SUMMARY ~~OF THE INVENTION~~

[0007] ~~In a first aspect, the~~The ~~invention~~ disclosure provides a **method of producing sialyloligosaccharides** from a liquid source that comprises sialyloligosaccharides, carbohydrates and minerals by subjecting the liquid source to a process comprising

a) heating the liquid source to a temperature of from about 67°C,

b) subjecting the heat-treated liquid source to

(i) a filtration step with a heat-resistant filter at a temperature of about 35 to about 95°C to produce a first retentate and first permeate, or

(ii) centrifugal separation to produce a light phase and a heavy phase and filtration of the light phase with a heat-resistant filter at a temperature of about 35 to about 95°C to produce a first retentate and first permeate,

c) subjecting the first permeate to nanofiltration, or nanofiltration and diafiltration, to produce a second retentate and second permeate, and

d) concentrating the second retentate,

to produce a sialyloligosaccharide-containing extract.

[0007a] In one aspect, there is provided a method of producing sialyloligosaccharides from a liquid source that comprises sialyloligosaccharides, carbohydrates and minerals by subjecting the liquid source to a process comprising

a) heating the liquid source to a temperature of from about 67°C,

b) subjecting the heat-treated liquid source to

i) a filtration step with a heat-resistant filter at a temperature of about 55 to about 90°C to produce a first retentate and first permeate, or

ii) centrifugal separation to produce a light phase and a heavy phase and ultrafiltration of the light phase at a temperature of about 50 to about 70°C to produce a first retentate and first permeate,

c) subjecting the first permeate to nanofiltration, or nanofiltration and diafiltration, to produce a second retentate and second permeate, and

d) concentrating the second retentate,

to produce a sialyloligosaccharide-containing extract.

[0007b] In another aspect, there is provided a sialyloligosaccharide-containing extract produced by the method as described herein.

[0008] In a further aspect the ~~invention~~ disclosure relates to **a sialyloligosaccharide-enriched composition**, comprising on a dry solids basis

- at least about 5% by weight total sialyloligosaccharides comprising
  - at least about 5% 3'-sialyllactose by weight of total sialyloligosaccharides,
  - at least about 2% 6'-sialyllactose by weight of total sialyloligosaccharides,
  - at least about 0.01% disialyllactose by weight of total sialyloligosaccharides,
  - at least about 0.01% sialyllactosamine by weight of total sialyloligosaccharides, and
- at least about 0.5% by weight free sialic acid,
- at least about 0.2% by weight neutral oligosaccharides,
- less than about 25% by weight protein and non-protein nitrogen,
- less than about 80% by weight sugars not including sialyllactose, and
- less than about 5% by weight ash.

[0008a] In another aspect, there is provided a nutritional formulation comprising a sialyloligosaccharide-containing extract produced by a method as described herein, or a sialyloligosaccharide-enriched composition as described herein.

[0009] In a further ~~aspect~~ example the ~~invention~~ disclosure relates to **a composition** for providing nutrition to an infant of less than 6 months of age comprising 3'-sialyllactose, 6'-

sialyllactose and sialyllactosamine in a ratio of from about 10:1:0.5 to about 1.5:1:0.03 3'-sialyllactose to 6'-sialyllactose.

[0010] In a further ~~aspect~~ example the ~~invention~~ disclosure relates to **a composition** for providing nutrition to an infant of from about 6 months to about 12 months of age comprising 3'-sialyllactose, 6'-sialyllactose and sialyllactosamine in a ratio of from about 10:1:0.5 to about 1.5:1:0.03 3'-sialyllactose to 6'-sialyllactose.

[0011] In a further ~~aspect~~ example the ~~invention~~ disclosure relates to **a composition** for providing nutrition to an infant of 12 months of age or over comprising 3'-sialyllactose, 6'-sialyllactose and sialyllactosamine in a ratio of from about 10:1:0.5 to about 1.5:1:0.03 3'-sialyllactose to 6'-sialyllactose.

[0011a] In another aspect, there is provided a composition for providing nutrition to an infant of less than 6 months of age, an infant of from about 6 months to about 12 months of age, or an infant of 12 months of age or over, wherein the composition is formulated to comprise 3'-sialyllactose and 6'-sialyllactose in a ratio of from 10:1 to about 1.5:1 3'-sialyllactose to 6'-sialyllactose, and at least about 0.001% sialyllactosamine on a dry solids basis.

[0012] In a further ~~aspect~~ example the ~~invention~~ disclosure relates to **method of treating** mild cognitive impairment associated with any one or more of age-related cognitive decline, or cognitive impairment associated with any one or more of Alzheimer's disease, vascular disease, frontotemporal lobar degeneration (FTLD), Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, cognitive impairment associated with schizophrenia, chemotherapy-induced neuropathy, Down's syndrome, Korsakoff's disease, cerebral palsy, epilepsy, neuronal ischemia, neuronal reperfusion injury, neuronal trauma, neuronal haemorrhage, neuronal infection, stroke, neuronal exposure to a toxic substance, age-related mental disorders, anxiety disorders, age-related depression, dementia associated with microvascular disorders (such as diabetes, hypotension, stroke induced vascular dementia, and obesity), dementia associated with a disorder of the immune system, dementia associated with a central nervous system (CNS) disorder, dementia associated with hypotension, dementia associated with obesity or vascular dementia comprising administering an effective amount of a sialyloligosaccharide-containing extract.

[0013] As used herein, “treating” includes preventing, delaying or ameliorating.

[0014] Any one or more of the following ~~embodiments~~ examples may relate to any of the ~~aspects~~ disclosure described herein or any combination thereof.

[0015] Preferably the liquid source comprises a combination of two or more sialyloligosaccharide-containing streams.

[0016] Preferably the liquid source comprises mother liquor as a by-product of lactose crystallisation, a dairy permeate, whey, whey permeate, or a combination of any two or more thereof.

[0017] Preferably the heat treatment is carried out at a temperature of from about 67 to about 100°C.

[0018] Preferably the heat treatment of the liquid source is carried out for about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 minutes, and suitable ranges may be selected from between any of these values.

[0019] Preferably the heat treatment of the liquid source precipitates about 2, 3, 4, 5, 6, 7, 8, 9 or 10% of the minerals present in the liquid source, and suitable ranges may be selected from between any of these values.

[0020] Preferably the first permeate comprises

less than about 96% of the minerals in the liquid source, and/or

less than about 20% of the protein in the liquid source.

[0021] Preferably the first retentate comprises

at least about 3% of the minerals in the liquid source, and/or

at least about 80% of the protein in the liquid source.

[0022] Preferably the filtration step results in a reduction in the amount of insoluble minerals and protein in the first permeate, when compared to the liquid source.

[0023] Preferably the filtration step of (b)(i) is carried out at about 50, 55, 60, 65, 70, 75, 80, 85 or 90°C to produce a first retentate and first permeate, and suitable ranges may be selected from between any of these values.

[0024] Preferably the heat resistant filter of (b)(i) is a ceramic filter.

[0025] Preferably the filtration step of (b)(ii) is carried out at about 55, 60, 65, or about 70°C to produce a first retentate and first permeate, and suitable ranges may be selected from between any of these values.

[0026] Preferably the filtration step of (b)(ii) is carried out using a heat resistant filter.

drying the sialyloligosaccharide-containing extract or crystallised sialyloligosaccharide-containing extract to produce a dried sialyloligosaccharide-containing extract.

[0047] Preferably the sialyloligosaccharide-containing extract comprises less than about 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4 3, 2, or 1% by weight protein and non-protein nitrogen, and suitable ranges may be selected from between any of these values.

[0048] Preferably the sialyloligosaccharide-containing extract comprises less than about 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1% by weight ash, and suitable ranges may be selected from between any of these values.

[0049] Preferably the sialyloligosaccharide-containing extract comprises, on a dry solids basis

- at least about 7% by weight sialyloligosaccharides,
- at least about 0.2% by weight neutral oligosaccharides,
- less than about 5% by weight protein and non-protein nitrogen,
- less than about 80% by weight non-sialyloligosaccharide sugars, and
- less than about 5% by weight ash.

[0050] Preferably the sialyloligosaccharide-containing extract comprises, on a dry solids basis

- at least about 5% by weight 3'-sialyllactose, and
- at least about 2% by weight 6'-sialyllactose.

[0051] In a further ~~aspect~~ example the ~~invention~~ disclosure relates to a sialyloligosaccharide-containing extract produced by any one of the methods described above.

[0052] Preferably the composition comprises, on a dry solids basis, a greater amount of 3'-sialyllactose relative to the amount of 6'-sialyllactose.

[0053] Preferably the composition comprises 3'-sialyllactose and 6'-sialyllactose in a ratio of from about 65:1 to about 1.5:1, from about 15:1 to about 1.5:1, or from about 8:1 to about 1.5:1, and suitable ranges may be selected from between any of these values.

[0054] In a further ~~aspect~~ example the ~~invention~~ disclosure relates to a nutritional formulation comprising a sialyloligosaccharide-containing extract produced by a method as

described above, or comprises a sialyloligosaccharide-enriched composition as described above.

[0055] Preferably the nutritional formulation is an infant formula, a follow-on formula, a growing-up formula, a paediatric formula, a human milk fortifier, a children's food or drink, a maternal supplement, a maternal nutritional formulation, a fermented food, a UHT milk, a UHT drinking yoghurt, an acidified milk drink, a UHT powder, a medical food, a sports nutritional formulation, or a formulation for senior or aged populations.

[0056] Preferably the nutritional formulation comprises one or more dairy lipids, one or more galactooligosaccharides, one or more probiotic strains, one or more protein hydrolysates, lactoferrin, a milk fat globular membrane (MFGM) formulation, other prebiotics, or a combination of any two or more thereof.

[0057] Preferably the nutritional formulation the dairy lipid comprises buttermilk, high fat whey protein concentrate, beta-serum, butter serum, or a combination of any two or more thereof.

[0058] Preferably the probiotic strain comprises a *Bifidobacterium lactis* or a *Lactobacillus rhamnosus* strain, or a combination of both *Bifidobacterium lactis* and *Lactobacillus rhamnosus*.

[0059] Preferably the *Bifidobacterium lactis* strain comprises *Bifidobacterium lactis* HN019 (DR10™).

[0060] Preferably the *Lactobacillus rhamnosus* strain comprises *Lactobacillus rhamnosus* HN001 (DR20™).

[0061] In a further ~~aspect~~ example the ~~invention~~ disclosure relates to a method of maintaining or increasing brain function or brain development, the method comprising administering (i) an effective amount of a sialyloligosaccharide-containing extract produced by the method as described above, or (ii) a nutritional formulation as described above, to a normal subject.

[0062] Preferably the sialyloligosaccharide-containing extract comprises

a) one or more structural glycoconjugates of sialic acid,

b) free sialic acid, or

c) both (a) and (b).

[0063] Preferably the extract, composition or nutritional formulation is administered to

a) prevent delayed brain development,
b) maintain the viability or function of neuronal cells,
c) maintain or increase cognitive development,
d) prevent delayed cognitive development,
e) maintain or increase cognitive performance,
f) prevent cognitive decline,
g) maintain or increase learning, memory or attention span,
h) prevent decline of learning ability, memory or attention span,
i) maintain or increase ability to cope with stress,
j) maintain or increase the level of neural cell adhesion molecule (NCAM),
k) prevent decline in brain function,
l) maintain or increase the level of gangliosides in the brain,
m) maintain or increase blood, nutrient or oxygen flow to the brain,
n) maintain or increase synaptogenesis,
o) maintain or increase the activity of the gut/brain axis,
p) maintain or increase neurogenesis in an older brain,
q) maintain or increase white matter volume, or
r) a combination of any two or more of a) to q).

[0064] Preferably the subject is a foetal, infant or child subject.

[0065] Preferably the extract, composition or nutritional formulation is administered to a mother during gestation to maintain or increase brain function or development in a foetal subject.

[0066] Preferably the extract, composition or formulation is administered to a breastfeeding mother to maintain or increase brain function or development in an infant subject.

[0067] In one ~~embodiment~~ example the administration of the composition of the ~~invention~~ disclosure to an individual leads to an increase in glycerophosphorylcholine (GPC) in the brain in that individual of at least about 10, 15, 20, 25, 30, 35, 40 or 50%, and suitable ranges may be selected from between any of these values.

[0068] In one ~~embodiment~~ example the administration of the composition of the ~~invention~~ disclosure to an individual leads to an increase in glutamine (Glu) in the brain in that individual of at least about 10, 15, 20, 25, 30, 35, 40 or 50%, and suitable ranges may be selected from between any of these values.

[0069] In one ~~embodiment~~ example the administration of the composition of the ~~invention~~ disclosure to an individual leads to an increase in myo inositol (Ins) in the brain in that individual of at least about 10, 15, 20, 25, 30, 35, 40 or 50%, and suitable ranges may be selected from between any of these values.

[0070] In one ~~embodiment~~ example the administration of the composition of the ~~invention~~ disclosure to an individual leads to a decrease in Lip09 in the brain in that individual of at least about 10, 15, 20, 25, 30, 35, 40 or 50%, and suitable ranges may be selected from between any of these values.

[0071] In one ~~embodiment~~ example the administration of the composition of the ~~invention~~ disclosure to an individual leads to a decrease in macromolecules (MM) in the brain in that individual of at least about 10, 15, 20, 25, 30, 35, 40 or 50%, and suitable ranges may be selected from between any of these values.

[0072] In one ~~embodiment~~ example the administration of the composition of the ~~invention~~ disclosure to an individual leads to an increase in N-Acetylaspartylglutamic acid (NAAG) in the brain in that individual of at least about 10, 15, 20, 25, 30, 35, 40 or 50%, and suitable ranges may be selected from between any of these values.

[0073] In one ~~embodiment~~ example the administration of the composition of the ~~invention~~ disclosure to an individual leads to an increase in the glutamate-glutamine complex (Glx) in the brain in that individual of at least about 10, 15, 20, 25, 30, 35, 40 or 50%, and suitable ranges may be selected from between any of these values.

[0074] In a further ~~aspect~~ example the ~~invention~~ disclosure relates to a method of treating mild cognitive impairment associated with any one or more of age-related cognitive decline, dementia, Alzheimer's disease, vascular disease, frontotemporal lobar degeneration (FTLD), dementia with Lewy bodies, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, cognitive impairment associated with schizophrenia, chemotherapy-induced neuropathy, Down's syndrome, Korsakoff's disease, cerebral palsy, epilepsy, neuronal ischemia, neuronal reperfusion injury, neuronal trauma, neuronal haemorrhage, neuronal infection, stroke, neuronal

exposure to a toxic substance, age-related mental disorders, anxiety disorders, age-related depression, dementia associated with microvascular disorders (such as diabetes, hypotension, stroke induced vascular dementia, and obesity), dementia associated with a disorder of the immune system, dementia associated with a central nervous system (CNS) disorder, dementia associated with hypotension, dementia associated with obesity or vascular dementia comprising administering an effective amount of a sialyloligosaccharide-containing extract produced by the method ~~of any one of claims 1 to 32~~as described herein, an extract or composition ~~of any one of claims 33 to 36 or 44 to 46~~as described herein, or a nutritional formulation ~~of any one of claims 37 to 43~~as described herein to a subject in need thereof.

[0075] In a further ~~aspect~~ example the ~~invention~~ disclosure relates to a sialyloligosaccharide-containing extract produced by a method ~~of any one of claims 1 to 32~~as described herein, a composition ~~of any one of claims 33 to 38 or 44 to 46~~as described herein, or a nutritional formulation ~~of any one of claims 37 to 43~~as described herein for treating mild cognitive impairment associated with any one or more of age-related cognitive decline, dementia, Alzheimer's disease, vascular disease, frontotemporal lobar degeneration (FTLD), dementia with Lewy bodies, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, cognitive impairment associated with schizophrenia, chemotherapy-induced neuropathy, Down's syndrome, Korsakoff's disease, cerebral palsy, epilepsy, neuronal ischemia, neuronal reperfusion injury, neuronal trauma, neuronal haemorrhage, neuronal infection, stroke, neuronal exposure to a toxic substance, age-related mental disorders, anxiety disorders, age-related depression, dementia associated with microvascular disorders (such as diabetes, hypotension, stroke induced vascular dementia, and obesity), dementia associated with a disorder of the immune system, dementia associated with a central nervous system (CNS) disorder, dementia associated with hypotension, dementia associated with obesity or vascular dementia.

[0076] In a further ~~aspect~~ example the ~~invention~~ disclosure relates to a the use of a sialyloligosaccharide-containing extract produced by the method ~~of any one of claims 1 to 32~~as described herein, or a composition ~~of any one of claims 33 to 36 or 44 to 46~~as described herein, in the manufacture of a formulation for treating mild cognitive impairment associated with any one or more of age-related cognitive decline, dementia, Alzheimer's disease, vascular disease, frontotemporal lobar degeneration (FTLD), dementia with Lewy bodies, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, cognitive impairment associated with schizophrenia, chemotherapy-induced

neuropathy, Down's syndrome, Korsakoff's disease, cerebral palsy, epilepsy, neuronal ischemia, neuronal reperfusion injury, neuronal trauma, neuronal haemorrhage, neuronal infection, stroke, neuronal exposure to a toxic substance, age-related mental disorders, anxiety disorders, age-related depression, dementia associated with microvascular disorders (such as diabetes, hypotension, stroke induced vascular dementia, and obesity), dementia associated with a disorder of the immune system, dementia associated with a central nervous system (CNS) disorder, dementia associated with hypotension, dementia associated with obesity or vascular dementia.

[0076a] In another aspect, there is provided use of a sialyloligosaccharide-enriched extract produced by the method as described herein, a sialyloligosaccharide-enriched composition as described herein, a nutritional formulation as described herein or a composition as described herein in the manufacture of a medicament for

- maintaining or increasing brain function or brain development in an otherwise healthy subject, or
- treating mild cognitive impairment associated with any one or more of age-related cognitive decline, dementia, Alzheimer's disease, vascular disease, frontotemporal lobar degeneration (FTLD), dementia with Lewy bodies, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, cognitive impairment associated with schizophrenia, chemotherapy-induced neuropathy, Down's syndrome, Korsakoff's disease, cerebral palsy, epilepsy, neuronal ischemia, neuronal reperfusion injury, neuronal trauma, neuronal haemorrhage, neuronal infection, stroke, neuronal exposure to a toxic substance, age-related mental disorders, anxiety disorders, age-related depression, dementia associated with microvascular disorders (such as diabetes, hypotension, stroke induced vascular dementia, and obesity), dementia associated with a disorder of the immune system, dementia associated with a central nervous system (CNS) disorder, dementia associated with hypotension, dementia associated with obesity or vascular dementia in a subject in need thereof.

[0076b] In another aspect, there is provided a sialyloligosaccharide-enriched extract produced by the method as described herein, a sialyloligosaccharide-enriched composition as described herein, a nutritional formulation as described herein or a composition as described herein for use in therapy.

[0077] It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7).

[0078] This ~~invention~~ disclosure may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, and any or all combinations of any two or more of said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which this ~~invention~~ disclosure relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

[0079] In this specification, where reference has been made to external sources of information, including patent specifications and other documents, this is generally for the purpose of providing a context for discussing the features of the present ~~invention~~ disclosure. Unless stated otherwise, reference to such sources of information is not to be construed, in any jurisdiction, as an admission that such sources of information are prior art or form part of the common general knowledge in the art.

[0080] The term "comprising" as used in this specification means "consisting at least in part of". When interpreting statements in this specification which include that term, the features, prefaced by that term in each statement, all need to be present but other features can also be present. Related terms such as "comprise" and "comprised" are to be interpreted in the same manner.

CLAIMS:

1. A method of producing sialyloligosaccharides from a liquid source that comprises sialyloligosaccharides, carbohydrates and minerals by subjecting the liquid source to a process comprising

a) heating the liquid source to a temperature of from about 67°C,

b) subjecting the heat-treated liquid source to

i) a filtration step with a heat-resistant filter at a temperature of about ~~35~~ 55 to about ~~95~~90°C to produce a first retentate and first permeate, or

ii) centrifugal separation to produce a light phase and a heavy phase and ultrafiltration of the light phase at a temperature of about 50 to about 70°C to produce a first retentate and first permeate,

c) subjecting the first permeate to nanofiltration, or nanofiltration and diafiltration, to produce a second retentate and second permeate, and

d) concentrating the second retentate,

to produce a sialyloligosaccharide-containing extract.

2. The method of claim 1, wherein the ultrafiltration step of (b)(ii) is carried out at about ~~50~~ 55 to about ~~90~~70°C or at about 60 to about 70°C to produce a first retentate and first permeate, and the ultrafiltration step of (b)(ii) is carried out using a heat resistant filter.

~~3. The method of claim 1 wherein the filtration step of (b)(i) is carried out at about 50 to about 70°C to produce a first retentate and first permeate.~~

~~4. The method of any one of claims 1 to 3 wherein the heat resistant filter is a ceramic filter.~~

~~5. The method of any one of claims 1 to 4 wherein the filtration step of (b)(ii) is carried out at about 55 to about 70°C to produce a first retentate and first permeate.~~

~~6. The method of any one of claims 1 to 5 wherein the filtration step of (b)(ii) is carried out at about 60 to about 70°C to produce a first retentate and first permeate.~~

~~7. The method of any one of claims 1 to 6 wherein the filtration step of (b)(ii) is carried out using a heat resistant filter.~~

~~8. The method of any one of claims 1 to 7 wherein the heat resistant filter is a ceramic filter.~~

~~9. The method of any one of claims 1 to 8 wherein the liquid source comprises a combination of two or more sialyloligosaccharide-containing streams.~~

~~10.~~3. The method of ~~any one of claims 1 to 9~~claim 1 or 2, wherein the liquid source comprises mother liquor as a by-product of lactose crystallisation, a dairy permeate, whey, whey permeate, or a combination of any two or more thereof.

~~11.~~4. The method of any one of claims 1 to ~~11~~ 3, wherein the heat treatment ~~is carried out at a temperature of from about 67 to about 100°C.~~of the liquid source

a) is carried out at a temperature of from about 67 to about 100°C,
b) is carried out for about 2 to about 20 minutes,
c) precipitates about 2% to about 10% of the minerals present in the liquid source, or
d) any combination of any two or more of a) to c).

~~12. The method of any one of claims 1 to 12 wherein the heat treatment of the liquid source is carried out for about 2 to about 20 minutes.~~

~~13. The method step of any one of claims 1 to 13 wherein the heat treatment of the liquid source precipitates about 2% to about 10% of the minerals present in the liquid source.~~

~~14. A method of any one of claims 1 to 14 wherein the first permeate comprises less than about 96% of the minerals in the liquid source, and/or less than about 20% of the protein in the liquid source.~~

~~15. A method of any one of claims 1 to 14 wherein the first retentate comprises at least about 3% of the minerals in the liquid source, and/or at least about 80% of the protein in the liquid source.~~

~~16. The method of any one of claims 1 to 16 wherein the filtration step results in a reduction in the amount of insoluble minerals and protein in the first permeate, when compared to the liquid source.~~

~~17. The method of any one of claims 1 to 17 wherein the heat resistant filter is a ceramic filter.~~

~~18. The method of claim 18 wherein the heat treated liquid source is subjected to ultrafiltration with a ceramic filter.~~

~~19.~~5. The method of any one of claims 1 to ~~19~~ 4, wherein the method comprises ~~including~~ at least one glycoside hydrolase or polymerase enzyme reaction.

~~20.~~6. The method of claim ~~20~~ 5, wherein

a) the first permeate of the filtration step from the heat-resistant filter,

b) the light phase,

c) the concentrated second retentate, or

d) a combination of any two or more of (a) to (c),

is subjected to the enzyme reaction, optionally wherein the enzyme comprises β-galactosidase.

~~21. The method of claim 20 or 21 wherein the enzyme reaction~~

- ~~increases the molecular size of at least one disaccharide in the source to produce at least one oligosaccharide, or~~
- ~~decreases the molecular size of at least one disaccharide in the source to produce one or more monosaccharides.~~

~~22. The method of any one of claims 20 to 22 wherein the enzyme comprises β-galactosidase.~~

~~23. The method of any one of claims 20 to 23 wherein the disaccharide comprises lactose.~~

~~24. The method of any one of claims 20 to 24 wherein the monosaccharide comprises galactose or glucose.~~

~~25. The method of any one of claims 20 to 25 wherein the oligosaccharide comprises one or more galactooligosaccharides.~~

~~26. The method of any one of claims 1 to 26 wherein the filtration of the light phase is ultrafiltration.~~

~~27. The method of claim 27 wherein the ultrafiltration is conducted at a temperature of from about 50 °C to about 70 °C, and preferably from about 55 °C to about 65 °C.~~

~~28.~~7. The method of any one of claims 1 to ~~28~~ 6, wherein the nanofiltration is conducted at a temperature of from about 25 °C to about 55 °C, ~~preferably~~ optionally about 50 °C.

~~29. The method of any one of claims 1 to 29 wherein the second permeate comprises soluble minerals and at least one monosaccharide or disaccharide.~~

~~30. The method of any one of claims 1 to 30 wherein the second retentate comprises~~

~~less than about 70% of the minerals in the first permeate, or~~

~~less than about 50% of the non-sialyloligosaccharide sugars in the first permeate.~~

~~31. The method of any one of claims 1 to 31 wherein the second retentate comprises~~

~~a) at least about 7% by weight sialyloligosaccharides,~~

~~b) at least about 0.2% by weight neutral oligosaccharides,~~

~~c) less than about 5% by weight protein and non-protein nitrogen,~~

~~d) less than about 80% by weight non-sialyloligosaccharide sugars, and~~

~~e) less than about 5% by weight ash.~~

~~32. The method of any one of claims 1 to 32 wherein the second permeate comprises~~

~~at least about 30% of the minerals in the first permeate, or~~

~~at least about 50% of the non-sialyloligosaccharide sugars in the first permeate.~~

~~33.~~8. The method of any one of claims 1 to ~~33~~ 7, wherein the second retentate is subjected to either nanofiltration or reverse osmosis to produce a third retentate.

~~34. The method of claim 34 wherein the concentrated second retentate, or the third retentate, is subjected to decolourisation.~~

~~35. The method of any one of claims 1 to 35 further comprising the steps of~~

- ~~subjecting the sialyloligosaccharide-containing extract to crystallisation to produce a crystallised sialyloligosaccharide-containing extract, and~~
- ~~drying the sialyloligosaccharide-containing extract or crystallised sialyloligosaccharide-containing extract to produce a dried sialyloligosaccharide-containing extract.~~

~~36.~~9. The method of any one of claims 1 to ~~36~~ 8, wherein the sialyloligosaccharide-containing extract comprises, on a dry solids basis

- at least about 7% by weight sialyloligosaccharides,
- at least about 0.2% by weight neutral oligosaccharides,
- less than about 5% by weight protein and non-protein nitrogen,

- less than about 80% by weight non-sialyloligosaccharide sugars, and
- less than about 5% by weight ash.

~~37.~~10. The method of any one of claims 1 to ~~37~~ 9, wherein the sialyloligosaccharide-containing extract comprises, on a dry solids basis

- at least about 5% by weight 3'-sialyllactose, and
- at least about 2% by weight 6'-sialyllactose.

~~38.~~11. A sialyloligosaccharide-containing extract produced by the method of any one of claims 1 to ~~38~~10.

~~39.~~12. A sialyloligosaccharide-enriched composition, comprising on a dry solids basis

- at least about 5% by weight total sialyloligosaccharides comprising
  - i) at least about 5% 3'-sialyllactose by weight of total sialyloligosaccharides,
  - ii) at least about 2% 6'-sialyllactose by weight of total sialyloligosaccharides,
  - iii) at least about 0.01% disialyllactose by weight of total sialyloligosaccharides,
  - iv) at least about 0.01% sialyllactosamine by weight of total sialyloligosaccharides, and
- at least about 0.5% by weight free sialic acid,
- at least about 0.2% by weight neutral oligosaccharides,
- less than about 25% by weight protein and non-protein nitrogen,
- less than about 80% by weight sugars not including sialyllactose, and
- less than about 5% by weight ash.

~~40.~~13. The composition of claim ~~40~~ 12 comprising, on a dry solids basis, a greater amount of 3'-sialyllactose relative to the amount of 6'-sialyllactose, optionally comprising 3'-sialyllactose and 6'-sialyllactose in a ratio of from about 65:1 to about 1.5:1, from about 15:1 to about 1.5:1, or from about 8:1 to about 1.5:1.

~~41. The composition of claim 40 or 41 comprising 3'-sialyllactose and 6'-sialyllactose in a ratio of from about 65:1 to about 1.5:1, from about 15:1 to about 1.5:1, or from about 8:1 to about 1.5:1.~~

~~42.~~14. A nutritional formulation comprising a sialyloligosaccharide-containing extract produced by a method of any one of claims 1 to ~~38~~10, or a sialyloligosaccharide-enriched composition of any one of claims ~~40~~ 11 to ~~42~~13.

~~43. The nutritional formulation of claim 43 wherein the nutritional formulation is an infant formula, a follow-on formula, a growing-up formula, a paediatric formula, a human milk fortifier, a children's food or drink, a maternal supplement, a maternal nutritional formulation, a fermented food, a UHT milk, a UHT drinking yoghurt, an acidified milk drink, a UHT powder, a medical food, a sports nutritional formulation, or a formulation for senior or aged populations.~~

~~44.~~15. The nutritional formulation of claim ~~43 or 44~~14, further comprising one or more dairy lipids, one or more galactooligosaccharides, one or more probiotic strains, one or more protein hydrolysates, lactoferrin, a milk fat globular membrane (MFGM) formulation, or a combination of any two or more thereof.

~~45. The nutritional formulation of claim 45 wherein the dairy lipid comprises buttermilk, high fat whey protein concentrate, beta-serum, butter serum, or a combination of any two or more thereof.~~

~~46.~~16. The nutritional formulation of claim ~~45~~ 14 or ~~46~~ 15, wherein the one or more probiotic strains comprises a *Bifidobacterium lactis* or a *Lactobacillus rhamnosus* strain, or a combination of both *Bifidobacterium lactis* and *Lactobacillus rhamnosus*, optionally wherein the *Bifidobacterium lactis* strain comprises *Bifidobacterium lactis* HN019 (DR10™), and/or optionally wherein the *Lactobacillus rhamnosus* strain comprises *Lactobacillus rhamnosus* HN001 (DR20™).

~~47. The nutritional formulation of claim 47 wherein the *Bifidobacterium lactis* strain comprises *Bifidobacterium lactis* HN019 (DR10™).~~

~~48. The nutritional formulation of claim 47 wherein the *Lactobacillus rhamnosus* strain comprises *Lactobacillus rhamnosus* HN001 (DR20™).~~

~~49.~~17. A composition for providing nutrition to an infant of less than 6 months of age, an infant of from about 6 months to about 12 months of age, or an infant of 12 months of age or over, wherein the composition is formulated to comprise ~~comprising~~ 3'-sialyllactose and 6'-sialyllactose in a ratio of from 10:1 to about 1.5:1 3'-sialyllactose to 6'-sialyllactose, and at least about 0.001% sialyllactosamine on a dry solids basis.

~~50. A composition for providing nutrition to an infant of from about 6 months to about 12 months of age comprising 3'-sialyllactose and 6'-sialyllactose in a ratio of from about 10:1 to about 1.5:1 sialyllactose to 6'-sialyllactose.~~

51. A composition for providing nutrition to an infant of 12 months of age or over comprising 3’-sialyllactose and 6’-sialyllactose in a ratio of from about 10:1 to about 1.5:1 sialyllactose to 6’-sialyllactose.

52. A sialyloligosaccharide-containing extract or composition produced by the method of any one of claims 1 to 38, or as claimed in any one of claims 40 to 42 or 50 to 52, or a nutritional formulation of any one of claims 43 to 49 for use in maintaining or increasing brain function or brain development.

53. A non therapeutic method of **maintaining or increasing brain function or brain development**, the method comprising administering an effective amount of a sialyloligosaccharide-containing extract produced by the method of any one of claims 1 to 39, a composition of any one of claims 40 to 42 or 50 to 52, or a nutritional formulation of any one of claims 43 to 49 in an otherwise healthy subject.

54. A method of claim 54 wherein the sialyloligosaccharide-containing extract comprises

a) one or more structural glycoconjugates of sialic acid,
b) free sialic acid, or
c) both (a) and (b).

55. The method of claim 54 or 55 wherein the extract, composition or nutritional formulation is administered to

a) treat or prevent delayed brain development,
b) maintain the viability or function of neuronal cells,
c) maintain or increase cognitive development,
d) prevent delayed cognitive development,
e) maintain or increase cognitive performance,
f) prevent cognitive decline,
g) maintain or increase learning, memory or attention span,
h) prevent decline of learning ability, memory or attention span,
i) maintain or increase ability to cope with stress,
j) prevent decline in brain function,
k) maintain or increase the level of gangliosides in the brain,
l) maintain or increase the level of neural cell adhesion molecule (NCAM),
m) maintain or increase blood, nutrient or oxygen flow to the brain,
n) maintain or increase synaptogenesis,

o) maintain or increase the activity of the gut/brain axis,

p) maintain or increase neurogenesis in an older brain,

q) maintain or increase white matter volume, or

r) a combination of any two or more of a) to q).

56. The method of any one of claims 54 to 56 wherein the subject is a foetal, infant or child subject.

57. The method of any one of claims 54 to 57 wherein the extract, composition or nutritional formulation is administered to a mother during gestation to maintain or increase brain function or development in a foetal subject.

58. The method of any one of claims 54 to 58 wherein the extract, composition or formulation is administered to a breastfeeding mother to maintain or increase brain function or development in an infant subject.

59. A method of treating mild cognitive impairment associated with any one or more of age-related cognitive decline, dementia, Alzheimer's disease, vascular disease, frontotemporal lobar degeneration (FTLD), dementia with Lewy bodies, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, cognitive impairment associated with schizophrenia, chemotherapy-induced neuropathy, Down's syndrome, Korsakoff's disease, cerebral palsy, epilepsy, neuronal ischemia, neuronal reperfusion injury, neuronal trauma, neuronal haemorrhage, neuronal infection, stroke, neuronal exposure to a toxic substance, age-related mental disorders, anxiety disorders, age-related depression, dementia associated with microvascular disorders (such as diabetes, hypotension, stroke induced vascular dementia, and obesity), dementia associated with a disorder of the immune system, dementia associated with a central nervous system (CNS) disorder, dementia associated with hypotension, dementia associated with obesity or vascular dementia comprising administering an effective amount of a sialyloligosaccharide-containing extract produced by the method of any one of claims 1 to 32, an extract or composition of any one of claims 33 to 36 or 44 to 46, or a nutritional formulation of any one of claims 37 to 43 to a subject in need thereof.

60. A sialyloligosaccharide-containing extract produced by a method of any one of claims 1 to 39, a composition of any one of claims 40 to 42 or 50 to 52, or a nutritional formulation of any one of claims 43 to 49 for treating mild cognitive impairment associated with any one or more of age-related cognitive decline, dementia, Alzheimer's disease, vascular disease, frontotemporal lobar degeneration (FTLD), dementia with Lewy bodies, Parkinson's disease, amyotrophic lateral

~~sclerosis, Huntington's disease, cognitive impairment associated with schizophrenia, chemotherapy-induced neuropathy, Down's syndrome, Korsakoff's disease, cerebral palsy, epilepsy, neuronal ischemia, neuronal reperfusion injury, neuronal trauma, neuronal haemorrhage, neuronal infection, stroke, neuronal exposure to a toxic substance, age-related mental disorders, anxiety disorders, age-related depression, dementia associated with microvascular disorders (such as diabetes, hypotension, stroke induced vascular dementia, and obesity), dementia associated with a disorder of the immune system, dementia associated with a central nervous system (CNS) disorder, dementia associated with hypotension, dementia associated with obesity or vascular dementia, infantile onset severe development delay (associated with mutations with the gene encoding for N-acetylneuraminic acid) and distal myopathy with rimmed vacuoles (DMRV).~~

~~61. Use of a sialyloligosaccharide-containing extract produced by the method of any one of claims 1 to 32, or a composition of any one of claims 33 to 36 or 44 to 46, in the manufacture of a formulation for treating mild cognitive impairment associated with any one or more of age-related cognitive decline, dementia, Alzheimer's disease, vascular disease, frontotemporal lobar degeneration (FTLD), dementia with Lewy bodies, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, cognitive impairment associated with schizophrenia, chemotherapy-induced neuropathy, Down's syndrome, Korsakoff's disease, cerebral palsy, epilepsy, neuronal ischemia, neuronal reperfusion injury, neuronal trauma, neuronal haemorrhage, neuronal infection, stroke, neuronal exposure to a toxic substance, age-related mental disorders, anxiety disorders, age-related depression, dementia associated with microvascular disorders (such as diabetes, hypotension, stroke induced vascular dementia, and obesity), dementia associated with a disorder of the immune system, dementia associated with a central nervous system (CNS) disorder, dementia associated with hypotension, dementia associated with obesity or vascular dementia or infantile onset severe development delay (associated with mutations with the gene encoding for N-acetylneuraminic acid) and distal myopathy with rimmed vacuoles (DMRV).~~

18. The nutritional formulation of any one of claims 14 to 16 or the composition of claim 17, wherein the nutritional formulation or composition comprises

- at least about 0.005% or 0.01% sialyllactosamine on a dry solids basis, and/or
- 3'-sialyllactose, 6'-sialyllactose and sialyllactosamine in a ratio of from about 10:1:0.5 to about 1.5:1:0.03.

19. Use of a sialyloligosaccharide-enriched extract produced by the method of any one of claims 1 to 10, a sialyloligosaccharide-enriched composition of any one of claims 11 to 13 or 17, a nutritional formulation of any one of claims 14 to 17 or 18 or a composition of claim 17 or 18 in the manufacture of a medicament for

- maintaining or increasing brain function or brain development in an otherwise healthy subject, or

- treating mild cognitive impairment associated with any one or more of age-related cognitive decline, dementia, Alzheimer's disease, vascular disease, frontotemporal lobar degeneration (FTLD), dementia with Lewy bodies, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, cognitive impairment associated with schizophrenia, chemotherapy-induced neuropathy, Down's syndrome, Korsakoff's disease, cerebral palsy, epilepsy, neuronal ischemia, neuronal reperfusion injury, neuronal trauma, neuronal haemorrhage, neuronal infection, stroke, neuronal exposure to a toxic substance, age-related mental disorders, anxiety disorders, age-related depression, dementia associated with microvascular disorders (such as diabetes, hypotension, stroke induced vascular dementia, and obesity), dementia associated with a disorder of the immune system, dementia associated with a central nervous system (CNS) disorder, dementia associated with hypotension, dementia associated with obesity or vascular dementia in a subject in need thereof.

20. The use of claim 19, wherein the extract, composition or nutritional formulation is to be administered to

a) treat or prevent delayed brain development,
b) maintain the viability or function of neuronal cells,
c) maintain or increase cognitive development,
d) prevent delayed cognitive development,
e) maintain or increase cognitive performance,
f) prevent cognitive decline,
g) maintain or increase learning, memory or attention span,
h) prevent decline of learning ability, memory or attention span,
i) maintain or increase ability to cope with stress,
j) prevent decline in brain function,
k) maintain or increase the level of gangliosides in the brain,
l) maintain or increase the level of neural cell adhesion molecule (NCAM),

m) maintain or increase blood, nutrient or oxygen flow to the brain,

n) maintain or increase synaptogenesis,

o) maintain or increase the activity of the gut/brain axis,

p) maintain or increase neurogenesis in an older brain,

q) maintain or increase white matter volume, or

r) a combination of any two or more of a) to q).

21. The use of claim 19 or 20, wherein

- the subject is a foetal, infant or child subject,
- the extract, composition or nutritional formulation is to be administered to a mother during gestation to maintain or increase brain function or development in a foetal subject, or
- the extract, composition or formulation is to be administered to a breastfeeding mother to maintain or increase brain function or development in an infant subject.

22. A sialyloligosaccharide-enriched extract produced by the method of any one of claims 1 to 10, a sialyloligosaccharide-enriched composition of any one of claims 11 to 13 or 17, a nutritional formulation of any one of claims 14 to 17 or 18 or a composition of claim 17 or 18 for use in therapy.