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Supplemental Data to The Impact of Processing on the Content and Composition of Extracellular Vesicles in Bovine Milk

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Batch	Fat (%)	Protein (%)	Lactose (%)	Total Solids (%)	Solids-not-fat (%)
M1 (n=9)	3.64 ± 0.01	3.14 ± 0.01	4.62 ± 0.01	12.50 ± 0.02	8.82 ± 0.01
M2 (n=9)	3.70 ± 0.01	3.16 ± 0.00	4.55 ± 0.01	12.53 ± 0.01	8.79 ± 0.01
Average	3.67 ± 0.04	3.15 ± 0.02	4.58 ± 0.04	12.51 ± 0.02	8.81 ± 0.02

Supplemental Table 1. Physical properties summary of raw bovine milk.

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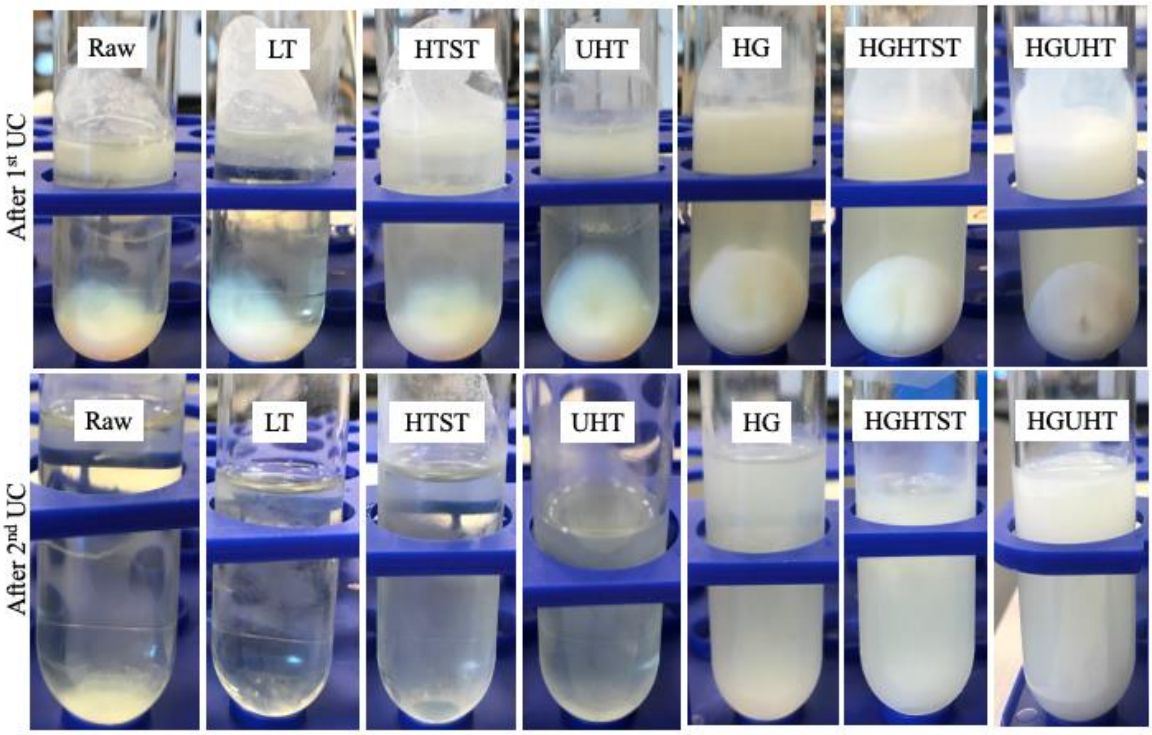

Supplemental Figure 1 Differences in turbidity across treatments after first and second UC spins for the EV isolation using a differential centrifugation protocol.

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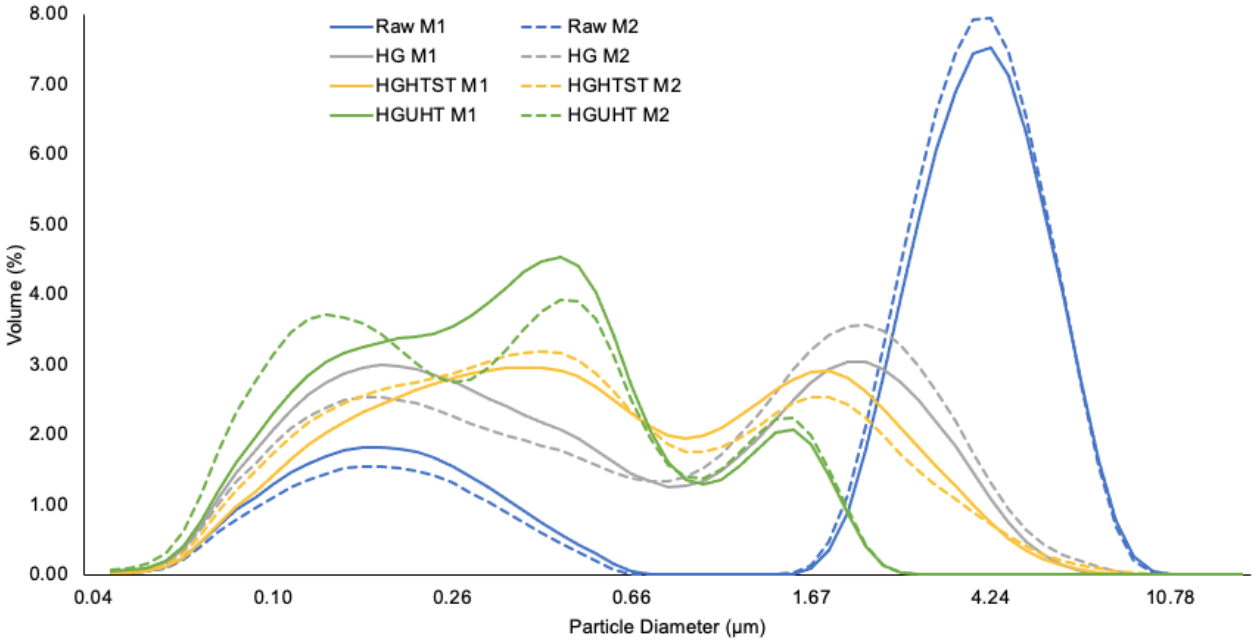

Supplemental Figure 2 Size distribution for homogenization validation (*n* = 24)**.** Raw milk displays the largest volume of particles with a peak in the volume % of particles with a diameter of 4.24 µm, while all homogenized milk group (HG, HGHTST, HGUHT) resulted in lower non-uniform peaks and smaller particle diameters. M1 = batch 1, M2 = batch 2.

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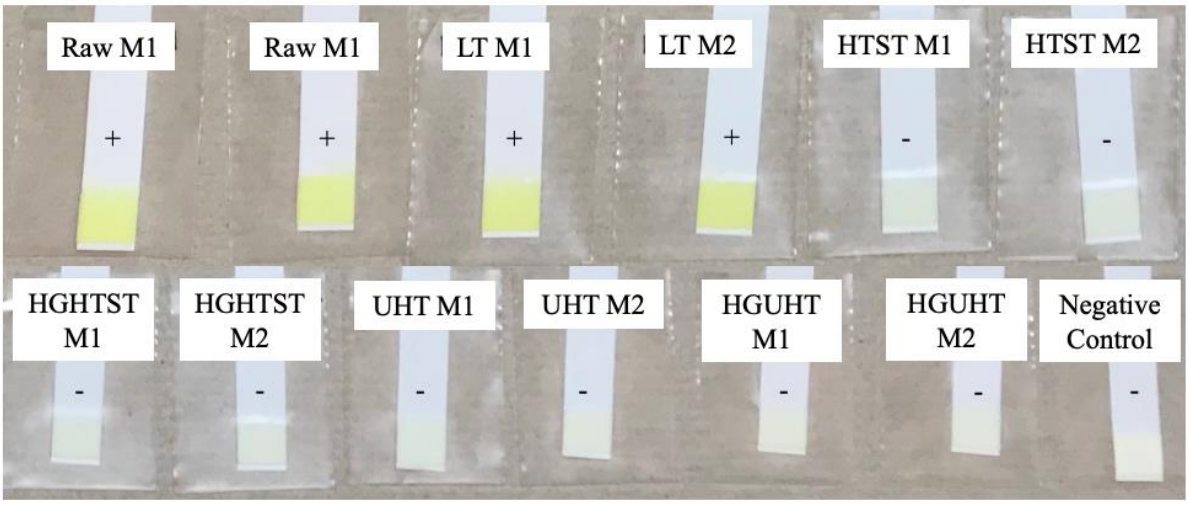

Supplemental Figure 3 ALP Test Results. ALP-inactivated milk samples (HTST, HGHTST, UHT, HGUHT) appear in white, while active ALP samples (raw, LT) appear in yellow.

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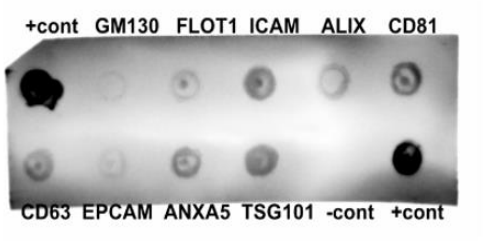
A

Antibody	Expected Result	Bovine Milk
GM130	-	-
FLOT1	+	+
ICAM	+	+
ALIX	+	+
CD81	+	+
CD63	+	+
EpCAM	-	-
ANXA5	+	+
TSG101	+	+

B



Supplemental Figure 4. Global characterization for EV markers. Expected and obtained results for the semiquantitative immunoblot of EVs from raw bovine milk (A). Semi-quantitative immunoblot of raw bovine milk EVs. +cont and -cont represents positive and negative controls, respectively. EpCAM and GM130 are markers of cellular contamination (B). TSG101, FLOT1, ANXA5, ALIX, CD63, CD81, ICAM are positive markers of EVs.

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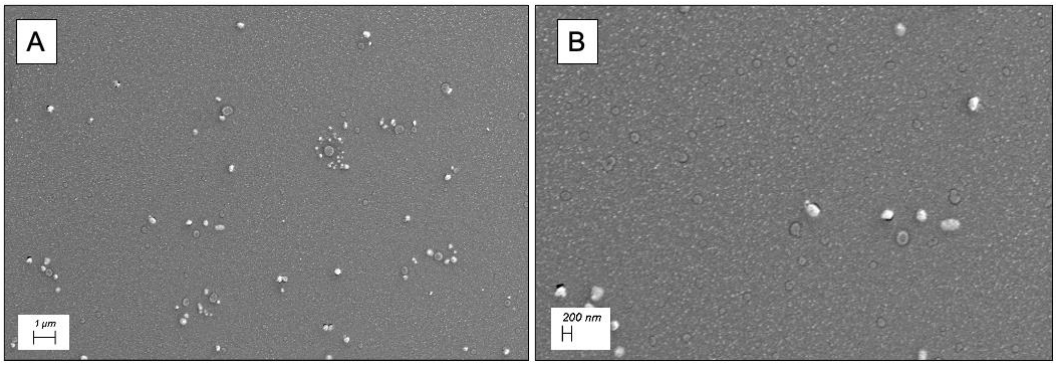

Supplemental Figure 5. Images of raw bovine milk extracellular vesicles (EVs) obtained by scanning electron microscopy (SEM). (A) Broad-field image, 1:3,500 dilution, 1 µm scale. Electron high tension = 10.00 kV, signal A = SE2, aperture size = 30.00um, working distance = 7.3 mm, magnification = 4.74 K X, specimen height = 2mm. (B) Close-up image, 1:3,500 dilution, 200nm scale. Electron high tension = 10.00 kV, signal A = SE2, aperture Size = 30.00um, working distance = 7.3 mm, Mag = 4.74 K X, specimen height = 2mm.