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(71) Applicant: DSM IP ASSETS B.V. [NL/NL]; Het Overloon 1, 6411 TE HEERLEN (NL).

(72) Inventors: FERRIER, Laurent Louis Maurice; Chemin du Pré-au-Seigneur 7, 1066 EPALINGES (FR). BERGONZELLI DEGONDA, Gabriela Elena; Chemin de Grandvignes 44, 1030 BUSSIGNY (CH). JANKOVIC, Ivana; Chemin de la Maraîche 26, 1802 CORSEAUX (CH).

(74) Agent: DSM INTELLECTUAL PROPERTY; P.O. Box 4, 6100 AA ECHT (NL).

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(54) Title: NUTRITIONAL COMPOSITION COMPRISING GOS AND HMOs

(57) Abstract: The present invention relates to a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs), or compositions comprising the combination, for use in treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL). The present invention also relates to the use of a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs), or compositions comprising the combination, for treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).



Nutritional composition comprising GOS and HMOs

FIELD OF THE INVENTION

The present invention relates to a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs), or compositions comprising the combination, use in treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL). The present invention also relates to the use of a combination of GOS and a mixture of HMOs, or compositions comprising the combination, for treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-FL and DFL or of LNT and 6'-SL.

BACKGROUND OF THE INVENTION

Anxiety disorders are a group of mental disorders characterised by feelings of anxiety and fear (*Diagnostic and Statistical Manual of Mental Disorders*, American Psychiatric Association, 5th ed., 2013). Anxiety often occurs with other mental disorders, particularly mood or depressive disorders. Medication options are benzodiazepines which are used for the short-term relief of severe anxiety, antidepressants, and beta-blockers to reduce some of physical symptoms, such as rapid heartbeats and shaking.

Mood or depressive disorders are a group of disorders involving primary disturbances of mood. These include depression, major depressive disorder (MDD or clinical depression), dysthymia, and bipolar disorder. Patients suffering from depression exhibit feelings of sadness, low mood and an aversion to activity and this mood can affect a person's thoughts, behaviour, feelings and sense of well-being. A depressed person may feel sad, anxious, empty, hopeless, worried, helpless, worthless, guilty, irritable, hurt or restless.

Major depressive disorder (MDD) is a disabling, severe mental disorder characterised by episodes of all-encompassing low mood, low self-esteem and loss of interest or pleasure in normally enjoyable activities. The illness tends to be chronic and repeated episodes are common. Other symptoms of MDD may include irritability or frustration, sleep disturbances, tiredness and lack of energy, changes in appetite, anxiety, agitation, restlessness, feelings of worthlessness or guilt, trouble thinking and concentrating, and unexplained physical problems, such as back pain or headaches. The disorder is a significant contributor to the global burden of disease and affects people in all communities across the world (Ferrari et al. *PLoS Med.* **10**, e1001547 (2013)). MDD is a highly prevalent psychiatric disorder with twin studies revealing

that up to 40 % of MDD cases are genetically determined (Kendler et al. *Psychol. Med.* **36**, 955, (2006)).

Although the exact causes of mood disorders are unknown, it is believed that a variety of factors may be involved, such as brain chemistry and physical brain differences, hormones, inherited traits and life events. This lack of knowledge of causation has made treatment difficult.

Many types of antidepressant medications are available to treat mood disorders that present with depression. Some available drugs include selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), norepinephrine and dopamine reuptake inhibitors (NDRIs), tricyclic antidepressants, monoamine oxidase inhibitors (MAOIs), and atypical antidepressants such as vortioxetine. However, despite the availability of numerous treatment options, individual response to antidepressant medication is suboptimal and variable. That is, not all individuals respond equally to a given antidepressant and some do not respond at all. As many as one half of patients do not receive adequate treatment and many respond partially or not at all to treatment. The presence of residual symptoms is also associated with a higher risk of recurrence, more chronic depressive episodes and a shorter duration between episodes. Guidelines for treatment recommend four possible strategies for managing non-response or partial response including: increasing the dose of the antidepressant drug; replacing the drug with a different antidepressant drug; augmenting the antidepressant therapy with a non-antidepressant agent; or combining the initial antidepressant with a second antidepressant.

Despite the lack of general efficacy, all of the available drugs have side effects with many having serious side effects. Therefore safer, nutrition-based options have also been investigated. One potential approach is the role of folate in central nervous system function because folate is a vitamin. Evidence suggests that folate can reduce depressive moods in certain patients; at least comparable to that of tricyclic antidepressants. Folate also appears to influence the rate of synthesis of tetrahydrobiopterin, a cofactor in the hydroxylation of phenylalanine and tryptophan, rate-limiting steps in the biosynthesis of dopamine, norepinephrine, and serotonin, neurotransmitters postulated to play a role in the pathogenesis of depression. In addition, methyltetrahydrofolate (MTHF) has been shown to bind to presynaptic glutamate receptors, where it may potentially modulate the release of other neurotransmitters, including the monoamines. However, folate appears to be best applicable to patients with certain genetic profiles (WO 2014/164882) and is probably not a solution for all patients.

Increasing evidence suggests that the intestinal microbiota also plays a key role in the generation of psychiatric disorders (Savignac et al. *Neurogastroenterol. Motil.* **26**, 1615 (2014)). The intestinal microbiota consists of a vast bacterial community that resides primarily in the

colon and lives in a symbiotic relationship with the host. The human gastrointestinal microbiota includes at least 1000 different species of bacteria, which collectively make up to 10^{14} bacterial cells, tenfold the number of human cells, and they encode 100-fold more unique genes than the human genome (Qin et al. *Nature* **464**, 59 (2010)). A bidirectional neurohumoral communication system, known as the gut-brain axis, integrates the host gut and brain activities.

This has led researchers to attempt using probiotics as a treatment option. Although data are limited, *Lactobacillus* and *Bifidobacterium* species have been shown to display potential therapeutic properties in psychiatric disorders (Dinan et al. *Biol. Psychiatry* **74**, 720 (2013)).

Some prebiotics may be another treatment option. 3'-SL and 6'-SL support normal behavioural responses in mice during stressor exposure, potentially through effects on the gut microbiota-brain axis (Tarr et al. *Brain Behav. Immun.* **50**, 166 (2015)). WO 2017/071715 and WO 2017/071716 disclose synthetic compositions for use in treating or improving an emotion and/or mood disorder in a patient characterised in that the compositions contain an effective amount of one or more neutral HMOs.

Gamma-aminobutyric acid (GABA) is the chief inhibitory neurotransmitter. It is present in the central nervous system (CNS), but is also present in high amounts in foods like crustaceans, plants and is naturally produced by certain bacteria. Therefore, GABA is naturally present in the gut.

Several gut bacteria have been shown to synthesize GABA (Wiley et al. *Mod. Trends Psychiatry* **32**, (2021)). There is clinical and preclinical evidence of the effect of exogenous GABA in relieving stress and promoting the sensation of feeling relaxed (Abdou et al. *BioFactors* **26**, 201 (2006)). Probiotics producing GABA have been shown to alleviate gut discomfort triggered by stress in preclinical studies (Pokusaeva et al. *Neurogastroenterol. Motil.* **29**, e12904 (2016)).

There is no nutritional solution shown or available to naturally increase the synthesis of GABA by the microbiota of a human over 7 years of age, in particular in treating and/or preventing stress and/or a mood disorder, for example in maintaining or restoring the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels. The present invention satisfies this need.

SUMMARY OF THE INVENTION

The present inventors surprisingly found that a combination of GOS and a mixture of HMOs can advantageously be used in treating and/or preventing stress and/or a mood disorder because the combination showed increased biosynthesis of GABA by the gut microbiota. The present inventors have found that these specific combinations are particularly effective in increasing biosynthesis of gamma-aminobutyric acid (GABA) by the gut. Thus, the invention

provides a way to increase the production of a metabolite (i.e. GABA) with known benefits on stress relief and relaxation. The mixture of HMOs consists or consists essentially of either 2'-FL and DFL or of LNT and 6'-SL. The impact of a combination of GOS and a mixture of HMOs on the biosynthesis of GABA by microbiota was not previously known. The combination is particularly effective in the restoration of the sensitivity of the bi-directional transmission pathways in the gut-brain axis to normal levels.

In the experiment at the basis of the present invention, a combination of GOS and either 2'-FL and DFL or LNT and 6'-SL resulted in increased biosynthesis of GABA by microbiota. Thus, the experiment indicates the role played by the combination in promoting the biosynthesis of GABA.

Thus, in a first aspect, the present invention provides a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs) for use in treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL). Suitably, the combination treats stress and/or a mood disorder by increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of the human over 7 years of age.

In another aspect, the invention provides the same combination as above for use in maintaining or restoring the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels in a human over 7 years of age.

In another aspect, the invention provides the same combination as above for use in increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of a human over 7 years of age.

In another aspect, the present invention provides a synthetic composition comprising a combination of GOS and a mixture of HMOs for use in treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-FL and DFL or of LNT and 6'-SL. Suitably, the composition treats stress and/or a mood disorder by increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of the human over 7 years of age. The synthetic composition is preferably a nutritional composition.

In another aspect, the invention provides the above mentioned synthetic or nutritional composition for use in maintaining or restoring the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels in a human over 7 years of age.

In another aspect, the invention provides the above mentioned synthetic or nutritional composition for use in increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of a human over 7 years of age.

In a further aspect, the present invention provides a method for treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, the method comprising administering to a human over 7 years of age a combination or a synthetic or nutritional composition mentioned above.

In a further aspect, the present invention provides a method for maintaining or restoring the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels in a human over 7 years of age, the method comprising administering to a human over 7 years of age a combination or a synthetic or nutritional composition mentioned above.

In a further aspect, the present invention provides a method for increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of a human over 7 years of age, the method comprising administering to a human over 7 years of age a combination or a synthetic or nutritional composition mentioned above.

In a further aspect, the present invention provides the use of a combination or a synthetic or nutritional composition mentioned above for treating and/or preventing stress and/or a mood disorder in a human over 7 years of age. Suitably, the combination treats stress and/or a mood disorder by increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of the human over 7 years of age.

In another aspect, the invention provides the use of a combination or a synthetic or nutritional composition mentioned above for maintaining or restoring the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels in a human over 7 years of age.

In another aspect, the invention provides the use of a combination or a synthetic or nutritional composition mentioned above for increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of a human over 7 years of age.

In another aspect, the invention provides a combination consisting of or consisting essentially of:

- galacto-oligosaccharides (GOS) and
- a mixture of human milk oligosaccharides (HMOs), wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).

In this regard, the combination of the present invention consists of or consists essentially of GOS, 2'-FL and DFL or GOS, LNT and 6'-SL.

In another aspect, the invention provides a synthetic composition comprising a combination that consists of or consists essentially of:

- 5 - galacto-oligosaccharides (GOS) and
- a mixture of human milk oligosaccharides (HMOs), wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).

10 The synthetic composition preferably does not contain prebiotics other than GOS and the HMOs mentioned above, and may contain other nutritionally acceptable ingredients that are necessary or suitable to provide a nutritional benefit to the consumer.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Relative abundance of GABA, expressed as peak area, after 24 hours of fermentation by toddler's microbiota of digested milk matrix supplemented by GOS, 2'-FL/DFL, LNT/6'-SL, GOS+2'-FL/DFL or GOS+LNT/6'-SL. Dots represent individual values, line is at the median, box are 25/75 percentiles and whiskers at min/max range. Different letters denote significant differences.

Figure 2: Relative abundance of GABA, expressed as the base-2 logarithm of the ratio between the value of GABA after 24 hours of fermentation by toddler's microbiota of digested milk matrix supplemented by GOS, 2'-FL/DFL, LNT/6'-SL, GOS+2'-FL/DFL or GOS+LNT/6'-SL, and the value of GABA obtained after fermentation of the digested milk matrix alone. Dots represent individual values, line is at the median, box are 25/75 percentiles and whiskers at min/max range. Different letters denote significant differences.

DETAILED DESCRIPTION OF THE INVENTION

25 The term "anxiety" refers to a feeling of unease, such as worry, nervousness, or fear, that can be mild or severe, particularly about something which is about to happen or may happen in the future.

30 The term "anxiety disorder" refers to a condition of intense, excessive and persistent worry and fear about everyday situations. The symptoms can interfere with daily activities such as schoolwork and relationships. There are several types of anxiety disorders, including generalized anxiety disorder, separation anxiety disorder, panic disorder, social anxiety disorder, and various phobia-related disorders.

The expression “baby food” means a foodstuff intended for particular nutritional use by infants or young children aged between one and less than three years of age during the first years of life.

The term “bipolar disorder” means a condition that causes extreme mood swings that include emotional highs (mania) and lows (depression). Mania can be characterised by euphoria, hyperactivity, over inflated ego and unrealistic optimism.

The term “depression” (also known as major depressive disorder) refers to a condition of persistently low mood (feeling sad, irritable, empty or loss of pleasure/interest). The symptoms can interfere with daily activities such as schoolwork and relationships.

“Dietary supplement” preferably means a nutritional composition that is intended to add to or supplement the diet and is different from conventional food. A dietary supplement can help to improve or maintain the overall health of the consumer, and can also help to meet the daily requirements of essential nutrients.

The term “effective amount” preferably means an amount of the combination or composition that provides the combination of GOS and the mixture of HMOs in a sufficient amount to render a desired treatment or prevention outcome in a subject. An effective amount can be administered in one or more doses to the subject to achieve the desired treatment or prevention outcome.

A “follow-up formula” or “follow-on formula” is given from the 6th month onwards to an infant. It constitutes the principal liquid element in the progressively diversified diet of this category of person.

The term “fortifier” refers to liquid or solid nutritional compositions suitable for fortifying or mixing with human milk, infant formula, growing-up milk or human breast milk fortified with other nutrients. Accordingly, the fortifier can be administered after dissolution in human breast milk, in infant formula, in growing-up milk or in human breast milk fortified with other nutrients or otherwise it can be administered as a stand-alone composition.

“Functional food” preferably means a food that offer health benefits beyond its nutritional value. Functional foods are also known as nutraceuticals.

The term “galacto-oligosaccharides” refers to a type of non-digestible fibre with prebiotic activity. GOS are formed via enzymatic (β -galactosidase) conversion of lactose. GOS generally comprise a chain of galactose units that arise through consecutive transgalactosylation reactions, with a terminal glucose unit, although a terminal galactose unit may be present instead. The degree of polymerization of GOS typically ranges from 2 to 8 monomeric units. The composition of the galacto-oligosaccharide fraction varies in chain length and type of linkage between the monomer units

The expression "growing-up milk" (or GUM) refers to a milk-based drink generally with added vitamins and minerals, that is intended for young children aged between one and less than three years of age or children between three and seven years of age.

The term "HMO" or "HMOs" refers to human milk oligosaccharide(s). These carbohydrates are highly resistant to enzymatic hydrolysis, indicating that they may display essential functions not directly related to their caloric value. It has especially been illustrated that they play a vital role in the early development of infants and young children, such as the maturation of the immune system. Many different kinds of HMOs are found in the human milk. Each individual oligosaccharide is based on a combination of glucose, galactose, sialic acid (N-acetylneuraminic acid), fucose and/or N-acetylglucosamine with many and varied linkages between them, thus accounting for the enormous number of different oligosaccharides in human milk - over 130 such structures have been identified so far. Almost all of them have a lactose moiety at their reducing end while sialic acid and/or fucose (when present) occupy terminal positions at the non-reducing ends. The HMOs can be acidic (e.g. charged sialic acid containing oligosaccharide) or neutral.

"Increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of a human over 7 years of age" means enhancing the biosynthesis of GABA by the gut microbiota of a human over 7 years of age who has been administered the combination or nutritional composition of the invention compared to the biosynthesis of GABA by the gut microbiota of a human over 7 years of age who has not been administered the combination or nutritional composition of the invention.

The expression "infant cereal composition" means a foodstuff intended for particular nutritional use by infants or young children aged between one and less than three years of age during the first years of life.

The expression "infant formula" as used herein refers to a foodstuff intended for particular nutritional use by infants during the first months of life and satisfying by itself the nutritional requirements of this category of person (Article 2(c) of the European Commission Directive 91/321/EEC 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae). It also refers to a nutritional composition intended for infants and as defined in Codex Alimentarius (Codex STAN 72-1981) and Infant Specialities (incl. Food for Special Medical Purpose). The expression "infant formula" encompasses both "starter infant formula" and "follow-up formula" or "follow-on formula".

"Maintaining the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels" means maintaining the bi-directional transmission pathways in the gut-brain axis of a human over 7 years of age at normal levels.

"Medical food" preferably means a food that is specially formulated and intended for the dietary management of a disease that has distinctive nutritional needs that cannot be met by normal diet alone.

The term "mood" refers to a positive or negative emotional state of varying intensity that changes in response to life's circumstances (Polak et al. in: *Nutrition for Brain Health and Cognitive Performance* (Eds. Best and Dye), Taylor and Francis, London, 95-112, (2015)). Mood, like emotion, is an affective state or 'a feeling' and has different aspects such as alertness/fatigue, anxiety, and stress. Moods can be distinguished from emotions in terms of duration and the (in)ability to describe the trigger. Moods are often undifferentiated, slow to change, can last from days to weeks, and are 'object-less' in that people may not know the cause or the source of the mood (Russell *Psychol. Rev.* **110**, 145 (2003)). Emotions have clear focus (i.e. a self-evident cause) and are short in duration, i.e. seconds to minutes. Moods and emotions are closely linked, it is common for bad moods to be accompanied by negative emotions, and good moods to accompany positive emotions.

The term "mood disorder" refers to a category of illnesses that describe a significant and serious change in mood. Mood disorders include: anxiety disorder, depression, bipolar disorder and SAD (seasonal affective disorder). In some embodiments, the mood disorder is anxiety disorder or depression. In some embodiments, the mood disorder is anxiety. In some embodiments, the mood disorder is depression.

The expression "nutritional composition" means a composition which nourishes a subject. This nutritional composition is usually to be taken orally or parenterally, and it usually includes a lipid or fat source and a protein source. A carbohydrate source may also be included. In one embodiment, the nutritional composition of the invention is a synthetic nutritional composition.

The term "prebiotic" means non-digestible carbohydrates that beneficially affect the host by selectively stimulating the growth and/or the activity of healthy bacteria such as bifidobacteria in the colon of humans (Gibson et al. *J. Nutr.* **125**, 1401 (1995)).

"Preventing" means to diminish the risk of onset or recurrence of a medical condition or disease. Both primary and secondary prevention are thus contemplated. "Primary prevention" means preventing a medical condition or disease before it occurs, and "secondary prevention" means preventing additional attacks of a medical condition or disease after the first attack has occurred. Preventing, therefore, includes the dietary or nutritional prophylaxis of the medical condition or disease by addressing nutritional needs of the person being treated. Preventing includes eliminating or minimising the risk of developing a medical condition or disease and reducing the risk of developing symptoms associated with the medical condition or disease.

The term "probiotic" means microbial cell preparations or components of microbial cells with a beneficial effect on the health or well-being of the host. (Salminen et al. *Trends Food Sci. Technol.* **10**, 107 (1999)). The microbial cells are generally bacteria or yeasts.

"Promoting relaxation" means enhancing the sensation of being relaxed, e.g. increasing calmness.

"Restoring the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels" means altering the bi-directional transmission pathways in the gut-brain axis of a human over 7 years of age suffering from a medical condition, disease or dysregulation of the transmission pathways in the gut-brain axis to normal levels.

The term "stress" means a state of emotional or psychological strain. Stress may be caused by adverse or demanding circumstances.

The term "stress resilience" means the ability to adapt successfully when faced with stress. Hence, the individual may avoid symptoms associated with stress.

The expression "synthetic combination" or "synthetic nutritional composition" means a mixture obtained by chemical and/or biological means, which can be chemically identical to the mixture naturally occurring in mammalian milks (i.e. the synthetic combination or synthetic composition is not breast milk).

"Treating" means to address a medical condition or disease with the objective of improving or stabilising an outcome in the person being treated or addressing an underlying nutritional need. Treating, therefore, includes the dietary or nutritional management of the medical condition or disease by addressing nutritional needs of the person being treated. Treating includes the elimination, reduction or amelioration of symptoms associated with the medical condition or disease.

All percentages are by weight unless otherwise stated.

Combination of GOS and selected HMOs

In one aspect, it is provided a combination consisting or consisting essentially of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs), wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).

In one embodiment, the mixture of HMOs consists or consists essentially of 2'-FL and DFL. In a particular embodiment, 2'-FL and DFL are present in the HMO mixture in a ratio 2'-FL:DFL of from 5:1 to 14:1, such as 5:1 to 12:1, 5:1 to 10:1, 6:1 to 10:1 or 8:1 to 10:1, preferably 6:1 to 10:1. In a particularly advantageous embodiment, this ratio is 9:1 or around 9:1. In another advantageous embodiment, the 2'-FL:DFL ratio is 7 ± 1 .

It is to be understood that when the expression “a mixture of HMOs consisting of or consisting essentially of 2'-FL and DFL” is used in the context of the present invention, such expression excludes the presence of HMOs different than 2'-FL and DFL in the mixture. In particular the mixture is devoid of N-acetylated oligosaccharides, sialylated oligosaccharides, fucosylated oligosaccharides other than 2'-FL and DFL and precursors of HMOs such as sialic acid or fucose. In some embodiments, the combination or nutritional composition is devoid of N-acetylated oligosaccharides, sialylated oligosaccharides, fucosylated oligosaccharides other than 2'-FL and DFL and precursors of HMOs such as sialic acid or fucose.

In another embodiment, the mixture of HMOs consists or consists essentially of LNT and 6'-SL. In a particular embodiment, the 6'-SL and the LNT in the HMO mixture are typically present in a ratio 6'-SL:LNT of from 3:1 to 1:3, such as 2:1 to 1:2 or 2:1 to 1:1. In a particularly advantageous embodiment, this ratio is 2:1 to 1:1, preferably this ratio is 1:1 or around 1:1.

It is to be understood that when the expression “a mixture of HMOs consisting of or consisting essentially of LNT and 6'-SL” is used in the context of the present invention, such expression excludes the presence of HMOs different than LNT and 6'-SL in the mixture. In particular the mixture is devoid of N-acetylated oligosaccharides, sialylated oligosaccharides, fucosylated oligosaccharides other than LNT and 6'-SL and precursors of HMOs such as sialic acid or fucose. In some embodiments, the combination or nutritional composition is devoid of N-acetylated oligosaccharides, sialylated oligosaccharides, fucosylated oligosaccharides other than LNT and 6'-SL and precursors of HMOs such as sialic acid or fucose.

When GOS, 2'-FL and DFL are combined, all three oligosaccharides are considered responsible for the effect, e.g. for treating and/or preventing stress and/or a mood disorder.

When GOS, LNT and 6'-SL are combined, all three oligosaccharides are considered responsible for the effect, e.g. for treating and/or preventing stress and/or a mood disorder.

Particularly, the GOS and the HMOs in the combination are typically present in a ratio GOS:HMO of from 5:1 to 1:1, such as 3:1 to 1:1 or, preferably, 2:1 to 1:1. In a particularly advantageous embodiment, this ratio is 1:1 or around 1:1.

It is to be understood that when the expression “a combination consisting of or consisting essentially of GOS, 2'-FL and DFL or GOS, LNT and 6'-SL” is used in the context of the present invention, such expression excludes the presence of prebiotics other than GOS, 2'-FL and DFL, or GOS, LNT and 6'-SL, in the combination.

Synthetic composition

Another aspect of the invention relates to a synthetic composition comprising the combination consisting or consisting essentially of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs), wherein the mixture of HMOs consists or

consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).

The synthetic composition can take any suitable form. For example, the composition can be in the form of a nutritional composition. A nutritional composition may contain other macronutrients such as proteins, lipids or other carbohydrates. The synthetic composition can also be an incomplete nutritional composition in unit dosage form, a pharmaceutical composition or other type of compositions such as patch, gel, cream.

A nutritional composition may contain sources of protein, lipids and/or digestible carbohydrates and can be in powdered or liquid forms. The composition can be designed to be the sole source of nutrition or a nutritional supplement. For emotion or mood disorder humans over 7 years of age, a nutritional supplement is preferred; especially a supplement which can form a meal or snack replacement. Preferably the nutritional composition is lactose-reduced or, better yet, lactose-free.

Suitable protein sources include milk proteins, soy protein, rice protein, pea protein and oat protein, or mixtures thereof. Milk proteins can be in the form of milk protein concentrates, whey protein or casein, or mixtures of both. Soy, rice, pea and oat protein can be in the form of protein isolated. The protein can be whole protein or hydrolysed protein, either partially hydrolysed or extensively hydrolysed. The protein can provide about 5 % to about 50 %, preferably about 10 % to 30 %, of the energy of the nutritional composition. The protein source preferably is not a source of carbohydrates such as lactose. Therefore, if a milk protein is used as the protein source, the milk protein is preferably lactose-reduced or lactose-free.

Suitable digestible carbohydrates include maltodextrin, hydrolysed or modified starch or corn starch, glucose polymers, corn syrup, corn syrup solids, tapioca, sucrose, and glucose, or mixtures thereof. Generally digestible carbohydrates provide about 35 % to about 75 %, preferably about 45 % to 70 %, of the energy of the nutritional composition. Preferably the digestible carbohydrate is free from lactose.

Suitable lipids include rapeseed oil, sunflower seed oil, palm oil, soy oil, milk fat, corn oil and soy lecithin. Long-chain poly unsaturated fatty acids (LC-PUFA), especially omega-3 fatty acids such as docosahexaenoic acid (DHA), can be included in the lipid source because they have anti-inflammatory properties. Suitable sources of LC-PUFA are plant oils, marine plankton oils, fungal oils, and fish oils. The lipid source can also include medium chain triglycerides (MCT). Fractionated coconut oils are a suitable source of medium chain triglycerides. The lipid source preferably provides about 5 % to about 25 % of the energy of the nutritional composition; for example about 10 % to 20 %.

The nutritional composition may also include vitamins and minerals. If the nutritional composition is intended to be a sole source of nutrition, it preferably includes a complete vitamin and mineral profile. Examples of vitamins include Vitamins A, B- complex (such as B1, B2, B6 and B12), C, D, E and K, niacin and acid vitamins such as pantothenic acid and folic acid and biotin. Examples of minerals include calcium, iron, zinc, magnesium, iodine, copper, phosphorus, manganese, potassium, chromium, molybdenum, selenium, nickel, tin, silicon, vanadium and boron.

The nutritional composition may also include a carotenoid such as lutein, lycopene, zeaxanthin, and beta-carotene. The total amount of carotenoid included can vary from about 0.001 µg/ml to about 10 µg/ml. Lutein can be included in an amount of from about 0.001 µg/ml to about 10 µg/ml, preferably from about 0.044 µg/ml to about 5 g/ml of lutein. Lycopene can be included in an amount from about 0.001 µg/ml to about 10 µg/ml, preferably about 0.0185 mg/ml to about 5 g/ml of lycopene. Beta-carotene can comprise from about 0.001 µg/ml to about 10 mg/ml, for example about 0.034 µg/ml to about 5 µg/ml of beta-carotene.

The nutritional composition may also contain various other conventional ingredients such as preservatives, emulsifying agents, thickening agents, buffers, fibres, probiotics, antioxidant/anti-inflammatory compounds including tocopherols, carotenoids, ascorbate/vitamin C, ascorbyl palmitate, polyphenols, glutathione, and superoxide dismutase (melon), other bioactive factors (e.g. growth hormones, cytokines, TFG-β), colorants, flavours, stabilisers and/or lubricants, and so forth.

In one particular embodiment, the nutritional composition comprises a probiotic, such as a probiotic bacterial strain. The probiotic microorganisms most commonly used are principally bacteria and yeasts of the following genera: *Lactobacillus spp.*, *Streptococcus spp.*, *Enterococcus spp.*, *Bifidobacterium spp.* and *Saccharomyces spp.* In some particular embodiments, the probiotic is a probiotic bacterial strain. In some specific embodiments, it is particularly *Bifidobacteria* and/or *Lactobacilli*. Suitable probiotic bacterial strains include *Lactobacillus rhamnosus* ATCC 53103 available from Valio Oy of Finland under the trademark LGG, *Lactobacillus rhamnosus* CGMCC 1.3724, *Lactobacillus paracasei* CNCM I-2116, *Lactobacillus johnsonii* CNCM I-1225, *Streptococcus salivarius* DSM 13084 sold by BLIS Technologies Limited of New Zealand under the designation KI2, *Bifidobacterium lactis* CNCM 1-3446 sold *inter alia* by the Chr. Hansen company of Denmark under the trademark Bb 12, *Bifidobacterium longum* ATCC BAA-999 sold by Morinaga Milk Industry Co. Ltd. of Japan under the trademark BB536, *Bifidobacterium breve* sold by Danisco under the trademark Bb-03, *Bifidobacterium breve* sold by Morinaga under the trade mark M-16V, *Bifidobacterium infantis* sold by Procter & Gamble Co. under the trademark Bifantis and *Bifidobacterium breve* sold by Institut Rosell (Lallemand) under the trademark R0070. Other exemplary prebiotics are

Lactobacillus casei strain Shirota, *B. infantis* 35624, *B. animalis* subsp. *lactis* BB-12, *B. lactis* Bi-07, *L. rhamnosus* GG, *L. rhamnosus* Lc705, *L. plantarum* DSM 9843, *L. plantarum* CECT7484, *L. plantarum* CECT7485, *L. acidophilus* NCFM, *L. fermentum* CECT5716, *B. breve* Bb99, *Propionibacterium freundenreichii* ssp. *Shermanii* JS, *P. acidilactici* CECT7483, *Streptococcus faecium*). The nutritional composition may contain from 10^3 to 10^{12} cfu of probiotic strain, more preferably between 10^7 and 10^{12} cfu such as between 10^8 and 10^{10} cfu of probiotic strain per g of composition on a dry weight basis. In one embodiment the probiotics are viable. In another embodiment the probiotics are non-replicating or inactivated. There may be both viable probiotics and inactivated probiotics in some other embodiments. Probiotic components and metabolites can also be added.

The nutritional composition may also comprise other types of oligosaccharide(s) (i.e. other than human milk oligosaccharides mentioned above) and/or a fibre(s) and/or a precursor(s) thereof. The other oligosaccharide and/or fibre and/or precursor thereof may be selected from the list comprising HMOs, fructo-oligosaccharides (FOS), inulin, (XOS), polydextrose and any combination thereof. They may be in an amount between 0 and 10% by weight of composition.

The nutritional composition can be in the form of a soluble powder, a liquid concentrate, or a ready-to-use formulation. Various flavours, fibres and other additives can also be present.

The nutritional composition can be prepared by any commonly used manufacturing techniques for preparing nutritional compositions in solid or liquid form. For example, the composition can be prepared from various feed solutions. A protein-in-fat feed solution can be prepared by heating and mixing the lipid source and then adding an emulsifier (e.g. lecithin), fat soluble vitamins, and at least a portion of the protein source while heating and stirring. A carbohydrate feed solution is also prepared by adding minerals, trace and ultra-trace minerals, thickening or suspending agents to water while heating and stirring. The resulting solution is held for 10 minutes with continued heat and agitation before adding carbohydrates (e.g. the GOS, the 2'-FL/DFL or LNT/6'-SL mixture neutral optionally digestible carbohydrate sources, further prebiotic and/or further HMOs). The resulting feed solutions are then blended together while heating and agitating and the pH adjusted to 6.6-7.0, after which the composition is subjected to high-temperature short-time processing during which the composition is heat treated, emulsified and homogenized, and then allowed to cool. Water soluble vitamins and ascorbic acid are added, the pH is adjusted to the desired range, if necessary, flavours are added, and water is added to achieve the desired total solid level.

For a liquid product, the resulting solution can then be aseptically packaged to form an aseptically packaged nutritional composition. In this form, the nutritional composition can be in

ready-to-feed or concentrated liquid form. Alternatively the composition can be spray dried and processed and packaged as a reconstitutable powder.

When the nutritional product is a ready-to-feed nutritional liquid, the total concentration of GOS+2'-FL+DFL or GOS+LNT+6'-SL in the liquid, by weight of the liquid, is from about 0.002 % to about 3.0 %, including from about 0.005 % to about 2 %, including from about 0.05 % to about 1.0 %. When the nutritional product is a concentrated nutritional liquid, the total concentration of GOS+2'-FL+DFL or GOS+LNT+6'-SL in the liquid, by weight of the liquid, is from about 0.004 % to about 6.0 %, including from about 0.01 % to about 4.0 %, including from about 0.1 % to about 2.0 %.

The synthetic composition of this invention can also be in a unit dosage form such as a capsule, tablet or sachet. For example, the composition can be formulated into single serve sachets containing GOS+2'-FL+DFL or GOS+LNT+6'-SL, especially if higher doses are to be administered (more than 3 g). Alternative the composition can be in a tablet form comprising GOS+2'-FL+DFL or GOS+LNT+6'-SL, and one or more additional components to aid formulation and administration, such as diluents, excipients, antioxidants, lubricants, colorants, binders, disintegrants, and the like. Suitable diluents, excipients, lubricants, colorants, binders, and disintegrants include polyethylene, polyvinyl chloride, ethyl cellulose, acrylate polymers and their copolymers, hydroxyethyl-cellulose, hydroxypropylmethyl-cellulose (HPMC), sodium carboxymethylcellulose, polyhydroxyethyl methacrylate (PHEMA), polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), polyethylene oxide (PEO), or polyacrylamide (PA), carrageenan, sodium alginate, polycarbophil, polyacrylic acid, tragacanth, methyl cellulose, pectin, natural gums, xanthan gum, guar gum, karaya gum, hypromellose, magnesium stearate, microcrystalline cellulose, and colloidal silicon dioxide. Suitable antioxidants are vitamin A, carotenoids, vitamin C, vitamin E, selenium, flavonoids, polyphenols, lycopene, lutein, lignan, coenzyme Q10 ("CoQ10") and glutathione.

The unit dosage forms, especially those in sachet form, can also include various nutrients including macronutrients.

The nutritional composition may be provided in chewable or effervescent tablet, or may be formulated in a palatable confection such as a confectionary bar, beverage, cereal, powder, gummy, chewing gum and the like.

For improving emotion and mood disorders in a human over 7 years of age, the amount of the combination of GOS+2'-FL+DFL or GOS+LNT+6'-SL required to be administered will vary depending upon factors such as the risk and severity of the disease, the age of the patient, the form of the composition, and other medications being administered to the patient. However, the required amount can be readily determined by a medical practitioner and would generally be in the range of about 20 mg to about 30 g per day, preferably about 50 mg to about 20 g per day,

or from about 100 mg to about 15 g per day, in certain embodiments from about 500 mg to about 10 g per day, preferably from about 1 g to about 7.5 g per day. During an initial treatment phase, the dosing can be higher, for example 100 mg to 30 g per day, preferably 500 mg to 15 g per day, more preferably 1 g to 10 g per day, in certain embodiments 2.5 g to 7.5 g per day.

- 5 During a secondary prevention phase, the dosing can be reduced, for example, to 20 mg to 20 g per day, preferably 100 mg to 10 g per day, more preferably 500 g to 7.5 g per day, in certain embodiments 750 mg to 5 g per day.

According to a particular embodiment, the invention relates to a synthetic composition comprising a combination of galacto-oligosaccharides (GOS) and a mixture of human milk
10 oligosaccharides (HMOs), wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL), wherein the synthetic composition is a nutritional composition with the proviso that nutritional compositions suitable or intended to use for infants and children up to 7 years of age are excluded. Typically, the following compositions are excluded: infant formula, a
15 starter infant formula, a follow-on or follow-up infant formula, a growing-up milk, a baby food, an infant cereal composition or a fortifier suitable for fortifying or mixing with human milk, infant formula, growing-up milk or human breast milk fortified with other nutrients. Accordingly, this particular embodiment relates to nutritional composition suitable or intended to use for a human over 7 years of age, preferably a teenager or adult, more preferably an adult. Examples of
20 nutritional compositions suitable or intended to use for a human over 7 years of age are dietary supplements, functional foods, medical foods, chewable or effervescent tablets, capsule formulations, compositions formulated in a palatable confection such as a confectionary bar, beverage, cereal, powder, gummy, chewing gum and the like.

The preferred and more preferred features of the combination disclosed above are also
25 applicable when the combination is comprised in a synthetic composition.

Use

In one aspect the present invention provides a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs) for use in treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, wherein the mixture
30 of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL). Preferably, the combination for the use consists or consists essentially of GOS and the mixture of HMOs.

The combination may be in the form of a synthetic or nutritional composition.

Accordingly, in another aspect, the present invention provides a synthetic or nutritional
35 composition comprising a combination of galacto-oligosaccharides (GOS) and a mixture of

human milk oligosaccharides (HMOs) for use in treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL). Preferably, the composition for the use comprises the combination
5 that consists or consists essentially of GOS and the mixture of HMOs.

There is clinical and preclinical evidence of the effect of exogenous GABA in relieving stress and promoting the sensation of feeling relaxed (Abdou *BioFactors* **26**, 201 (2006)). Probiotics producing GABA have been shown to alleviate gut discomfort triggered by stress in preclinical studies (Pokusaeva et al. *Neurogastroenterol. Motil.* **29**, e12904 (2016)). The effect
10 of oral GABA administration on stress is reviewed by Hepsomali *et al.* (Hepsomali et al. *Front. Neurosci.* **14**, 923 (2020)). GABA has been shown to reduce salivary cortisol in humans (Kanehira et al. *J. Nutr. Sci. Vitaminol.* **57**, 9 (2011)). However, no nutritional solution has previously been shown to naturally increase the synthesis of GABA by the microbiota of a human over 7 years of age exposed to the nutritional solution.

15 Treating includes the elimination, reduction or amelioration of symptoms associated with the medical condition or disease.

In some embodiments, treating improves the stress resilience of a human over 7 years of age.

Symptoms of stress that may be eliminated, reduced or ameliorated include anxiety,
20 distress, depression, heightened sensitivity to painful stimuli, and impaired neuronal development or neuronal functioning. Suitably, the treatment may reduce the amount of cortisol in the saliva, which is a measure of stress (Jessop et al. *Stress*, **11**, 1 (2008); Bi et al. *J. Anim. Physiol. Anim. Nutr.* **104**, 590 (2020); Kanehira et al. *J. Nutr. Sci. Vitaminol.* **57**, 9 (2011)).

In some embodiments, the mood disorder is anxiety disorder or depression.

25 Symptoms of anxiety disorder that may be eliminated, reduced or ameliorated include finding it hard to concentrate, difficulty sleeping, irritability, crying, feeling nervous, restlessness or loss of appetite. Suitably, the treatment may reduce the anxiety subscale score in Quality of Life (QoL) questionnaire.

Symptoms of depression that may be eliminated, reduced or ameliorated include finding
30 it hard to concentrate, difficulty sleeping, irritability, crying, anxiety, restlessness, loss of appetite or feeling sad, tearful, empty or hopeless.

Suitably, the combination or composition according to the invention treats stress and/or a mood disorder by promoting relaxation in the human over 7 years of age.

Suitably, the combination or composition according to the invention treats stress and/or a mood disorder by increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of the human over 7 years of age. Many species of *Lactobacillus* and *Bifidobacteria*, including *Bifidobacterium adolescentis*, are able to synthesise and secrete the potent inhibitory neurotransmitter gamma-aminobutyric acid (GABA) which is known to be involved in reducing stress, anxiety and depression.

Accordingly, in another aspect, the invention provides a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs) for use in increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).

In another aspect, the invention provides a synthetic or nutritional composition comprising a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs) for use in increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).

Suitably, the biosynthesis of GABA by the gut microbiota of the human over 7 years of age is increased from 1.1 to 20-fold, such as by 1.2 to 11-fold, preferably by 4-fold to 11-fold. Suitably, the biosynthesis of GABA by the gut microbiota of the human over 7 years of age is increased by at least 1.1-fold, such as by 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold or 20-fold.

Suitably, the base-2 logarithm of the ratio between the value of GABA for the combination or composition according to the invention and the value of GABA for a corresponding milk control is increased by from 0.2 to 5, suitably from 0.25 to 3.8.

The amount of GABA secreted (i.e. GABA biosynthesis) by the gut microbiota of the human over 7 years of age may be measured by methods known in the art. For example, the method disclosed herein may be used (see Example 2). Suitably, the amount of GABA in fresh faecal samples from a human over 7 years of age fed the combination or nutritional composition according to the invention may be compared to samples from a human over 7 years of age not exposed to a combination or nutritional composition according to the invention (Altaib et al. *Microorganisms* **9**, 378 (2021)).

The gut-brain axis consists of bidirectional communication between the central nervous system (CNS) and the enteric nervous system (ENS), linking emotional and cognitive centres of

the brain with peripheral intestinal functions. The autonomic nervous system, hypothalamic-pituitary-adrenal (HPA) axis, and nerves within the gastrointestinal (GI) tract, all link the gut and the brain, allowing the brain to influence intestinal activities, including activity of functional immune effector cells; and the gut to influence mood, cognition, and mental health. Recent
5 advances in research have described the importance of gut microbiota in influencing these interactions. This interaction between microbiota and gut-brain axis appears to be bidirectional, namely involving signalling from gut-microbiota to brain and from brain to gut-microbiota by means of neural, endocrine, immune, and humoral links (e.g. by means of the vagus nerve). In clinical practice, evidence of microbiota-GBA interactions comes from the association of
10 dysbiosis with central nervous disorders (i.e. autism, anxiety-depressive behaviours) and functional gastrointestinal disorders.

Clinical, epidemiological, and immunological evidence suggest that the enteric microbiota influences the gut-brain axis (i.e., mental state, emotional regulation, neuromuscular function, and regulation of the HPA). For example, several mood disorders, such as anxiety
15 disorder, depression, and autism spectrum disorders now have well-established links to functional GI disruptions and GI diseases (e.g., irritable bowel syndrome) often involve psychological comorbidities associated with alteration of the gut microbiome.

Without wishing to be bound by theory, by altering the microbiota to increase biosynthesis of GABA, amongst other impacts, the combination according to the invention may
20 be capable of influencing neurotransmission in the paraventricular hypothalamus, the central nucleus of the amygdala, and the bed nucleus of the stria terminalis. All three of these regions are involved in the processing of emotions related to anxiety and mood.

Accordingly, in some embodiments, the combination or the synthetic or nutritional composition according to the invention maintains or restores the functionality of the bi-
25 directional transmission pathways in the gut-brain axis to healthy levels in a human over 7 years of age.

In another aspect, the invention provides a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs) for use in maintaining or restoring the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy
30 levels in a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).

In another aspect, the invention provides a synthetic or nutritional composition comprising a combination of galacto-oligosaccharides (GOS) and a mixture of human milk
35 oligosaccharides (HMOs) for use in maintaining or restoring the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels in a human over 7 years of age,

wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).

In a further aspect, the present invention provides a method for treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, the method comprising administering to a human over 7 years of age a combination according to the invention or a synthetic composition according to the invention.

In a further aspect, the present invention provides a method for maintaining or restoring the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels in a human over 7 years of age, the method comprising administering to a human over 7 years of age a combination according to the invention or a synthetic composition according to the invention.

In a further aspect, the present invention provides a method for increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of a human over 7 years of age, the method comprising administering to a human over 7 years of age a combination according to the invention or a synthetic composition according to the invention.

In some embodiments of the methods and uses of the invention, the combination or composition according to the invention is administered to the human over 7 years of age in an effective amount.

In a further aspect, the present invention provides the use of a combination according to the invention or a synthetic composition according to the invention for treating and/or preventing stress and/or a mood disorder in a human over 7 years of age. Suitably, the combination treats stress and/or a mood disorder by increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of the human over 7 years of age.

In another aspect, the invention provides the use of a combination according to the invention or a synthetic composition according to the invention for maintaining or restoring the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels in a human over 7 years of age.

In some embodiments of the methods and uses of the invention, the combination or the composition according to the invention is used for the treatment of stress and/or a mood disorder.

In some embodiments of the methods and uses of the invention, the combination or the composition according to the invention is used for the prevention of stress and/or a mood disorder.

In some embodiments of the methods and uses of the invention, the combination or the composition according to the invention is used for the treatment and/or prevention of stress.

In some embodiments of the methods and uses of the invention, the combination or the composition according to the invention is used for the treatment and/or prevention of a mood disorder.

The preferred and more preferred features of the combination or the synthetic composition comprising the combination disclosed above are also applicable for the combinations or the synthetic compositions suitable for the therapeutic use.

In another aspect of the invention, the use or method is non-therapeutic. Suitably, the use or method is for increasing the production of gamma-aminobutyric acid (GABA) by the gut microbiota of a healthy human over 7 years of age. As opposed to the therapy which is concerned with bringing the body from a pathological state (disease) back to a normal state, the non-therapeutic method or use is practised on an individual with a normal, healthy state, even though that individual may feel some discomfort, but that discomfort will not likely develop a pathological state or medical condition. Therefore, the non-therapeutical use or method targets a performance improvement of the body from the starting point of a normal state. Accordingly, the invention also relates to a method is for increasing the production of gamma-aminobutyric acid (GABA) by the gut microbiota in a healthy human over 7 years of age, the method comprising administering to the human over 7 years of age a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs), wherein the mixture of HMOs consists or consists essentially of 2'-FL and DFL or of LNT and 6'-SL, or administering a synthetic composition comprising the combination.

The preferred and more preferred features of the combination or the synthetic composition comprising the combination disclosed above are also applicable for the combinations or the synthetic compositions suitable for the non-therapeutic use.

Target population

In some embodiments of the methods and uses of the invention mentioned above, the combination or the composition administered to a child over 7 years of age.

In some embodiments of the methods and uses of the invention mentioned above, the combination or the composition is administered to a teenager/adolescent.

In some embodiments of the methods and uses of the invention mentioned above, the combination or composition is administered to an adult.

Preferably, the target population is teenagers or adults, more preferably adults.

EXAMPLES

Example 1

A milk matrix (a standard milk follow-up formula) was subjected to oral, gastric and small intestinal digestion procedures according to the standardized INFOGEST 2.0 method (Brodkorb et al. *Nature Protocols* **14**, 991 (2019)). Then *in vitro* fermentation was achieved for 24 hours in reactor containing:

- 10% of the digested milk matrix,
- 80% of fermentation medium containing microbiota derived from fresh faecal samples, recovered from 3 healthy toddlers aged 3.2-3.3 years,
- 10% of the test ingredients, consisting of
 - Buffer only;
 - Galacto-oligosaccharides at the final concentration of 2 g/l;
 - Blend of 2'-FL and DFL (ratio 9:1), at a final concentration of 2 g/l;
 - Blend of LNT and 6'-SL (ratio 2:1), at a final concentration of 2 g/l;
 - A combination of (i) GOS and (ii) the blend of 2'-FL and DFL (ratio 9:1), both at a final concentration of 2 g/l;
 - A combination of (i) GOS and (ii) the blend of LNT and 6'-SL (ratio 2:1), both at a final concentration of 2 g/l.

Each combination was tested in triplicate. After 24 hours, fermentation medium was collected for metabolomics analysis.

The untargeted LC-MS/MS was carried out using a Thermo Scientific Vanquish LC coupled to Thermo Q Exactive HF MS. An electrospray ionization interface was used as ionization source. Analysis was performed in negative and positive ionization mode. The UPLC was performed using a slightly modified version of the protocol described by Doneanu in the publication entitled *UPLC/MS Monitoring of Water-Soluble Vitamin Bs in Cell Culture Media in Minutes*, Waters Application Note, 2011, (<https://www.waters.com/webassets/cms/library/docs/720004042en.pdf>). Peak areas were extracted using Compound Discoverer 3.1 (Thermo Scientific). In addition to the automatic compound extraction by Compound Discoverer 3.1, a manual extraction of compounds included in an in-house library was performed using Skyline 21.1 (MacCoss Lab Software).

For each blend, we calculate $\log_2(\text{GABA}(\text{blend})/\text{GABA}(\text{milk}))$ that is, the base-2 logarithm of the ratio between the value of GABA for the blend and the value of GABA for milk.

Mixture linear models with random intercepts were used to assess the effect of the blends on the level of each metabolite.

The goodness of fit was assessed using the marginal and the conditional R². The marginal R² (marginal coefficient of determination) represents the variance explained by the fixed effects while the conditional R² is interpreted as a variance explained by the entire model, including both fixed and random effects. In addition, we inspected the standardized residuals of each model to check whether outliers in the data were potentially of concern.

The ratio of the between-cluster variance to the total variance is called the Intraclass Correlation (ICC) was generally between 0.6 and 0.8. This can also be interpreted as the correlation among observations within the same cluster (donor).

P-values, approximated with the Kenward-Roger method, were used to determine which blends were significantly associated with the outcome.

Exposure of microbiome to digested milk matrix, in presence of either GOS, a blend of 2'-FL and DFL or a blend of LNT and 6'-SL, did not trigger any effect on GABA abundance (Figure 1). Conversely, the presence of a blend comprising GOS plus HMOs (2'-FL/DFL or LNT/6'-SL) strongly increased the synthesis of GABA by the microbiota compared to single ingredients (Figure 2). The amount of GABA secreted was higher upon exposure with GOS+2'-FL/DFL than GOS+LNT/6'-SL.

Example 2 – Nutritional composition

A ready-to-feed nutritional composition is prepared from water, maltodextrin, corn syrup, sugar, milk protein concentrate, vegetable oil (canola, high oleic sunflower and corn), soy protein isolate, acacia gum, flavours, GOS+2'-FL+DFL or GOS+LNT+6'-SL, potassium citrate, magnesium phosphate, cellulose gel and gum, calcium carbonate, sodium ascorbate, soy lecithin, choline bitartrate, calcium phosphate, alpha-tocopheryl acetate, ascorbic acid, carrageenan gum, ferric pyrophosphate, flavours, sweeteners (Stevia), vitamin A palmitate, niacinamide, vitamin D3, calcium pantothenate, manganese sulphate, copper sulphate, pyridoxine hydrochloride, thiamine hydrochloride, beta carotene, riboflavin, chromium chloride, folic acid, biotin, potassium iodide, phytonadione, sodium selenite, sodium molybdate, vitamin B12.

The composition provides a nutritional supplement which is a good source of protein, low in fat, vitamins, minerals and antioxidants. Further, the composition promotes the growth of beneficial intestinal bacteria and improve gut barrier function.

Example 3 – Capsule composition

A capsule is prepared by filling about 1 g of GOS+2'-FL+DFL or GOS+LNT+6'-SL into a 000 gelatine capsule using a filling machine. The capsules are then closed. The GOS+2'-FL+DFL or GOS+LNT+6'-SL is in free-flowing powder form.

CLAIMS

1. A combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs) for use in treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).
2. A synthetic composition comprising a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs) for use in treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).
3. The combination for the use according to claim 1 or the synthetic composition for the use according to claim 2, wherein the combination or the composition maintains or restores the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels.
4. The combination for the use according to claim 1 or 3 or the synthetic composition for the use according to claim 2 or 3, wherein the combination or the composition increases the production of gamma-aminobutyric acid (GABA) by the gut microbiota of the human over 7 years of age and thereby treats stress and/or a mood disorder in the human over 7 years of age.
5. The combination for the use according to any of the claims 1, 3 or 4, or the synthetic composition for the use according to any one of the claims 2 to 4, wherein the mood disorder is anxiety or depression.
6. The combination for the use according to any of the claims 1 or 3 to 5, or the synthetic composition for the use according to any one of the claims 2 to 5, wherein the mixture of HMOs consists or consists essentially of 2'-FL and DFL.
7. The combination or the composition for the use according to claim 6, wherein the ratio of 2'-FL to DFL is 6:1 to 10:1 by weight.
8. The combination for the use according to any of the claims 1 or 3 to 5, or the synthetic composition for the use according to any one of the claims 2 to 5, wherein the mixture of HMOs consists or consists essentially of LNT and 6'-SL.
9. The combination or the composition for the use according to claim 8, wherein the ratio of LNT to 6'-SL is 2:1 to 1:1 by weight.
10. A method for treating and/or preventing stress and/or a mood disorder in a human over 7 years of age, the method comprising administering to a human over 7 years of age an

effective amount of a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs) or a synthetic composition comprising an effective amount of said combination, as defined in any of the claims 1 to 9.

11. A method for maintaining or restoring the functionality of the bi-directional transmission pathways in the gut-brain axis to healthy levels in a human over 7 years of age, the method comprising administering to a human over 7 years of age an effective amount of a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs) or a synthetic composition comprising an effective amount of said combination, as defined in any of the claims 1 to 9.
12. A method for increasing production of gamma-aminobutyric acid (GABA) by the gut microbiota of a human over 7 years of age, the method comprising administering to a human over 7 years of age an effective amount of a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs) or a synthetic composition comprising an effective amount of said combination, as defined in any of the claims 1 to 9.
13. A synthetic composition comprising a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs), wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL), wherein the synthetic composition is a nutritional composition with the proviso that the following compositions are excluded: infant formula, a starter infant formula, a follow-on or follow-up infant formula, a growing-up milk, a baby food, an infant cereal composition or a fortifier suitable for fortifying or mixing with human milk, infant formula, growing-up milk or human breast milk fortified with other nutrients.
14. The synthetic composition according to claim 13, wherein the combination consists of or consists essentially of GOS and the HMOs.
15. The synthetic composition according to claim 13 or 14, wherein the mixture of HMOs consists or consists essentially of 2'-FL and DFL.
16. The synthetic composition according to claim 15, wherein the ratio of 2'-FL to DFL is 6:1 to 10:1 by weight.
17. The synthetic composition according to claim 13 or 14, wherein the mixture of HMOs consists or consists essentially of LNT and 6'-SL.
18. The synthetic composition according to claim 17, wherein the ratio of LNT to 6'-SL is 2:1 to 1:1 by weight.
19. A non-therapeutic method for increasing the production of gamma-aminobutyric acid (GABA) by the gut microbiota in a healthy human over 7 years of age, the method comprising

administering to a healthy human over 7 years of age a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs), wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).

- 5 20. A non-therapeutic method for increasing the production of gamma-aminobutyric acid (GABA) by the gut microbiota in a healthy human over 7 years of age, the method comprising administering to a healthy human over 7 years of age a synthetic composition comprising a combination of galacto-oligosaccharides (GOS) and a mixture of human milk oligosaccharides (HMOs), wherein the mixture of HMOs consists or consists essentially of 2'-fucosyllactose (2'-
10 FL) and difucosyllactose (DFL) or of lacto-N-tetraose (LNT) and 6'-sialyllactose (6'-SL).
21. The combination for the use according to any of the claims 1 or 3 to 9, the synthetic composition for the use according to any one of the claims 2 to 9, or the method according to any of the claims 10 to 12, 19 or 20, wherein the human over 7 years of age is an adult.

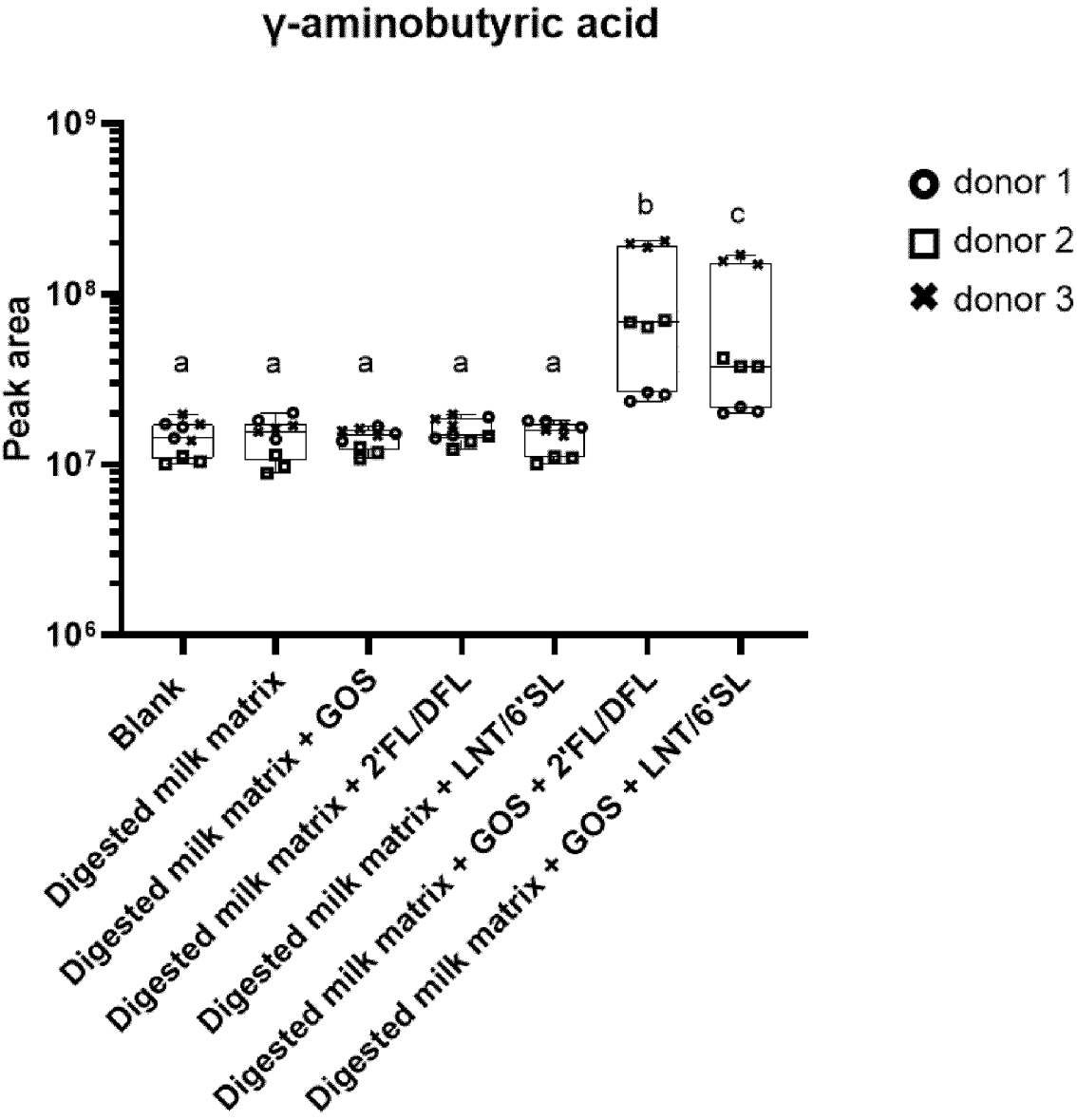


Figure 1

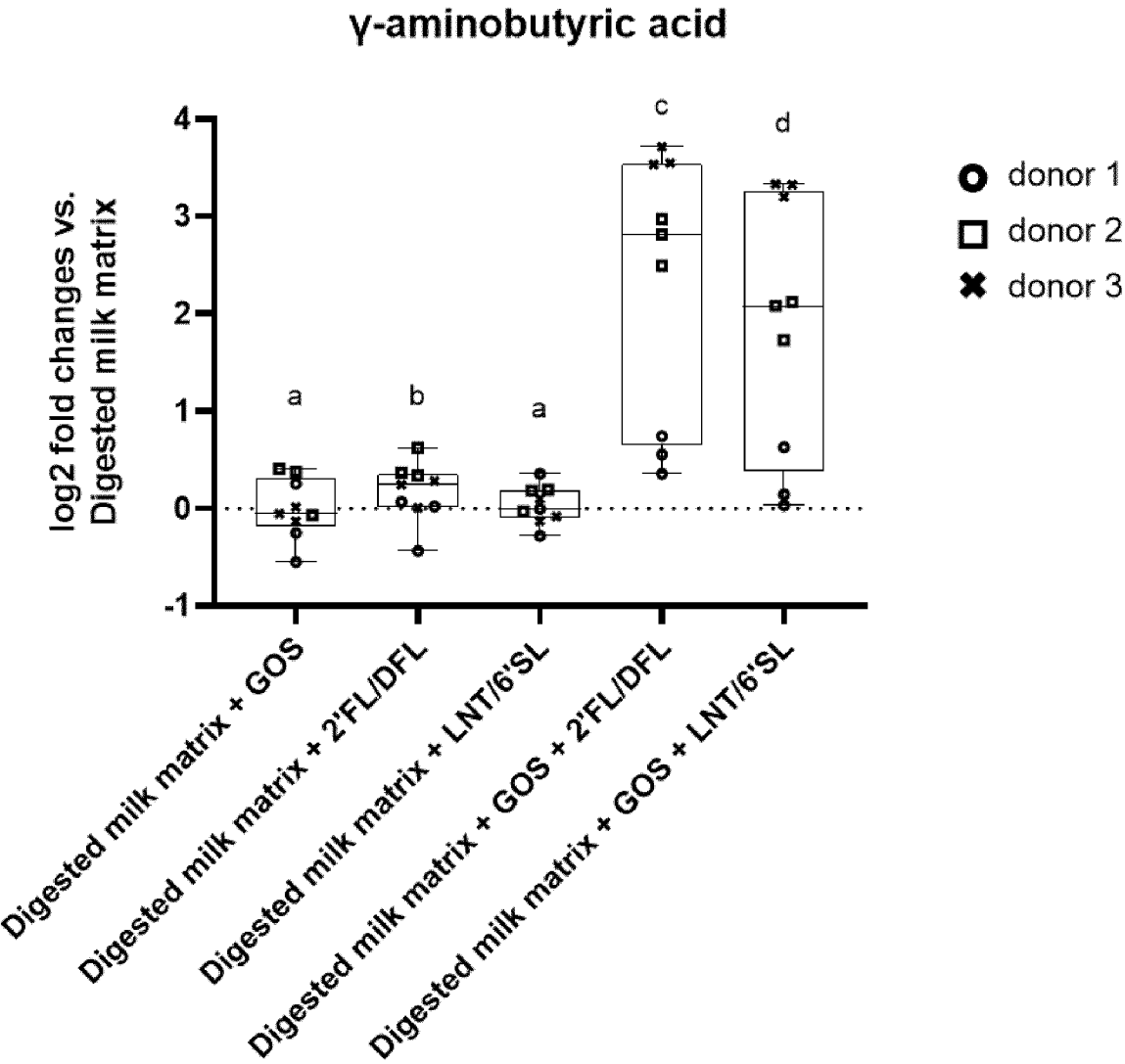


Figure 2

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2023/063199

A. CLASSIFICATION OF SUBJECT MATTER		
INV. A61K31/702 A61P25/22 A61P25/24 A23L33/21		
ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) A61K A61P A23L		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HEPSOMALI PIRIL ET AL: "Effects of Oral Gamma-Aminobutyric Acid (GABA) Administration on Stress and Sleep in Humans: A Systematic Review", FRONTIERS IN NEUROSCIENCE, vol. 14, 17 September 2020 (2020-09-17), XP093002386, DOI: 10.3389/fnins.2020.00923 abstract, discussion : paragraph stress ----- -/--	1-21
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 17 August 2023		Date of mailing of the international search report 28/08/2023
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Venturini, Francesca

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2023/063199

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Y	PAIVA IGOR HENRIQUE R ET AL: "The role of prebiotics in cognition, anxiety, and depression", EUROPEAN NEUROPSYCHOPHARMACOLOGY, ELSEVIER SCIENCE PUBLISHERS BV , AMSTERDAM, NL, vol. 34, 30 March 2020 (2020-03-30), pages 1-18, XP086143513, ISSN: 0924-977X, DOI: 10.1016/J.EURONEURO.2020.03.006 [retrieved on 2020-03-30] conclusions; paragraph [02.3] - paragraph [02.4]; figure 1; tables 1,2 -----	1-21
Y	AL-KHAFAJI ALIA H ET AL: "The potential of human milk oligosaccharides to impact the microbiota-gut-brain axis through modulation of the gut microbiota", JOURNAL OF FUNCTIONAL FOODS, ELSEVIER BV, NL, vol. 74, 23 September 2020 (2020-09-23), XP086272909, ISSN: 1756-4646, DOI: 10.1016/J.JFF.2020.104176 [retrieved on 2020-09-23] paragraphs [01.3], [0002], [0003], [0004]; figure 2 -----	1-21
Y	US 2021/268005 A1 (HAUSER JONAS [CH] ET AL) 2 September 2021 (2021-09-02) claims; examples ----- -/--	1-21

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International application No

PCT/EP2023/063199

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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