

CLAIMS

1. A method for purifying sialylated oligosaccharides that were produced by microbial fermentation or *in-vitro* biocatalysis, the method comprising the steps of
 - i) separating biomass from the fermentation broth;
 - ii) removing cations from the fermentation broth or reaction mixture;
 - iii) removing anionic impurities from the fermentation broth or reaction mixture; and
 - iv) removing compounds having a molecular weight lower than that of the sialylated oligosaccharide to be purified from the fermentation broth or reaction mixture.
2. The method according to claim 1, further comprising one or more steps selected from the group consisting of
 - v) Increasing the concentration of the sialylated oligosaccharide;
 - vi) removing non-desired oligosaccharides;
 - vii) removing colorants;
 - viii) removing endotoxins;
 - ix) sterilizing; and
 - x) spray-drying or crystallizing the sialylated oligosaccharide.
3. The method according to claim 1 or 2, wherein the sialylated oligosaccharide is a sialylated human milk oligosaccharide, preferably selected from the group consisting of 3'-SL, 6'-SL, LST-a, LST-b, LST-c, 3-F-SL, DS-LNT and F-LST-b.
4. The method according to any one of claims 1 to 3, wherein removing biomass from the fermentation broth is performed by subjecting the fermentation broth to an ultrafiltration, preferably to an ultrafiltration removing the biomass and compounds having a molecular weight of ≥ 500 kDa from the fermentation broth, more preferably to an ultrafiltration removing the biomass and compounds having a molecular weight of ≥ 150 kDa from the fermentation broth, and most preferably to an ultrafiltration removing the biomass and

compounds having a molecular weight of ≥ 100 kDa from the fermentation broth.

5. The method according to any one of claims 1 to 4, wherein removing cations from the fermentation broth is performed by cation exchange chromatography.
- 5 6. The method according to any one of claims 1 to 5, wherein removing anionic impurities from the fermentation broth is performed by anion exchange chromatography.
7. The method according to any one of claims 1 to 6, wherein removing
10 compounds having a molecular weight lower than that of the sialylated oligosaccharide to be purified is performed by cross-flow filtration.
8. The method according to any one of claims 1 to 7, wherein the concentration of the sialylated oligosaccharide to be purified is increased by nanofiltration or evaporation of the solvent.
9. The method according to any one of claims 1 to 87, wherein removing
15 colorants is performed by treating the fermentation broth/solution containing the desired sialylated oligosaccharide with activated carbon.
10. The method according to any one of claims 1 to 9, wherein removing endotoxins is performed by filtration of the solution containing the desired oligosaccharide through a 6 kDa filter or a 3kDa filter.
- 20 11. The method according to any one of claims 1 to 10, wherein sterilizing the solution is performed by filtration of the solution through a 0.2 μm filter.
12. The method according to any one of claims 1 to 11 further comprising a step of SMB chromatography.
13. The method according to any one of claims 1 to 12 further comprising a step
25 of electrodialysis.

14. A preparation of a sialylated oligosaccharide, wherein said sialylated oligosaccharide has been purified by a method according to any one of claims 1 to 13.
- 5 15. The preparation according to claim 14, wherein the sialylated oligosaccharide is present in the preparation in a purity of $\geq 80\%$ by weight.
16. The preparation according to claim 14 or 15, wherein the preparation is a fluid or a powder, preferably of a powder wherein the particles are amorphous or crystalline particles.
- 10 17. Use of the preparation according to any one of claims 14 to 16 for manufacturing a nutritional composition, preferably an infant formula.
18. A nutritional composition comprising at least one sialylated oligosaccharide, wherein said at least one sialylated oligosaccharide has been produced by microbial fermentation or biocatalysis, and wherein said nutritional composition is a medicinal formulation, a dietary supplement, a dairy drink or
15 an infant formula.
19. A nutritional composition containing at least 5 HMOs, wherein said at least 5 HMOs are selected from the group consisting of 2'-fucosyllactose, 3-fucosyllactose, lacto-*N*-tetraose, lacto-*N*-neotetraose, lacto-*N*-fucopentaose I, 3'-sialyllactose and 6'-sialyllactose.
- 20 20. A nutritional composition according to claim 18 or 19, wherein the nutritional composition contains at least one sialylated HMO and at least one neutral HMO and a probiotic microorganism.
21. A spray-dried, GMO-free powder consisting essentially of a sialylated oligosaccharide with a purity of $> 80\%$ by dry-weight and possessing less than
25 10% water by weight.
22. The spray-dried powder product according to claim 21, wherein the product is selected from the group consisting of 3'-sialyllactose, 6'-sialyllactose, and a mixture of 3'-sialyllactose and 6'-sialyllactose.

23. The spray-dried powder product according to claim 22, wherein the spray-dried powder essentially consists of a mixture of 3'-sialyllactose, 6'-sialyllactose and one or more neutral HMOs, said one or more neutral HMOs being selected from the group consisting of 2'-fucosyllactose, 3-fucosyllactose, lacto-
5 N-tetraose, lacto-*N*-neotetraose, lacto-*N*-fucopentaose I.