Electronic Supplementary Information (ESI)

ESI Table 1. Sterol determination in human milk: calibration curves with addition of matrix.

	Colostrum		Transition		1 month HM		3 month HM		6 month HM	
	Calibration curve	**r**	**Calibration curve**	**r**	**Calibration curve**	**r**	**Calibration curve**	**r**	**Calibration curve**	**r**
					Cholesterol (0-128.70 µg/assay)					
Coastal	$y=1.1053x+24.034^{a,x}$	0.9964	$y=1.1321x+21.768^{a,x}$	0.9968	$y=1.3342x+15.491^{a,y}$	0.9983	$y=1.3342x+15.491^{a,y}$	0.9983	$y=1.1915x+13.875^{a,x}$	0.9991
Central	$y=0.9647x+9.941^{b,x}$	0.9984	$y=1.3525x+17.311^{a,y}$	0.9905	$y=1.0008x+15.634^{b,x}$	0.9997	$y=1.1738x+12.101^{b,y}$	0.9996	$y=1.1738x+12.101^{a,y}$	0.9996
					Desmosterol (0-4.90 µg/assay)					
Coastal	$y=1.3717x+1.4556^{a,x}$	0.9990	$y=1.2895x+2.1992^{a,x}$	0.9978	$y=0.9639x+1.6683^{a,y}$	0.9903	$y=0.4798x+1.1377^{a,z}$	0.9980	$y=1.4119x+1.0625^{a,x}$	0.9994
Central	$y=1.1789x+1.1939^{b,x,z}$	0.9937	$y=1.5714x+0.6938^{b,y}$	0.9946	$y=1.2953x+1.3737^{b,x,y}$	0.9977	$y=1.5714x+0.6938^{b,y}$	0.9936	$y=1.4166x+0.4342^{a,y}$	0.9980
					Lathosterol (0-1.59 µg/assay)					
Coastal	$y=0.9944x+0.0427^{a,x}$	0.9997	$y=1.0421x+0.0458^{a,y}$	0.9994	$y=1.1225x+0.0292^{a,y}$	0.9975	$y=0.8128x+0.0393^{a,z}$	0.9976	$y=1.0747x+0.0656^{a,y}$	0.9994
Central	$y=0.8363x+0.0435^{b,x,z}$	0.9962	$y=1.1099x+0.0286^{a,y}$	0.9958	$y=0.8966x+0.0447^{b,z}$	0.9998	$y=1.0591x+0.0569^{b,y}$	0.9972	$y=0.9972x+0.0562^{b,y}$	0.9985
					Lanosterol (0-0.40 µg/assay)					
Coastal	$y=1.0819x+0.0462^{a,w}$	0.9985	$y=1.0935x+0.0932^{a,w}$	0.9976	$y=1.0935x+0.0932^{a,w}$	0.9976	$y=0.7356x-0.002^{a,x,z}$	0.9932	$y=1.2593x+0.068^{a,z}$	0.9979
Central	$y=0.7356x-0.002^{a,x,z}$	0.9932	$y=1.3501x+0.0493^{b,y}$	0.9908	$y=0.9887x+0.061^{a,x,z}$	0.9979	$y=1.1614x+0.0494^{b,x,y}$	0.9908	$y=1.1127x+0.0347^{b,x,y}$	0.9970
					Campesterol (0-0.24 µg/assay)					
Coastal	$y=1.3949x+0.0389^{a,x}$	0.9998	$y=1.5429x+0.0504^{a,x}$	0.9993	$y=1.5429x+0.0504^{a,x}$	0.9993	$y=1.3949x+0.0389^{a,x}$	0.9998	$y=1.5382x+0.0592^{a,x}$	0.9954
Central	$y=1.05x+0.0503^{a,w}$	0.9990	$y=1.9136x+0.0364^{b,x}$	0.9983	$y=1.2889x+0.0504^{b,y}$	0.9962	$y=2.0476x+0.0446^{b,x}$	0.9984	$y=1.8357x+0.0364^{b,x}$	0.9963
					Stigmasterol (0-0.08 µg/assay)					
Coastal	$y=1.9885x+0.0231^{a,x}$	0.9944	$y=1.9885x+0.0231^{a,x}$	0.9944	$y=2.176x+0.0144^{a,x}$	0.9927	$y=1.6704x+0.0102^{a,x}$	0.9940	$y=1.8812x+0.0188^{a,x}$	0.9966
Central	$y=1.1101x+0.023^{b,x}$	0.9521	$y=1.5548x+0.014^{b,x}$	0.9971	$y=1.5548x+0.0193^{b,x}$	0.9732	$y=1.5548x+0.0193^{a,x}$	0.9732	$y=1.5548x+0.014^{a,x}$	0.9971
					β-Sitosterol (0-0.12 µg/assay)					
Coastal	$y=1.2236x+0.0268^{a,x}$	0.9966	$y=1.2236x+0.0268^{a,x}$	0.9966	$y=1.2236x+0.0268^{a,x}$	0.9966	$y=1.1992x+0.0314^{a,x}$	0.9968	$y=1.1992x+0.0314^{a,x}$	0.9968
Central	$y=0.6698x-0.004^{b,x}$	0.9950	$y=0.8151x+0.022^{b,y}$	0.9921	$y=0.9x+0.0168^{b,y,z}$	0.9864	$y=0.9x+0.0168^{b,y,z}$	0.9864	$y=0.7519x+0.0195^{b,x,y}$	0.9941

y = sterol area/IS area; x = µg sterol/µg IS; r = linear correlation coefficient. HM = human milk. Different superscript letters denote significant differences ($p < 0.05$) in the slope for each sterol in different geographical area in the same lactation stage (a-b), and in different lactation stage in the same geographical area (x-z).

ESI Table 2. Sterol contents in human milk and bioaccessible fractions (expressed as μg/100 g of HM) and their bioaccessibility.

Sterols	Colostrum			1 month			3 months		
	HM	BF	BA	HM	BF	BA	HM	BF	BA
Cholesterol*	20.53 ± 0.70 ^{a,x}	9.46 ± 0.92 ^{a,x}	46.05 ± 4.50 ^{a,x}	12.50 ± 0.13 ^{a,y}	9.12 ± 0.40 ^{a,x}	72.97 ± 3.17 ^{a,y}	10.50 ± 0.19 ^{a,z}	6.14 ± 0.38 ^{a,y}	58.49 ± 3.65 ^{a,z}
Desmosterol*	0.90 ± 0.05 ^{b,x}	0.32 ± 0.04 ^{b,x}	35.25 ± 4.58 ^{b,x}	1.54 ± 0.01 ^{b,y}	0.55 ± 0.02 ^{b,y}	35.63 ± 1.34 ^{b,x}	1.98 ± 0.05 ^{b,z}	0.30 ± 0.02 ^{b,x}	15.13 ± 0.89 ^{b,y}
Lathosterol	34.23 ± 2.27 ^{c,x}	8.97 ± 0.91 ^{c,x}	26.21 ± 2.64 ^{c,x}	33.21 ± 1.92 ^{c,x}	11.44 ± 1.39 ^{c,x}	34.44 ± 4.19 ^{b,y}	53.52 ± 1.94 ^{c,y}	9.76 ± 1.75 ^{c,x}	18.23 ± 3.28 ^{b,z}
Lanosterol	35.50 ± 3.07 ^{c,x}	8.54 ± 0.60 ^{c,x}	24.07 ± 1.68 ^{c,d,x}	64.30 ± 3.30 ^{c,y}	37.67 ± 2.56 ^{c,y}	58.59 ± 3.98 ^{c,e,y}	62.41 ± 5.13 ^{c,y}	18.99 ± 2.35 ^{c,z}	30.43 ± 3.76 ^{c,x}
Σ Animal sterols*	21.51 ± 0.71 ^{a,x}	9.79 ± 0.96 ^{a,x}	45.54 ± 4.47 ^{a,x}	14.14 ± 0.14 ^{d,y}	9.72 ± 0.42 ^{a,x}	68.73 ± 2.96 ^{a,y}	12.59 ± 0.25 ^{d,z}	6.47 ± 0.39 ^{a,y}	51.38 ± 3.06 ^{a,x}
Campesterol	24.15 ± 1.57 ^{c,x}	7.07 ± 0.80 ^{c,x}	29.26 ± 3.32 ^{b,c,x}	28.33 ± 1.66 ^{c,y}	18.93 ± 0.61 ^{c,y}	97.21 ± 3.11 ^{d,y}	25.40 ± 1.07 ^{c,x,y}	9.22 ± 1.26 ^{c,z}	36.28 ± 4.97 ^{c,x}
Stigmasterol	6.98 ± 0.19 ^{c,x}	1.47 ± 0.22 ^{c,x}	20.98 ± 3.22 ^{d,x}	6.21 ± 0.57 ^{c,x,y}	3.78 ± 0.28 ^{c,y}	66.18 ± 4.84 ^{a,e,y}	4.83 ± 0.75 ^{c,y}	n.d	n.d
β-Sitosterol	15.94 ± 0.56 ^{c,x}	2.37 ± 0.68 ^{c,x}	14.90 ± 4.29 ^{e,x}	21.59 ± 1.80 ^{c,y}	7.67 ± 0.61 ^{c,y}	41.80 ± 3.34 ^{b,y}	12.65 ± 0.97 ^{c,x}	12.57 ± 0.56 ^{c,z}	99.32 ± 4.40 ^{d,z}
Σ Plant sterols	47.07 ± 2.05 ^{c,x}	10.62 ± 1.04 ^{c,x}	22.56 ± 2.22 ^{d,x}	55.63 ± 3.09 ^{c,y}	30.96 ± 1.88 ^{c,y}	55.65 ± 3.37 ^{c,y}	42.88 ± 2.79 ^{c,x}	25.05 ± 1.92 ^{c,z}	58.41 ± 4.48 ^{a,y}
Σ Total sterols*	21.55 ± 0.71 ^{a,x}	9.80 ± 0.96 ^{a,x}	45.49 ± 4.46 ^{a,x}	14.19 ± 0.14 ^{d,y}	9.75 ± 0.42 ^{a,x}	68.68 ± 2.95 ^{a,y}	12.64 ± 0.24 ^{d,z}	6.49 ± 0.39 ^{a,y}	51.36 ± 3.10 ^{a,x}
Ratio animal/plant sterols	457	922	2.0	254	314	1.2	294	258	0.9

Values are expressed as mean ± standard deviation of three replicates for HM analysis and four replicates for BF analysis. *expressed as mg/100 g of HM. HM: human milk. BF: bioaccessible fraction. BA: bioaccessibility, calculated as [sterol content in BF/sterol content in HM] x 100. Different superscript letters denote significant differences ($p < 0.05$) in the same column (a-f) and in the same line (x-z) for each sample (HM, BF, or BA). n.d = not detected. Σ Animal sterols: sum of cholesterol, desmosterol, lathosterol and lanosterol. Σ Plant sterols: sum of campesterol, stigmasterol, and β-sitosterol.