CLAIMS

What is claimed is:

1. A method of isolating exosomes from a milk sample, the method comprising:

    a. centrifuging the milk sample under conditions suitable to separate fats from one or more other components of the milk sample, and discarding a fat layer and pellet separated by the centrifugation of the milk sample, thereby producing a first supernatant;

    b. repeating step (a) on the first supernatant one or more times to produce a second supernatant;

    c. filtering the second supernatant using a 0.45 to 0.22 μm filter to produce a third supernatant;

    d. adding EDTA to the third supernatant to between 30 and100 mM and maintaining the resulting mixture at between 37° C and 42° C for between 60 and 120 minutes, thereby forming a fourth supernatant comprising solubilized casein; and

    e. separating exosomes from solubilized casein in the fourth supernatant by tangential flow filtration to obtain a casein-depleted exosomal retentate.

2. The method of claim 1, wherein step (d) comprises maintaining the resulting mixture at 37 °C for 60-120 minutes.

3. The method of claim 1, wherein the milk sample is a mammalian milk sample.

4. The method of claim 4, wherein the milk sample is unpasteurized.

5. The method of claim 1, wherein step (b) is repeated 1-5 times.

6. The method of claim 1, wherein steps (a)-(c) and (e) are performed at 4 degrees Celsius.

7. The method of claim 1, wherein (a) comprises centrifuging the milk sample at 2,000 to 3,000 rcf.

8. The method of claim 1, wherein (a) comprises centrifuging the milk sample at 2,500 rcf.

9. The method of claim 1, wherein step (a) is repeated 1-3 times.

10. The method of claim 1, wherein step (b) comprises repeating step (a) at least twice on the first supernatant.

11. The method of claim 11, wherein the step (a) comprises centrifuging the milk sample at 13,500 to 15,500 rcf for 45-75 minutes.

12. The method of claim 11, wherein the step (a) comprises centrifuging the milk sample at 14,500 rcf for about 60 minutes.

13. The method of claim 11, wherein the step (b) comprises centrifuging the first supernatant at 24,800 to 26,800 rcf for 45-75 minutes.

14. The method of claim 14, wherein the step (b) comprises centrifuging the first supernatant at about 25,800 rcf for about 60 minutes.

15. The method of claim 11, wherein the step (b) is repeated 1-3 times, with each repetition being performed on the supernatant from the immediately preceding repetition.

16. The method of claim 1, wherein step (c) comprises filtering the second supernatant through one or more filters in series ranging from a 0.45 μm filter to a 0.22 μm filter.

17. The method of claim 17, wherein step (c) comprises filtering the second supernatant through a 0.45 μm filter followed by filtering the resulting filtrate through a 0.22 μm filter.

18. The method of claim 1, wherein the tangential flow filtration of step (e) is performed using an ultrafiltration membrane with a cutoff ranging from 250 kDa to 750 kDa.

19. The method of claim 18, wherein the tangential flow filtration of (e) is performed using a 500 kDa ultrafiltration membrane.

20. The method of claim 1, wherein the tangential flow filtration of step (e) is performed at a flow rate of 5 to 15 mL per minute.

21. The method of claim 19, wherein the tangential flow filtration of step (e) is performed at a flow rate of about 10 mL per minute.

22. The method of claim 1, wherein the method yields an exosomal concentrate that is at least 7.5 percent, 8 percent, 8.5 percent, 9 percent, 9.5 percent, 10 percent, 10.5 percent, 11 percent, 11.5 percent, 12 percent, 12.5 percent, 13 percent, 13.5 percent, 14 percent, 14.5 percent, 15 percent, 15.5 percent, 16 percent, 16.5 percent, 17 percent, 17.5 percent, 18 percent, 18.5 percent, 19 percent, 19.5 percent, or at least 20 percent of the starting volume of milk.

23. The method of claim 1, further comprising loading the exosomes in the casein-depleted exosomal retentate with one or more cargos.

24. A formulation comprising exosomes, wherein the exosomes are obtained from casein-depleted exosomal retentate of claim 1.

25. An isolated exosome according to claim 1 for use in therapy.

26. The method of claim 23, wherein the one or more cargos are therapeutic cargos.