filter. In one example, the depth filter comprises a filter of about 1 micron to about 5 microns.

[0017] In one example, the lactase is removed via filtration. In one example, the lactase is removed via filtration through a filter with a pore size of about 50,000 Daltons. In one example, the composition is further filtered through one or more additional filters. In some examples, the one or more additional filters comprises a membrane with a pore size of about 2,000 to about 3,000 Daltons. In some examples, the one or more additional filters comprises a membrane with a pore size of ≤ 600 Daltons. In some examples, the composition is filtered through both a filter comprising a membrane of about 2,000 to about 3,000 Daltons followed by filtration through a membrane of ≤600 Daltons.

[0017a] In one aspect, the present disclosure refers to a method of making a purified human milk oligosaccharide composition comprising:

(a) mixing human milk permeate with lactase under conditions suitable for the digestion of lactose in the human milk permeate to create a permeate/lactases mixture, wherein the pH of the permeate is adjusted to a pH of about 4.3 to about 4.7 prior to or concurrent with the addition of the lactase, and wherein the permeate is heated to a temperature of about 45°C to about 55°C prior to or concurrent with the addition of the lactase;

(b) removing the lactase from the permeate/lactase mixture by ultrafiltration through a membrane having a pore size of less than or equal to 50,000 Daltons, thereby creating an ultrafiltered mixture, wherein prior to the ultrafiltration, the permeate/lactase mixture is cooled to a temperature of about 20°C to about 30°C and clarified through a depth filter; and

(c) filtering the ultrafiltered mixture with a nanofiltration membrane to purify and concentrate human milk oligosaccharides, wherein the nanofilter comprises a membrane having a pore size of ≤ 600 Daltons,

thereby making a purified human milk oligosaccharide composition.

[0018] In some examples, purified HMO compositions made by the methods of the current disclosure are provided. In some examples, the purified HMO composition has a reduced level of lactose and minerals compared to permeate. In some examples, the purified HMO composition comprises less than about 5.0% w/w lactose. In some examples, the HMO composition comprises the mineral profile of Table 1. In one example, the purified HMO composition comprises an HMO concentration of about 0.5% to about 7.5% HMO. In some examples, the purified HMO composition comprises an HMO concentration of about 1.0% to about 2.0% HMO. In some examples, the purified HMO composition comprises an HMO concentration of about 2.0% to about 4.0% HMO. In some examples, the purified HMO composition comprises an HMO concentration of about 4.0% to about 5.0% HMO. In some examples, the purified HMO composition comprises an HMO concentration of about 5.0% to about 7.5% HMO. In some examples, the purified HMO composition comprises an HMO concentration of about 5.0% w/w HMO. In one example, the HMO profile made according to the methods described herein comprises the HMO profile as shown in Figure 5 (E and F).

[0019] In some examples, provided herein are methods for administering the purified HMO composition to a subject in need thereof. In some examples, provided herein is a method for treating or preventing NEC in a subject in need thereof. In some examples, a method for decreasing systemic inflammation is provided by administering the purified HMO composition made by the methods described herein. In some examples, a method for treating or preventing infection in a subject in need thereof is provided. In some examples, a method for treating or preventing a viral or bacterial infection by administering the purified HMO composition made by the methods described

herein is provided. In some examples, the bacterial infection is a *Clostridium difficile* infection. In some examples, the viral infection is a norovirus or a rotavirus.

[0020] In some examples, the purified HMO composition is administered before, during or after an additional pharmaceutical or therapeutic agent. In some examples, the purified HMO composition is administered before during or after a fecal, organ or bone marrow transplant. In some examples, the purified HMO composition is administered before during or after an antibiotic, antiviral, or antifungal treatment regimen. In some examples, the purified HMO composition is administered before during or after a probiotic composition. In some examples, the purified HMO composition is administered before during or after chemotherapy and/or radiation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] **Figure 1** shows a schematic of an exemplary HMO production process.

[0022] **Figure 2** shows a schematic of an alternative HMO production process.

[0023] **Figure 3** shows a schematic of the process used to produce 20x concentrated permeate from ≥8x concentrated permeate from ≥8x concentrated permeate.

[0024] **Figure 4** shows (A) a schematic of the process used to formulate the purified HMO composition and (B) the process used to pasteurize and fill the purified HMO composition

[0025] **Figure 5** shows the results of HPAEC-PAD chromatography of neutral (A, C, and E) and sialylated (B, D and F) HMOs from pooled donor milk (A and B), human milk permeate (C and D) and purified HMO compositions (E and F).

[0026] **Figure 6** shows the global untargeted metabolomics of serum, feces and urine from adults administered an HMO obtained using LC/MS/MS and Polar LC. Results show parenteral HMO and HMO breakdown products detected in (A) serum, (B) urine, (C) feces and (D) milk.

[0027] **Figure 7** shows (A) the metabolic pathway of eicosanoids obtained using LC/MS/MS and Polar LC and (B and C) the levels of the eicosanoid metabolites over time in subjects ingesting the purified HMO compositions made by the methods of the invention.

[0028] **Figure 8** shows the serum levels of sphingolipid metabolites using LC/MS/MS and Polar LC over time in subjects ingesting the purified HMO compositions made by the methods of the invention.

What is claimed is:

1. A method of making a purified human milk oligosaccharide composition comprising:

(a) mixing human milk permeate with lactase under conditions suitable for the digestion of lactose in the human milk permeate to create a permeate/lactases mixture, wherein the pH of the permeate is adjusted to a pH of about 4.3 to about 4.7 prior to or concurrent with the addition of the lactase, and wherein the permeate is heated to a temperature of about 45°C to about 55°C prior to or concurrent with the addition of the lactase;

(b) removing the lactase from the permeate/lactase mixture by ultrafiltration through a membrane having a pore size of less than or equal to 50,000 Daltons, thereby creating an ultrafiltered mixture, wherein prior to the ultrafiltration, the permeate/lactase mixture is cooled to a temperature of about 20°C to about 30°C and clarified through a depth filter; and

(c) filtering the ultrafiltered mixture with a nanofiltration membrane to purify and concentrate human milk oligosaccharides, wherein the nanofilter comprises a membrane having a pore size of ≤ 600 Daltons,

thereby making a purified human milk oligosaccharide composition.

2. The method of claim 1 wherein the pH is adjusted to 4.5 prior to the addition of the lactose.

3. The method of claim 1 wherein the permeate is heated to about 50°C prior to adding the lactase.

4. The method of claim 1 wherein lactase is added in step (a) at about 0.1% to about 0.5% weight/weight.

5. The method of claim 4 wherein lactase is added at about 0.1% weight/weight.

6. The method of claim 1 wherein the permeate/lactase mixture is cooled to a temperature of about 25°C.

7. The method of claim 1 wherein the depth filter is about 1 to about 5 micron filter.

8. The method of claim 1 wherein the concentration of human milk oligosaccharides is at least 5% weight/weight.