resulting in various forms of paralysis and therefore can be a serious, potentially fatal, disease.

[0005] Many human milk banks use pasteurization during human milk processing. As applied to human milk, pasteurization typically involves heating the milk at 62.5°C for 30 minutes ("Holder pasteurization"). *See* Guidelines of the Establishment and Operation of a Donor Human Milk Bank, Human Milk Banking Association of North America, 2015. Methods that eliminate *B. Cereus* and *C. Botulinum* employ more extreme processing conditions. These extreme processing conditions, as well as the conditions employed for Holder pasteurization, often have a detrimental effect on the quality of the sterilized human milk. Namely, the process irreversibly damages nutritional proteins, lipids, and carbohydrates (e.g., oligosaccharides). Therefore, although the sterilized human milk is cleared of pathogens and is safe for consumption, the nutritional content is also substantially altered, such that the benefits rendered by consumption of the sterilized human milk are lost. Moreover, some processes used to reduce *B. Cereus* and *C. Botulinum* content include direct injection of heated steam into the milk, which risks introduction of contaminants and necessitates the downstream separation of water from the milk.

[0006] Accordingly, there is a need for a human milk sterilization process that reduces or eliminates harmful pathogens, such as *B. Cereus* and *C. Botulinum*, while also maintaining the nutritional qualities of the human milk.

4. SUMMARY

[0006a] In one aspect, provided herein is a method of sterilizing an unpasteurized volume of human milk to produce a sterilized human milk product, the method comprising:

homogenizing an unpasteurized volume of human milk;

heating the homogenized human milk volume in a tube to within a temperature range of 130˚ C and 150˚ C while flowing the human milk through the tube, the tube heated by flowing heating fluid in a direction counter to the direction of flow of the human milk, wherein the heating fluid is in contact with an exterior surface of the tube;

holding the temperature of the human milk volume within the temperature range for a duration of 3 to 15 seconds; and

cooling the heated human milk volume to produce the sterilized human milk product, wherein the protein digestibility corrected amino acid score (PDCAAS) of the sterilized human milk product is greater than or equal to 70 (w/w%).

[0007] The present disclosure provides a method for the sterilization and generation of a sterilized human milk product. Raw human milk is provided from at least one donor. The raw human milk may have both *B. Cereus* and *C. Botulinum.* The raw human milk is provided to a closed in-line sterilization system to generate the sterilized human milk product. The raw human milk passes through at least one bacterial clarifier, and is further separated into a cream fraction and skim fraction. The skim fraction can then be further concentrated to generate a retentate. A standardized milk product is engineered by combining a portion of the cream fraction and a portion of the retentate to achieve desired concentrations of components and/or nutrients. In some embodiments, the standardized milk product is fortified with additional components and/or nutrients. The standardized milk product is further sterilized through a countercurrent heat exchange process. Rapidly elevating the

temperature of the standardized milk product to a target temperature and holding it at the target temperature for a duration of time eliminates pathogen content while also maintaining nutritional quality of the sterilized milk product. Importantly, the standardized milk product of the present disclosure exhibits at least a 12 Log reduction of *C. Botulinum* content and a greater than 1000 Log reduction of *B. Cereus*. The standardized milk product is rendered safe for consumption by adults, adolescents, and in particular, for premature infants.

[0008] Accordingly, disclosed herein is a method of sterilizing human milk to produce a sterilized human milk product. The method comprises receiving, by a sterilizer located within a closed in-line system, as an input, a human milk sample; flowing the human milk sample through a tube of the sterilizer in a first direction at a first flow rate; heating the human milk sample in the tube by flowing heating fluid in a second direction at a second flow rate, wherein the heating fluid is in contact with an exterior surface of the tube; and cooling, by the sterilizer, the heated human milk sample.

[0009] In some examples, heating the human milk sample in the tube comprises: raising a temperature of the human milk sample to within a temperature range of 130° C and 150° C; and holding the temperature of the human milk sample within the temperature range for a duration of 3 to 15 seconds. In some examples, heating the human milk sample in the tube comprises: raising a temperature of the human milk sample to within a temperature range of 135° C and 145° C; and holding the temperature of the human milk sample within the temperature range for a duration of 6 to 14 seconds. In some examples, heating the human milk sample in the tube comprises: raising a temperature of the human milk sample to within a temperature range of 138 C and 142° C; and holding the temperature of the human milk sample within the temperature range for a duration of 8 to 13 seconds. In some examples, heating the human milk sample in the tube comprises: raising a temperature of the human milk sample to within a temperature range of 140° C and 141° C; and holding the temperature of the human milk sample within the temperature range for a duration of 12 to 13 seconds.

[0010] In various examples, a length of the portion of the tube and the first flow rate of the engineered human milk sample are each previously chosen to hold the temperature of the engineered human milk within the temperature range for the duration.

[0011] In some examples, the first tube of the pharmaceutical grade sterilizer has a diameter between 0.25 inches and 10 inches. In some examples, the first flow rate of the

engineered human milk sample through the tube of the pharmaceutical grade sterilizer is between 0.25 gallons per minute and 25 gallons per minute. In some examples, heating the human milk sample in the tube comprises: preheating the human milk sample to a first temperature between 80°C and 100°C in a first portion of the tube; and heating the human milk sample to a second temperature between 130° C and 150° C in a second portion of the tube.

[0012] In some examples, the human milk sample received by the pharmaceutical grade sterilizer is non-homogenized. In some examples, the human milk sample is homogenized prior to being received by the sterilizer or homogenized after being cooled by the sterilizer.

[0013] In various examples, the method of sterilizing human milk to produce a sterilized human milk product comprises a step, prior to the sterilizer receiving the human milk sample, of eliminating pathogens from a raw human breast milk sample through one or more clarification processes. In some examples, the method further comprises: separating a clarified human milk sample into a cream sample and a skim sample; generating a retentate by performing a concentration process on the skim sample; and engineering a standardized human milk sample to produce an engineered human milk product by combining a portion of the cream sample with a portion of the obtained retentate. In some examples, the method of combining a portion of the cream sample with a portion of the obtained retentate comprises: detecting an initial characteristic of the cream sample; comparing the detected initial characteristic of the cream sample to a target characteristic for the engineered human milk product; and determining the portion of the cream sample and the portion of the obtained retentate based on the comparison.

[0014] In various examples the method further comprises a step subsequent to cooling of the heated engineered human milk, the step comprising packaging, through an aseptic process, the sterilized human milk product. In various examples, the sterilizer is one of a commercial grade sterilizer or a pharmaceutical grade sterilizer.

[0015] In some examples, the sterilized human milk product has at least 63 calories per 100 milliliters of the sterilized human milk product. In some examples, the sterilized human milk product has a total fat content of at least 3.0 g/100 mL (3 wt%).

[0016] In various examples, the sterilized human milk product retains above 88% of immunoglobulin A (IgA) in comparison to a raw human breast milk sample that the sterilized

human milk product derives from. In various examples, the sterilized human milk product retains above 77% of immunoglobulin M (IgM) in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 93% of immunoglobulin G (IgG) in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 64% of anti-trypsin in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 81% of lactoferrin in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 78% of lysozyme in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 72% of lactalbumin in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 92% of Alpha Casein in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 92% of Beta Casein in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 92% of Kappa Casein in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In other example, the sterilized human milk product retains above 87% of osteopontin in comparison to a raw human breast milk sample that the sterilized human milk product derives from.

[0017] In various examples, the sterilized human milk product retains above 92% of total human milk oligosaccharides in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 90% of fucosylated human milk oligosaccharides in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 90% of 2'-fucosyllactose in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 90% of 3'-fucosyllactose in comparison to a raw human breast milk sample that

the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 90% of sialylated human milk oligosaccharides in comparison to a raw human breast milk sample that the sterilized human milk product derives from. In various examples, the sterilized human milk product retains above 90% of non-fucosylated human milk oligosaccharides in comparison to a raw human breast milk sample that the sterilized human milk product derives from.

[0018] Accordingly, also disclosed herein is a closed, in-line processing system. The system comprises a sterilizer comprising: a tube configured to flow a human milk sample in a first direction at a first flow rate; and a container that a portion of the tube resides within, wherein the container is configured to flow a heating fluid in contact with an exterior surface of the portion of the tube in a second direction at a second flow rate to heat the human milk sample flowing through the portion of the first tube to a predetermined temperature for a duration.

[0019] In some examples, the predetermined temperature is within a temperature range of 130° C and 150° C and the predetermined duration is between 3 and 15 seconds. In some examples, the predetermined temperature is within a temperature range of 135° C and 145° C and the predetermined duration is between 6 and 14 seconds. In some examples, the predetermined temperature is within a temperature range of 138° C and 142° C and the predetermined duration is between 8 and 13 seconds. In some examples, the predetermined temperature is within a temperature range of 140° C and 141° C and the predetermined duration is between 12 and 13 seconds. In various examples, the tube of the sterilizer is a spiral tube, wherein the duration that the human milk sample is held at the predetermined temperature is dependent on a number of spirals of the spiral tube. In some examples, the first flow rate of the human milk sample through the tube of the pharmaceutical grade sterilizer is between 0.25 gallons per minute and 25 gallons per minute.

[0020] In various examples, the system further comprises: one or more bacterial clarifiers, each bacterial clarifier configured to eliminate pathogens from a raw human breast milk sample; a milk separator in fluid communication with the last in series of the at least one bacterial clarifier, the milk separator configured to separate a clarified human milk sample into a cream sample and a skim sample; a milk concentrator in fluid communication with the milk separator, the milk concentrator further configured to generate a retentate by performing a reverse osmosis process on the skim sample received from the milk separator; and a milk

combiner configured to combine a portion of the cream sample from the milk separator and a portion of the retentate from the milk concentrator.

[0021] In some examples, the milk standardizer is in fluid communication with the sterilizer, and a homogenizer is not located in the closed, in-line processing system prior to the sterilizer. In some examples, the system is capable of undergoing a clean-in-place (CIP) method of cleaning. In some examples, the sterilizer is a pharmaceutical grade sterilizer. In some examples, the sterilizer is a commercial grade sterilizer.

[0022] Accordingly, also disclosed herein is a sterilized human milk product comprising: a bacterial aerobic plate count of less than 10 CFUs per gram of the sterilized human milk product; and a concentration of lactoferrin between 0.81 grams per liter to 13.28 grams per liter of the sterilized human milk product. In some examples, the sterilized human milk product further comprises a concentration of lysozyme between 0.012 grams per liter to 0.105 grams per liter of the sterilized human milk product. In some examples, the sterilized human milk product further comprises a concentration of lactalbumin between 1.87 grams per liter to 2.68 grams per liter of the sterilized human milk product. In some examples, the sterilized human milk product further comprises a concentration of anti-trypsin between 0.057 grams per liter to 0.40 grams per liter of the sterilized human milk product. In some examples, the sterilized human milk product further comprises a concentration of human milk oligosaccharides between 8.8 grams per liter and 20.0 grams per liter of the sterilized human milk product. In some examples, the sterilized human milk product further comprises a concentration of 2'fucosyllactose between 0.72 grams per liter and 4.3 grams per liter of the sterilized human milk product.

[0023] In some examples, the sterilized human milk product has, per 100 milliliters: (a) 63 to 67 calories, (b) 30 to 34 calories from fat, (c) 1.2 to 1.8 grams of saturated fat, (d) 10 to 16 mg of cholesterol, (e) 10 to 20 mg of sodium, (f) 4 to 10 grams of total carbohydrate, (g) 4 to 7 grams of sugars, (h) 1.2 to 1.6 grams of protein (i) 160 to 200 International Units of vitamin A, and (j) 27 to 35 mg of calcium. In some examples, the sterilized human milk product has, per 100 milliliters, greater than 63 calories. For example, the sterilized human milk product may have 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 calories per 100 milliliters of the sterilized human milk product.

[0024] In some examples, the sterilized human milk product has a reduced amount of *Bacillus Cereus* as compared to the amount of *Bacillus Cereus* in a raw human breast milk sample that the sterilized human milk product was sterilized from, wherein the reduced amount is at least a 500 log reduction of *Bacillus Cereus*. In some examples, the sterilized human milk product has a reduced amount of *Clostridium Botulinum* as compared to the amount of *Clostridium Botulinum* in the raw human breast milk sample that the sterilized human milk product was sterilized from, wherein the reduced amount is at least a 12 log reduction of *Clostridium Botulinum*.

[0025] In some examples, the sterilized human milk product is included in an aseptically packaged product. In some examples, the aseptically packaged product is packaged in one of a bottle, booster cup, paper carton, paper brick, or a pouch.

[0026] In some examples, the sterilized human milk product is included in an extended shelf life packaged product. In some examples, the extended shelf life packaged product is packaged in one of a bottle, booster cup, paper carton, paper brick, or a pouch.

[0027] Various objects, features, examples and advantages of the present disclosure will become more apparent from the following detailed description of the examples of the disclosure, along with the accompanying drawings in which like numerals represent like components.

5. BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The accompanying drawing(s), which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

[0029] FIG. 1A depicts a flow process within a system for processing raw human breast milk to generate a sterilized human milk product, in accordance with an embodiment of the present invention.

[0030] FIG. 1B depicts devices of an optional bacterial clarifier (synonymously, milk clarifier) of the system, in accordance with an embodiment of the present invention.

[0031] FIG. 1C depicts devices of an optional milk standardizer of the system, in accordance with an embodiment of the present invention.

[0032] FIG. 2 depicts a system diagram of the milk sterilizer within the closed in-line sterilization system, in accordance with an embodiment of the present invention.

[0033] FIG. 3 depicts a graph of the milk product temperature while flowing through the milk sterilizer, in accordance with an embodiment of the present invention.

CLAIMS

What is claimed is:

1. A method of sterilizing an unpasteurized volume of human milk to produce a sterilized human milk product, the method comprising:

homogenizing an unpasteurized volume of human milk;

heating the homogenized human milk volume in a tube to within a temperature range of 130˚ C and 150˚ C while flowing the human milk through the tube, the tube heated by flowing heating fluid in a direction counter to the direction of flow of the human milk, wherein the heating fluid is in contact with an exterior surface of the tube;

holding the temperature of the human milk volume within the temperature range for a duration of 3 to 15 seconds; and

cooling the heated human milk volume to produce the sterilized human milk product, wherein the protein digestibility corrected amino acid score (PDCAAS) of the sterilized human milk product is greater than or equal to 70 (w/w%).

2. The method of claim 1, wherein a length of the tube and the flow rate of the human milk are each chosen to hold the temperature of the human milk within the temperature range for the duration.

3. The method of claim 1, wherein the tube has a diameter between 0.25 and 10 inches.

4. The method of claim 1, wherein the flow rate of the human milk through the tube is between 0.25 gallons per minute and 25 gallons per minute.

5. The method of claim 1, further comprising a step, prior to homogenizing the human milk volume, of:

eliminating *Bacillus Cereus* and/or *Clostridium Botulinum* spores from the homogenized milk volume through one or more centrifugal clarification processes to obtain a clarified human milk volume.

6. The method of claim 5, further comprising, after eliminating *Bacillus Cereus* and/or *Clostridium Botulinum* spores through one or more centrifugal clarification processes and prior to heating the human milk volume, steps of:

separating the clarified human milk volume into a cream sample and a skim sample;

generating a retentate by performing a concentration process on the skim sample; and

engineering a standardized human milk volume by combining a portion of the cream sample with a portion of the generated retentate.

7. The method of claim 6, wherein combining a portion of the cream sample with a portion of the obtained retentate comprises:

detecting an initial characteristic of the cream sample;

comparing the detected initial characteristic of the cream sample to a target characteristic for the standardized human milk volume; and

determining the portion of the cream sample and the portion of the generated retentate based on the comparison.

8. The method of claim 1, further comprising subsequent to producing the sterilized human milk product, a step of packaging, through an aseptic process, the sterilized human milk product.