

Tempering of cocoa butter and chocolate using minor lipidic components

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Supplementary Information

Supplementary Table 1: Fatty acid profile of unrefined cocoa butter and monoacylglycerol additives determined by gas-liquid chromatography.

Cocoa Butter		GMO		GMP		GMS	
Fatty Acid	Area %	Fatty Acid	Area %	Fatty Acid	Area %	Fatty Acid	Area %
C16:0	25.40	C16:0	6.45	C14:0	1.24	C16:0	11.10
C18:0	37.30	C18:0	1.81	C16:0	56.94	C18:0	88.33
C18:1	33.54	C18:1	81.8	C18:0	40.52	C20:0	0.57
C18:2	2.51	C18:2	7.1	C20:0	0.43		
C20:0	1.24						

Supplementary Table 2: Phosphorus concentrations in cocoa butter before and after neutralization and bleaching as determined by AOCS Official Method Ca 12-55. Unrefined average represents the mean of 3 replicates.

Before Degumming:	Unrefined Average	Neutralized	Bleached
Mass of CB (g)	3.13	3.08	3.12
Absorbance Trial 1	0.1737	0.0053	0.0089
Absorbance Trial 2	0.1734	0.0059	0.0098
Absorbance Trial 3	0.1740	0.0057	0.0102
Average Absorbance	0.1737	0.0056	0.0096
Phosphorus Content (mg)	0.0283	-0.0003	0.0004
Phosphorus %	0.0088	-0.0004	-0.0001
Equivalent Phosphatides %	0.2629	-0.0109	-0.0042

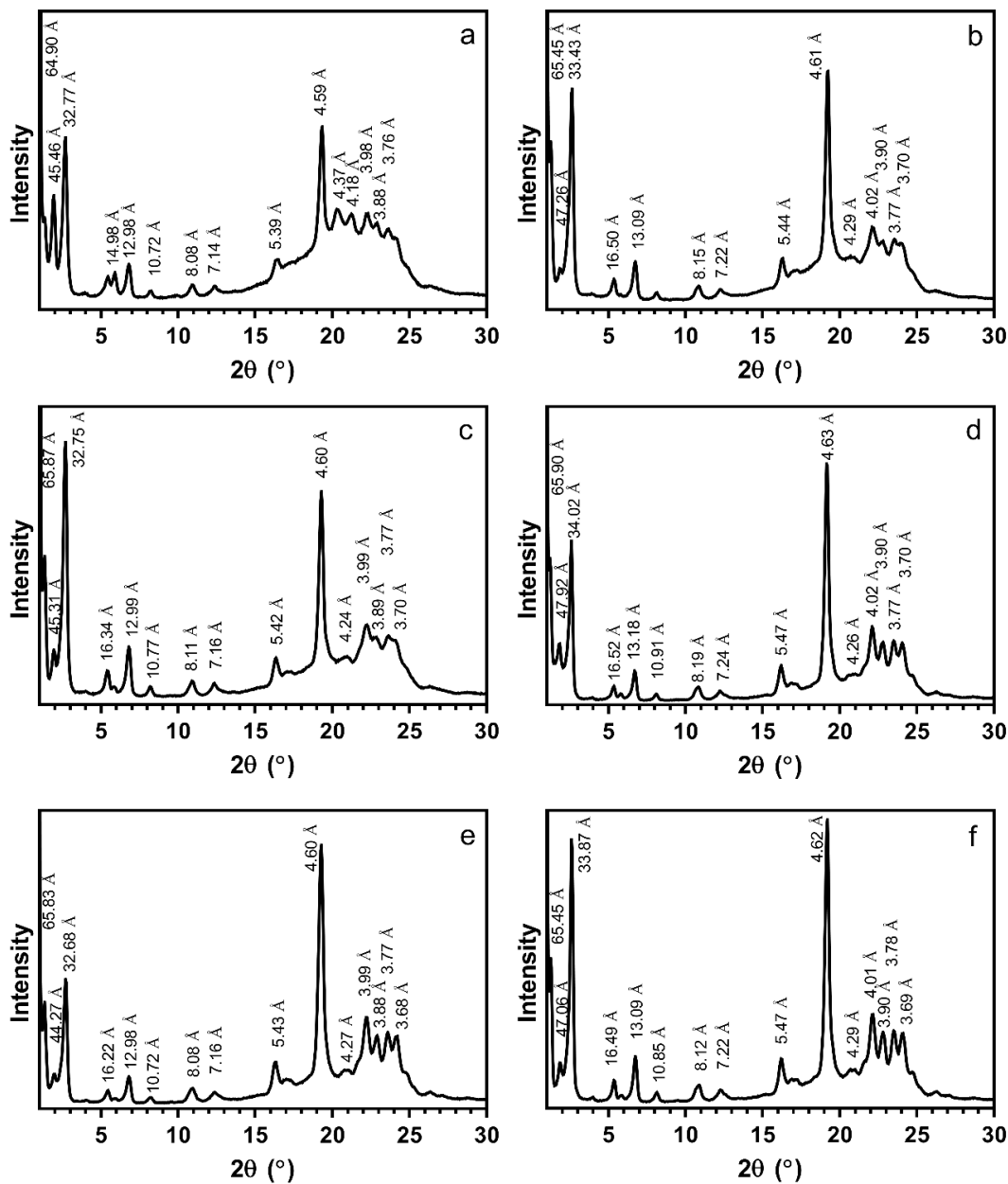
Supplementary Table 3: Free fatty acid concentrations in cocoa butter before and after neutralization and bleaching as determined by AOCS Official Method Ca 5a-40. Unrefined average represents the mean of 3 replicates.

Trial	Unrefined Average	Neutralized	Bleached
CB Mass (g)	28.20	28.39	29.70
NaOH (mL)	3.23	0.21	0.12
% as Oleic acid	1.646	0.106	0.058
% as Lauric acid	1.168	0.075	0.041
% as Palmitic acid	1.495	0.096	0.053

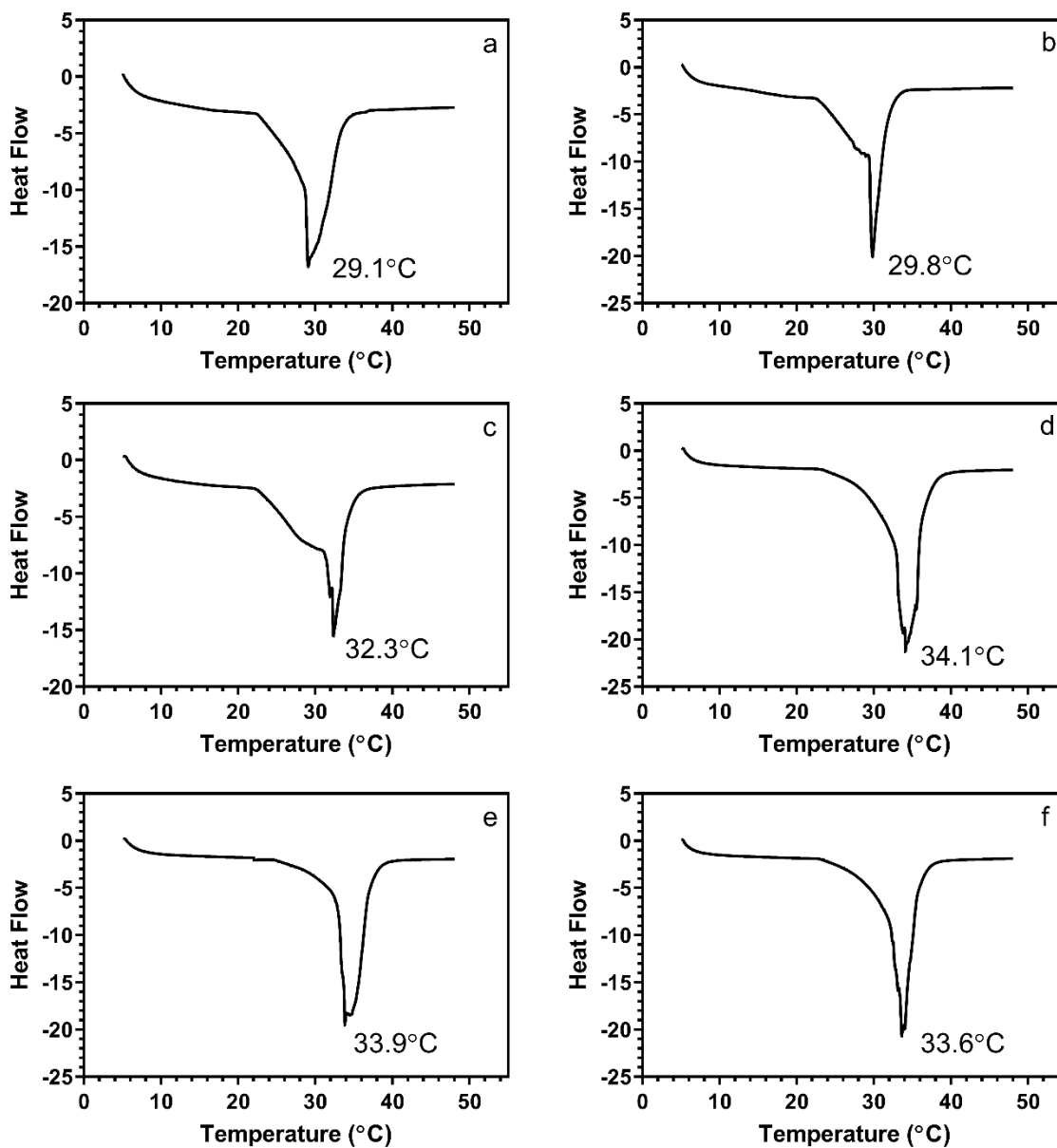
Supplementary Table 4: Characteristic wide-angle powder X-ray diffraction short spacings for cocoa butter in crystal polymorphic forms of IV, V and IV.

Polymorph	Short-spacing (Å)*				
	IV	4.35 (s)	4.15 (s)	3.97 (m)	3.81 (m)
V	4.58 (vs)	3.98 (m)	3.87 (m)	3.75 (m)	3.67 (m)
VI	4.61 (vs)	4.06 (m)	3.96 (m)	3.88 (m)	3.71 (s)

*vs, s, m, w represents “very strong”, “strong”, “medium” and “weak” peak intensities.



Supplementary Figure 1: Powder X-ray diffraction patterns for refined cocoa butter with added minor lipidic components. Samples included refined CB with added 0.5% (w/w) of (a) glycerol monostearate, (b) glycerol monopalmitate, (c) glycerol monooleate, (d) stearic acid, (e) palmitic acid, and (f) oleic acid. Samples were crystallized statically at 23°C for 24 hours



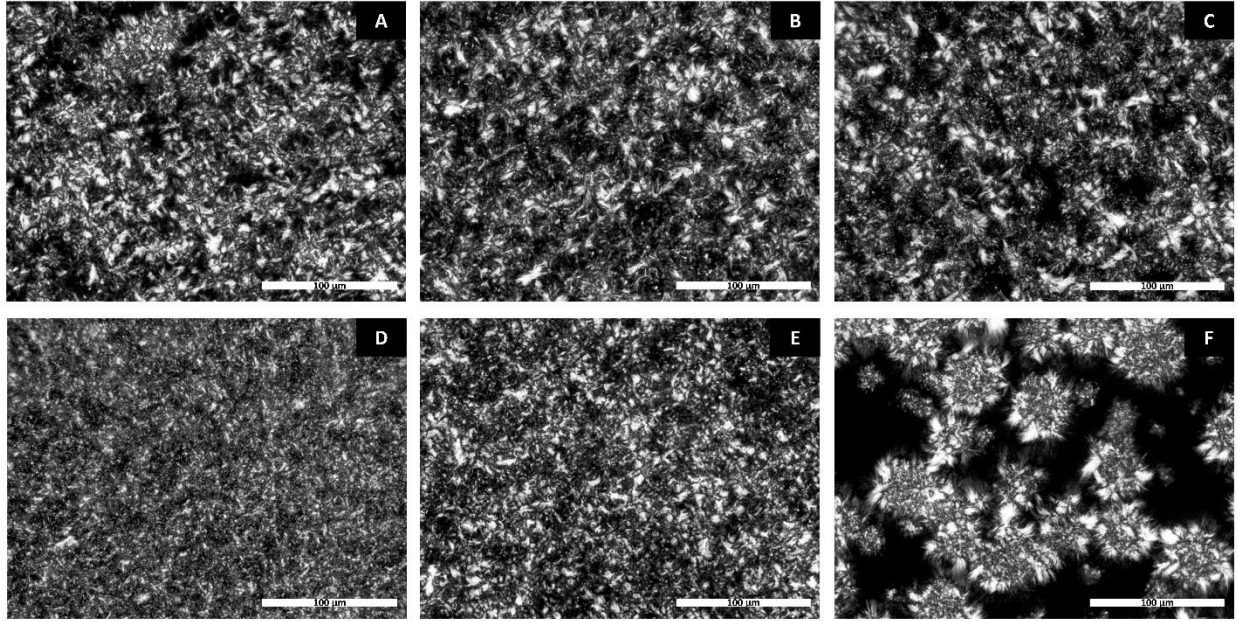
Supplementary Figure 2: Differential scanning calorimetry melting thermograms and peak melting temperatures for refined cocoa butter with added minor lipidic components. Samples included refined CB with added 0.5% (w/w) of (a) glycerol monostearate and (b) glycerol monopalmitate, (c) glycerol monooleate, (d) stearic acid, (e) palmitic acid and (f) oleic acid. Samples were crystallized statically at 23°C for 24 hours and scanned using a heating rate of 5°C/min

Supplementary Table 5: Avrami constants and crystallization half-times calculated by nonlinear regression ($SFC = SFC_{\max}(1 - e^{-k_A t^n})$) of samples crystallized statically at 23°C (n=3, ± standard error). Values with the same superscript letter within a row are not statistically different (P>0.05).

Sample	Unrefined	Refined	DMPC	DPPE
k _A (min ⁻ⁿ)	1.91±0.43E-09 ^a	5.58±1.85E-07 ^a	3.57±1.79E-06 ^b	3.52±1.90E-11 ^a
n	3.51 ± 0.06 ^{a,b}	2.67 ± 0.06 ^a	3.23 ± 0.71 ^{a,b}	4.88 ± 0.17 ^c
SFC _{max} (%)	49.1 ± 0.2 ^a	59.5 ± 0.2 ^b	56.9 ± 0.8 ^c	55.4 ± 0.3 ^{c,d}
Half-time (min)	281.9 ± 6.0 ^a	200.6 ± 3.7 ^{b,c}	112.3 ± 6.5 ^d	141.7 ± 3.2 ^{d,e}

Supplementary Table 5 continued

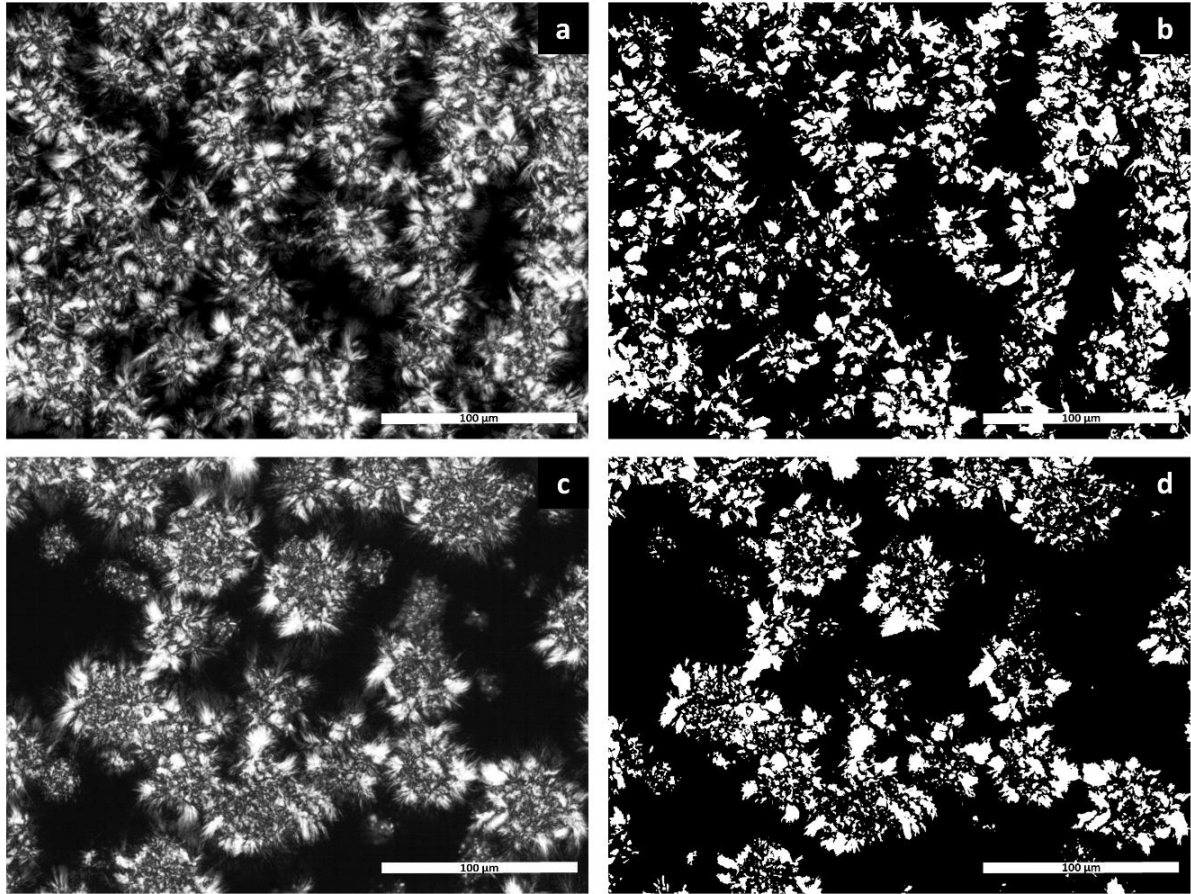
Sample	GMS	GMP	GMO	Stearic	Palmitic	Oleic
k _A (min ⁻ⁿ)	2.15±1.33E-10 ^a	2.65±1.22E-09 ^a	1.70±1.50E-08 ^a	3.34±2.65E-09 ^a	4.36±2.66E-08 ^a	4.39±0.77E-08 ^a
n	4.23 ± 0.25 ^{b,c,d}	3.64 ± 0.15 ^{a,c}	3.66 ± 0.22 ^{a,c}	3.92 ± 0.18 ^{a,c}	3.33 ± 0.16 ^{a,d}	3.19 ± 0.04 ^{a,d}
Y _{max} (%)	55.9 ± 0.3 ^{c,d}	55.0 ± 0.4 ^{c,d}	54.6 ± 0.3 ^{c,d}	54.7 ± 0.3 ^{c,d}	54.2 ± 0.3 ^d	56.3 ± 0.5 ^{c,d}
Half-time (min)	217.6 ± 8.2 ^b	226.5 ± 7.8 ^b	164.8 ± 4.3 ^{e,f}	163.1 ± 3.4 ^{e,f}	170.5 ± 4.6 ^{e,f}	183.8 ± 1.8 ^{c,f}



Supplementary Figure 3: Polarized light microscopy images of refined cocoa butter with added minor lipidic components. Samples included refined CB with added 0.5% (w/w) of (a) glycerol monostearate and (b) glycerol monopalmitate, (c) glycerol monooleate, (d) stearic acid, (e) palmitic acid and (f) oleic acid. Samples were crystallized statically at 23°C for 24 hours. The horizontal magnification bar at the bottom of the images corresponds to 100μm.

Supplementary Table 6: Box-counting fractal dimension of thresholded 8-bit grayscale polarized light micrographs for all samples used in this study. The intensity and contrast of the grayscale images were auto-levelled and thresholding was carried out automatically using a grayscale level of 128.

Trial	1	2	3	4	5	6	Average	SE
Unrefined	1.581	1.591	1.630	1.702	1.644	1.628	1.629	0.018
Bleached	1.824	1.787	1.778	1.694	1.715	1.732	1.755	0.020
DMPC	1.708	1.725	1.725	1.710	1.716	1.752	1.723	0.007
DPPE	1.809	1.799	1.710	1.674	1.627	1.689	1.718	0.029
GMS	1.719	1.740	1.620	1.609	1.736	1.689	1.685	0.024
GMP	1.694	1.648	1.704	1.694	1.645	1.622	1.668	0.014
GMO	1.785	1.691	1.627	1.598	1.630	1.624	1.659	0.028
Stearic	1.615	1.603	1.650	1.729	1.584	1.555	1.623	0.025
Palmitic	1.568	1.612	1.685	1.782	1.782	1.729	1.693	0.036
Oleic	1.567	1.434	1.695	1.743	1.704	1.695	1.639	0.048



Supplementary Figure 4: Polarized light microscopy images of refined cocoa butter with added minor lipidic components. The 8-bit grayscale micrographs with 256 shades of gray were automatically thresholded using a grayscale level of 128. Here we show the conversion of the grayscale image of (a) refined cocoa butter into its thresholded version (b), as well as the conversion of the grayscale image of (c) refined cocoa butter with added 0.5% oleic acid to its thresholded counterpart (d). Before thresholding, intensity levels and contrast were auto-levelled. These thresholded images were then used for the box-counting analysis. The horizontal magnification bar at the bottom of the images corresponds to 100 μ m.