w/v of the mixture;

wherein the sugar is metabolisable by the probiotic microorganism;
wherein the sugar is 1 % w/v to 20% w/v of the mixture; and
wherein the probiotic microorganism is inoculated at about 10^5 CFU per mL to less than about 10^8 CFU per mL of the culture.

Advantageously, the fermented culture has a beneficial impact on the gut microbiome, especially of the elderly as shown by promoting beneficial microbes, supressing potentially harmful microbes and promoting elevated levels of beneficial bacterial metabolites such as the short chain fatty acids acetic, propionic and butyric acids. The fermented culture which when freeze dried, and hence can be used as a powder with extended shelf life and application, retained and can even exhibit enhanced beneficial properties.

In some embodiments, the fermentation step is performed at about 25 °C to about 50 °C.

In some embodiments, the fermentation step is performed under stirring of about 100 rpm to about 800 rpm.

In some embodiments, the fermentation step is performed for at least 24 h.

It was found that through a fermentation in the presence of sugar for at least 24 h, sugar can be utilized by the probiotic bacterium and/or yeast to at least maintain their viability during storage and/or when the probiotics is delivered to the gut of a host when consumed. If complex carbohydrates are used, the carbohydrates can be reduced to oligosaccharides and/or monosaccharides such as glucose and fructose, which provides for a stable probiotics viability.

In some embodiments, the grain flour is selected from teff flour, wheat flour, oat flour, rice flour, corn flour, barley flour, sorghum flour, rye flour, millet flour, triticale flour, amaranth flour, buckwheat flour, quinoa flour, sugar cane fibre/bagasse or a combination thereof.

In some embodiments, the grain flour is oat flour, wherein the oat flour is derived from simple oat, germinated oat or a mixture thereof.

In some embodiments, the sugar is selected from a monosaccharide, disaccharide, oligosaccharide, polysaccharide, or a combination thereof.

In some embodiments, the sugar is derived from an animal source or a plant source.

In some embodiments, the sugar is selected from glucose, fructose, trehalose, inulin, molasses, honey, or a combination thereof.

In some embodiments, the sugar in the fermented culture is less than about 50% relative to the mixture.

In some embodiments, the probiotic bacterium is about 0.2 % w/v to about 2% v/v of the mixture.

In some embodiments, the probiotic bacterium is inoculated at about 10^7 CFU per mL of the mixture.

In some embodiments, the probiotic bacterium and/or yeast is selected from *L. fermentum, L. bulgaricus, L. acidophilus, L. brevis, L. plantarum, L. rhamnosus, L. caucasicus, L helveticus, L. lactis, L. reuteri, L. casei., B. infantis, B. bifidum, S. thermophiles, E. faecium*, *L. amylovorus, L. paracasei, S. cerevisiae, S. boulardii,* or a combination thereof*.*

In some embodiments, the probiotic bacterium is *L. fermentum.*

In some embodiments, a pH of the fermented culture after at least 24 h is about 4.

In some embodiments, the method further comprises a step of pasteurising the grain flour, sugar, or the mixture thereof.

In some embodiments, the fermentation process further comprises a step of storing the fermented culture.

In some embodiments, the fermented culture is stored at about 4 °C.

In some embodiments, the fermented culture is stored for at least 10 days.

In some embodiments, the fermented culture is freeze dried.

In some embodiments, a viable count of probiotic bacterium in the culture after inoculating is about 6 log CFU/mL to about 7 log CFU/mL.

In some embodiments, a viable count of probiotic bacterium in the culture after fermenting is about 7 log CFU/mL to about 8 log CFU/mL.

In some embodiments, a viable count of probiotic bacterium in the fermented culture after storing is about 7 log CFU/mL to about 8 log CFU/mL.

In some embodiments, the fermented culture has a β-glucan content which is substantially not altered when compared to the mixture.

In some embodiments, the fermented culture comprises glucose of about 5 g/L to about 7 g/L, fructose of about 9 g/L to about 10 g/L, lactic acid of about 2 g/L to about 4 g/L, and acetic acid of about 0.6 g/L to about 0.7 g/L.

The present invention also provides a probiotic composition, comprising:

a) a grain flour;

b) a sugar; and

c) a probiotic bacterium and/or yeast;

wherein the grain flour is about 5 % w/v to about 30 % w/v of the composition;

wherein the sugar is about 1 % w/v to about 20% w/v of the composition.

In some embodiments, the grain flour is oat flour.

In some embodiments, the sugar is selected from glucose, fructose, trehalose, inulin, molasses, honey, or a combination thereof.

In some embodiments, a viable count of probiotic bacterium in the composition is about 7 log CFU/mL to about 8 log CFU/mL.

In some embodiments, a viable count of probiotic bacterium in the composition after exposure to gastric and/or intestinal conditions is about 7 log CFU/mL to about 8 log CFU/mL.

In some embodiments, the viable count of probiotic bacterium in the composition before exposure to gastric and/or intestinal conditions is substantially the same as after exposure to gastric and/or intestinal conditions.

In some embodiments, the probiotic composition further comprises glucose of about 5 g/L to about 7 g/L.

In some embodiments, the probiotic composition further comprises fructose of about 9 g/L to about 10 g/L.

In some embodiments, the probiotic composition further comprises lactic acid of about 2 g/L to about 4 g/L.

In some embodiments, the probiotic composition further comprises acetic acid of about 0.6 g/L to about 0.7 g/L.

In some embodiments, the probiotic composition further comprises a phenolic compound selected from gallic acid, 4-hydrooxybenzoic acid, chlorogenic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, quercetin, or a combination thereof.

In some embodiments, the probiotic composition has a total phenolic content of about 0.6 mg GAE/g to about 0.8 mg GAE/g.

In some embodiments, the probiotic composition has a gallic acid content of about 3 µg/g to about 5 µg/g.

In some embodiments, the probiotic composition has a 4-hydroxybenzoic acid content of about 0.7 µg/g to about 0.9 µg/g.

In some embodiments, the probiotic composition has a catechin content of about 1.8 µg/g to about 2.6 µg/g.

In some embodiments, the probiotic composition has a vanillic acid content of about 0.2 µg/g to about 0.7 µg/g.

In some embodiments, the probiotic composition has a caffeic acid content of about 0.6 µg/g to about 0.9 µg/g.

In some embodiments, the probiotic composition has a p-coumaric acid content of about 0.45 µg/g to about 1.5 µg/g.

In some embodiments, the probiotic composition has a ferulic acid content of about 0.5 µg/g to about 0.8 µg/g.

In some embodiments, the probiotic composition has a sinapic acid content of about 1.5 µg/g to about 2.3 µg/g.

In some embodiments, the probiotic composition has a quercetin content of about 5.2 µg/g to about 5.6 µg/g.

In some embodiments, the probiotic composition has an antioxidant activity of at least 60 nmol TE/mg.

In some embodiments, the probiotic composition further comprises β-glucan at about 2 g/100 g DW to about 3 g/100 g DW.

In some embodiments, the probiotic composition has an absence of dairy product.

The present invention also provides a use of the probiotic composition as a fermented beverage or a freeze dried powder which is reconstitutable in an aqueous medium.

Brief description of the drawings

Embodiments of the present invention will now be described, by way of non-limiting example, with reference to the drawings in which:

Figure 1 is a plot showing the survival of *L. fermentum* PC1 in the fermented culture during exposure to simulated gastrointestinal conditions. Results are expressed as Log CFU/mL in mean±SD. P values were calculated using t-test. The statistics are presented by labelling lowercase letter 'a, b, c'. The different superscripted letters indicate significant ($P < 0.05$) differences between each other.

Figure 2 is a plot showing the concentration of glucose, fructose, lactic acid and acetic acid during fermentation and storage. Results are presented as g/L in mean±SD. P values were calculated using t-test. The statistics are presented by labelling lowercase letter 'a, b, c, d, e'. The different superscripted letters indicate significant ($P < 0.05$) differences between each other.

Figure 3 is a plot showing the change of total antioxidant activity and phenolic acid content in fermented culture during fermentation and storage. (A) Total antioxidant activity are expressed as nmol Trolox equivalents per mg sample (nmol TE/mg). (B) Total phenolic acids are expressed as mg gallic acid equivalents per g sample (mg GAE/g). Results are presented as mean±SD. P values were calculated using t-test. The statistics are presented by labelling lowercase letter 'a, b, c, d'. The different superscripted letters indicate significant ($P < 0.05$) different between each other.

Figure 4 is a plot showing the concentration of β-glucan in fermented culture during fermentation and storage. Results are presented as g/100 g DW (dry weight) in mean±SD.

Figure 5 is a plot of probiotic compositions and their pH at various stages of the process.

Figure 6 is a plot of probiotic compositions and their probiotic bacterium viable counts at various stages of the process.

Figure 7 is a plot of probiotic compositions and their yeast viable count at various stages of the process.

Figure 8 is a plot of probiotic compositions and their beta-glucan concentration at various stages of the process.

Figure 9A-C are plots showing the total antioxidant concentrations of compositions of the present invention. 5A is the total antioxidants (residue; insoluble); 5B is the total antioxidants (supernatant; soluble); and 5C is the total antioxidants (residue and supernatant).

Figure 10A-C are plots showing the total phenolic content of compositions of the present invention. 6A is the total phenolic content (residue; insoluble); 6B is the total phenolic content (supernatant; soluble); and 6C is the total phenolic content (residue and supernatant).

Figure 11 is a plot of viable count of enterics bacteria in the simulated gut 48 h after addition of fresh and freeze dried probiotic compositions.

Figure 12 is a plot of viable count of *L. fermentum* in the simulated gut 48 h after addition of fresh and freeze dried probiotic compositions.

Figure 13 is a plot of acetic acid concentration in the simulated gut 48 h after addition of fresh and freeze dried probiotic compositions.

Figure 14 is a plot of propanoic acid concentration in the simulated gut 48 h after addition of fresh and freeze dried probiotic compositions.

Figure 15 is a plot of butyric acid concentration in the simulated gut 48 h after addition of fresh and freeze dried probiotic compositions.

Figure 16 is a plot of viable count of *L. fermentum* in the simulated gut 0 h after addition of fresh and freeze dried probiotic compositions.

Detailed description

The present invention is predicated on the understanding that probiotics such as *L. fermentum* PC1 can have a good capacity for attenuating inflammation, working as an oral adjuvant and influencing the gut microbes. It can tolerate bile salts and low pH, and survive passage through the digestive tract when dosed at the high dose, but less well at a low dose. Furthermore, the addition of a prebiotic can enhance the survival of probiotics. In particular, preliminary studies showed that a significant decrease of the

β-glucan content was found in oats when no sugar was added in the fermentation, most probably because the β-glucan is a selective substrate of lactobacilli.

Without wanting to be bound by theory, the inventors believe that a grain based probiotic food (such as an oat-based probiotic food) with both improved probiotic viability and bioactive ingredients can be developed if sugars are added to promote the growth of the probiotic strain and enhance fermentation. With the target to develop a healthy functional food, honey was used as a sugar source for the probiotic strain, because honey can have prebiotic activity and also contains antioxidant and oligosaccharides, and therefore is a suitable ingredient of functional foods. Moreover, as it has been used in old and modern medical practice due to its antimicrobial, anti-inflammation and wound-healing properties, its acceptance as a healthy product by the general populace has commercial imperative.

In particular, it is believed that the viability of *L. fermentum* PC1 during *in vitro* digestion and storage can be improved through the use of oats and honey as a delivery matrix fermented with *L. fermentum* PC1. Additionally, such fermentation can enhance and maintain the bioactive ingredients in the end product. To demonstrate this, viability testing, the sugar consumption and organic acid production were monitored during fermentation and storage of the oat based composition. The bioactive ingredients including antioxidant potential, total phenolic acid content, phenolic composition and β-glucan were also evaluated. Additionally, the impact of the probiotic composition on the gut microbes was demonstrated for both the fresh and the freeze dried probiotic composition.

The present invention provides a fermentation process, comprising:

a) mixing a grain flour and a sugar in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the grain flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the sugar is about 1 % w/v to about 20% w/v of the mixture.

The present invention provides a fermentation process, comprising:

a) mixing a grain flour and a sugar in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the grain flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the sugar is about 1 % w/v to about 20% w/v of the mixture.

The present invention provides a fermentation process, comprising:

a) mixing a grain flour and a sugar in an aqueous medium to form a mixture;

b) inoculating a probiotic yeast in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the grain flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the sugar is about 1 % w/v to about 20% w/v of the mixture.

The fermented culture can be made suitable for drinking as a non-dairy fermented beverage, which can deliver fermentation components with both improved probiotic and bioactive properties to a consumer. The fermented culture can be freeze dried as a powder with inherent longer stability and which can be reconstituted for use in foods or as a beverage.

In some embodiments, the method is a liquid state fermentation process. Liquid state fermentation is performed in tanks, which can reach 1,001 to 2,500 cubic metres at an industrial scale. Liquid culture can be useful for the growing of unicellular organisms such as bacteria or yeasts. To achieve liquid aerobic fermentation, there may be a need to constantly supply the microorganism with oxygen, which is generally done via stirring the fermentation media. Accurately managing the synthesis of the desired metabolites may requires regulating temperature, soluble oxygen, ionic strength and pH and control nutrients.

In some embodiments, the grain flour is selected from teff flour, wheat flour, oat flour, rice flour, corn flour, barley flour, sorghum flour, rye flour, millet flour, triticale flour, amaranth flour, buckwheat flour, quinoa flour, sugar cane/bagasse or a combination thereof. In some embodiments, the grain flour is oat flour. In some embodiments, the oat flour is derived from whole grain oat. In some embodiments, oat flour is derived from simple oat, germinated oat or a mixture thereof. Germinated oat can have higher nutritional content than non-germinated or simple oat, which may include an increase in phenolics, flavonoids, soluble proteins, and γ-aminobutyric acid. The extracts from sprouted oats can exhibit an increased antioxidant activity, which can contribute to the nutritional value of oats as a consumed food.

In some embodiments, the grain flour is about 5 %w/v to about 20 %w/v relative to the mixture. In other embodiments, the concentration is about 5 %w/v to about 18 %w/v, about 5 %w/v to about 16 %w/v, about 5 %w/v to about 15 %w/v, about 5 %w/v to about 14 %w/v, about 5 %w/v to about 12 %w about 6 %w/v to about 12

%w/v, about 8 %w/v to about 12 %w/v,/v, or about 10 %w/v to about 12 %w/v. In other embodiments, the concentration is about 10 %w/v.

In some embodiments, the sugar is selected from a monosaccharide, disaccharide, oligosaccharide, polysaccharide, or a combination thereof. Also within this scope are sugar sources, mixtures or byproducts, either refined or unrefined. Monosaccharides include glucose, fructose, and galactose. Disaccharides are molecules composed of two monosaccharides joined by a glycosidic bond. Some examples are sucrose (table sugar) (glucose + fructose), lactose (glucose + galactose), and maltose (two molecules of glucose). Oligosaccharides or polysaccharides include starch (a glucose polymer found in plants). In some embodiments, the sugar is selected from glucose, fructose, or a combination thereof. Other types of sugars that can be used includes molasses, trehalose, inulin, honey, or a combination thereof.

In some embodiments, the sugar is derived (or obtained) from a natural source such as a plant or an animal. In some embodiments, the sugar is derived from an animal source or a plant source. In other words, the sugar is not synthesised in a chemical setting. Examples of sugars from a natural source includes honey, fruits, sugarcane, sugar beet, malted grain, corn and milk.

In some embodiments, the sugar is derived (or obtained) from honey; i.e. sugar is not extracted from honey, but rather honey is used as a whole as the sugar source. Honey is a sweet, viscous food substance made by honey bees and some related insects, such as stingless bees. Bees produce honey from the sugary secretions of plants (floral nectar) or from secretions of other insects (such as honeydew), by regurgitation, enzymatic activity, and water evaporation. Honey bees store honey in wax structures called honeycombs, whereas stingless bees store honey in pots made of wax and resin. Honey gets its sweetness from the monosaccharides fructose and glucose, and has about the same relative sweetness as sucrose (table sugar). A study of 20 different honeys found that their sugar contents comprise fructose at about 28% to about 41%, and glucose at about 22% to about 35%. Honey can also comprise maltose at about 7%, sucrose at about 1%, water at about 17%, higher sugars at about 1.5% and oligosaccharides at up to 8%, ash at about 0.2%. Organic acids comprise most of the acids in honey, accounting for 0.17–1.17% of the mixture, with gluconic acid formed by the actions of glucose oxidase as the most prevalent. Minor amounts of other organic acids are present, consisting of formic, acetic, butyric, citric, lactic, malic, pyroglutamic, propionic, valeric, capronic, palmitic, and succinic, among many others. Honey also comprises other nutrients, such as vitamins (riboflavin (B_2), niacin (B_3), pantothenic

acid (B_5), vitamin B_6, folate (B_9), vitamin C) and minerals (calcium, iron, magnesium, phosphorus, potassium, sodium, zinc).

In some embodiments, the honey is a floral honey. In other embodiments, the floral honey is a blended honey, polyfloral honey, or monofloral honey. An example of polyfloral honey is wildflower honey. In some embodiments, the honey is honeydew honey. In other embodiments, the honey is selected from crystallised honey, pasteurised honey, raw honey, strained honey, filtered honey, ultrasonicated honey, creamed honey, dried honey, comb honey, chunk honey, honey decoction, or baker's honey. In other embodiments, the honey is Sardinian honey, blended Australian eucalyptus honey, or a combination thereof.

In some embodiments, the sugar is about 1 % w/v to about 20 %w/v relative to the mixture. In some embodiments, the sugar is about 1 % w/v to about 18 %w/v, about 1 % w/v to about 16 %w/v, about 1 % w/v to about 14 %w/v, about 1 % w/v to about 12 %w/v, about 1 % w/v to about 10 %w/v, about 9 %w/v, about 1 % w/v to about 8 %w/v, about 1 % w/v to about 7 %w/v, about 1 % w/v to about 6 %w/v, about 1 % w/v to about 5 %w/v, about 2 % w/v to about 5 %w/v, or about 3 % w/v to about 5 %w/v. In other embodiments, the concentration is about 3 %w/v.

As the sugar in the mixture is metabolised by the probiotic bacterium, the amount of sugar in the fermented culture is decreased. In some embodiments, the sugar in the fermented culture is less than about 50% relative to the mixture. In other embodiments, the amount of residual sugar is about 45%, 40%, 35%, 30%, 25%, 20% or 15% relative to the amount of sugar in the mixture.

Accordingly, in some embodiments, the fermentation process, comprising:

a) mixing a grain flour and a sugar derived from honey in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the grain flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the honey is about 1 % w/v to about 20% w/v of the mixture.

In some embodiments, the fermentation process, comprising:

a) mixing a oat flour and a sugar derived from honey in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the honey is about 1 % w/v to about 20% w/v of the mixture.

In some embodiments, the fermentation process, comprising:

a) mixing a oat flour and a sugar derived from honey in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the honey is about 1 % w/v to about 20% w/v of the mixture.

In some embodiments, the fermentation process, comprising:

a) mixing an oat flour and a sugar derived from honey in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the honey is about 1 % w/v to about 20% w/v of the mixture.

In some embodiments, the fermentation process, comprising:

a) mixing an oat flour and a sugar derived from honey in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the oat flour is about 10 % w/v of the mixture;

wherein the honey is about 3 % w/v of the mixture.

The term 'aqueous medium' used herein refers to a water based solvent or solvent system, and which comprises of mainly water. Such solvents can be either polar or non-polar, and/or either protic or aprotic. Solvent systems refer to combinations of solvents which resulting in a final single phase. Both 'solvents' and 'solvent systems' can include, and is not limited to, acetic acid, ethylene glycol, diethylene glycol or water. Water based solvent or solvent systems can also include dissolved ions, salts and molecules such as amino acids, proteins, sugars and phospholipids. Such salts may be, but not

limited to, sodium chloride, potassium chloride, ammonium acetate, magnesium acetate, magnesium chloride, magnesium sulfate, potassium acetate, potassium chloride, sodium acetate, sodium citrate, zinc chloride, HEPES sodium, calcium chloride, ferric nitrate, sodium bicarbonate, potassium phosphate and sodium phosphate.

In some embodiments, the aqueous medium is purified water. In other embodiments, the aqueous medium is filtered water.

The probiotic bacterium is introduced (or inoculated) into the mixture to form a culture. In some embodiments, the probiotic bacterium and/or yeast is selected from *L. fermentum, L. bulgaricus, L. acidophilus, L. brevis, L. plantarum, L. rhamnosus, L. caucasicus, L helveticus, L. lactis, L. reuteri, L. casei., B. infantis, B. bifidum, S. thermophiles, E. faecium*, *L. amylovorus, L. paracasei, S. cerevisiae, S. boulardii,* or a combination thereof*.* In some embodiments, the probiotic bacterium is selected from *L. fermentum, L. bulgaricus, L. acidophilus, L. brevis, L. plantarum, L. rhamnosus, L. caucasicus, L helveticus, L. lactis, L. reuteri, L. casei., B. infantis, B. bifidum, S. thermophiles, E. faecium*, *L. amylovorus, L. paracasei,* or a combination thereof*.* In other embodiments, the probiotic bacterium is *L. fermentum*. In some embodiments, the probiotic yeast is selected from *S. cerevisiae, S. boulardii,* or a combination thereof*.*

In some embodiments, the probiotic bacterium is about 0.2 % v/v to about 2% v/v relative to the mixture. In other embodiments, the concentration is about 0.4 % v/v to about 2% v/v, about 0.6 % v/v to about 2% v/v, about 0.8 % v/v to about 2% v/v, about 1 % v/v to about 2% v/v, about 1 % v/v to about 1.8% v/v, about 1 % v/v to about 1.6% v/v, about 1 % v/v to about 1.5% v/v, about 1 % v/v to about 1.4% v/v, about 1 % v/v to about 1.3% v/v, about 1 % v/v to about 1.2% v/v, or about 1 % v/v to about 1.1% v/v. In other embodiments, the concentration is about 1 %v/v.

In some embodiments, the probiotic bacterium is inoculated at about 10^7 cells per mL of the mixture. In other embodiments, the probiotic bacterium is inoculated at about 10^7 CFU per mL of the mixture. In microbiology, colony-forming unit (CFU) is a measure of viable bacterial or fungal numbers. Unlike in direct microscopic counts where all cells, dead and living, are counted, CFU measures viable cells. In other embodiments, the concentration is about 10^5 CFU per mL to about 10^{10} CFU per mL, about 10^6 CFU per mL to about 10^{10} CFU per mL, about 10^7 CFU per mL to about 10^{10} CFU per mL, about 10^7 CFU per mL to about 10^9 CFU per mL, or about 10^7 CFU per mL to about 10^8 CFU per mL.

In some embodiments, the fermentation process, comprising:

a) mixing an oat flour and a sugar derived from honey in an aqueous medium to form a mixture;
b) inoculating a probiotic bacterium in the mixture to form a culture; and
c) fermenting the culture to form a fermented culture;
wherein the oat flour is about 10 % w/v of the mixture;
wherein the honey is about 3 % w/v of the mixture; and
wherein the probiotic bacterium is about 1 % v/v relative to the mixture.

In some embodiments, the fermentation process, comprising:
a) mixing an oat flour and a sugar derived from honey in an aqueous medium to form a mixture;
b) inoculating a probiotic bacterium in the mixture to form a culture; and
c) fermenting the culture to form a fermented culture;
wherein the oat flour is about 10 % w/v of the mixture;
wherein the honey is about 3 % w/v of the mixture;
wherein the probiotic bacterium is about 1 % v/v relative to the mixture; and
wherein the probiotic bacterium is *L. fermentum*.

In some embodiments, the fermentation step is performed at about 25 °C to about 50 °C. In other embodiments, the temperature is about 25 °C to about 45 °C, about 25 °C to about 45 °C, about 25 °C to about 40 °C, about 30 °C to about 40 °C, or about 35 °C to about 40 °C. In other embodiments, the temperature is about 37 °C.

In some embodiments, the fermentation step is performed under stirring of about 100 rpm to about 800 rpm. In other embodiments, the stirring is about 100 rpm to about 700 rpm, about 100 rpm to about 600 rpm, about 100 rpm to about 500 rpm, about 100 rpm to about 400 rpm, about 100 rpm to about 300 rpm, or about 100 rpm to about 200 rpm. In other embodiments, the stirring is about 150 rpm.

In some embodiments, the fermentation step is performed for at least 24 h. In other embodiments, the duration is at least 28 h, 32 h, 36 h, 40 h, 44 h, 48 h, 52 h, 56 h, 60 h, 64 h, 68 h, or 72 h. It was found that the fermentation time of at least 24 h is particularly advantageous for residual sugars to accumulate, which can possibly assist in the continuous metabolic activity of the probiotics in fermented foods, and enhance the survival of probiotics in acidic environments.

In some embodiments, a pH of the fermented culture after at least 24 h is about 4. In other embodiments, the pH after 72 h is about 3.9. In some embodiments, the pH is

about 3.5 to about 4.5, about 3.5 to about 4.4, about 3.5 to about 4.3, about 3.5 to about 4.2, about 3.5 to about 4.1, or about 3.5 to about 4.

The fermented culture can be fermented under aerobic conditions, or can be fermented under facultative anaerobic or strict anaerobic conditions with oxygen in the ppm level.

In some embodiments, the fermentation process comprises:

a) mixing a oat flour and a sugar in an aqueous medium to form a mixture;
b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and
c) fermenting the culture for at least 24 h to form a fermented culture;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the mixture;
wherein the sugar is about 1 % w/v to about 20% w/v of the mixture.

In some embodiments, the fermentation process comprises:

a) mixing a grain flour and a sugar derived from honey in an aqueous medium to form a mixture;
b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and
c) fermenting the culture for at least 24 h to form a fermented culture;

wherein the grain flour is about 5 % w/v to about 30 % w/v of the mixture;
wherein the honey is about 1 % w/v to about 20% w/v of the mixture.
In some embodiments, the method further comprises a step of pasteurising the grain flour, sugar, or mixture thereof. The grain flour and sugar can be separately pasteurised before mixing to form the mixture. The grain flour can for example be pasteurised by autoclave. The sugar can for example be pasteurised by heating it to at least 80 °C for at least 10 min.

In some embodiments, the method further comprises a step of storing the fermented culture. The storing of the fermented culture can occur in an undisturbed condition. When stored in an undisturbed condition, the culture is not subjected to a mixing force. The probiotic bacterium is allowed to naturally settle due to gravity.

In some embodiments, the fermentation process, comprising:

a) mixing an oat flour and a sugar derived from honey in an aqueous medium to form a mixture;
b) inoculating a probiotic bacterium in the mixture to form a culture;
c) fermenting the culture for at least 24 h to form a fermented culture; and
d) storing the fermented culture;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the mixture;
wherein the honey is about 1 % w/v to about 20% w/v of the mixture.

In some embodiments, the fermented culture is storable at about 4 °C. In some embodiments, the fermented culture is storable for at least 10 days.

In other embodiments, the fermented culture is stored undisturbed for at least 1 day at 4 °C. In other embodiments, the duration is at least 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 12 days, or 14 days. In other embodiments, the fermented culture is stored undisturbed for at least 14 day at 4 °C. The storage further allows the residual sugars and acids in the fermented culture to reach equilibrium to support the metabolic activity of the probiotics.

In some embodiments, the fermented culture is freeze dried and stored at about 4 °C. The freeze dried powder can have inherently longer shelf life and can be reconstituted for use in foods or as a beverage.

In some embodiments, a viable count of probiotic bacterium in the culture after inoculating is about 6 log CFU/mL to about 7 log CFU/mL.

In some embodiments, a viable count of probiotic bacterium in the culture after fermenting is about 6 log CFU/mL to about 7 log CFU/mL. In other embodiments, the probiotic bacterium in the culture is about 6 log CFU/mL to about 8 log CFU/mL after at least 24 h. In other embodiments, the probiotic bacterium in the culture is about 7 log CFU/mL to about 8 log CFU/mL after at least 24 h. In other embodiments, the probiotic bacterium in the culture is about 7.1 log CFU/mL to about 8 log CFU/mL, about 7.2 log CFU/mL to about 8 log CFU/mL, about 7.3 log CFU/mL to about 8 log CFU/mL, about 7.3 log CFU/mL to about 7.9 log CFU/mL, about 7.3 log CFU/mL to about 7.8 log CFU/mL, about 7.3 log CFU/mL to about 7.7 log CFU/mL, about 7.3 log CFU/mL to about 7.6 log CFU/mL, or about 7.3 log CFU/mL to about 7.5 log CFU/mL.

In some embodiments, the viable count of probiotic bacterium in the fermented culture is increased by about 2% relative to the inoculation concentration. In other embodiments, the viable count is increased by about 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%.

In some embodiments, a viable count of probiotic bacterium in the fermented culture after storing is about 6 log CFU/mL to about 7 log CFU/mL. The storage can be for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days,

12 days, or 14 days. In other embodiments, the probiotic bacterium in the fermented culture is about 6 log CFU/mL to about 8 log CFU/mL. In other embodiments, the probiotic bacterium in the fermented culture is about 7 log CFU/mL to about 8 log CFU/mL. In other embodiments, the probiotic bacterium in the fermented culture is about 7.1 log CFU/mL to about 8 log CFU/mL, about 7.2 log CFU/mL to about 8 log CFU/mL, about 7.3 log CFU/mL to about 8 log CFU/mL, about 7.3 log CFU/mL to about 7.9 log CFU/mL, about 7.3 log CFU/mL to about 7.8 log CFU/mL, about 7.3 log CFU/mL to about 7.7 log CFU/mL, about 7.3 log CFU/mL to about 7.6 log CFU/mL, or about 7.3 log CFU/mL to about 7.5 log CFU/mL.

In some embodiments, the viable count of probiotic bacterium in the fermented culture after fermentation and after storage is maintained. In some embodiments, a viable count of probiotic bacterium in the composition after storage for at least 10 days is about 7 log CFU/mL to about 8 log CFU/mL. In some embodiments, the viable count of probiotic bacterium in the fermented culture after storage is increased by about 2% relative to the inoculation concentration. In other embodiments, the viable count is increased by about 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%.

In some embodiments, the fermented culture and/or stored culture comprises glucose of about 5 g/L to about 7 g/L. In some embodiments, the fermented culture and/or stored culture comprises glucose of about 5 g/L to about 7 g/L after at least 24 h. In some embodiments, the fermented culture and/or stored culture comprises glucose of about 5 g/L to about 7 g/L after storing for at least 1 day. In other embodiments, the concentration is about 5 g/L to about 6.8 g/L, about 5 g/L to about 6.6 g/L, about 5 g/L to about 6.5 g/L, about 5 g/L to about 6.4 g/L, about 5 g/L to about 6.2 g/L, about 5 g/L to about 6 g/L, about 5 g/L to about 5.8 g/L, or about 5 g/L to about 5.6 g/L. In other embodiments, the concentration is about 5.4 g/L.

In some embodiments, the fermented culture and/or stored culture comprises fructose of about 9 g/L to about 10 g/L. In some embodiments, the fermented culture and/or stored culture comprises fructose of about 9 g/L to about 10 g/L after at least 24 h. In other embodiments, the concentration is about 9 g/L to about 9.9 g/L, about 9 g/L to about 9.8 g/L, about 9 g/L to about 9.7 g/L, about 9 g/L to about 9.6 g/L, about 9 g/L to about 9.5 g/L, or about 9 g/L to about 9.4 g/L. In other embodiments, the concentration is about 9.4 g/L.

In some embodiments, the fermented culture and/or stored culture further comprises lactic acid of about 2 g/L to about 4 g/L. In some embodiments, the fermented culture and/or stored culture further comprises lactic acid of about 2 g/L to about 4 g/L after at

least 24 h. In some embodiments, the fermented culture and/or stored culture further comprises lactic acid of about 2 g/L to about 4 g/L after storing for at least 1 day. In other embodiments, the concentration is about 2.2 g/L to about 4 g/L, about 2.4 g/L to about 4 g/L, about 2.5 g/L to about 4 g/L, about 2.6 g/L to about 4 g/L, about 2.8 g/L to about 4 g/L, about 2.8 g/L to about 3.8 g/L, about 2.8 g/L to about 3.6 g/L, about 2.8 g/L to about 3.5 g/L, about 2.8 g/L to about 3.4 g/L, or about 2.8 g/L to about 3.2 g/L. In other embodiments, the concentration is about 3 g/L.

In some embodiments, the fermented culture and/or stored culture further comprises acetic acid of about 0.5 g/L to about 0.7 g/L. In some embodiments, the fermented culture and/or stored culture further comprises acetic acid of about 0.5 g/L to about 0.7 g/L after at least 24 h. In some embodiments, the fermented culture and/or stored culture further comprises acetic acid of about 0.5 g/L to about 0.7 g/L after storing for at least 1 day. In other embodiments, the concentration is about 0.52 g/L to about 0.7 g/L, about 0.54 g/L to about 0.7 g/L, about 0.55 g/L to about 0.7 g/L, about 0.56 g/L to about 0.7 g/L, about 0.58 g/L to about 0.7 g/L, about 0.6 g/L to about 0.7 g/L, about 0.6 g/L to about 0.68 g/L, about 0.6 g/L to about 0.66 g/L, about 0.6 g/L to about 0.65 g/L, or about 0.6 g/L to about 0.64 g/L. In other embodiments, the concentration is about 0.66 g/L.

In some embodiments, the fermentation process comprises:

a) mixing a oat flour and a sugar in an aqueous medium to form a mixture;
b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and
c) fermenting the culture for at least 24 h to form a fermented culture;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the mixture;
wherein the sugar is about 1 % w/v to about 20% w/v of the mixture;
wherein the fermented culture comprises lactic acid of about 2 g/L to about 4 g/L; and
wherein the fermented culture comprises acetic acid of about 0.6 g/L to about 0.7 g/L.

In some embodiments, the fermentation process comprises:

a) mixing a grain flour and a sugar derived from honey in an aqueous medium to form a mixture;
b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and
c) fermenting the culture for at least 24 h to form a fermented culture;

wherein the grain flour is about 5 % w/v to about 30 % w/v of the mixture;
wherein the honey is about 1 % w/v to about 20% w/v of the mixture;
wherein the fermented culture comprises lactic acid of about 2 g/L to about 4 g/L; and

wherein the fermented culture comprises acetic acid of about 0.6 g/L to about 0.7 g/L.

In some embodiments, the fermentation process comprises:

a) mixing a oat flour and a sugar in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and

c) fermenting the culture for at least 24 h to form a fermented culture;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the sugar is about 1 % w/v to about 20% w/v of the mixture;

wherein the fermented culture comprises glucose of about 5 g/L to about 7 g/L;

wherein the fermented culture comprises fructose of about 9 g/L to about 10 g/L;

wherein the fermented culture comprises lactic acid of about 2 g/L to about 4 g/L; and

wherein the fermented culture comprises acetic acid of about 0.6 g/L to about 0.7 g/L.

In some embodiments, the fermentation process comprises:

a) mixing a grain flour and a sugar derived from honey in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium and/or yeast in the mixture to form a culture; and

c) fermenting the culture for at least 24 h to form a fermented culture;

wherein the grain flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the honey is about 1 % w/v to about 20% w/v of the mixture;

wherein the fermented culture comprises glucose of about 5 g/L to about 7 g/L;

wherein the fermented culture comprises fructose of about 9 g/L to about 10 g/L;

wherein the fermented culture comprises lactic acid of about 2 g/L to about 4 g/L; and

wherein the fermented culture comprises acetic acid of about 0.6 g/L to about 0.7 g/L.

The antioxidant concentration refers to the total antioxidant content in the sample. This includes soluble and insoluble residues. In some embodiments, after fermenting for 3 days, the total antioxidant concentration is maintained. In some embodiments, after fermenting for 3 days, the total antioxidant concentration is increased. In some embodiments, after fermentation for 3 days, the fermented culture has a total antioxidant concentration of about 4 nmol/µl (mM) to about 20 nmol/µl. In other embodiments, the concentration is about 4 nmol/µl to about 18 nmol/µl, about 4 nmol/µl to about 16 nmol/µl, about 4 nmol/µl to about 14 nmol/µl, about 4 nmol/µl to about 12 nmol/µl, about 4 nmol/µl to about 10 nmol/µl, about 4 nmol/µl to about 8 nmol/µl, or about 4 nmol/µl to about 6 nmol/µl.

In some embodiments, the fermented culture and/or stored culture has an antioxidant activity of at least 60 nmol TE/mg. Antioxidant activity refers to the extent of inhibition of oxidation by restraining oxidative chain reactions. The antioxidant activity can be measured in Trolox equivalent. The Trolox equivalent antioxidant capacity (TEAC) assay measures the antioxidant capacity of a given substance, as compared to the standard, Trolox. Alternatively, antioxidant capacity can be measured using the ABTS ((2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) Decolorization Assay. Other antioxidant capacity assays which use Trolox as a standard include the diphenylpicrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC) and ferric reducing ability of plasma (FRAP) assays. In this regard, the antioxidant concentration can be different from the antioxidant activity. For example, insoluble residues which are included in the concentration measurement may not have activity (or until it is broken down). Soluble residues may also be complexed such that their antioxidant property cannot be directly measured. In some embodiments, the fermented culture and/or stored culture has an antioxidant activity of at least 60 nmol TE/mg after at least 24 h. In some embodiments, the fermented culture and/or stored culture has an antioxidant activity of at least 60 nmol TE/mg after storing for at least 1 day. In other embodiments, the antioxidant activity is at least 65 nmol TE/mg, 70 nmol TE/mg, 75 nmol TE/mg, 80 nmol TE/mg, 85 nmol TE/mg, 90 nmol TE/mg, or 95 nmol TE/mg. In other embodiments, the antioxidant activity is at least 75 nmol TE/mg after 48 h. In other embodiments, the antioxidant activity is at least 95 nmol TE/mg after 14 days.

In some embodiments, the fermented culture and/or stored culture comprises phenolic compounds selected from gallic acid, 4-hydrooxybenzoic acid, chlorogenic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, quercetin, or a combination thereof. In other embodiments, the phenolic compounds are selected from gallic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, or a combination thereof. In other embodiments, the fermented culture and/or stored culture comprises phenolic compounds selected from gallic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, or a combination thereof. In other embodiments, the phenolic compounds in the fermented culture and/or stored culture is characterised by an increase relative to the unfermented culture of at least 40%. In other embodiments, the increase is at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%. In other embodiments, gallic acid is increased by at least 55%. In other embodiments, catechin is increased by at least 90%. In other embodiments, vanillic acid is increased by at least 65%. In other embodiments, caffeic acid is increased by at least 100%. In other embodiments, p-coumaric acid is increased by at least 100%. In other embodiments, ferulic acid is increased by at least 100%. In other embodiments, sinapic acid is increased by at least 40%.

In some embodiments, the phenolic compounds in the fermented culture after storage is characterised by a decrease relative to the fermented culture of about 5% to about 60%. In other embodiments, the phenolic compounds in the fermented culture after storage of 14 days is characterised by a decrease relative to the fermented culture of about 5% to about 60%. In other embodiments, the decrease is about 5% to about 55%, about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, or about 5% to about 20%. In other embodiments, 4-hydroxybenzoic acid is decreased by about 10%. In other embodiments, vanillic acid is decreased by about 55%. In other embodiments, caffeic acid is decreased by about 20%. In other embodiments, p-coumaric acid is decreased by about 50%. In other embodiments, ferulic acid is decreased by about 9%.

In some embodiments, after fermenting for 3 days, the total phenolic content is maintained. In some embodiments, after fermenting for 3 days, the total phenolic content is increased. In some embodiments, after fermenting for 3 days, the fermented culture has a total phenolic content of about 1 mg GAE/g to about 5 mg GAE/g.

In some embodiments, the fermented culture and/or stored culture has a total phenolic content of about 0.4 mg GAE/g (mg gallic acid equivalents per g sample) to about 3 mg GAE/g. In some embodiments, the fermented culture and/or stored culture has a total phenolic content of about 0.4 mg GAE/g to about 2.8 mg GAE/g, about 0.4 mg GAE/g to about 2.6 mg GAE/g, about 0.4 mg GAE/g to about 2.4 mg GAE/g, about 0.4 mg GAE/g to about 2.2 mg GAE/g, about 0.4 mg GAE/g to about 2 mg GAE/g, about 0.4 mg GAE/g to about 1.8 mg GAE/g, about 0.4 mg GAE/g to about 1.6 mg GAE/g, about 0.4 mg GAE/g to about 1.4 mg GAE/g, about 0.4 mg GAE/g to about 1.2 mg GAE/g, about 0.4 mg GAE/g to about 1 mg GAE/g, about 0.4 mg GAE/g to about 0.8 mg GAE/g, about 0.5 mg GAE/g to about 0.8 mg GAE/g, about 0.6 mg GAE/g to about 0.8 mg GAE/g after at least 24 h. In some embodiments, the fermented culture and/or stored culture has a total phenolic content of about 0.4 mg GAE/g to about 3 mg GAE/g after storing for at least 1 day. In other embodiments, the total phenolic content is about 0.4 mg GAE/g to about 2.8 mg GAE/g, about 0.4 mg GAE/g to about 2.6 mg GAE/g, about 0.4 mg GAE/g to about 2.4 mg GAE/g, about 0.4 mg GAE/g to about 2.2 mg GAE/g, about 0.4 mg GAE/g to about 2 mg GAE/g, about 0.4 mg GAE/g to about 1.8 mg GAE/g, about 0.4 mg GAE/g to about 1.6 mg GAE/g, about 0.4 mg GAE/g to about 1.4 mg GAE/g, about 0.4 mg GAE/g to about 1.2 mg GAE/g, about 0.4 mg GAE/g to about 1 mg GAE/g, about 0.4 mg GAE/g to about 0.8 mg GAE/g, about 0.5 mg GAE/g to about 0.8 mg GAE/g, about 0.6 mg GAE/g to about 0.78 mg GAE/g, about 0.6 mg GAE/g to about 0.76 mg GAE/g, about 0.6 mg GAE/g to about 0.75 mg GAE/g, about 0.6 mg

GAE/g to about 0.74 mg GAE/g, about 0.6 mg GAE/g to about 0.72 mg GAE/g, about 0.6 mg GAE/g to about 0.7 mg GAE/g, or about 0.6 mg GAE/g to about 0.68 mg GAE/g. In other embodiments, the total phenolic content is about 0.62 mg GAE/g after 72 h. In other embodiments, the total phenolic content is about 0.68 mg GAE/g during storage for 14 days.

In some embodiments, the fermented culture and/or stored culture has a gallic acid content of about 3 μg/g to about 5 μg/g. In other embodiments, the fermented culture and/or stored culture has a gallic acid content of more than about 3 μg/g after at least 24 h. In other embodiments, the fermented culture and/or stored culture has a gallic acid content of more than about 4.5 μg/g after storing for at least 1 day. In other embodiments, the fermented culture and/or stored culture has a gallic acid content of about 3.2 μg/g to about 5 μg/g, about 3.4 μg/g to about 5 μg/g, about 3.5 μg/g to about 5 μg/g, about 3.6 μg/g to about 5 μg/g, about 3.8 μg/g to about 5 μg/g, about 4 μg/g to about 5 μg/g, about 4.2 μg/g to about 5 μg/g, about 4.4 μg/g to about 5 μg/g, or about 4.6 μg/g to about 5 μg/g.

In some embodiments, the fermented culture and/or stored culture has a 4-hydroxybenzoic acid content of about 0.7 μg/g to about 0.9 μg/g. In other embodiments, the fermented culture and/or stored culture has a 4-hydroxybenzoic acid content of less than about 0.9 μg/g after at least 24 h. In other embodiments, the fermented culture and/or stored culture has a 4-hydroxybenzoic acid content of less than about 0.9 μg/g after storing for at least 1 day. In other embodiments, the fermented culture and/or stored culture has a 4-hydroxybenzoic acid content of about 0.72 μg/g to about 0.9 μg/g, about 0.74 μg/g to about 0.9 μg/g, about 0.76 μg/g to about 0.9 μg/g, about 0.78 μg/g to about 0.9 μg/g, about 0.8 μg/g to about 0.9 μg/g, about 0.8 μg/g to about 0.88 μg/g, or about 0.8 μg/g to about 0.86 μg/g.

In some embodiments, the fermented culture and/or stored culture has a chlorogenic acid content which is substantially not altered (or is maintained) when compared to the mixture. In other embodiments, the chlorogenic acid content is about 1.4 μg/g to about 1.6 μg/g, about 1.42 μg/g to about 1.6 μg/g, about 1.44 μg/g to about 1.6 μg/g, about 1.46 μg/g to about 1.6 μg/g, about 1.48 μg/g to about 1.6 μg/g, about 1.5 μg/g to about 1.6 μg/g, about 1.5 μg/g to about 1.58 μg/g, or about 1.5 μg/g to about 1.56 μg/g.

In some embodiments, the fermented culture and/or stored culture has a catechin content of about 1.8 μg/g to about 2.6 μg/g. In other embodiments, the fermented culture and/or stored culture has a catechin content of more than about 1.8 μg/g after

at least 24 h. In other embodiments, the fermented culture and/or stored culture has a catechin content of more than about 2 μg/g after storing for at least 1 day. In other embodiments, the fermented culture and/or stored culture has a catechin content of about 1.85 μg/g to about 2.6 μg/g, about 1.9 μg/g to about 2.6 μg/g, about 1.95 μg/g to about 2.6 μg/g, about 2 μg/g to about 2.6 μg/g, about 2.05 μg/g to about 2.6 μg/g, about 2.1 μg/g to about 2.6 μg/g, about 2.15 μg/g to about 2.6 μg/g, about 2.2 μg/g to about 2.6 μg/g, about 2.25 μg/g to about 2.6 μg/g, about 2.3 μg/g to about 2.6 μg/g, about 2.35 μg/g to about 2.6 μg/g, about 2.4 μg/g to about 2.6 μg/g, about 2.4 μg/g to about 2.55 μg/g, or about 2.4 μg/g to about 2.5 μg/g.

In some embodiments, the fermented culture and/or stored culture has a vanillic acid content of about 0.2 μg/g to about 0.7 μg/g. In other embodiments, the fermented culture and/or stored culture has a vanillic acid content of more than about 0.3 μg/g after at least 24 h. In other embodiments, the fermented culture and/or stored culture has a vanillic acid content of less than about 0.3 μg/g after storing for at least 14 days. In other embodiments, the fermented culture and/or stored culture has a vanillic acid content of about 0.2 μg/g to about 0.65 μg/g, about 0.2 μg/g to about 0.6 μg/g, about 0.2 μg/g to about 0.55 μg/g, about 0.2 μg/g to about 0.5 μg/g, about 0.2 μg/g to about 0.45 μg/g, about 0.2 μg/g to about 0.4 μg/g, about 0.2 μg/g to about 0.35 μg/g, or about 0.3 μg/g to about 0.35 μg/g.

In some embodiments, the fermented culture and/or stored culture has a caffeic acid content of about 0.6 μg/g to about 0.9 μg/g. In other embodiments, the fermented culture and/or stored culture has a caffeic acid content of more than about 0.6 μg/g after at least 24 h. In other embodiments, the fermented culture and/or stored culture has a caffeic acid content of more than about 0.6 μg/g after storing for at least 1 day. In other embodiments, the fermented culture and/or stored culture has a caffeic acid content of about 0.6 μg/g to about 0.85 μg/g, about 0.6 μg/g to about 0.8 μg/g, about 0.6 μg/g to about 0.75 μg/g, about 0.6 μg/g to about 0.7 μg/g, or about 0.65 μg/g to about 0.7 μg/g.

In some embodiments, the fermented culture and/or stored culture has a p-coumaric acid content of about 0.45 μg/g to about 1.5 μg/g. In other embodiments, the fermented culture and/or stored culture has a p-coumaric acid content of more than about 0.45 μg/g after at least 24 h. In other embodiments, the fermented culture and/or stored culture has a p-coumaric acid content of more than about 0.6 μg/g after storing for at least 1 day. In other embodiments, the fermented culture and/or stored culture has a p-coumaric acid content of about 0.45 μg/g to about 1.4 μg/g, about 0.45 μg/g to about 1.3 μg/g, about 0.45 μg/g to about 1.2 μg/g, about 0.45 μg/g to about 1.1 μg/g, about

0.45 μg/g to about 1 μg/g, about 0.45 μg/g to about 0.9 μg/g, about 0.45 μg/g to about 0.8 μg/g, about 0.5 μg/g to about 0.7 μg/g, or about 0.6 μg/g to about 0.7 μg/g.

In some embodiments, the fermented culture and/or stored culture has a ferulic acid content of about 0.5 μg/g to about 0.8 μg/g. In other embodiments, the fermented culture and/or stored culture has a ferulic acid content of more than about 0.6 μg/g after at least 24 h. In other embodiments, the fermented culture and/or stored culture has a ferulic acid content of more than about 0.6 μg/g after storing for at least 1 day. In other embodiments, the fermented culture and/or stored culture has a ferulic acid content of about 0.52 μg/g to about 0.8 μg/g, about 0.54 μg/g to about 0.8 μg/g, about 0.56 μg/g to about 0.8 μg/g, about 0.58 μg/g to about 0.8 μg/g, about 0.6 μg/g to about 0.8 μg/g, about 0.6 μg/g to about 0.78 μg/g, about 0.6 μg/g to about 0.76 μg/g, about 0.6 μg/g to about 0.74 μg/g, about 0.6 μg/g to about 0.72 μg/g, or about 0.6 μg/g to about 0.7 μg/g.

In some embodiments, the fermented culture and/or stored culture has a sinapic acid content of about 1.5 μg/g to about 2.3 μg/g. In other embodiments, the fermented culture and/or stored culture has a sinapic acid content of more than about 1.5 μg/g after at least 24 h. In other embodiments, the fermented culture and/or stored culture has a sinapic acid content of more than about 2 μg/g after storing for at least 1 day. In other embodiments, the fermented culture and/or stored culture has a sinapic acid content of about 1.55 μg/g to about 2.3 μg/g, about 1.6 μg/g to about 2.3 μg/g, about 1.65 μg/g to about 2.3 μg/g, about 1.7 μg/g to about 2.3 μg/g, about 1.75 μg/g to about 2.3 μg/g, about 1.8 μg/g to about 2.3 μg/g, about 1.85 μg/g to about 2.3 μg/g, about 1.9 μg/g to about 2.3 μg/g, about 1.95 μg/g to about 2.3 μg/g, about 2 μg/g to about 2.3 μg/g, about 2 μg/g to about 2.25 μg/g, about 2 μg/g to about 2.2 μg/g, or about 2.1 μg/g to about 2.2 μg/g.

In some embodiments, the fermented culture and/or stored culture has a quercetin content of about 5.2 μg/g to about 5.6 μg/g. In other embodiments, the fermented culture and/or stored culture has a quercetin content of more than about 5.2 μg/g after at least 24 h. In other embodiments, the fermented culture and/or stored culture has a quercetin content of more than about 5.4 μg/g after storing for at least 1 day. In other embodiments, the fermented culture and/or stored culture has a quercetin content of about 5.25 μg/g to about 5.6 μg/g, about 5.3 μg/g to about 5.6 μg/g, about 5.35 μg/g to about 5.6 μg/g, about 5.4 μg/g to about 5.6 μg/g, about 5.45 μg/g to about 5.6 μg/g, or about 5.5 μg/g to about 5.6 μg/g.

In some embodiments, the fermented culture and/or stored culture has a β-glucan content which is substantially not altered when compared to the mixture. In some embodiments, the fermented culture and/or stored culture has a β-glucan content which is maintained when compared to the mixture. In other embodiments, the β-glucan concentration is about 2 g/100 g dry weight (DW) to about 3 g/100 g DW. In other embodiments, the β-glucan concentration is about 2 g/100 g DW to about 2.9 g/100 g DW, about 2 g/100 g DW to about 2.8 g/100 g DW, about 2 g/100 g DW to about 2.7 g/100 g DW, about 2 g/100 g DW to about 2.6 g/100 g DW, about 2 g/100 g DW to about 2.5 g/100 g DW, or about 2.1 g/100 g DW to about 2.5 g/100 g DW.

In some embodiments, the fermentation process comprises:

a) mixing an oat flour and a sugar derived from honey in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the honey is about 1 % w/v to about 20% w/v of the mixture;

wherein the fermented culture has a ferulic acid content of about 0.5 µg/g to about 0.8 µg/g.

In some embodiments, the fermentation process comprises:

a) mixing an oat flour and a sugar derived from honey in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the honey is about 1 % w/v to about 20% w/v of the mixture;

wherein the fermented culture has a ferulic acid content of about 0.5 µg/g to about 0.8 µg/g;

wherein the fermented culture has a gallic acid content of about 3 µg/g to about 5 µg/g.

In some embodiments, the fermentation process comprises:

a) mixing an oat flour and a sugar derived from honey in an aqueous medium to form a mixture;

b) inoculating a probiotic bacterium in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the mixture;

wherein the honey is about 1 % w/v to about 20% w/v of the mixture;

wherein the fermented culture has a ferulic acid content of about 0.5 µg/g to about 0.8 µg/g;
wherein the fermented culture has a gallic acid content of about 3 µg/g to about 5 µg/g;
wherein the fermented culture has a catechin content of about 1.8 µg/g to about 2.6 µg/g.

In some embodiments, the method further comprises a step of heat treating the fermented culture. The fermented culture can be heat treated at about 100 °C. The fermented culture can be heat treated for a period of about 5 min to about 30 min, about 5 min to about 25 min, about 5 min to about 50 min, about 5 min to about 15 min, or about 5 min to about 10 min. The heat treatment kills the probiotics, but does not cause degradation of the antioxidant, phenolic compound and/or beta-glucan. This facilitates the distribution of a nutritional drink without cold chain logistics.

The present invention also provides a probiotic composition as prepared by the method as disclosed herein.

The present invention also provides a probiotic composition, comprising:
a) a grain flour;
b) a sugar; and
c) a probiotic bacterium;
wherein the grain flour is about 5 % w/v to about 30 % w/v of the composition;
wherein the sugar is about 1 % w/v to about 20% w/v of the composition.

The probiotic composition refers to the fermented culture and/or stored culture, and which is suitable for consumption.

In some embodiments, the probiotic composition comprises:
a) a oat flour;
b) a sugar; and
c) a probiotic bacterium;
wherein the oat flour is about 5 % w/v to about 30 % w/v of the composition;
wherein the sugar is about 1 % w/v to about 20% w/v of the composition.

In some embodiments, the probiotic composition comprises:
a) a grain flour;
b) a sugar derived from honey; and
c) a probiotic bacterium;
wherein the grain flour is about 5 % w/v to about 30 % w/v of the composition;

wherein the honey is about 1 % w/v to about 20% w/v of the composition.

In some embodiments, the probiotic composition comprises:

a) a oat flour;

b) a sugar; and

c) a probiotic bacterium;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the composition;

wherein the sugar is about 1 % w/v to about 20% w/v of the composition;

wherein the probiotic composition comprises lactic acid of about 2 g/L to about 4 g/L; and

wherein the probiotic composition comprises acetic acid of about 0.6 g/L to about 0.7 g/L.

In some embodiments, the probiotic composition comprises:

a) a grain flour;

b) a sugar derived from honey; and

c) a probiotic bacterium;

wherein the grain flour is about 5 % w/v to about 30 % w/v of the composition;

wherein the honey is about 1 % w/v to about 20% w/v of the composition;

wherein the probiotic composition comprises lactic acid of about 2 g/L to about 4 g/L; and

wherein the probiotic composition comprises acetic acid of about 0.6 g/L to about 0.7 g/L.

In some embodiments, the probiotic composition comprises:

a) a oat flour;

b) a sugar; and

c) a probiotic bacterium;

wherein the oat flour is about 5 % w/v to about 30 % w/v of the composition;

wherein the sugar is about 1 % w/v to about 20% w/v of the composition;

wherein the probiotic composition comprises glucose of about 5 g/L to about 7 g/L;

wherein the probiotic composition comprises fructose of about 9 g/L to about 10 g/L;

wherein the probiotic composition comprises lactic acid of about 2 g/L to about 4 g/L; and

wherein the probiotic composition comprises acetic acid of about 0.6 g/L to about 0.7 g/L.

In some embodiments, the probiotic composition comprises:

a) a grain flour;

b) a sugar derived from honey; and

c) a probiotic bacterium;

wherein the grain flour is about 5 % w/v to about 30 % w/v of the composition;
wherein the honey is about 1 % w/v to about 20% w/v of the composition;
wherein the probiotic composition comprises glucose of about 5 g/L to about 7 g/L;
wherein the probiotic composition comprises fructose of about 9 g/L to about 10 g/L;
wherein the probiotic composition comprises lactic acid of about 2 g/L to about 4 g/L; and
wherein the probiotic composition comprises acetic acid of about 0.6 g/L to about 0.7 g/L.

In some embodiments, a viable count of probiotic bacterium in the composition is about 6 log CFU/mL to about 7 log CFU/mL. In other embodiments, the viable count is about 6 log CFU per mL to about 8 log CFU per mL, about 6.2 log CFU per mL to about 8 log CFU per mL, about 6.4 log CFU per mL to about 8 log CFU per mL, about 6.6 log CFU per mL to about 8 log CFU per mL, about 6.8 log CFU per mL to about 8 log CFU per mL, about 7 log CFU per mL to about 8 log CFU per mL, about 7 log CFU per mL to about 7.8 log CFU per mL, or about 7 log CFU per mL to about 7.6 log CFU per mL.

The probiotic composition can be formed from the fermented culture, with or without storage. In some embodiments, the viable count of probiotic bacterium in the composition with storage is substantially the same as before storage.

In some embodiments, a viable count of probiotic bacterium in the composition after exposure to gastric and/or intestinal conditions is about 6 log CFU/mL to about 7 log CFU/mL. In some embodiments, the viable count of probiotic bacterium in the composition before exposure to gastric and/or intestinal conditions is substantially the same as after exposure to gastric and/or intestinal conditions.

In some embodiments, the probiotic composition is capable of maintaining a viable count of about 6 CFU/mL to about 8 log CFU/mL under gastric digestion conditions. In some embodiments, the probiotic composition is capable of maintaining a viable count of about 6 CFU/mL to about 8 log CFU/mL under gastric digestion conditions after at least 24 h. In some embodiments, the probiotic composition is capable of maintaining a viable count of about 6 CFU/mL to about 8 log CFU/mL under gastric digestion conditions after storing for at least 1 day. In other embodiments, the viable count is about 7 log CFU/mL to about 8 log CFU/mL. In other embodiments, the viable count is about 7.1 log CFU/mL to about 8 log CFU/mL, about 7.2 log CFU/mL to about 8 log CFU/mL, about 7.3 log CFU/mL to about 8 log CFU/mL, about 7.3 log CFU/mL to about

7.9 log CFU/mL, about 7.3 log CFU/mL to about 7.8 log CFU/mL, about 7.3 log CFU/mL to about 7.7 log CFU/mL, about 7.3 log CFU/mL to about 7.6 log CFU/mL, or about 7.3 log CFU/mL to about 7.5 log CFU/mL.

For example, stimulated digestion conditions can be a pH of about 3 for 2 hours. Simulated salivary digestion conditions can be human α-amylase at about 75 U/ml for about 2 min at pH 7. Simulated gastric digestion conditions can be porcine pepsin at about 2000 U/ml for 2 h at pH 3. Simulated intestinal digestion conditions can be porcine trypsin (100 U per ml), bovine chymotrypsin (25 U per ml), porcine pancreatic lipase (2000 U per ml), and fresh bile salts (10 mM) for about 2 h at pH 7. In some embodiments, the digestion is performed under simulated salivary digestion conditions, simulated gastric digestion conditions, simulated intestinal digestion conditions, or a combination thereof. In other embodiments, the digestion is performed at 37 °C.

In some embodiments, the probiotic composition is capable of maintaining a viable count of about 6 CFU/mL to about 8 log CFU/mL under intestinal digestion conditions. In some embodiments, the probiotic composition is capable of maintaining a viable count of about 6 CFU/mL to about 8 log CFU/mL under intestinal digestion conditions after at least 24 h. In some embodiments, the probiotic composition is capable of maintaining a viable count of about 6 CFU/mL to about 8 log CFU/mL under intestinal digestion conditions after storing for at least 1 day. In other embodiments, the viable count is about 7 log CFU/mL to about 8 log CFU/mL. In other embodiments, the viable count is about 7.1 log CFU/mL to about 8 log CFU/mL, about 7.2 log CFU/mL to about 8 log CFU/mL, about 7.3 log CFU/mL to about 8 log CFU/mL, about 7.3 log CFU/mL to about 7.9 log CFU/mL, about 7.3 log CFU/mL to about 7.8 log CFU/mL, about 7.3 log CFU/mL to about 7.7 log CFU/mL, about 7.3 log CFU/mL to about 7.6 log CFU/mL, or about 7.3 log CFU/mL to about 7.5 log CFU/mL.

In some embodiments, the probiotic composition further comprises glucose of about 5 g/L to about 7 g/L. In other embodiments, the concentration is about 5 g/L to about 6.8 g/L, about 5 g/L to about 6.6 g/L, about 5 g/L to about 6.5 g/L, about 5 g/L to about 6.4 g/L, about 5 g/L to about 6.2 g/L, about 5 g/L to about 6 g/L, about 5 g/L to about 5.8 g/L, or about 5 g/L to about 5.6 g/L. In other embodiments, the concentration is about 5.4 g/L.

In some embodiments, the probiotic composition further comprises fructose of about 9 g/L to about 10 g/L. In other embodiments, the concentration is about 9 g/L to about 9.9 g/L, about 9 g/L to about 9.8 g/L, about 9 g/L to about 9.7 g/L, about 9 g/L to about

9.6 g/L, about 9 g/L to about 9.5 g/L, or about 9 g/L to about 9.4 g/L. In other embodiments, the concentration is about 9.4 g/L.

In some embodiments, the probiotic composition further comprises lactic acid of about 2 g/L to about 4 g/L. In other embodiments, the concentration is about 2.2 g/L to about 4 g/L, about 2.4 g/L to about 4 g/L, about 2.5 g/L to about 4 g/L, about 2.6 g/L to about 4 g/L, about 2.8 g/L to about 4 g/L, about 2.8 g/L to about 3.8 g/L, about 2.8 g/L to about 3.6 g/L, about 2.8 g/L to about 3.5 g/L, about 2.8 g/L to about 3.4 g/L, or about 2.8 g/L to about 3.2 g/L. In other embodiments, the concentration is about 3 g/L.

In some embodiments, the probiotic composition further comprises acetic acid of about 0.6 g/L to about 0.7 g/L. In other embodiments, the concentration is about 0.5 g/L to about 0.7 g/L, about 0.52 g/L to about 0.7 g/L, about 0.54 g/L to about 0.7 g/L, about 0.55 g/L to about 0.7 g/L, about 0.56 g/L to about 0.7 g/L, about 0.58 g/L to about 0.7 g/L, about 0.6 g/L to about 0.7 g/L, about 0.6 g/L to about 0.68 g/L, about 0.6 g/L to about 0.66 g/L, about 0.6 g/L to about 0.65 g/L, or about 0.6 g/L to about 0.64 g/L. In other embodiments, the concentration is about 0.66 g/L.

The probiotic composition can have antioxidants comprising free phenolic acids and/or bound phenolic acids. In most cases, bound phenolic acids are covalent bonded with polysaccharides, proteins, or lipids, or hydrophobically interacted such that their release from the food matrix is hindered. Bound phenolic acids can be released to impart bioactivity, the release can for example be via acid hydrolysis, base hydrolysis and/or enzymatic. The chemical bonds between the substances can thus be destroyed, releasing bound phenolic substances. In the embodiments disclosed herein, alkaline hydrolysis was used to extract the bound phenolic substances, but is not limited to as such. The extraction would provide a more complete estimation of the polyphenols within the sample.

For example, free phenolic acids can be selected from Beta-carotenol, Eugenol, 6-Gingerol, Chrysin, Apigenin, Coumarin, Epigallocatechin, Spermidine, 6-Paradol, Cinnamic acid, p-Coumaric acid, Methoxyphenylacetic acid, 2-Hydroxybenzoic acid, Vanillic acid, Ascorbic acid, Ferulic acid (or dimers thereof such as 5,5’-diferulic, 8-o-4-diferulic, 8,5’-diferulic acids), Caffeic acid, Sinapic acid, Gallic acid, pyrogallol, Catechin, Quercetin, Chlorogenic acid, Rutin, p-hydroxybenzoic acid, p-Coumaric acid, Avenanthramide 2c, Avenanthramide 2p, Avenanthramide, Protocatechuic acid, Dihydroxybenzoic acid, Tocopherol, Protocatchuic acid, Proanthocyanidin dimer, Proanthocyanidin trimer, Kaempherol, Isorhamnetin, Gamma-oryzanol, or a combination thereof. For example, phenolic acids that can be derived from bound

phenolic acids include Ferulic acid, p-Coumaric acid, Dimers of ferulic acid (5,5'-diferulic, 8-o-4-diferulic, 8,5'-diferulic acids), Gallic acid, Chlorogenic acid, Caffeic acid, Vanillic acid, Sinapic acid, Catechin, Rutin, Quercetin, or a combination thereof.

In some embodiments, the probiotic composition comprises an antioxidant selected from Ferulic acid, Caffeic acid, p-Coumaric acid, Sinapic acid, Dimers of ferulic acid (5,5'-diferulic, 8-o-4-diferulic, 8,5'-diferulic acids), or a combination thereof.

In some embodiments, the probiotic composition has an antioxidant activity of at least 60 nmol TE/mg. In other embodiments, the antioxidant activity is at least 65 nmol TE/mg, 70 nmol TE/mg, 75 nmol TE/mg, 80 nmol TE/mg, 85 nmol TE/mg, 90 nmol TE/mg, or 95 nmol TE/mg.

In some embodiments, the probiotic composition further comprises phenolic compounds selected from gallic acid, 4-hydrooxybenzoic acid, chlorogenic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, quercetin, or a combination thereof. In other embodiments, the phenolic compounds are selected from gallic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, or a combination thereof.

In some embodiments, the probiotic composition has a total phenolic content of about 0.4 mg GAE/g to about 3 mg GAE/g. In other embodiments, the total phenolic content is about 0.4 mg GAE/g to about 2.8 mg GAE/g, about 0.4 mg GAE/g to about 2.6 mg GAE/g, about 0.4 mg GAE/g to about 2.4 mg GAE/g, about 0.4 mg GAE/g to about 2.2 mg GAE/g, about 0.4 mg GAE/g to about 2 mg GAE/g, about 0.4 mg GAE/g to about 1.8 mg GAE/g, about 0.4 mg GAE/g to about 1.6 mg GAE/g, about 0.4 mg GAE/g to about 1.4 mg GAE/g, about 0.4 mg GAE/g to about 1.2 mg GAE/g, about 0.4 mg GAE/g to about 1 mg GAE/g, about 0.4 mg GAE/g to about 0.8 mg GAE/g, about 0.5 mg GAE/g to about 0.8 mg GAE/g, about 0.6 mg GAE/g to about 0.78 mg GAE/g, about 0.6 mg GAE/g to about 0.76 mg GAE/g, about 0.6 mg GAE/g to about 0.75 mg GAE/g, about 0.6 mg GAE/g to about 0.74 mg GAE/g, about 0.6 mg GAE/g to about 0.72 mg GAE/g, about 0.6 mg GAE/g to about 0.7 mg GAE/g, or about 0.6 mg GAE/g to about 0.68 mg GAE/g.

In some embodiments, the probiotic composition has a gallic acid content of about 3 µg/g to about 5 µg/g. In other embodiments, the probiotic composition has a gallic acid content of about 3.2 µg/g to about 5 µg/g, about 3.4 µg/g to about 5 µg/g, about 3.5 µg/g to about 5 µg/g, about 3.6 µg/g to about 5 µg/g, about 3.8 µg/g to about 5 µg/g,

about 4 µg/g to about 5 µg/g, about 4.2 µg/g to about 5 µg/g, about 4.4 µg/g to about 5 µg/g, or about 4.6 µg/g to about 5 µg/g.

In some embodiments, the probiotic composition has a 4-hydroxybenzoic acid content of about 0.7 µg/g to about 0.9 µg/g. In other embodiments, the probiotic composition has a 4-hydroxybenzoic acid content of about 0.72 µg/g to about 0.9 µg/g, about 0.74 µg/g to about 0.9 µg/g, about 0.76 µg/g to about 0.9 µg/g, about 0.78 µg/g to about 0.9 µg/g, about 0.8 µg/g to about 0.9 µg/g, about 0.8 µg/g to about 0.88 µg/g, or about 0.8 µg/g to about 0.86 µg/g.

In some embodiments, the probiotic composition has a chlorogenic acid content of about 1.4 µg/g to about 1.6 µg/g, about 1.42 µg/g to about 1.6 µg/g, about 1.44 µg/g to about 1.6 µg/g, about 1.46 µg/g to about 1.6 µg/g, about 1.48 µg/g to about 1.6 µg/g, about 1.5 µg/g to about 1.6 µg/g, about 1.5 µg/g to about 1.58 µg/g, or about 1.5 µg/g to about 1.56 µg/g.

In some embodiments, the probiotic composition has a catechin content of about 1.8 µg/g to about 2.6 µg/g. In other embodiments, the probiotic composition has a catechin content of about 1.85 µg/g to about 2.6 µg/g, about 1.9 µg/g to about 2.6 µg/g, about 1.95 µg/g to about 2.6 µg/g, about 2 µg/g to about 2.6 µg/g, about 2.05 µg/g to about 2.6 µg/g, about 2.1 µg/g to about 2.6 µg/g, about 2.15 µg/g to about 2.6 µg/g, about 2.2 µg/g to about 2.6 µg/g, about 2.25 µg/g to about 2.6 µg/g, about 2.3 µg/g to about 2.6 µg/g, about 2.35 µg/g to about 2.6 µg/g, about 2.4 µg/g to about 2.6 µg/g, about 2.4 µg/g to about 2.55 µg/g, or about 2.4 µg/g to about 2.5 µg/g.

In some embodiments, the probiotic composition has a vanillic acid content of about 0.2 µg/g to about 0.7 µg/g. In other embodiments, the probiotic composition has a vanillic acid content of about 0.2 µg/g to about 0.65 µg/g, about 0.2 µg/g to about 0.6 µg/g, about 0.2 µg/g to about 0.55 µg/g, about 0.2 µg/g to about 0.5 µg/g, about 0.2 µg/g to about 0.45 µg/g, about 0.2 µg/g to about 0.4 µg/g, about 0.2 µg/g to about 0.35 µg/g, or about 0.3 µg/g to about 0.35 µg/g.

In some embodiments, the probiotic composition has a caffeic acid content of about 0.6 µg/g to about 0.9 µg/g. In other embodiments, the probiotic composition has a caffeic acid content of about 0.6 µg/g to about 0.85 µg/g, about 0.6 µg/g to about 0.8 µg/g, about 0.6 µg/g to about 0.75 µg/g, about 0.6 µg/g to about 0.7 µg/g, or about 0.65 µg/g to about 0.7 µg/g.

In some embodiments, the probiotic composition has a p-coumaric acid content of about 0.45 µg/g to about 1.5 µg/g. In other embodiments, the probiotic composition has a p-coumaric acid content of about 0.45 µg/g to about 1.4 µg/g, about 0.45 µg/g to about 1.3 µg/g, about 0.45 µg/g to about 1.2 µg/g, about 0.45 µg/g to about 1.1 µg/g, about 0.45 µg/g to about 1 µg/g, about 0.45 µg/g to about 0.9 µg/g, about 0.45 µg/g to about 0.8 µg/g, about 0.5 µg/g to about 0.7 µg/g, or about 0.6 µg/g to about 0.7 µg/g.

In some embodiments, the probiotic composition has a ferulic acid content of about 0.5 µg/g to about 0.8 µg/g. In other embodiments, the probiotic composition has a ferulic acid content of about 0.52 µg/g to about 0.8 µg/g, about 0.54 µg/g to about 0.8 µg/g, about 0.56 µg/g to about 0.8 µg/g, about 0.58 µg/g to about 0.8 µg/g, about 0.6 µg/g to about 0.8 µg/g, about 0.6 µg/g to about 0.78 µg/g, about 0.6 µg/g to about 0.76 µg/g, about 0.6 µg/g to about 0.74 µg/g, about 0.6 µg/g to about 0.72 µg/g, or about 0.6 µg/g to about 0.7 µg/g.

In some embodiments, the probiotic composition has a sinapic acid content of about 1.5 µg/g to about 2.3 µg/g. In other embodiments, the probiotic composition has a sinapic acid content of about 1.55 µg/g to about 2.3 µg/g, about 1.6 µg/g to about 2.3 µg/g, about 1.65 µg/g to about 2.3 µg/g, about 1.7 µg/g to about 2.3 µg/g, about 1.75 µg/g to about 2.3 µg/g, about 1.8 µg/g to about 2.3 µg/g, about 1.85 µg/g to about 2.3 µg/g, about 1.9 µg/g to about 2.3 µg/g, about 1.95 µg/g to about 2.3 µg/g, about 2 µg/g to about 2.3 µg/g, about 2 µg/g to about 2.25 µg/g, about 2 µg/g to about 2.2 µg/g, or about 2.1 µg/g to about 2.2 µg/g.

In some embodiments, the probiotic composition has a quercetin content of about 5.2 µg/g to about 5.6 µg/g. In other embodiments, the probiotic composition has a quercetin content of about 5.25 µg/g to about 5.6 µg/g, about 5.3 µg/g to about 5.6 µg/g, about 5.35 µg/g to about 5.6 µg/g, about 5.4 µg/g to about 5.6 µg/g, about 5.45 µg/g to about 5.6 µg/g, or about 5.5 µg/g to about 5.6 µg/g.

In some embodiments, the probiotic composition further comprises β-glucan at about 2 g/100 g DW to about 3 g/100 g DW. In other embodiments, the β-glucan concentration is about 2 g/100 g DW to about 2.9 g/100 g DW, about 2 g/100 g DW to about 2.8 g/100 g DW, about 2 g/100 g DW to about 2.7 g/100 g DW, about 2 g/100 g DW to about 2.6 g/100 g DW, about 2 g/100 g DW to about 2.5 g/100 g DW, or about 2.1 g/100 g DW to about 2.5 g/100 g DW.

In some embodiments, probiotic composition further comprises an additive. The additive can be added at step a) or at step c) of the process. The additive can be added for

improving the coloration of the resultant culture, or stability of the culture. The additive can also be added to improve the taste profile and/or nutritional value of the culture. The additive can also be added to increase the amount of antioxidant and/or phenolic compounds in the culture. For example, the additive can be a vitamin, fibre, or a combination thereof. In some embodiments, the additive is a vitamin mix, sugarcane fibre, or a combination thereof. The vitamin can be vitamin A, vitamin B2, vitamin C, vitamin D3, vitamin E, or a combination thereof.

In some embodiments, the probiotic composition comprises retinol (Vitamin A) of about 500 IU to about 1500 IU, about 500 IU to about 1400 IU, about 500 IU to about 1300 IU, about 500 IU to about 1200 IU, about 500 IU to about 1100 IU, about 500 IU to about 1000 IU, about 500 IU to about 900 IU, about 600 IU to about 900 IU, about 700 IU to about 900 IU, or about 800 IU to about 900 IU.

In some embodiments, the probiotic composition comprises riboflavin (Vitamin B2) of about 10 mg to about 80 mg, about 10 mg to about 70 mg, about 10 mg to about 60 mg, about 10 mg to about 50 mg, about 20 mg to about 50 mg, or about 20 mg to about 40 mg.

In some embodiments, the probiotic composition comprises ascorbic acid (Vitamin C) of about 100 mg to about 1000 mg, about 100 mg to about 900 mg, about 100 mg to about 800 mg, about 100 mg to about 700 mg, about 100 mg to about 600 mg, about 200 mg to about 600 mg, about 300 mg to about 600 mg, about 400 mg to about 600 mg, or about 400 mg to about 500 mg.

In some embodiments, the probiotic composition comprises cholecalcifrol (Vitamin D3) of about 10 µg to about 100 µg, about 10 µg to about 90 µg, about 10 µg to about 80 µg, about 10 µg to about 70 µg, about 10 µg to about 60 µg, about 10 µg to about 50 µg, about 10 µg to about 40 µg, about 10 µg to about 30 µg, or about 20 µg to about 30 µg.

In some embodiments, the probiotic composition comprises alpha tocopherol (Vitamin E) of about 10 mg to about 1000 mg, about 10 mg to about 900 mg, about 10 mg to about 800 mg, about 10 mg to about 700 mg, about 10 mg to about 600 mg, about 10 mg to about 500 mg, about 10 mg to about 400 mg, about 10 mg to about 300 mg, about 10 mg to about 200 mg, or about 10 mg to about 100 mg.

In some embodiments, the additive can be a flavouring. In other embodiments, the flavouring can be an artificial flavouring, natural flavouring, or a nature identical

flavouring. Examples of artificial flavouring can be Manzanate (Apple), Diacetyl or acetylpropionyl or acetoin (Buttery), Isoamyl acetate (Banana), Benzaldehyde (Bitter almond, cherry), Cinnamaldehyde (Cinnamon), Ethyl propionate (Fruity), Methyl anthranilate (Grape), Limonene (Orange), Ethyl decadienoate (Pear), Allyl hexanoate (Pineapple), Ethyl maltol (Sugar, cotton candy), 2,4-Dithiapentane (Truffle), Ethylvanillin (Vanilla), Methyl salicylate (Wintergreen), or a combination thereof. Acids can also be added (such as ascorbic acid, citric acid, malic acid, tartaric acid) to further enhance the flavouring. Examples of natural flavouring includes extracts derived from raspberry, lemon, orange, vanilla, coconut, caramel, peppermint, strawberry, chocolate, almond, apple, banana, apricot, blackcurrent, blueberry, cherry, coffee, cranberry, elderflower, ginger, grapefruit, hazelnut, honey, lavender, lime, liquorice, lychee, mango, maple, passion fruit, peach, pecan, pineapple, pistachio, plum, rhubarb, rose, rum, or a combination thereof.

In some embodiments, the additive can be a colorant. The colorant can be an artificial colorant, natural colorant, or a nature identical colorant. Examples of natural colorants include, Carotenoids (E160, E161, E164), chlorophyllin (E140, E141), anthocyanins (E163), betanin (E162), Annatto (E160b), Caramel coloring (E150a-d), Carmine (E120), Elderberry juice (E163), Lycopene (E160d), Paprika (E160c), Turmeric/curcumin (E100), or a combination thereof. Examples of artificial colorants include E numbers within the range of 102-143, such as E104 (Quinoline yellow), E122 (Carmoisine), E124 (Ponceau 4R), E131 (Patent blue V), E142 (Green S), or a combination thereof.

In some embodiments, the probiotic composition has an additive concentration of about 0.5 %w/v to about 4 % w/v relative to the probiotic composition. In other embodiments, the concentration is about 0.5 %w/v to about 3.5 % w/v, about 0.5 %w/v to about 3 % w/v, about 0.5 %w/v to about 2.5 % w/v, about 0.5 %w/v to about 2 % w/v, about 0.5 %w/v to about 1.5 % w/v, or about 1 %w/v to about 1.5 % w/v.

In some embodiments, the probiotic composition has an absence of dairy product.

For example, as disclosed herein, *Lactobacillus fermentum* PC1 can be used to ferment oats with added honey to develop a probiotic beverage with enhanced bioactive ingredients. The viable lactobacilli were enumerated during the fermentation and storage at 4 °C, as well as after exposure to simulated gastrointestinal tract conditions. Good survival was noted both during storage as well as when exposed to the in vitro digestive tract conditions. Comparative analysis of the antioxidant activity, total phenolic content and phenolic composition indicated fermentation improved the total antioxidant capacity and phenolic acid concentration. An increase of more than 50% of

gallic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid and ferulic acid was observed in the methanol extracts. Moreover, no significant decrease in the β-glucan content was noted during fermentation and storage. In conclusion, this fermented culture has great potential to be formed as a functional food with enhanced probiotic survival and increased bioactive ingredients.

For example, the probiotic composition can be provided as a liquid mixture, or can be provided as a solid. The solid can be a powder, which is formable by, for example, freeze drying techniques. The powder can be dissolved in water by a user to reconstitute the probiotic composition as a drink.

For example, it is shown that oat flour with added honey promoted the growth and maintained the survival of the probiotic, *L. fermentum* PC1, both during fermentation and storage. The viable count of PC1 was stable (7.32 log CFU/mL) during storage at 4 °C for at least 14 days. The survival of the PC1 in the fermented oat exposed to simulated gastrointestinal conditions was significantly enhanced compared to control cells. Furthermore, it was apparent that the PC1 was metabolically active during storage at 4 °C for at least 14 days since the content of sugars and acid production continued to change. Moreover, there were improvements in antioxidant capacity and phenolic content, and no significant decrease of β-glucan. The main phenolic components that were detected in higher amounts in the methanol extracts were gallic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid and sinapic acid. Thus, the fermentation of oat flour with added honey and *L. fermentum* PC1 could be a potentially valuable probiotic food with both improved levels of probiotic and bioactive components. Other approaches have not achieve improvements in both probiotic and bioactive properties, or only focused on one aspect.

Examples

Materials, enzymes, and strain

HPLC-grade formic acid, acetonitrile and Folin–Ciocalteau reagent as well as phenolic acid standards including gallic acid, chlorogenic acid, Catechin, 4-hydroxybenzoic acid, caffeic acid, vanillic acid, p-coumaric acid, sinapic acid, ferulic acid and quercetin were all purchased from Sigma-Aldrich (Singapore). Whole grain oat flour from Bob's Red Mill was purchased from Lazada online shop in Singapore. *L. fermentum* PC1 (FII511400) was obtained from Patricia Conway. Human α-amylase (A1031), porcine pepsin (P6887), porcine trypsin (T4799), bovine chymotrypsin (C4129), porcine pancreatic lipase (L0382), and fresh bile salts (B8756) were purchased from Sigma-Aldrich (Singapore). DeMann-Rogosa-Sharpe (MRS) broth and agar were obtained from Sigma-Aldrich (Singapore) and prepared according to the manufacturer's instructions.

Fermentation conditions

Dry oat flour was autoclaved at 121°C for 10 min. Honey from Sardinia (Miele Di Sardegna honey) was suspended in distilled water (3g honey in 90 mL distilled water) and pasteurized at 80 °C for 10 min. The diluted honey was added aseptically to the sterilized oat flour to yield a final concentration of 10% oats (w/v) and 3% honey (w/v). This mixture was heated to 80 ℃ for 10 min with regularly stirring in a thermostatically controlled water bath to ensure homogenization. The mixture was cooled to room temperature before inoculation.

Overnight-grown *L. fermentum* PC1 strain in MRS (pH 6.2±0.2) was inoculated into the oat and honey mixture at 1% (v/v) to yield an initial concentration of about 10^7 per mL. The mixture was fermented in screw cap bottles (250 mL) at 37 °C, 150 rpm for 72 h, and subsequently stored at 4 °C for 14 days. Samples were taken daily during fermentation and then after 10 and 14 days of storage, and analyzed for viable count of lactobacilli, pH values, and bioactive compounds as well as viable counts after exposure to simulated digestive tract conditions.

Enumeration of viable *L. fermentum* PC1

Viable lactobacilli in the fermented culture were quantified using the standard plate count method. In brief, 1 mL of fermented culture was used to make 10-fold serial dilutions in PBS. Aliquots of 10 µL of appropriate dilutions were plated in triplicate on MRS agar plates using the drop plate method. The plates were incubated at 37 ℃ for 48 h. Colonies were counted and recorded as log CFU (colony forming units) per mL.

Impact of simulated digestive tract conditions on survival of *L. fermentum* PC1 in fermented oat

Fermented oat products were exposed to conditions which simulated oral, gastric and small intestinal digestion conditions according to a published method in Minekus M. et al.; 2014; Food & Function; 5:1113-1124 with slight modifications, the reference of which is incorporated herein. *L. fermentum* PC1 48 h secondary culture grown in MRS was washed and re-suspended in PBS to about 10^7 per mL and used as control. In summary, samples were initially combined with simulated salivary fluid with a final concentration of human α-amylase of 75 U per mL, and the mixture was incubated for 2 min at pH 7, followed by the addition of simulated gastric fluid with final concentration of porcine pepsin of 2000 U per ml and pH 3, and incubated for 2 h. The mixture was then combined with simulated intestinal fluid and incubated for another 2 h after pH adjustment to 7, and with final concentrations of the following enzymes: porcine trypsin (100 U per mL), bovine chymotrypsin (25 U per mL), porcine pancreatic lipase (2000 U

per mL), and fresh bile salts (10mM). All three steps were performed at 37 °C. Aliquots (100 µL) of undigested samples, and samples taken after simulated gastric phase and intestinal phase were serially diluted. Aliquots (10 µL) of appropriate dilutions were plated on MRS agar plates using the drop plate method for enumeration of viable lactobacilli.

Glucose, fructose, lactic acid and acetic acid assay

Samples taken throughout the experiment were centrifuged at 16000×g for 30 min, then supernatants diluted 3 or 4 times in 5 mM H_2SO_4 prior to filtration using a 0.45 µm membrane (PES, VWR). Glucose, fructose, lactic acid and acetic acid concentrations in the supernatant were determined using an Ultra-Fast Liquid Chromatography (Shimadzu) equipped with a refractor index detector (RID-10A). An Aminex® HPX-87H column (Bio-Rad, Singapore) was used for the separation with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min according to the manufacturer's instructions. The temperature of the column oven and RID were set at 50 °C and 45 °C, respectively. Samples (20 µL) were injected in duplicate for each independent experiment. Concentration of glucose, fructose, lactic acid and acetic acid were calculated according to a standard curve prepared using concentrations ranging from 0.625 to 20 g/L.

Ultrasound-assisted extraction of phenolic compounds

Samples were extracted using a slightly modified variant of the published ultrasound-assisted extraction method in Călinoiu LF. Et al.; 2019; Antioxidants; 8:372, the reference of which is incorporated herein. Firstly, 40.0 ±0.1 mg of freeze-dried sample was accurately weighed, and 1.8 mL of hexane was added to remove fats. The mixtures were vortexed for 30 s, sonicated for 10 min, and vortexed for another 30 min. Then, the mixture was centrifuged for 15 min at 8000×g, the supernatant was discarded, and the wet samples were dried for 30 min at 30 °C in a fume hood. These dried samples were extracted by adding 80:20 methanol: water (1.5 mL), and vortexed until fully suspended and held in a sonic bath for 1 h at 40 °C. Samples were then vortexed for another 20 min prior to being centrifuged for 15 min at 10000×g. This extraction was repeated one more time and the supernatants were combined and evaporated to dryness. The dried extracts were reconstituted in 0.2 mL 80% methanol, vortexed for 5 min and then centrifuged in 10000×g for 20 min prior to analyses of antioxidant activity, total phenolic content and HPLC analysis of phenolic compounds.

Determination of antioxidant activity and total phenolic content

Total antioxidant activity was analyzed by Total Antioxidant Capacity Assay Kit (Sigma-Aldrich, MAK187) according to the manufacturer's instructions. Briefly, 5 µL of the methanol extracts were mixed with Cu^{2+} reagent and incubated in darkness at room

temperature for 90 minutes, and the absorbance was measured at 570 nm using a microplate reader (Bio-Rad, Benchmark Plus Microplate Spectrophotometer System). Trolox solutions ranging from 0 to 20 nmol per well were used to prepare a standard curve. The antioxidant activity was expressed as nmol Trolox equivalents per mg sample (nmol TE/mg).

Total phenolic content was analyzed according to the Folin-Ciocalteu method with modification in Călinoiu LF. Et al.; 2019; Antioxidants; 8:372, the reference of which is incorporated herein. Briefly, 20 µL phenolic extract was mixed with 10 µL Folin-Ciocalteu's reagent for 5 min. Then, 30 µL 20% Na_2CO_3 (w/v) and 140 µL of distilled water were added to the solution to reach a final volume of 200 µL. The mixture was incubated in the dark for 60 min at 300 rpm at room temperature. The plate was centrifuged at 200 ×g and 120 µL samples of supernatant from each well were transferred to a new plate, and the absorbance was read at 760 nm with a microplate reader (Bio-Rad, Benchmark Plus Microplate Spectrophotometer System). A standard curve was prepared using a series of concentrations of gallic acid ranging from 0 to 8.4 µg per well. The results were expressed as mg gallic acid equivalents per g sample (mg GAE/g).

HPLC analysis of phenolic compounds

The HPLC analyses were carried out using an Agilent 1290 Infinity LC system coupled with photodiode array detector. Separation was performed at 25°C on a ZORBAX RRHD SB-C18 column (1.8 µm, 2.1 mm × 150 mm) (Agilent Technologies, Singapore). Two solvents were used for the mobile phase: 0.1% formic acid in distilled water (v/v) (solvent A) and 0.1% formic acid in acetonitrile (v/v) (solvent B). The following optimized gradient elution (expressed in % B) was used: 0–2 min, 5% B; 2–6 min, 5–14% B; 6–38 min, 14–40% B; 38–40 min; 40-90% B; 40–42 min, 90–5% B; 40–45 min, 5% B. Aliquots (20 µL) of phenolic extracts from each time point were injected into the column. The flow rate was 0.3 mL/min, and detection was performed at 280 nm. Phenolic acids were identified by comparing their retention times and UV visibility with the standards under same analysis conditions. Quantitation was based on linear calibration curves of phenolic acid standards prepared using concentrations ranging from 0.78125 to 100 mg/L. All measurements were performed in triplicate and all the samples were injected in duplicate. The final concentrations of phenolic acids were expressed as µg/g.

Analysis of β-glucan

The β-glucan content in the fermented oat product was quantified using the Mixed Link (1-3, 1-4) Beta Glucan kit (Megazyme International, Bray, Ireland) with modifications

of method B. In brief, 30 mg (±1%) of freeze-dried fermented oat product was weighed to 0.1 mg precision and transferred into a 2 mL plastic screw cap tube. Firstly, the sample was extracted with 1.75 mL of 50% (v/v) aqueous ethanol to remove free sugars and fats. The extraction was repeated two additional times and the supernatant after centrifugation was discarded. Secondly, the pellet was suspended in 1.0 mL of sodium phosphate buffer (20 mM, pH 6.5) and the tube was incubated at 50°C for 5 min. Thirdly, 50 µL of lichenase (2.5 U) was added and the tube was vortexed and incubated for 1 h at 50 °C with stirring at 300 rpm. Then, 0.5 mL of sodium acetate buffer (200 mM, pH 4.0) was added and the mixture was vigorously mixed. After that, the tubes were centrifuged for 10 min at 10,000×g. Aliquots (25 µL) were transferred into 2 mL test tubes, and β-glucosidase (25 µL, 0.05 U) in 50 mM sodium acetate buffer (pH 4.0) was added and then the tubes were incubated at 50°C for 10 min. Finally, GOPOD Reagent (0.75 mL) was added to each tube prior to incubation at 50°C for a further 20 min. Glucose concentrations in the samples were measured at 510 nm against a reagent blank using SPECTRONIC 200 (Thermo Scientific, Singapore). Reagent blanks and D-glucose standards of 1mg/mL were included in duplicate. For every independent assay, the test was carried out in duplicate with a reaction blank. The final β-glucan content was expressed as g/100 g DW (dry weight).

Simulated gut model for studying the impact on the intestinal microbes

The simulated gut model utilised an ex-vivo microcosm system and donor fecal samples from elderly subjects. Fecal sample of 30 g was added to 120 ml of Wilkins-Chalgren Anaerobe Broth (WC media; Thermo Fisher Scientific, USA) to obtain a 20% fecal slurry. WC media, probiotic culture, grain and fecal slurry 50%, making up a total volume of 10 ml in each tube. The tubes were incubated in anaerobic conditions at 37°C, with rotary agitation of 120 rpm for 48 h. After 48 h of incubation, aliquots of 1000 µl of each duplicate sample were harvested for enumeration of viable counts and quantification of SCFAs.

Enumeration of viable counts

Viable lactobacilli and enteric bacteria from harvested samples were quantified using the standard plate count method. Briefly, 1 ml of harvested sample was used to make 10-fold serial dilutions in 0.1M PBS. Aliquots of 10 µl of appropriate dilutions were plated in triplicate on selective agar plates using the drop plate method. MacConkey agar plates for enumeration of enteric bacteria were incubated at 37°C for 24 h, while Rogosa agar plates for enumeration of lactobacilli were incubated anaerobically at 37°C for 48 h. The number of CFU for each dilution were counted and recorded as log CFU/ml.

Quantification of SCFAs in fecal samples

Fecal SCFAs were extracted using an extraction procedure with minor modifications (Garcia-Villalba et al., 2012). Stock solutions of acetic acid, propionic acid, lactic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, caproic acid, heptanoic acid and 4-methyl valeric acid (200 mM) (Sigma-Aldrich, USA) in ethyl acetate were prepared for each individual SCFA and stored at -20C before further use. Briefly, 25 µl of 10% phosphoric acid (Sigma-Aldrich, USA) was added to 500 µl of harvested fecal sample to adjust the pH to about 2.0. Samples were vortexed thoroughly, and 5 µl of 50 mM 4-methyl valeric acid was added as internal standard (IS) to a final concentration of 500 µM. Then, 500 µl of ethyl acetate was added to extract SCFAs, vortexed for 10 min and centrifuged at 17 949 x *g* for 10 min. Lastly, 50 µl of organic extracts (upper phase) were transferred into GC glass vials for GC-MS analysis while the remaining organic extracts were transferred in storage vials and kept in -20°C. SCFAs were quantified using a GC-MS method consisting of an Agilent 7890A GC system (Agilent Technologies, USA) coupled to an Agilent 5975C mass selective detector.

Statistical analysis

All the experiments were performed at least as 3 independent experiments, each analyzed in duplicate. The results are expressed as mean ± SD. Statistical analyses were carried out using either the Student's t test or One-way ANOVA in R (version 3.6.3). P values<0.05 were considered statistically significant. Correlations between total phenolic content and antioxidant activity were determined using Pearson's correlation. Correlation coefficient r >0.5 is considered to show a strong positive correlation.

Example 1: *L. fermentum* PC1 growth during fermentation

Whole grain oat flour was used as a delivery vehicle for *L. fermentum* PC1 by fermenting 10% oat flour supplemented with 3% honey in distilled water with no additional ingredients. *L. fermentum* PC1 was inoculated around 10^7 cell per mL. The growth profile shown in Table 1 demonstrated that there was a significant increase of viable PC1 cells during the first 24 h (7.96 log CFU/mL) as compared to 0 h (7.12 log CFU/mL), with a slight decrease after 48 h and 72 h to 7.28 log CFU/mL and 7.38 log CFU/mL, respectively. The viable count remained relatively stable during storage at 4 ℃ for 10 days (7.40 log CFU/mL) and 14 days (7.32 log CFU/mL). With this increase of cell growth, there was a significant decrease of pH from 6.26 to 4.12 after 24 h, and a further decrease to 3.93 at 72 h. The pH of the fermented culture was constant during storage at 4 °C, which reflects the noted stability of organoleptic properties of the product. The stability was probably due to the buffering capacity of other compounds produced in the fermented culture, such as acetic acid, lactic acid and phenolic compounds. These results demonstrated that oat flour and honey supported the growth

of *L. fermentum* PC1 and maintained viability during storage at 4 ℃, with levels above the recommended concentration of 10^6–10^7 CFU per mL.

Table 1. Viability of *L. fermentum* PC1 and pH value in the fermentation product

Parameter	Fermentation and storage time	Viable count
Viable count (Log CFU/mL)	0 h (day 0)	7.12±0.04[a]
	24 h (day 1)	7.96±0.05[b]
	48 h (day 2)	7.28±0.03[c]
	72 h (day 3)	7.38±0.01[d]
	storage 10-day at 4 ℃ (day 13)	7.40±0.03[d]
	storage 14-day at 4 ℃ (day 17)	7.32±0.06[c]
pH	0 h (day 0)	6.26±0.02[a]
	24 h (day 1)	4.12±0.01[b]
	48 h (day 2)	4.05±0.01[c]
	72 h (day 3)	3.93±0.02[d]
	storage 10-day at 4 ℃ (day 13)	3.94±0.01[d]
	storage 14-day at 4 ℃ (day 17)	3.96±0.01[d]

Note: Results are presented as mean±SD. Values in the column with different superscript letters (a-d) are significant different ($P<0.05$).

Similarly, probiotic *L*. *casei* fermented with different oat substrates including simple and germinated oat, can give a final cell growth from 6.3 to 7.12 log CFU/mL. Different fermentation factors including the percent of oats, sugar content, inoculum size and fermentation time can influence the growth and stability of lactic acid bacteria in these products. Earlier studies showed that with 5.5% oats, 1.25% sucrose and 5% inoculum (9.34 log CFU/mL) and a short fermentation time of 8 h, a high growth of 10.4 log CFU/mL *L. plantarum* ATCC 8014 was obtained, however there was a reduction of viability of about 0.5 log CFU/mL at 14 days and 0.9 log CFU/mL at 21 days during storage at 4 °C. For the development of functional foods, not only high cell viability reached during fermentation is critical for maintaining the function of the probiotic and the desired level of acid production, but the stability of viable cells during storage is also important for maintaining the quality of the products. The results demonstrated that this oat flour and honey fermented culture has great potential for the delivery of viable *L. fermentum* PC1 cells. In particular, through a combination of a longer fermentation period and the use of honey, the complex carbohydrates in honey can be preferentially utilized by the probiotics to at least maintain their viability.

Example 2: Survival of *L. fermentum* PC1 in simulated digestive tract conditions

The tolerance to gastrointestinal conditions is important for the function of probiotic strains in the gut. There are many different food matrices that have been investigated as probiotic carriers, but only a few studies have evaluated the effect of gastrointestinal conditions on the survival capacity of probiotics. With the aim to improve the tolerance

of *L. fermentum* PC1 under gastrointestinal conditions, we investigated the survival rate of *L. fermentum* PC1 in the fermented oat product using a standardized *in vitro* method for simulating conditions in the digestive tract. The results indicate that both fermented cultures harvested at 72 h, and product stored at 4 ℃ for 14 days had significantly higher viable count and recovery rate as compare to control *L. fermentum* PC1 culture grown in MRS and resuspended in PBS (Figure 1). For the control *L. fermentum* PC1 48 h culture suspended in PBS, a viable count of 5.22±0.28 log CFU/mL was obtained after simulated saliva and gastric conditions, while the subsequent intestinal simulated conditions resulted in no detectable viable cells (<3 log CFU/mL). In contrast, for *L. fermentum* PC1 fermented oat product, viable counts of 7.57± 0.05 log CFU/mL and 7.55±0.03 log CFU/mL after exposure to simulated gastric and intestinal conditions were observed, respectively. For fermented oat product stored at 4 ℃ for 14 days, viable counts of 7.48±0.04 log CFU/mL and 7.46±0.03 log CFU/mL after exposure to simulated gastric and intestinal conditions were observed, respectively (Fig .1).

Without wanting to be bound by theory, the high buffering capacity of oat flour and honey could probably be one important factor contributing to the high survival rate of the PC1. It is believed that the protection effect depends on the probiotic strains, food matrices used and fermentation conditions. For example, the inclusion of milk has been shown to significantly improved survival of probiotic lactobacilli when exposed to simulated digestive tract conditions. This is consistent with an earlier clinical study showing that drinking milk enhanced the survival of probiotic strains and raised the pH in the stomach. Another study used legumes with *L. plantarum* 299v and obtained a recovery rate of above 80% for lentils and around 40% for mung beans. The difference in recovery rate is specific for the strains and the food matrices used. Moreover, sugar residues after fermentation could be another important factor that contributes to the survival rate noted in the present invention, since it has been shown that survival of lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars.

Example 3: Sugars and organic acids content during fermentation and storage

The compositions of sugars and organic acids are important indicators of the metabolic state of probiotics during fermentation and storage. Since lactic acid is the major end-product of carbohydrate metabolism by lactic acid bacteria, the observed decrease in glucose and fructose, and increase of lactic acid during fermentation was expected. In addition, we observed an increase in the concentration of acetic acid during fermentation (Figure 2). There was a significant decrease of glucose from 9.82 ±0.03 g/L at 0 h to 6.65±0.07 g/L at 24 h, and a further decrease to 5.61±0.07 g/L at the end of fermentation (72 h) with 5.38±0.09 g/L after 14 days of storage (Figure 2) at 4 ℃. The concentration of fructose decreased from 10.6±0.03 g/L at 0 h to 9.39±0.17 g/L after

24 h, with no significant decrease during further fermentation and storage. The concentration of lactic acid significantly increased from 0 g/L at 0 h to 2.04±0.09 g/L at 24 h, and further increased to 2.85±0.08 g/L after 72 h fermentation. There was a slight increase of lactic acid to 3.06±0.11 g/L after 14 days of storage at 4 ℃ (Figure 2). The concentration of acetic acid increased from 0 g/L at 0 h to 0.51±0.04 g/L after 24 h, increased further to 0.62±0.02 g/L by 3 days of fermentation and to 0.66±0.03 g/L after storage at 4 ℃ for 14 days (Figure 2). The observed sugar consumption and acid production were consistent with the growth of the lactobacillus and decrease of pH value. The change of sugar and acids will contribute to the flavor and taste of the final product. A substantial amount of residual glucose and fructose (>50%) in the fermented cultures was observed, even after 3 days of fermentation. Moreover, a further decrease of glucose and an increase of lactic and acetic acids were observed in the product during storage at 4 ℃, which implies there was on-going metabolic activity of the *Lactobacillus* PC1 strain. The presence of residual sugars can possibly assist in the continuous metabolic activity of the probiotics in fermented foods, and enhance the survival of lactobacilli in acidic environments. This is consistent with the stable viability of the PC1 strain during storage and in simulated gastrointestinal conditions. This observation also suggests that metabolizable sugar (such as glucose and/or fructose) could be one factor contributing to survival of the PC1 during storage and simulated gastrointestinal conditions. A concentration of about 1% to about 5% w/v was found to be acceptable.

Example 4: Antioxidant activity and total phenolic acid content during fermentation and storage

The health benefits of oats and honey have been associated with the presence of antioxidant capacity. An enhanced antioxidant capacity and related bioactive compounds such as phenolic acids after fermentation has been reported previously. In this example, 80% aqueous methanol was used to extract the methanol soluble antioxidant and phenolic components in the fermented oat product. An increased antioxidant activity was shown using the Cu^{2+} reagent-based antioxidant assay and results expressed as nmol Trolox equivalents per mg sample (nmol TE/mg) (Figure 3A). The antioxidant activity increased significantly in the fermented culture after both 24 h (63.8 ± 2.76 nmol TE/mg) and 48 h (76.4± 4.51 nmol TE/mg), compared with that measured at 0 h (57.9± 3.52 nmol TE/mg). There was no significant increase at 72 h (76.9 ± 3.49 nmol TE/mg) as compared with 48 h (76.4± 4.51 nmol TE/mg). Interestingly, there was a significant increase in antioxidant activity during storage at 4 ℃, and it reached 95.7± 8.07 nmol TE/mg after 14 days of storage (Figure 3A), which further supports the suggestion that the probiotic strain was metabolically active during the storage period.

In agreement with the increase of total antioxidant capacity, an increase in the total phenolic content was observed from 0.534±0.03 mg GAE/g at 0 h to 0.628±0.01 mg GAE/g after 72 h fermentation (Figure 3B). The total phenolic content continued to increase to 0.678±0.01 mg GAE/g during storage for 14 days at 4 °C (Figure 3B) as did the total antioxidant activity. Phenolic compounds are one of the most important natural antioxidants in oats and honey, consequently the observed increase of both total phenolic content and total antioxidant capacity beneficial for human consumption. Pearson's correlation coefficient (r) between antioxidant activity and total phenolic content was 0.88. The strong positive correlation suggests that phenolic acid components present in the methanol extracts have a major contribution to the antioxidant activity of the fermented oat product. Similarly, solid-state fermentations of oats with fungi or other lactic acid bacteria have been shown to improve the phenolic composition and antioxidant activity of oats. For example, improved bioavailability of phenolic acids in barley and oats was observed during fermentation with 8 probiotic strains. The improvement of phenolic acids can be strain dependent, with a reported dramatic increase of free phenolic acids (more than 25 folds) for three probiotic strains, *L. acidophilus, L. johnsonii* and *L. reuteri*. An 83% increase of total phenolic content was observed in another solid-state yeast fermentation study. Other probiotic fermentation studies show that increased antioxidant activity of plant based food is due to an increased release or synthesis of antioxidant compounds during fermentation. The possible enzymes, such as glycoside hydrolase, cellulose, esterase, β-glucosidases, produced by strains during fermentation can enhance the availability of phenolics and other antioxidant compounds. During fermentation, these enzymes could potentially release esterified and insoluble-bound phenols in a time-dependent manner, because the enzyme production is dependent on fermentation time. The present data indicates that the increased total phenolic content and antioxidant activity were dependent on fermentation time. Parameters including fermentation time can be optimized to enhance the total antioxidant capacity and total phenolic content. These results support the finding that fermentation is an effective way to enhance the total antioxidant capacity of the probiotic product.

Example 5: Changes in phenolic composition during fermentation

Significantly enhanced total and specific phenolic compounds in oats during solid state fermentation have been reported in several studies, however, there is limited studies of the improvement of specific phenolic compounds in liquid state fermentation. To assess the effect of *L. fermentum* PC1 fermentation on the bioavailability of specific phenolic acids in the fermented oat product, the concentrations of 10 phenolic acids in the methanol soluble extract during fermentation and storage were analyzed by HPLC. As shown in Table 2, the phenolic composition was influenced by fermentation. Of the 10

identified phenolic compounds, the levels of gallic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid and sinapic acid increased during fermentation, with the highest relative increase occurring after 72 h fermentation (gallic acid +58.65%, catechin +92.35%, vanillic acid +67.17%, caffeic acid +117.08, p-coumaric acid +197.87, ferulic acid +116.35, sinapic acid +49.20%). There were no significant changes in the concentration of 4-hydroxybenzoic acid, chlorogenic acid and quercetin during fermentation. There were significant decreases in the concentration of 4-hydroxybenzoic acid (-10.37%), vanillic acid (-55.02%), caffeic acid (-21.41%), p-coumaric acid (-52.81%), ferulic acid (-9.02%) during storage for 14 days at 4 °C, but not below the initial value noted for caffeic acid, p-coumaric acid and ferulic acid in the control at 0 h. Since the production and activity of possible enzymes involved in the liberation of phenolic compounds is dependent on fermentation time, the changes of phenolic acid production can be explained by the changes of enzyme production and stability. Moreover, the modulation of phenolic content is highly depended on the microorganisms used, since the enzymes responsible for the metabolism of phenolic compounds may be only express in specific strains. The improvement of phenolic acids (caffeic, p-coumaric, ferulic and sinapic acids) can vary between different probiotic strains, and changes can be associated with the different amounts of bacterial feruloyl esterase in different strains. The decrease of phenolic acids during storage can be related to the decline of available nutrient and accumulation of waste compounds in the product. The observed phenolic compounds in this example are mostly in line with other reports, but the concentrations of some phenolic acids differ from other findings. For example, ferulic acid was reported as the major component in several studies, but the concentration of ferulic acid detected here was lower than the level reported in other studies. Since the phenolic composition varies between different oat products and extraction methods, this could be due to different oat flour and extraction method used. In addition, the added honey also contributed to the phenolic composition of our product. Overall, it is demonstrated that the fermented samples had higher concentrations of several individual phenolic acids as compared with the 0h non-fermented control.

Table 2. Phenolic compounds analysis during fermentation and storage

Phenolic compound	0h	24h	48h	72h	Storage 10-day	Storage 14-day
Gallic acid	2.93±0.08^{a}	3.28±0.12^{b}	3.62±0.06^{c}	4.64±0.24^{d}	4.81±0.24^{d}	4.76±0.35^{d}
4-Hydroxybenzoic acid	0.94±0.08^{a}	0.91±0.06^{a}	0.88±0.08^{a}	0.90±0.03^{a}	0.83±0.05^{b}	0.81±0.05^{b}
Chlorogenic acid	1.52±0.23^{a}	1.49±0.07^{a}	1.57±0.10^{a}	1.55±0.11^{a}	1.54±0.02^{a}	1.53±0.07^{a}
Catechin	1.27±0.30^{a}	1.93±0.27^{b}	2.22±0.11^{b}	2.43±0.23^{c}	2.37±0.22^{c}	2.43±0.23^{c}

Vanillic acid	0.34±0.01[a]	0.38±0.07[a]	0.47±0.05[b]	0.56±0.04[c]	0.48±0.13[b]	0.25±0.06[d]
Caffeic acid	0.39±0.19[a]	0.65±0.01[b]	0.73±0.04[c]	0.85±0.06[d]	0.68±0.02[e]	0.67±0.02[e]
p-Coumaric acid	0.45±0.05[a]	0.47±0.02[a]	1.24±0.26[b]	1.33±0.16[b]	0.82±0.16[c]	0.63±0.07[d]
Ferulic acid	0.32±0.20[a]	0.60±0.01[b]	0.58±0.05[b]	0.70±0.08[c]	0.61±0.07[c]	0.64±0.11[c]
Sinapic acid	1.42±0.34[a]	1.56±0.13[a]	1.82±0.29[a]	2.12±0.28[b]	2.15±0.19[b]	2.14±0.20[b]
Quercetin	5.08±0.15[a]	5.25±0.11[a]	5.37±0.09[a]	5.47±0.18[a]	5.43±0.19[a]	5.45±0.17[a]

Note: results are expressed as mean±SD µg/g, Values in the same row followed by different superscript letters (a–e) indicate significant differences ($p < 0.05$) between days of fermentation and storage.

Example 6: Content of β-glucan during fermentation

Another main bioactive component in oats, β-glucan, has cholesterol-lowering effects at dietary intake levels of at least 3 g per day, and may reduce the risk of cardiovascular disease. It has been reported that there was a loss of β-glucan during harsh processing, such as excessive heat and shearing (Zhu et al. 2016). Therefore, it was of interest in the present study to follow the β-glucan levels during the fermentation and storage to ensure the β-glucan was not lost. There was no change in the β-glucan content during the first 24 h of fermentation with 2.54±0.09 g/100 g DW as compared to 0 h (2.54±0.185 g/100 g DW). There was a slight decrease ($P>0.05$) to 2.36±0.135 g/100 g DW after 3 days of fermentation, and this level was maintained at 2.26± 0.270 g/100 g DW during storage at 4 ℃ for 14 days (Figure 4). These results are in agreement with another study that investigated the effect of fermentation on β-glucans in oat sourdough (Lu et al. 2019). These workers reported that the total β-glucan content decreased slightly during fermentation and was stabilized when the lactic acid bacteria counts were almost stable. β-glucan is selectively utilized by bifidobacteria and lactobacilli in the gut (Jaskari, Kontula, Siitonen, Jousimies-Somer, Mattila-Sandholm and Poutanen 1998), and thereby produce short chain fatty acids (SCFA) which are linked to beneficial physiological effects (Simpson and Campbell 2015). Consequently, it is important to retain stable levels of the β-glucan to ensure the fermented oat product has the capacity to support growth of potentially beneficial bacteria and the production of SCFAs.

Example 7: Compositions of grain flour and sugar

Table 3. Compositions

S/N	**10g grain (10% w/v)**	**3g carbon source (3%w/v)**	**Strain used**	**Volume**
1	Whole Grain (WG) Oat flour	Glucose	*L. amylovorus*	100ml

2	WG Oat flour	Glucose	*L. rhamnosus*	100ml
3	WG Oat flour	Glucose	*L. paracasei* PCB 003	100ml
4	WG Oat flour	Glucose	LAB from Kefir (MK1_105_003)	100ml
5	WG Oat flour	Glucose	*S. cerevisiae*	100ml
6	WG Oat flour	Glucose	*S. boulardii*	100ml
7*	WG Oat flour	Glucose	*L. fermentum* PC1	100ml
8*	WG Oat flour	Sardinia Honey	*L. fermentum* PC1	100ml
9	Brown rice flour	Glucose	*L. fermentum* PC1	100ml
10	Brown rice flour	Sardinia Honey	*L. fermentum* PC1	100ml
11	WG Oat + vitamin mix (1.5g)	Sardinia Honey	*L. fermentum* PC1	100ml
12	WG Oat + sugarcane fibre (1.5g)	Sardinia Honey	*L. fermentum* PC1	100ml
13	Buckwheat flour	Sardinia Honey	*L. fermentum* PC1	100ml

* denotes heat treatment (100 °C for 10 min) after day 3 of fermentation

As shown in Figure 5, the pH of fermented mixture decreased over time from D0 (start of fermentation) to D3 (end of fermentation). The pH of these fermented mixture were maintained at D17 (after 14 days of storage) since D3. Condition 11 had an initial starting pH of 4.27 and did not decrease drastically over time from D0 to D3. This could be due to the addition of vitamin pre-mix into the mixture.

As shown in Figure 6, the levels of viable lactobacilli generally increased over time from D0 (start of fermentation) to D3 (end of fermentation) and these levels were relatively maintained at D17 (after 14 days of storage). This observation coincides with the decrease in pH over time and the correlation between low pH and the survival of lactobacilli could be deduced. However, this observation was not represented in conditions 1, 9 and 11. Despite the low pH which could aid the survival and growth of the respective lactobacilli, it could be that these specific lactobacilli will require a more specific and unique environment to grow in, one that could not be provided for by the fermented mixture conditions. As for conditions 7 and 8, no viable lactobacilli counts were observed at D17 as the fermented mixtures were heat-treated after D3.

Figure 7 shows that the levels of viable yeast cells increased from D0 (start of fermentation) to D3 (end of fermentation) and maintained at D17 (after 14 days of storage).

Figure 8 shows Beta-glucan content generally increase over time from D0 (start of fermentation) to D3 (end of fermentation) and maintained at D17 (after 14 days of storage). It has been established that beta-glucan is selectively utilized by bifidobacteria and lactobacilli in the gut, and thereby produce short chain fatty acids which are linked to beneficial physiological effects to the host. Interestingly, conditions 9, 10 and 13 had close to zero beta-glucan content over time from D0 to D17. Conditions 9 and 10 used brown rice flour, while condition 13 used buckwheat flour instead of wholegrain oats. It could be possible that these sources of flour had undergone harsh processing treatments such as excessive heat and shearing, resulting in the loss of beta-glucan.

Figure 9A-C shows the resultant total antioxidant content of these compositions at day 0, at day 3 (after fermentation) and at day 17 (after 14 days storage).

Figure 9A shows the total antioxidant activity from the insoluble fractions, reported in nmol Trolox equivalents per ul of sample (nmol/ul), generally increased over time from D0 (start of fermentation) to D3 (end of fermentation) and maintained at D17 (after 14 days of storage). However, conditions 12 and 13 showed a decrease in total antioxidant activity from the insoluble fractions over time. Figure 9B shows the total antioxidant activity from the soluble fractions, reported in nmol Trolox equivalents per ul of sample (nmol/ul), generally decreased over time from D0 (start of fermentation) to D3 (end of fermentation) and to D17 (after 14 days of storage). However, conditions 11 and 13 showed an increase in total antioxidant activity from the soluble fractions over time. Figure 9C shows the total antioxidant activity reported in nmol Trolox equivalents per ul of sample (nmol/ul) were generally maintained across most conditions over time from D0 (start of fermentation) to D3 (end of fermentation) and to D17 (after 14 days of storage). However, conditions 11 and 13 showed an increase in total antioxidant activity over time. The increase in total antioxidant activity is due to the increase in antioxidant activity from the soluble fraction, rather than from the insoluble fractions.

Figure 10A-C shows the resultant total phenolic content of compositions in Table 2 at day 0, at day 3 and at day 17.

In agreement with the antioxidant activity results, Figure 10A shows the total phenolic content from the insoluble fractions, reported in mg Gallic Acid Equivalents per g of sample (mg GAE/g), generally increased over time from D0 (start of fermentation) to D3 (end of fermentation) and maintained at D17 (after 14 days of storage). However, conditions 12 and 13 showed a decrease in total phenolic content from the insoluble fractions over time. Similarly, Figure 10B shows the total phenolic content from the

soluble fractions, reported in mg Gallic Acid Equivalents per g of sample (mg GAE/g), generally decreased over time from D0 (start of fermentation) to D3 (end of fermentation) and to D17 (after 14 days of storage). However, conditions 11 and 13 showed an increase in total phenolic content from the soluble fractions over time. Figure 10C shows the total phenolic content (soluble and insoluble), reported in mg Gallic Acid Equivalents per g of sample (mg GAE/g), were generally maintained across most conditions over time from D0 (start of fermentation) to D3 (end of fermentation) and to D17 (after 14 days of storage). The decrease of phenolic acids during storage as seen in condition 12 could be related to the decline of available nutrient and accumulation of waste compounds in the product. Conditions 11 and 13 showed an increase total phenolic content over time. The increase total phenolic content is due to the increase in phenolic content from the soluble fraction, rather than from the insoluble fractions.

Several probiotic fermentation studies have shown that the increase of phenolic content and antioxidant activity of fermented plant-based food was due to an increased release or synthesis of antioxidant compounds during fermentation. The possible enzymes, such as glycoside hydrolase, cellulose, esterase, β-glucosidases, produced by strains during fermentation could enhance the availability of phenolics and other antioxidant compounds. Therefore, this could potentially explain the increase in phenolic content and antioxidant activity in condition 11 and 13.

It should also be noted that improvement of phenolic content was strain dependent.

Further, with heat treatment (S/N 7 and 8), no detrimental effect on the beta glucan (Figure 8), antioxidant (Figure 9A-C) or phenolic content (Figure 10A-C) was observed. This points towards a viable room temperature, stable nutritional product since after heat treatment is will kill the probiotic but the bioactive content will not be damaged. This allows for a distribution chain that doesn't require cold storage.

Example 8: Evaluation of fresh and freeze dried fermented oats beverage on the intestinal microbiome of the elderly using honey and other carbohydrate sources added to the oats prior to fermentation

Parameters tested for the fermentation:

S/N	**Oat**	**3g carbon source in 87 ml ddH_20 (3% w/v)**	**Total volume**
1	3g glucan rich Oat (3%)	Sardinia Honey	100 ml
2	10g Whole grain (WG) Oat (10%)	Sardinia Honey	100 ml

3	10g WG Oat (10%)	Blended Australian eucalyptus Honey	100 ml
4	10g WG Oat (10%)	Molasses	100 ml
5	10g WG Oat (10%)	Glucose	100 ml
6	10g WG Oat (10%)	Trehalose	100 ml
7	10g WG Oat (10%)	Inulin	100 ml

These compositions were inoculated with *L. fermentum* PC1. Part of the sample is evaluated fresh while the other part is freeze dried and evaluated. The results are shown in Figure 11 to 15, and are compared against a positive control having only the fecal sample with no added carbon source such as honey for sugar and no added oat.

For comparison, Figure 16 shows the counts at the start of the microcosm in fermented compositions to show the impact on the gut microbes at 0h. The fermented compositions are fermented for 3 days. It can be seen that the impact of various carbohydrates on the probiotic is similar to the Sardinian honey.

The impact of the fermented oats on the gut microbiome was evaluated and shown to have a beneficial impact with a decrease in potentially harmful enteric Gram negative bacteria and an elevation in Gram positive potentially beneficial lactobacilli. There was also an increase in the potentially beneficial short chain fatty acids such as acetic, propionic and butyric acid. These findings demonstrate that both freshly fermented oats as well as the freeze dried material are beneficial. This extends the applications of the material to a powder in a sachet to be reconstituted prior to use and hence a greater shelf life. Also the benefits were noted for the two types of honeys as well as the molasses, glucose, trehalose and inulin. Trehalose in the freeze dried material was found to have enhanced benefits.

The results show that various carbohydrates can be used. The fermented oats not only enhances survival of the probiotic in the fermented culture but also has a benefit on the gut microbiome. Freeze dried material also has the beneficial impact on the gut microbiome.

<u>Conclusion</u>

In summary, it is shown that oat flour with added honey promoted the growth and maintained the survival of *L. fermentum* PC1 both during fermentation and storage. The viable count of PC1 was stable (7.32 log CFU/mL) during storage at 4 ℃ for at least 14 days. The survival of the PC1 in the fermented oat exposed to simulated gastrointestinal

conditions was significantly enhanced compared to control cells. Furthermore, it was apparent that the PC1 was metabolically active during storage at 4 ℃ for at least 14 days since the content of sugars and acid production continued to change. Moreover, there were improvements in antioxidant capacity and phenolic content, and no significant decrease of β-glucan. The main phenolic components that were detected in higher amounts in the methanol extracts were gallic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid and sinapic acid. Thus, it is demonstrated that fermentation of oat flour with added honey and *L. fermentum* PC1 could be a potentially valuable probiotic food with both improved levels of probiotic and bioactive components.

It will be appreciated that many further modifications and permutations of various aspects of the described embodiments are possible. Accordingly, the described aspects are intended to embrace all such alterations, modifications, and variations that fall within the spirit and scope of 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 integers or steps.

Throughout this specification and the claims which follow, unless the context requires otherwise, the phrase "consisting essentially of", and variations such as "consists essentially of" will be understood to indicate that the recited element(s) is/are essential i.e. necessary elements of the invention. The phrase allows for the presence of other non-recited elements which do not materially affect the characteristics of the invention but excludes additional unspecified elements which would affect the basic and novel characteristics of the method defined.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Claims

1. A fermentation process, comprising:

a) mixing a grain flour and a sugar in an aqueous medium to form a mixture;

b) inoculating a probiotic microorganism selected from probiotic bacterium and/or probiotic yeast in the mixture to form a culture; and

c) fermenting the culture to form a fermented culture;

wherein the grain flour is 5 % w/v to 30 % w/v of the mixture;

wherein the sugar is metabolisable by the probiotic microorganism;

wherein the sugar is 1 % w/v to 20% w/v of the mixture; and

wherein the probiotic microorganism is inoculated at about 10^5 CFU per mL to less than about 10^8 CFU per mL of the culture.

2. The fermentation process according to claim 1, wherein the fermentation step is performed at 25 °C to 50 °C.

3. The fermentation process according to claim 1 or 2, wherein the fermentation step is performed under stirring of 100 rpm to 800 rpm.

4. The fermentation process according to any one of claims 1 to 3, wherein the fermentation step is performed for at least 72 h.

5. The fermentation process according to any one of claims 1 to 4, wherein the grain flour is selected from teff flour, wheat flour, oat flour, rice flour, corn flour, barley flour, sorghum flour, rye flour, millet flour, triticale flour, amaranth flour, buckwheat flour, quinoa flour or a combination thereof.

6. The fermentation process according to any one of claims 1 to 4, wherein the mixture further comprises sugar cane fibre/bagasse.

7. The fermentation process according to any one of claims 1 to 6, wherein the grain flour is oat flour, wherein the oat flour is derived from non-germinated oat, germinated oat or a mixture thereof.

8. The fermentation process according to any one of claims 1 to 7, wherein the sugar is selected from a monosaccharide, disaccharide, oligosaccharide, polysaccharide, or a combination thereof.

9. The fermentation process according to any one of claims 1 to 8, wherein the sugar is derived from an animal source or a plant source.

10. The fermentation process according to any one of claims 1 to 9, wherein the sugar is selected from glucose, fructose, trehalose, inulin, molasses, honey, or a combination thereof.

11. The fermentation process according to any one of claims 1 to 10, wherein the sugar in the fermented culture is less than 50% relative to the mixture.

12. The fermentation process according to any one of claims 1 to 11, wherein the probiotic bacterium is 0.2 % v/v to 2% v/v of the culture.

13. The fermentation process according to any one of claims 1 to 12, wherein the probiotic bacterium is inoculated at about 10^7 CFU per mL of the culture.

14. The fermentation process according to any one of claims 1 to 13, wherein the probiotic bacterium and/or yeast is selected from *L. fermentum, L. bulgaricus, L. acidophilus, L. brevis, L. plantarum, L. rhamnosus, L. caucasicus, L helveticus, L. lactis, L. reuteri and L. casei. B. infantis, B. bifidum, S. thermophiles, E. faecium*, *L. amylovorus, L. paracasei, S. cerevisiae, S. boulardii,* or a combination thereof.

15. The fermentation process according to any one of claims 1 to 14, wherein the probiotic bacterium is *L. fermentum.*

16. The fermentation process according to any one of claims 1 to 15, wherein a pH of the fermented culture after at least 24 h is about 4.

17. The fermentation process according to any one of claims 1 to 16, further comprising a step of pasteurising the grain flour, sugar, or the mixture thereof.

18. The fermentation process according to any one of claims 1 to 17, further comprising a step of storing the fermented culture.

19. The fermentation process according to claim 18, wherein the fermented culture is stored at about 4 °C.

20. The fermentation process according to claim 18 or 19, wherein the fermented culture is stored for at least 10 days.

21. The fermentation process according to any one of claims 1 to 20, further comprising a step of freeze drying the fermented culture.

22. The fermentation process according to any one of claims 1 to 21, wherein a viable count of probiotic bacterium in the culture after inoculating is 6 log CFU/mL to 7 log CFU/mL.

23. The fermentation process according to any one of claims 1 to 22, wherein a viable count of probiotic bacterium in the culture after fermenting is 7 log CFU/mL to 8 log CFU/mL.

24. The fermentation process according to any one of claims 1 to 23, wherein a viable count of probiotic bacterium in the fermented culture after storing is 7 log CFU/mL to 8 log CFU/mL.

25. The fermentation process according to any one of claims 1 to 24, wherein the fermented culture has a β-glucan content which is substantially not altered when compared to the mixture.

26. The fermentation process according to any one of claims 1 to 25, wherein the fermented culture comprises glucose of 5 g/L to 7 g/L, fructose of 9 g/L to 10 g/L, lactic acid of 2 g/L to 4 g/L, and acetic acid of 0.6 g/L to 0.7 g/L.