

CLAIMS

1. A method of preparing a sugar reduced product from a biomass comprising:
 - i) treating the biomass with fermentation enzymes to reduce the sugar
5 concentration; and
 - ii) post-treating the material obtained by step i) to further reduce the sugar concentration.
2. The method of claim 1, wherein step i) comprises fermentation of the biomass
10 with one or more bacteria selected from lactic acid, acetic acid, propionic acid and bifido bacteria.
3. The method of claim 2, wherein the fermentation is anaerobic.
- 15 4. The method of claim 2 or claim 3, wherein the bacteria are removed after step i) or step ii).
5. The method of any one of claims 1 to 4, wherein the fermentation enzymes are produced by one or more of lactic acid, acetic acid, propionic and bifido bacteria
20 cultured in biomass before step i).
6. The method of any one of claims 1 to 5, wherein post-treating comprises one or more of the following:
 - i) microwaving,
 - 25 ii) heating,
 - iii) exposure to high frequency sound waves (ultrasound), and
 - iv) exposure to high hydrostatic pressure.
7. The method of claim 6, wherein post-treating increases the activity of the
30 fermentation enzymes.
8. The method of claim 6, wherein heating comprises high temperature short time (HTST).
- 35 9. The method of claim 8, wherein HTST is at about 100°C for about 5 to about 20 seconds.

10. The method of any one of claims 1 to 7, wherein the post-treating comprises microwaving for at least 1 minute.
- 5 11. The method of claim 6, wherein high hydrostatic pressure comprises treatment with 150 to 800 MPa.
12. The method of any one of claims 1 to 11, wherein the sugar in the material obtained in step i) is reduced by about 10 to about 70 % compared to the biomass.
- 10 13. The method of any one of claims 1 to 12, wherein the sugar in the material obtained in step ii) is reduced by about 5 to about 50% compared to the sugar in the material obtained in step i).
- 15 14. The method of any one of claims 1 to 11, wherein the sugar in the material obtained by step ii) is reduced by at least 30%, or at least 40%, or at least 50%, or at least 60% compared to the biomass.
- 20 15. The method of any one claims 1 to 14, wherein the sugar is one or more or all of sucrose, glucose, fructose and lactose.
16. The method of any one of claims 1 to 15, wherein the sugar is sucrose.
17. The method of any one of claims 1 to 16, wherein the concentration of an oligosaccharide is increased in the material obtained by step ii) compared to the biomass.
- 25 18. The method of claim 17, wherein the oligosaccharide is selected from one or more or all of:
- 30 i) a gluco-oligosaccharide,
ii) a fructo-oligosaccharide,
iii) a isomalto-oligosaccharide; and
iv) galactooligosaccahrde.
- 35 19. The method of claim 18, wherein the isomalto-oligosaccharide is panose.

20. The method of any one of claims 1 to 19, wherein the concentration of a polysaccharide is increased in the material obtained by step ii) compared to the biomass.
- 5 21. The method of claim 20, wherein the polysaccharide is selected from one or more of: dextran, levan and inulin type fructans.
22. The method of any one of claims 1 to 21, wherein the concentration of one or more of: mannitol, isomaltose and isomaltotriose is increased in the material obtained
10 by step ii) compared to the biomass.
23. The method of any one of claims 1 to 22, wherein the fermentation enzymes were produced by one or more of lactic acid, acetic acid, propionic acid and bifido bacteria.
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24. The method of any one of claims 1 to 23, wherein fermentation enzymes comprise one or more or all of:
i) glycosyltransferase,
ii) glycosidase or aryl glycosidase,
20 iii) pectinase,
iv) esterase,
v) decarboxylase,
vi) tannase, and
vii) oxidoreductase.
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25. The method of claim 24, wherein the glycosyltransferase is selected from one or more or all of:
i) dextranucrase,
ii) levansucrase,
30 iii) inulosucrase
iv) alternansucrase,
v) fructosyltransferases, and
vi) β -galactosidase.
- 35 26. The method of claim 24, wherein the oxidoreductase is mannitol dehydrogenase.

27. The method of any one of claims 1 to 26, wherein the concentration of carotenoid is increased in the material obtained by step ii) compared to the biomass.
28. The method of any one of claims 2 to 27, wherein the lactic acid bacteria is from
5 one or more of the Genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*,
Streptococcus, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*,
Fructobacillus, *Fructobacillus Sporolactobacillus*, *Tetragenococcus*, *Vagococcus* and
Weissella.
- 10 29. The method of claim 28, wherein the lactic acid bacteria is selected from one or
more of *Leuconostoc mesenteroides*, *Lactobacillus reuteri*, *Lactobacillus gasseri* and
Lactococcus lactis.
- 15 30. The method of claim 29, wherein the lactic acid bacteria is *Leuconostoc*
mesenteroides.
31. The method of claim 30, wherein the *Leuconostoc mesenteroides* is selected
from:
i) ATCC 8293;
20 ii) NRRL B-512F;
iii) BF1 deposited under V17/021729 on 25 September 2017 at the National
Measurement Institute Australia; and
iv) BF2 deposited under V17/021730 on 25 September 2017 at the National
Measurement Institute Australia.
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32. The method of any one of claims 2 to 31, wherein the acetic acid bacteria is
Acetobacteraceae.
33. The method of claim 32, wherein the *Acetobacteraceae* is *Gluconacetobacter*.
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34. The method of any one claims 2 to 33, wherein fermentation is for at least 24
hours.
35. The method of any one claims 2 to 34, wherein fermentation is at a pH of about
35 5 to about 6.

36. The method of any one of claims 1 to 35, wherein the biomass is a plant material selected from one or more of: a fruit, vegetable, grass, nut, legume or grass.
37. The method of claim 36 where the plant material is selected from one or more of: juice, juice concentrate, puree, reconstituted fruit or vegetable powder, rehydrated dried fruit pieces, sugary fraction of fruit and vegetable processing, milk, milk concentrate, whey, permeate, retentate, juice, juice concentrate, puree, whole or chopped plant material.
38. The method of any one of claims 1 to 36, wherein the biomass is selected from: animal milk, animal milk concentrate or a product produced thereof.
39. The method of claim 36 or claim 37, wherein the fruit is from a family selected from one or more of: *Arecaceae*, *Myrtaceae*, *Rosaceae*, *Musaceae*, *Ericaceae*, *Saxifragaceae*, *Cucurbitaceae*, *Nightshade*, *Capparaceae*, *Adoxaceae*, *Vitaceae*, *Rutaceae*, *Actinidiaceae*, *Sapindaceae*, *Anacardiaceae*, *Moraceae*, *Oleaceae*, *Cactaceae*, *Passifloraceae*, *Bromeliaceae*, *Cactaceae*, *Lythraceae*, *Polygonaceae*, *Oxalidaceae* and *Caesalpinioidae*.
40. The method of claim 39, wherein the family is *Rosaceae*.
41. The method of claim 40, wherein the *Rosaceae* is an apple.
42. The method of claim 36, wherein the fruit is grape or orange.
43. The method of claim 36 or claim 37, wherein the vegetable is from a family selected from one or more of: *Brassicaceae*, *Amaryllidaceae*, *Asparagaceae*, *Polygonaceae*, *Compositae*, *Amaranthaceae*, *Chenopodiaceae*, *Cucurbitaceae*, *Leguminosae*, *Malvaceae*, *Convolvulaceae*, *Solanaceae* and *Umbelliferae*.
44. The method of claim 36 or claim 37, wherein the grass is from the family *Poaceae*.
45. The method of any one of claims 1 to 44, wherein one of more of the following is added before, during or after step i):
- calcium,

- ii) nitrogen,
- iii) phosphate,
- iv) maltose, and
- v) isomaltose.

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46. The method of any one of claims 1 to 45, wherein the method does not comprise the addition of sucrose.

47. The method of any one of claims 1 to 46, further comprising pre-treating the biomass before step i).

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48. The method of claim 47, wherein pre-treating comprises one or more of:

- i) microwaving,
- ii) heating,
- iii) exposure to high frequency sound waves (ultrasound),
- iv) exposure to high hydrostatic pressure,
- v) pulse electric field processing, and
- vi) exposure to shockwaves.

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49. The method of any one of claims 1 to 48, wherein the product from step i) is combined with a juice or a juice base before step ii).

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50. A method of preparing a sugar reduced product from carrot biomass comprising treating the biomass with fermentation enzymes to reduce the sugar concentration and increase the carotenoid concentration.

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51. The method of claim 50, wherein the fermentation enzymes are from *Leuconostoc mesenteroides* or *Lactobacillus gasseri*.

52. The method of claim 51, wherein treating with fermentation enzymes comprises fermentation.

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53. A method of preparing fermentation enzymes for reducing the sugar concentration of a biomass comprising:

i) inoculating the biomass with one or more bacteria selected from lactic acid, acetic acid, propionic acid and bifido bacteria which have previously been cultured in biomass,

ii) fermenting for a sufficient time for fermentation enzymes to be produced,

5 iii) removing the bacteria or isolating fermentation enzymes secreted by the bacteria or removing the bacteria.

54. A sugar reduced product produced by the method of any one of claims 1 to 52.

10 55. The product of claim 54, wherein the product is juice, juice concentrate, milk, milk concentrate, puree, fruit and/or vegetable pieces or a powder.

56. The product of claim 55, wherein the juice or concentrate is apple juice or apple concentrate.

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57. The product of any one of claims 54 to 56, wherein the product comprises about 40 to about 80 g/L of total sugar.

20 58. The product of any one of claims 54 to 57, wherein the total sugar in the product is reduced by about 30% to about 60% compared to the biomass.

59. The product of any one of claims 54 to 58, wherein the product is a pro-biotic and/or pre-biotic.

25 60. The product of claim 59, wherein the product is a food, supplement, or animal feed.

61. The product of claim 54, wherein the product is a sweetener.

30 62. The product of claim 61, wherein the sweetener is low sugar.