CLAIMS

1. A method for the purification of an oligosaccharide of interest from a fermentation broth, the method comprises:
   - providing a fermentation broth which contains the oligosaccharide of interest, biomass, microbial cells and carbohydrates other than the oligosaccharide of interest;
   - removing the microbial cells from the fermentation broth, thereby providing a process stream by centrifugation and/or at least one filtration step;
   - subjecting the process stream to a first filtration step using a nanofiltration membrane, thereby providing a filtrate which contains the oligosaccharide of interest;
   - subjecting the filtrate to a second filtration step using a nanofiltration membrane, thereby providing a retentate which contains the oligosaccharide of interest; and
   - removing salts from the process stream using electrodialysis thereby providing a purified preparation of the oligosaccharide of interest

   - wherein the oligosaccharide of interest is a neutral human milk oligosaccharide (HMO) or a sialylated HMO and

   - wherein the method does not comprise an ion exchange step.

2. The method according to claim 1, wherein the microbial cells are removed from the fermentation broth by subjecting the fermentation broth to at least one centrifugation step and/or to at least one filtration step which is a microfiltration step.

3. The method according to claim 1 or 2, wherein the at least one filtration step is a microfiltration, preferably a microfiltration using a membrane which has a molecular weight cut-off of about 500 kDa., more preferably a molecular

~~weight cut-off of about 150 kDa.~~

~~3.~~4. The method according to claim 2, wherein the at least one filtration step is a microfiltration using a membrane which has a molecular weight cut-off of 150 kDa.

~~4.~~5. The method according to claim ~~3~~2 to 4, wherein the process stream is subjected to at least one step of ultrafiltration, using a membrane having a molecular weight cut-off of ~~about~~ 50 kDa, preferably a molecular weight cut-off of ~~about~~ 30 kDa, more preferably a molecular weight cut-off of 10 kDa.

~~5.~~6. The method according to any one of claims 1 to ~~5~~4, wherein the nanofiltration membrane used in the first filtration step has a molecular weight cut-off of between ~~about~~ 700 Dalton and ~~about~~ 3,000 Dalton, preferably a molecular weight cut-off of ~~about~~ 1,000 and ~~about~~ 2,000 Dalton.

~~6.~~7. The method according to any one of claims 1 to ~~6~~5, wherein the nanofiltration membrane used in the second filtration step has a molecular weight cut-off between 100 Dalton and 1,000 Dalton, preferable a molecular weight cut-off of ~~about~~ 150 Dalton and ~~about~~ 500 Dalton, more preferably a molecular weight cut-off of ~~about~~ 200 and ~~about~~ 300 Dalton.

~~7.~~8. The method according to any one of claims 1 to 6, wherein the oligosaccharide of interest is ~~a neutral oligosaccharide or a sialylated oligosaccharide, preferably a neutral HMO or a sialylated HMO, more preferably a~~ HMO selected from the group consisting of 2'-fucosyllactose, 3-fucosyllactose, 2',3-difucosyllactose, lacto-*N*-triose II, lacto-*N*- tetraose, lacto-*N*-neotetraose, lacto-*N*-fucopentaose I, lacto-*N*- neofucopentaose, lacto-*N*-fucopentaose II, lacto-*N*-fucopentaose III, lacto-*N*-fucopentaose V, lacto-*N*-neofucopentaose V, lacto-*N*- difucohexaose I, lacto-*N*-difucohexaose II, 6'-galactosyllactose, 3'- galactosyllactose, lacto-*N*-hexaose and lacto-*N*-neohexaose, , lacto-*N*-*neo*hexaose, para-lacto-*N*-hexaose, para-lacto-*N*-

*neo*hexaose, difucosyl-lacto-*N*-*neo*hexaose 3'-sialyllactose, 6'-sialyllactose, lacto-*N*-sialylpentaose a, lacto-*N*-sialylpentaose b, lacto-*N*-sialylpentaose c, fucosyl-lacto-*N*-sialylpentaose a ,fucosyl-lacto-*N*-sialylpentaose b, fucosyl-lacto-*N*-sialylpentaose c, disialyl-lacto-*N*-tetraose, disialyl-lacto-*N*-fucopentaose, sialyllacto-*N*-tetraose a, sialyllacto-*N*-tetraose, sialyllacto- *N*-tetraose c, 3-fucosyl-3'-sialyllactose, 3-fucosyl-6'-sialyllactose, 3-fucosyl-sialyllactose, disialyl-lacto-*N*-tetraose and fucosyl-LST b, and lacto-*N*-*neo*difucohexaose I.

8.9. The method according to any one of claims 1 to 7, wherein the process stream is subjected to the second filtration step such that

i) the amount of salt in the retentate is < 10%-wt., preferably < 5 %-wt., more preferably ≤ 2 %-wt., and/or

ii) the conductivity is between 0.5 and 10.0 mS/cm^2, preferably between 1 and 8 mS/cm^2, more preferably between 1.5 and 4.0 mS/cm^2.

10. The method according to any one of claims 1 to 89, wherein the process stream is subjected to a concentration step., ~~preferably~~

11. The method according to claim 10 wherein the process stream is subjected to a concentration step by reverse osmosis and/or an additional nanofiltration step.,

9.12. The method according to claim 11 wherein the process stream is subjected ~~preferably by~~ to a nanofiltration step using a nanofiltration membrane which has a molecular weight cut-off in the range of 200 to 300 Da.

10.13. The method according to claim 10 to 129, wherein the concentration step is performed

- by nanofiltration at a temperature of < 80 °C, preferably < 50°C, more preferably 4 °C to 45 °C, more preferably 10 °C to 40 °C, even more preferably 15 to 30 °C, most preferably 15 to 20 °C; and/or

- by reverse osmosis at a temperature of 20 °C to 50 °C, more preferably 30 °C to 45 °C, most preferably 35 °C to 45 °C; and/or
- at a pressure between > 5 bar and < 50 bar, preferably at a pressure between > 10 bar and < 40 bar, more preferably at a pressure between > 15 and < 30 bar.

~~11.~~14. The method according to claim ~~9 or~~ 10 to 13 ~~10~~, wherein the process stream is subjected to a concentration step such that the concentration of the oligosaccharide of interest is ≥ 100 g/L, preferably ≥ 150 g/L, more preferably ≥ 200 g/L.

~~12.~~15. The method according to any one of claims 1 to 14~~11~~, wherein the process stream is subjected to a step of removing colorants, ~~preferably~~ by treating the process stream with activated charcoal.

~~13.~~16. The method according to claim 15 ~~12~~, wherein the step of removing colorants is performed

i) before or after the step of diafiltration;
ii) before or after the step of concentrating the process stream; and/or
iii) before or after the step of electrodialysis.

~~14.~~17. The method according to any one of claims 1 to ~~13~~16, wherein the electrodialysis is an electrodialysis under neutral conditions or an electrodialysis under acidic conditions.

~~15.~~18. The method according to any one of claims 1 to ~~14~~17, wherein the process stream after removal of salts contained therein

i) comprises an amount of salt that is < 1 %-wt., preferably < 0.5 %-wt., more preferably < 0.2 %-wt.; and/or
ii) has a conductivity of between 0.05 and 1.0 mS/cm^2, preferably between 0.1 and 0.5 mS/cm^2, more preferably between 0.2 and 0.4 mS/cm^2.

~~16.~~19. The method according to any one of claims 1 to ~~15~~18, wherein the process stream is spray-dried, ~~preferably~~ spray dried

i) at an inlet temperature in the range of 110-150°C, ~~preferably 120-140°C, more preferably 125-135°C. The~~ and an outlet temperature can be in the range of 60-80°C, ~~preferably 65-70°C;~~ and/or,~~.~~

ii) at a concentration of the oligosaccharide of interest in the process stream of 5-60 %-wt.~~., preferably 10-50 %-wt., more preferably 15-45% %-wt~~.

~~17.~~20. The method according to any one of the preceding claims, wherein the purity of the oligosaccharide of interest in the purified preparation is ≥ 80 %-wt., preferably of ≥ 85 %-wt., more preferably ≥ 90 %-wt., with respect to the dry matter of the purified preparation.