Supplementary information

The supplementary materials are organized in two general parts: in the first, we describe additional experimental procedures, whereas in the second, we present complementary experimental results.

The experimental procedures are listed as follows: Isolation of polyphenols from Quillaja Dry (section 1), determination of total polyphenols (section 2), HPLC analysis of bile salts, saponins and polyphenols (section 3), gas chromatography (GC) (section 4), procedure for quantitative analysis of the GC data (section 5), atomic absorption spectrometry (section 6) and cryogenic transmission electron microscopy (section 7).

Complementary experimental results are presented for: fatty acid composition of the studied fats and oils (Table S1), HPLC of bile salts, saponins and polyphenols (Figure S1-S4), GC analysis after lipolysis of cocoa butter (Figure S5), degree of fat lipolysis (Figure S6), solubilization of FA in the permeate after lipolysis of sunflower oil and lard emulsions (Figure S7), distribution of saturated FA, as a function of C_{Ca} (Figure S8), solubilization of monoglycerides, as a function of QD concentration (Figure S9), HPLC of saponins in QD extract and Supersap (Figure S10).

S1. Isolation of polyphenols from Quillaja Dry.

The polyphenols in Quillaja Dry were isolated by dialysis through a 10 000 Da cellulose membrane (Slide-A-Lyzer, cat. № 66810, Thermo Scientific). The dialysis cassette was filled with 10 mL of 10 wt % Quillaja Dry (QD) solution, the concentration being calculated in respect to QD solids. This concentrated QD solution had a very dark-brown colour. This cassette was then inserted in 200 mL water, supplemented with 0.1 g/L NaN_3 (to prevent microbial growth). Dialysis was performed for 4 days, which resulted in an orange-coloured dialysate and a dark-orange solution inside the cassette, the colour coming from the polyphenols present in QD.

S2. Determination of total polyphenols.

Total polyphenols were determined by the Folin-Ciocalteau (FC) method, which is based on a chemical oxidation of the phenols and reduction of the reagent (a mixture of tungsten and molybdenum oxides). The products of the metal oxide reduction have a blue colour that exhibits a broad light absorption with a maximum at 765 nm. The absorption at that wavelength is proportional to the concentration of phenols. By measuring the absorption of a series of standard solutions of gallic acid we prepared a calibration curve, which was then used to determine the concentration of polyphenols in QD or its dialysate.

We placed 60 µL sample or a gallic acid calibration standard (in deionized water) in a 10-ml vial. Then, we added 4.74 ml water, followed by 300 µL FC reagent. We swirled to mix and incubated for ≈ 5 min at room temperature. At the end we added 900 µL sodium carbonate solution, mixed and incubated 2 h at room temperature. Around 2 ml of the final

solution were transferred to a polycarbonate cuvette and absorbance was measured at 765 nm in a UNICAM spectrophotometer. The absorbance of the blank was subtracted from all readings.

S3. High pressure liquid chromatography (HPLC). The analysis was performed on a Shimadzu (Japan) apparatus, equipped with two high-pressure mixing binary gradient pumps (LC-20AD), DGU-14A four-line membrane degasser, SIL-10ADvp autosampler, CTO-10ASvp wide temperature range column oven, and SPD-10Avp UV-VIS detector. The separation was carried out on 25cm x 4.6 mm i.d., 5 μm particle size, Ascentis® C18 column (Supelco, USA) and a guard column Ascentis® C18 Supelguard™ Guard Cartridge (5 μm particle size, 2 cm x 4 mm i.d.). All samples were filtered through 200 nm filters (section 2.4) and kept at 37 ºC prior injection.

(A) Separation of bile salts.

For the separation of bile salts we developed a new procedure, in which we used gradient elution with 1.5 vol. % formic acid in water (A) and 1.5 vol. % formic acid in acetonitrile (B). The total flow rate was kept at 1 ml/min for the whole time of the analysis (35 min). The system was run with the following gradient program: from 40 % B to 75 % B for 20 min, from 75 % B to 40 % B for the next 2 min and constant flow at 40 % B for 13 min. The sample injection volume was 20 μL. The UV-VIS detector was set at λ = 200 nm and the column temperature was 37 ºC.

All major peaks in the bile extract have been separated by this procedure, see Figure S1. However, the identification of these peaks was difficult, as only some of them had retention times close to our standards, Figure S2. Thus, we analysed the change in the area of the main bile peaks in the presence and in the absence of Quillaja extract.

(B) Separation of saponins.

The mobile phases used in this analysis are 1.5 vol. % formic acid in water (A) and 1.5 vol. % formic acid in acetonitrile (B). The gradient performed was as follows: 0-30 min, linear increase from 37 to 55 % organic phase (B); 30-32 min, linear decrease to 37 % B. The column was allowed to equilibrate for 13 min prior to the next injection. The total flow rate was 1 mL/min, at T = 37 ºC. The saponins were detected by measuring the absorbance at 203 nm. The sample injection volume was 20 μL. The separation of saponins in the Quillaja Dry extract is presented in Figure S3.

(C) Separation of polyphenols.

The analysis was carried out with a flow rate of 1 mL/min and the mobile phase consisted of a gradient mixture of eluent A (formic acid 1.5 vol. % in water) and eluent B (acetonitrile). The following gradient of eluents was used: 0–47 min, from 5 to 30 % eluent B, 47-50 min, from 30 to 5 % eluent B and finally 5 min with 5% eluent B. The UV-VIS

detector was set at 280 nm and injected sample volume was 20 μL. An illustrative chromatogram of the polyphenols in QD is presented in Figure S4.

S4. Gas chromatography (GC) procedure.

The GC analyses were performed on a TRACE GC apparatus (ThermoQuest, Italy) equipped with autosampler AS 2000, both being donated kindly by Unilever R&D Laboratory in Colworth, UK (SEAC division). We used a capillary column Quadrex, USA, with the following specification: 5 % phenyl methylpolysiloxane, 10 m length, i.d.. 0.53 mm, 0.1 μm film thickness. Cold on-column injection was used, at a secondary cooling time of 0.3 min. The injection volume was 1 μL. The oven was programmed as follows: start at 120 ºC, hold 2 min, ramp 1 to 325 ºC at 10 ºC/min, ramp 2 to 345 ºC at 5 ºC/min, hold 5 min. The flame-ionization detector (FID) temperature was set to 350 ºC. The carrier gas was helium, set at a constant pressure flow mode (60 kPa).

Illustrative chromatogram, obtained in real experiment by the procedure described above, is presented in Figure S5. The different peaks were identified using the standard substances, described in the Materials section. Hexadecanol (cetanol) was used as internal standard, at a concentration of 0.15 mg/mL.

S5. Procedure for quantitative analysis of the GC data. The chromatograms obtained by the GC analysis were analysed using peak area integration. The concentration of the different FA, MG, TG and cholesterol was calculated from the internal standard, by the following equation:

$$C_X = A_X \frac{C_{IS}}{A_{IS}} \tag{2}$$

where C_X is the component concentration, A_X is the component peak area, C_{IS} is the concentration of the internal standard, and A_{IS} is the peak area of the internal standard. The experiments performed with standard solutions, containing known quantities of FA, MG, TG and cholesterol, showed that correction factors of 1.16 and 1.90 are needed for the FA and the TG, respectively. No correction factor was needed for the MG and no DG.

S6. Atomic absorption spectrometry (AAS) for determination of Ca^{2+} ions.

Because the AAS determination of calcium ions interferes with other ions, the Quillaja dry extract (QD) was dissolved in an electrolyte solution containing 1.37 M NaCl, 0.1 M KCl, 25 mM Na_2EDTA and 2 g/L NaN_3 (as in the *in vitro* experiments), whereas the polyphenol isolate was measured directly in water. A Perkin Elmer Analyst 400 flame atomic absorption spectrometer was used for the determination of calcium in the obtained solution. A calcium hollow cathode lamp was used as a specific radiation source. The sample was measured directly in air/acetylene flame under optimal instrumental parameters.

We prepared standard solutions of Ca^{2+} by dissolving known amounts of calcium dichloride in an electrolyte solution, which had the same composition as the one used for QD, or in water (for the polyphenol isolate). In this way, we prepared matrix matched standards. A calibration curve was prepared by measuring the absorption of the standard solutions. The obtained dependence of the absorbance on the Ca^{2+} concentration was linear and was used to calculate the calcium in QD and in the polyphenols isolate.

S7. Cryogenic transmission electron microscopy (cryo-TEM). The cryo-samples were prepared using a CryoPlunge 3 unit (Gatan Instruments) using a double blot technique. Briefly, 3µl of the studied solution was pipetted onto 15 s plasma etched, 400 mesh holey carbon grid (Agar Scientific), held in the plunge chamber at approx 90 % humidity. The samples were blotted from both sides for 0.5, 0.8, or 1.0 s. The samples were then plunged into liquid ethane, at a temperature of -170˚C. The grids were blotted to remove the excess ethane, then transferred under liquid nitrogen at -170˚ C to cryo-TEM specimen holder (Gatan 626). The samples were examined with Jeol 2100 TEM, operated at 200 kV, and imaged using Gatan Ultrascan 4000 camera and DigitalMicrograph software (Gatan).

Table S1. Fatty acid composition of the studied fats and oils, as determined by GC. These percentages are defined as the moles of given fatty acid, normalized by the total moles of all visible fatty acids in the GC chromatogram. The fatty acid composition obtained after complete hydrolysis of the TG to glycerol with alcoholic NaOH is abbreviated "To Gly", whereas the composition obtained after our in vitro lipolysis is abbreviated "After Lip". Note that the lipolysis affects predominantly 1 and 3 positions in the TG which explains the different results in the columns "To Gly" and "After Lip" for given fat.

Fatty acids, molar %	SFO		Lard		Cocoa butter	
	To Gly	After Lip	To Gly	After Lip	To Gly	After Lip
Myristic acid (C14:0)	0	0	1.7	0	0	0
Palmitoleic acid (C16:1)	0	0	2.0	0	0	0
Palmitic acid (C16:0)	7.8	10.9	31.3	15.8	28.6	37.5
Oleic + linoleic acid (C18:1,2)	86.6	82.4	43.9	58.6	33.9	15.0
Stearic acid (C18:0)	5.6	6.7	21.1	25.6	37.5	47.5
Saturated FA (SFA)	13.4	17.6	54.1	41.4	66.1	85.0
Unsaturated FFA (UFA)	86.6	82.4	43.9	58.6	33.9	15.0

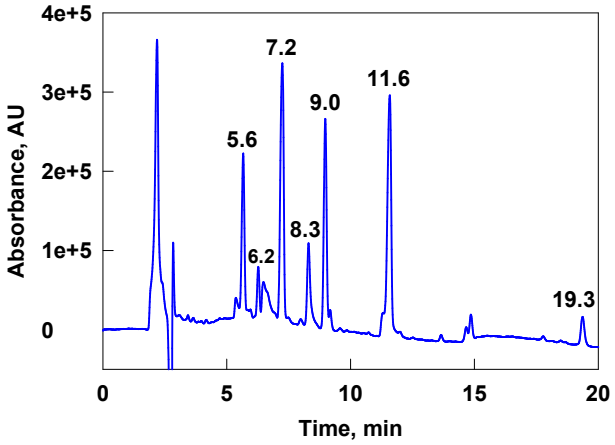

Figure S1. Typical HPLC chromatogram of the bile salts and acids present in the permeate, which is obtained after filtration of the lipolysis solution through 200 nm filter. The major bile salts and acids elute at 5.6, 6.2, 7.2, 8.3, 9.0, 11.6 and 19.3 min (see Figure S2).

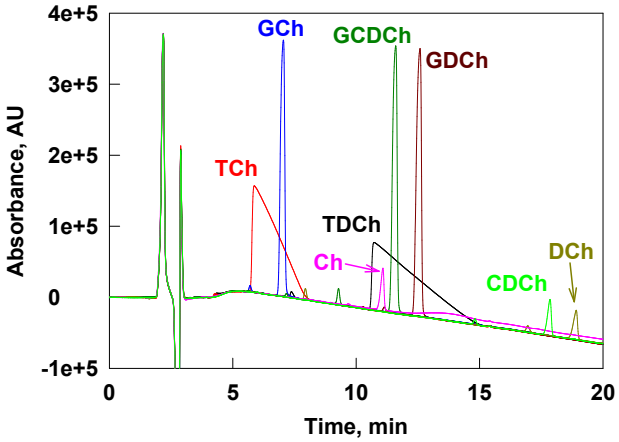

Figure S2. HPLC chromatograms of single bile salt and bile acid standards, plotted on the same graph, as a function of the retention time: Taurocholic acid (TCh, 6.2 min), glycocholic acid (GCh, 7 min), taurodeoxycholic acid (TDCh, 10.7 min), cholic acid (Ch, 11 min), glycochenodeoxycholic acid (GCDCh, 11.5 min), glycodeoxycholic acid (GDCh, 12.6 min), chenodeoxycholic acid (CDCh, 17.8 min) and deoxycholic acid (DCh, 18.8 min).

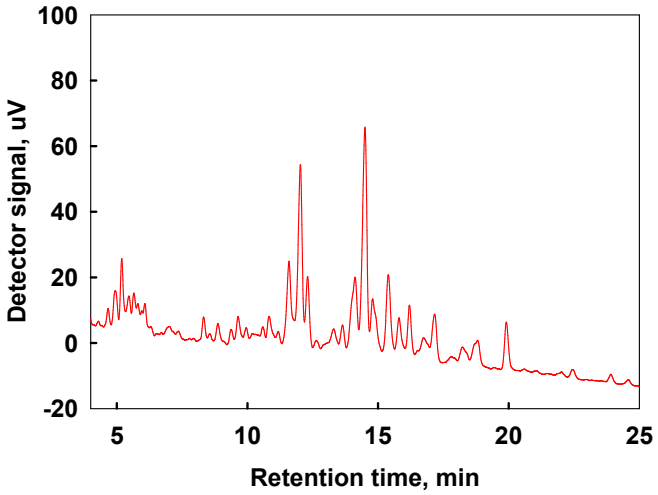

Figure S3. HPLC chromatogram of the saponins in Quilaja Dry (QD) extract.

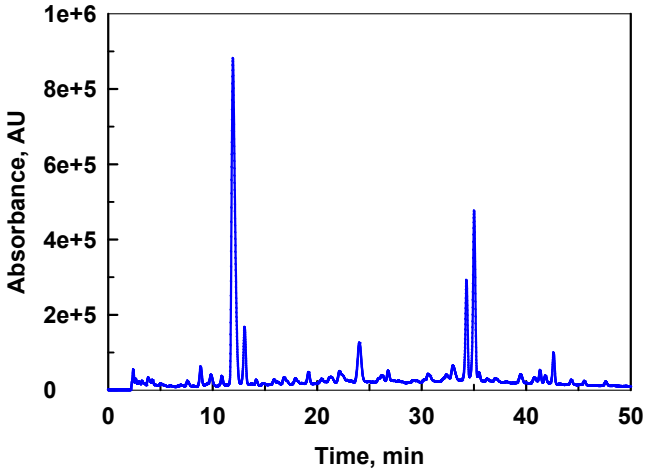

Figure S4. HPLC chromatogram of the polyphenols in Quillaja Dry (QD) extract.

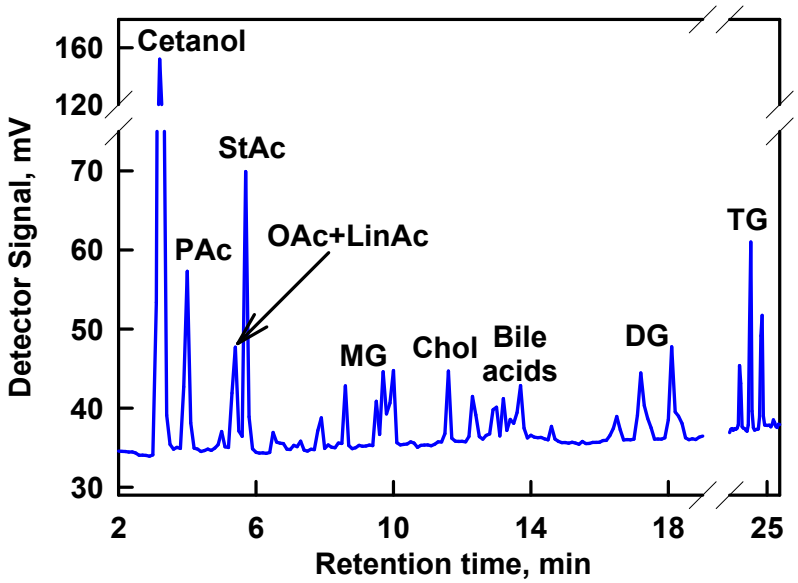

Figure S5. GC signal, as a function of retention time, for cocoa butter emulsion, after its *in vitro* lipolysis and extraction of the lipophilic components with chloroform. The different peaks were identified using the respective standard substances.

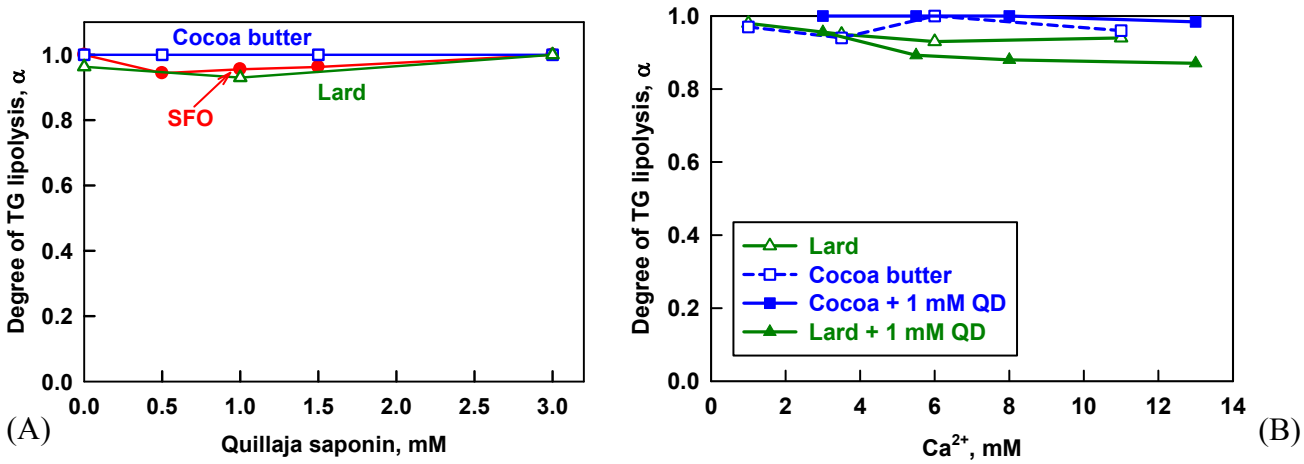

Figure S6. Degree of TG lipolysis, α, as a function of: (A) Quillaja saponin concentration for emulsions of sunflower oil (red circles), cocoa butter (blue square) and lard (green triangle); (B) Ca^{2+} concentration for emulsions of lard (green triangles) or cocoa butter (blue squares), in presence (full symbols) or in absence (empty symbols) of Quillaja Dry. The presence of Ca^{2+} ions in QD is accounted for in (B). The experimental data are from single experiments.

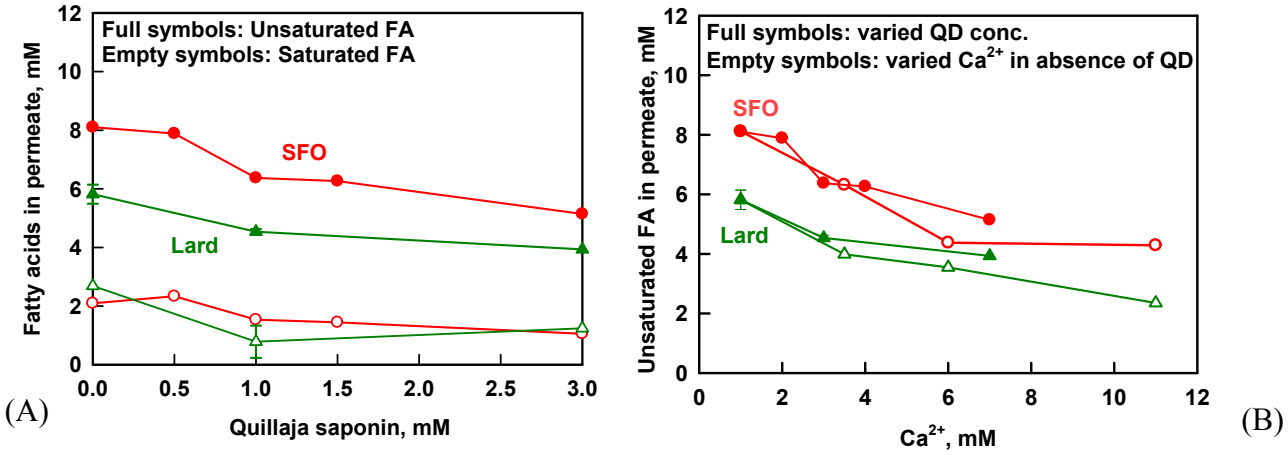

Figure S7. (A) Concentration of the solubilized saturated (full symbols) and unsaturated (empty symbols) free FA, as a function of QD concentration, obtained after lipolysis of sunflower oil (red circles) and lard (green triangles). The concentration of free FA in the whole sample (before filtration) for SFO is 3.7 mM for the saturated and 8.5 mM for the unsaturated acids, while for lard is 2.7 mM for the saturated and 5.8 mM for the unsaturated acids. (B) Concentration of the solubilized unsaturated free FA, as a function of calcium concentration for experiments performed at different QD concentrations (full symbols) or in absence of QD and varying C_{Ca} (empty symbols). Note that 1 mM of saponins in QD extract corresponds to the addition of 2 mM Ca^{2+} ions. The data are obtained after lipolysis of sunflower oil emulsions (red circles) and lard emulsions (green triangles) and are averaged from (at least) two separate experiments.

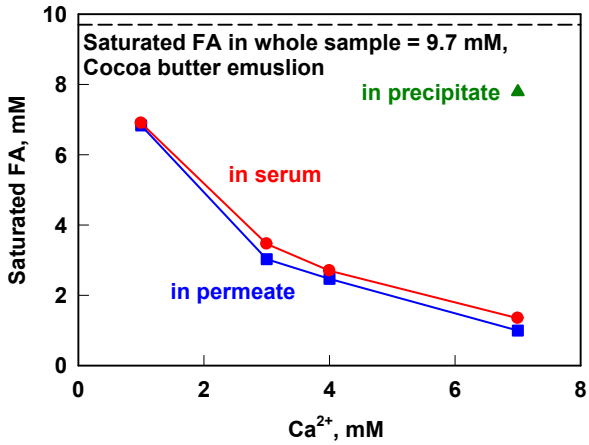

Figure S8. Molar concentration of solubilized saturated fatty acids in the permeate after filtration (blue squares), in the serum (red circles), and in the precipitate after centrifugation (green triangle), as a function of Ca^{2+} concentration, obtained after lipolysis of cocoa butter emulsion. The concentration of saturated FA in the whole sample (before filtration or centrifugation) is 9.7 mM. The concentration of Ca^{2+} is varied by using different concentrations of QD. The experimental data are from single experiments.

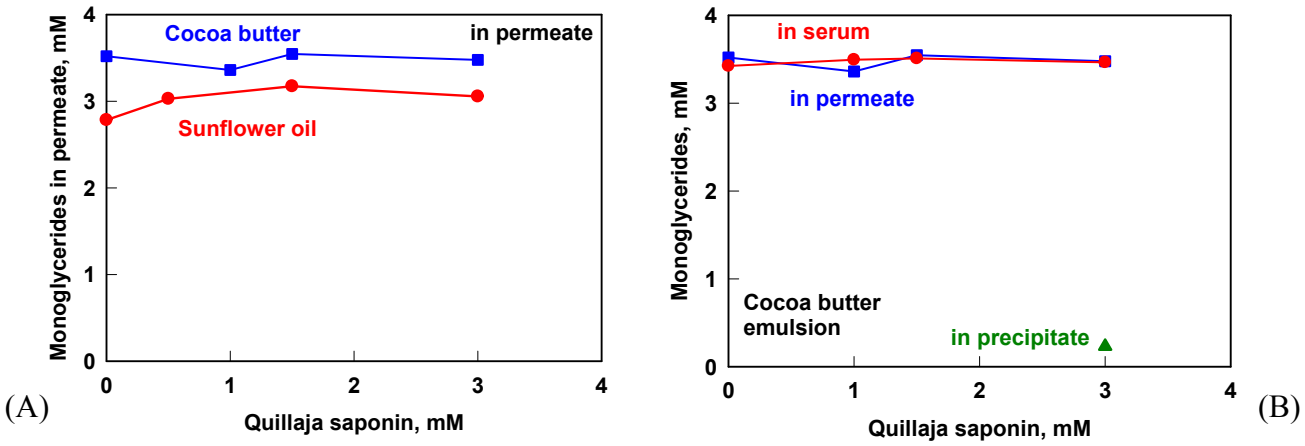

Figure S9. (A) Molar concentration of monoglycerides in the permeate for cocoa butter (blue squares) and sunflower oil (red circles), as a function of the Quillaja saponin concentration. (B) Molar concentration of solubilized monoglycerides in the permeate (blue squares), in the serum (red circles) and in the precipitate (green triangles), as a function of Quillaja saponin concentration, obtained after lipolysis of cocoa butter. All data are from single experiments.

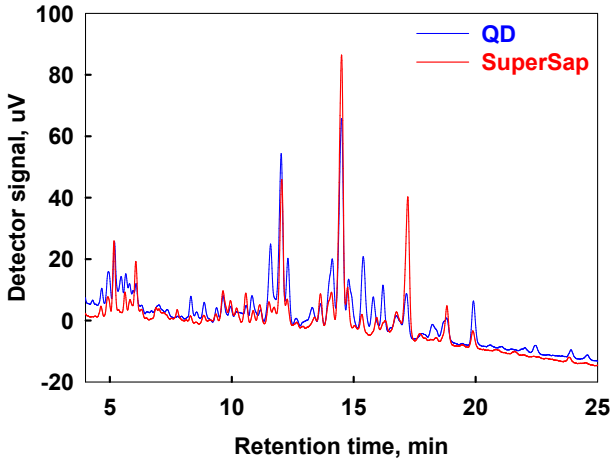

Figure S10. HPLC chromatogram of the saponins in QD extract (blue curve) and highly purified SuperSap extract (red curve).