Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supporting Information

A One-shot Double-slice Selection NMR Method for Biphasic Systems

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Experimental

Sample Preparations:

Model Biphasic Sample: 50 mg of sucrose and 53 mg of 4-chromanone were dissolved in 1 ml of D_2O and 1 ml of $CDCl_3$, respectively. First, 250 μL of the 4-chromamone-$CDCl_3$ solution was transferred to a 5-mm NMR tube. Then, 250 μL of 4-chromanone-D_2O solution was carefully and slowly added to the tube to form a biphasic sample.

In-tube extraction tea samples: For the tea pure solvent extraction samples, 25 mg of cryo-ground tea leaves (Watermelon Lime Zinger Caffeine Free Herbal Tea made by Celestial Seasonings, Inc. Boulder CO, USA and Benner Green Tea Original by Aldi, Inc. Batavia, IL) were added to 3 ml of D_2O and $CDCl_3$, respectively. These were mixed well. The mixtures were then filtered using a 45 μm sterile syringe filter before transferring to a 5-mm NMR tube.

For the biphasic tea samples, 250 μL $CDCl_3$ and 250uL of D_2O were added into a 5-mm NMR tube, then ~6.5mg of cryo-ground tea leaves were transferred into the tube and mixed well with the solvents by shaking. The NMR tube containing the biphasic sample was centrifuged with a hand crank centrifuge until a well separated biphasic sample formed.

Cold and hot brew coffee samples: For the coffee extractions, two vials were prepared by combining ~500 mg of ground coffee beans (Organic Rainforest Blend Whole Bean Coffee by San Francisco Bay Coffee, Lincoln, CA, USA) with 10 mL of D_2O; the vials were shaken/swirled to ensure contact of the D_2O and the coffee grounds. The cold brew coffee vial was then left at room temperature for 24hrs with occasional swirling. The hot brew coffee vial was brought to a boil, and left to boil with occasional swirling for two minutes. The contents of both vials were transferred to polystyrene centrifuged tubes and centrifuged at ~3000 RCF for 25 minutes. The liquid contents were decanted into fresh containers, leaving the coffee grinds behind in the tubes. 250 μL of $CDCl_3$ were added to two NMR tubes, followed by the addition of 250 μL of the D_2O coffee samples (separate tubes for each brew method). The samples were gently inverted and shaken repeatedly to perform the extraction. The tubes were then placed in centrifuge tubes with home-made foam inserts and centrifuged at ~3000 RCF for 30 minutes until well-separated biphasic samples formed.

NMR instrument: A Bruker AVIII 400 MHz NMR spectrometer with a BBFO probe and Bruker NEO 600 MHz spectrometer with a TCI cryoprobe were used in this study. All experiments were carried out at 300 K.

In a one-shot double-slice selection experiment, alternative polarity of the gradient pulses (±3.43 G/cm) were used to improve the spectral properties such as phasing and baseline lineshapes.

A presat TOCSY pulse with the mlev spin-lock scheme was used by replacing the 90° pulse for magnetization preparation with a selective ReBurp pulse in our investigation.

The r.f. power of ReBurp.1000 pulse for a given pulse width can be calculated by the pulse programs or calibrated by observing the minimum signal at the zero-offset frequency. The ReBurp.1000 excitation profile was measured by selecting a CH_2 group of 4-chromanone in $CDCl_3$ using a Bruker "selzg" pulse program and ramping the offset frequency from -5000 Hz to +5000 Hz with a step length of 50 Hz.

The number of scans to be collected was determined based upon obtaining sufficient signal-to-noise. For the model biphasic sample on a 400 MHz instrument with a conventional BBFO probe, the number of scans was 16. For the biphasic tea and coffee samples using cryoprobe on a 600 MHz instrument, the number of scans were either 32 or 64.

Pulse Programs (Bruker Avance NMR Spectrometer):

OSDS-1D:

```
;one-shot double-slice
;avance-version
;1D sequence
;
;$CLASS=HighRes
;$DIM=1D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

#include <Avance.incl>
#include <Grad.incl>

"acqt0=-p1*2/3.1416"
"spw2=plw1/((p12*90.0)/(p1*totrot2))*((p12*90.0)/(p1*totrot2))*(integfac2*integfac2)"

"spoffs2=cnst11"
"l0=1"

1 ze
2 30m
  20u pl1:f1
  d1
  50u UNBLKGRAD
 if "l0%2==1"
 {
  5u gron0
  (p12:sp2 ph1):f1
  5u groff
 }
 else
 {
  5u gron1
  (p12:sp2 ph1):f1
  5u groff
 }
  50u BLKGRAD
  d20
  "l0=l0+1"
  go=2 ph31
  30m mc #0 to 2 F0(zd)
  20u BLKGRAD
exit

ph1=0 0 2 2 1 1 3 3
ph31=0 2 2 0 1 3 3 1

;pl1 : f1 channel - power level for pulse (default)
;p1 : f1 channel -  high power pulse
;p12 : f1 channel - Reburp.1000 pulse [1 - 2ms]
;d1 : relaxation delay; 1-5 * T1
;NS: 1 * n, total number of scans: NS * TD0

;$Id: zg,v 1.9 2006/11/10 10:56:44 ber Exp $
```

OSDS-TOCSY

```
;osds-mlevphpr
;avance-version (12/01/11)
;homonuclear Hartman-Hahn transfer using MLEV17 sequence
;   for mixing
;using two power levels for excitation and spinlock
;phase sensitive
```

```
;
;A. Bax & D.G. Davis, J. Magn. Reson. 65, 355-360 (1985)
;
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"p5=p6*.667"
"p7=p6*2"
"d11=30m"
"d12=20u"
"d13=4u"

"in0=inf1"

"d0=in0/2-p1*2/3.1416-4u"

"spw2=plw1/(((p12*90.0)/(p1*totrot2))*((p12*90.0)/(p1*totrot2))*(integfac2*integfac2))"
"spoffs2=cnst12"


"SCALEF=p7*2/p5"
"FACTOR1=((d9-p17*2)/(p6*64+p5))/SCALEF"
"l1=FACTOR1*SCALEF"


1 ze
2 d11
3 d12 pl9:f1
  d1 cw:f1 ph29
  d13 do:f1
  50u UNBLKGRAD
  d12 pl1:f1
  5u gron0
  (p12:sp2 ph1):f1
  5u groff
  d0
  4u pl10:f1
  (p17 ph26)
                                                                ;begin MLEV17
4 (p6 ph22 p7 ph23 p6 ph22)
  (p6 ph24 p7 ph25 p6 ph24)
  (p6 ph24 p7 ph25 p6 ph24)
  (p6 ph22 p7 ph23 p6 ph22)
  (p6 ph24 p7 ph25 p6 ph24)
  (p6 ph24 p7 ph25 p6 ph24)
  (p6 ph22 p7 ph23 p6 ph22)
  (p6 ph22 p7 ph23 p6 ph22)
  (p6 ph24 p7 ph25 p6 ph24)
  (p6 ph22 p7 ph23 p6 ph22)
  (p6 ph22 p7 ph23 p6 ph22)
  (p6 ph24 p7 ph25 p6 ph24)
  (p6 ph22 p7 ph23 p6 ph22)
  (p6 ph22 p7 ph23 p6 ph22)
  (p6 ph24 p7 ph25 p6 ph24)
  (p6 ph24 p7 ph25 p6 ph24)
  (p5 ph23)
  lo to 4 times l1
                                                                ;end MLEV17
  (p17 ph26)
  50u BLKGRAD
  go=2 ph31
  d11 mc #0 to 2 F1PH(calph(ph1, +90) & calph(ph29, +90), caldel(d0, +in0))
exit
```

```
ph1=0 2 2 0 1 3 3 1
ph22=3 1 3 1 0 2 0 2
ph23=0 2 0 2 1 3 1 3
ph24=1 3 1 3 2 0 2 0
ph25=2 0 2 0 3 1 3 1
ph26=0 2 0 2 1 3 1 3
ph29=0
ph31=0 2 2 0 1 3 3 1


;pl1 : f1 channel - power level for pulse (default)
;pl9 : f1 channel - power level for presaturation
;pl10: f1 channel - power level for TOCSY-spinlock
;p1 : f1 channel -  90 degree high power pulse
;p5 : f1 channel -  60 degree low power pulse
;p6 : f1 channel -  90 degree low power pulse
;p7 : f1 channel - 180 degree low power pulse
;p12 : f1 channel - Reburp.1000 ulse [1-2ms]
;p17: f1 channel - trim pulse                [2.5 msec]
;d0 : incremented delay (2D)
;d1 : relaxation delay; 1-5 * T1
;d9 : TOCSY mixing time
;d11: delay for disk I/O                     [30 msec]
;d12: delay for power switching                [20 usec]
;d13: short delay                            [4 usec]
;l1: loop for MLEV cycle: (((p6*64) + p5) * l1) + (p17*2) = mixing time
;inf1: 1/SW = 2 * DW
;in0: 1/(1 * SW) = 2 * DW
;nd0: 1
;ns: 8 * n
;ds: 16
;td1: number of experiments
;FnMODE: States-TPPI, TPPI, States or QSEQ


;Processing

;PHC0(F1): 180
;PHC1(F1): -180
;FCOR(F1): 1



;$Id: mlevphpr,v 1.12 2012/01/31 17:49:27 ber Exp $
```

Proton Image of Model Biphasic Sample:

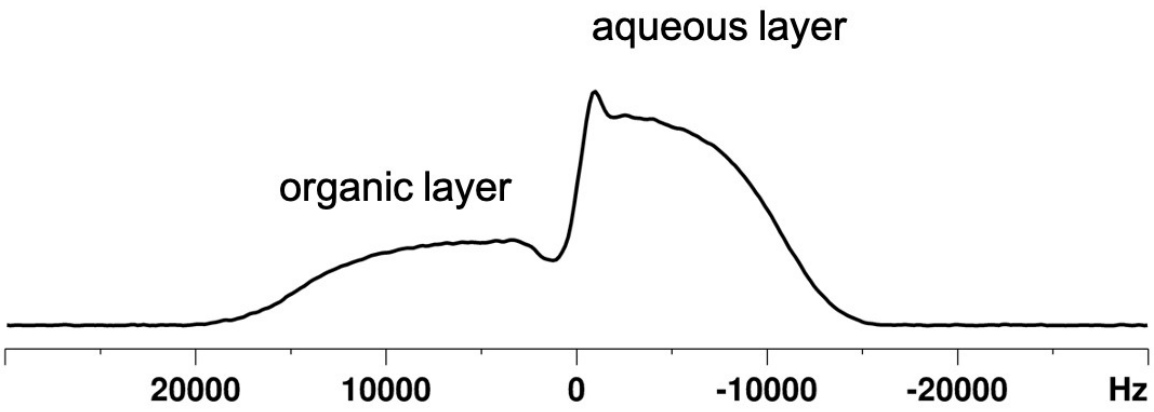

Fig. S1. 1D ^{1}H image of model biphasic sample of sucrose in D_2O and 4 chromanone in $CDCl_3$. The image was obtained using a gradient echo pulse program with an echo time of 2 ms. The frequency axis at zero hertz corresponds to the centre of NMR coil where the interface of the biphasic sample is located. The organic and aqueous layers are labelled in the profiles. The gradient strength is 3.43 G/cm.

ReBurp Pulse Simulations:

The Shaped Pulse Tool of Bruker's TopSpin software was used to calculate the excitation profiles and simulate magnetization trajectories of the ReBurp pulses. The following parameters were chosen to generate a ReBurp pulse for the simulation:

Excitation mode: Universal 180
Excitation Type: Refocusing
Rotation: 180
Bandwidth Factor: 5.81
Size: 1024

First, the excitation profile of a ReBurp pulse at a given pulse width was calculated, then the positive and negative effective offsets were obtained from the profile. The magnetization trajectories were simulated by setting a three-step simulation from the negative offset to the positive offset.

Table S1. Bandwidths, Effective offsets, Effective bandwidths of the ReBurp[a] Pulse

Pulse width	Band width	Ω_{eff}	$\Delta\nu_{eff}$	z	Δz
(μs)	(Hz)	(Hz)	(Hz)	(mm)	(mm)
1000	6264	±2700	1030	1.8	0.7
1200	5220	±2243	860	1.5	0.6
1400	4474	±1928	737	1.3	0.5
1600	3915	±1684	645	1.2	0.4
1800	3483	±1498	573	1.0	0.4
2000	3132	±1346	516	0.9	0.4

[a]Effective ReBurp pulse offsets and bandwidth for excitation are obtained from simulations using the shaped pulse tool (Bruker TopSpin). *z* and *Δz* are estimated using the calibrated gradient strength of 3.43 G/cm and $^{\Omega_{eff}}$and $^{\Delta\nu_{eff}}$.

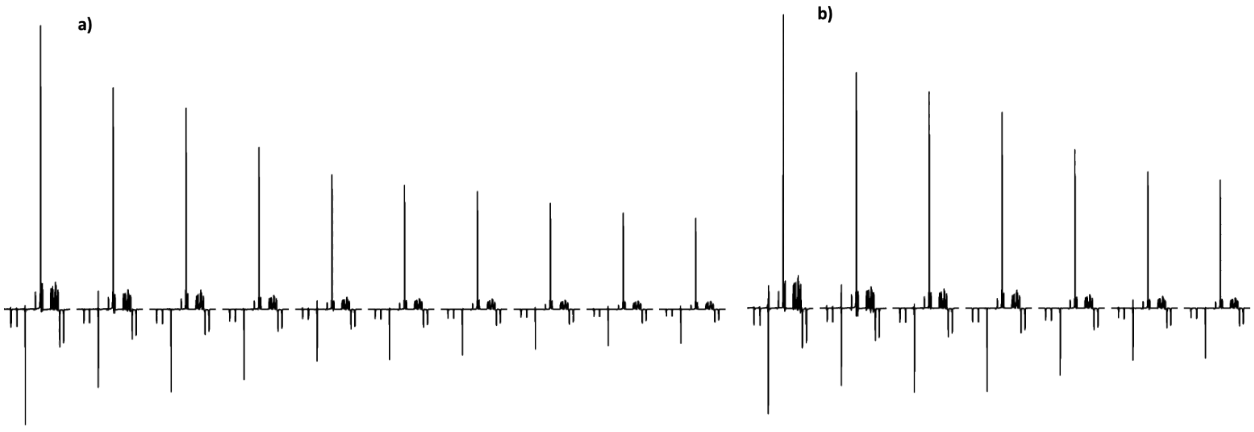

Fig. S2. One-shot double-slice NMR signals of the model biphasic sample as a function of pulse width (a) from 800 μs (left) to 2600 μs. (right) with an increment of 200 μs at a gradient strength of 3.43 G/cm. The one-shot double-slice NMR signals measured as a function of gradient strength (b) from 4% (left) to 10% (right) with a step length of 1%. The pulse width was set to 1200 us and the gradient strength at 100% is 51.1 G/cm.

^{1}H Spectral Assignments of BGT, CHT, and HBC

The presat ^{1}H spectra of BGT, CHT, and HBC biphasic samples are given in Figs. S2, S3, and S4, in which the peaks are numerically labeled and their assignments based on the reports in the literatures are given in Table S2, S3, and S4.

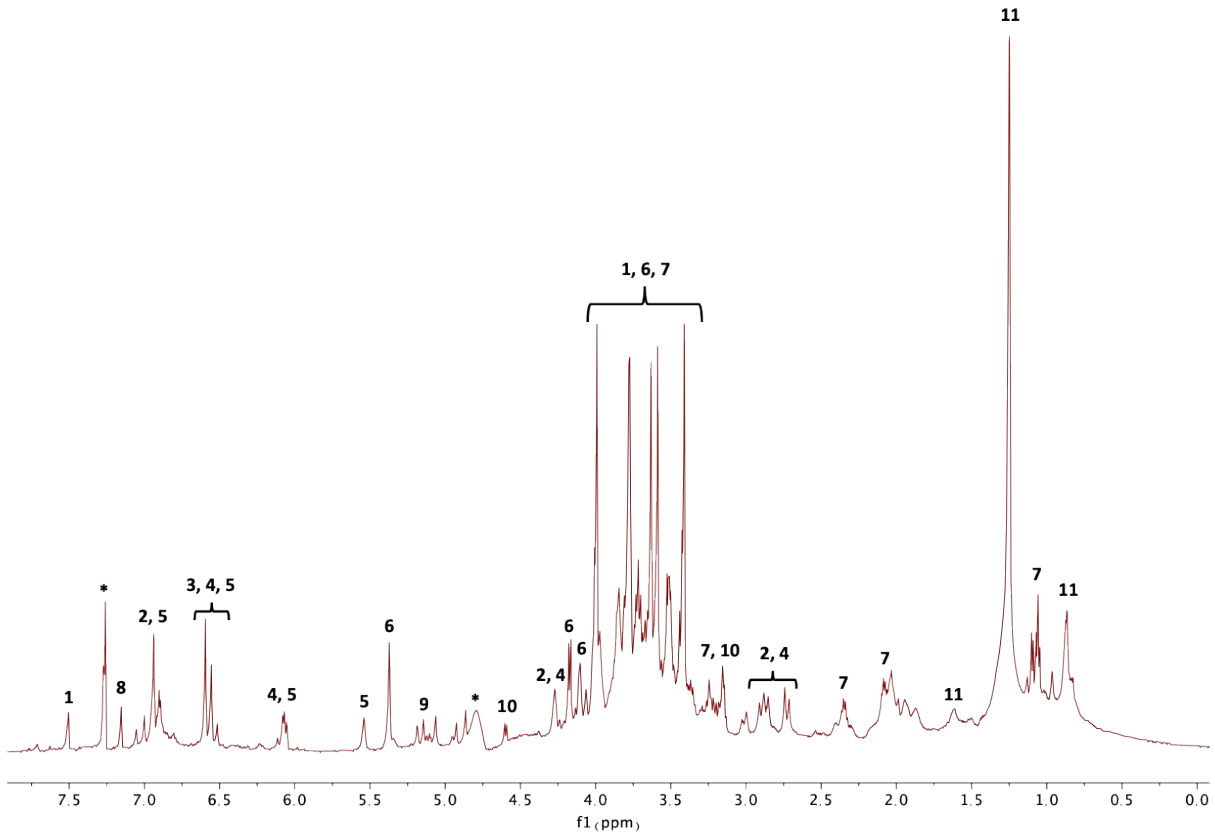

Fig. S3. ^{1}H spectrum of the BGT biphasic sample.

Table S2: Chemical shift assignment of BGT

Peak Label	Assignment	Chemical shift, δ (ppm)
1	Caffeine[1]	7.50 (s), 3.99 (s), 3.59 (s), 3.41 (s)
2	Epicatechin (EC)[2]	6.95, 4.27, 2.87, 2.73
3	Gallocatechin (GC)[3]	6.52 (s)
4	Epigallocatechin (EGC)[3]	6.59 (s), 6.07, 4.27, 2.87, 2.73
5	Epigallocatechin gallate (EGCG)[3]	6.90, 6.55 (s), 6.07, 5.54
6	Sucrose[1a, 3]	5.37 (d), 4.17, 4.06, 3.85 (m), 3.78, 3.63, 3.51 (m)
7	Theanine[1]	3.72 (t), 3.16 (m), 2.33 (m), 2.08 (m), 1.06 (t)
8	Gallic acid[1b]	7.15 (s)
9	α-glucose[1]	5.18 (d)
10	β-glucose[1b]	4.60 (d), 3.23 (m)
11	Unsaturated fatty acid[4]	1.61, 1.25 (s), 0.87 (m)

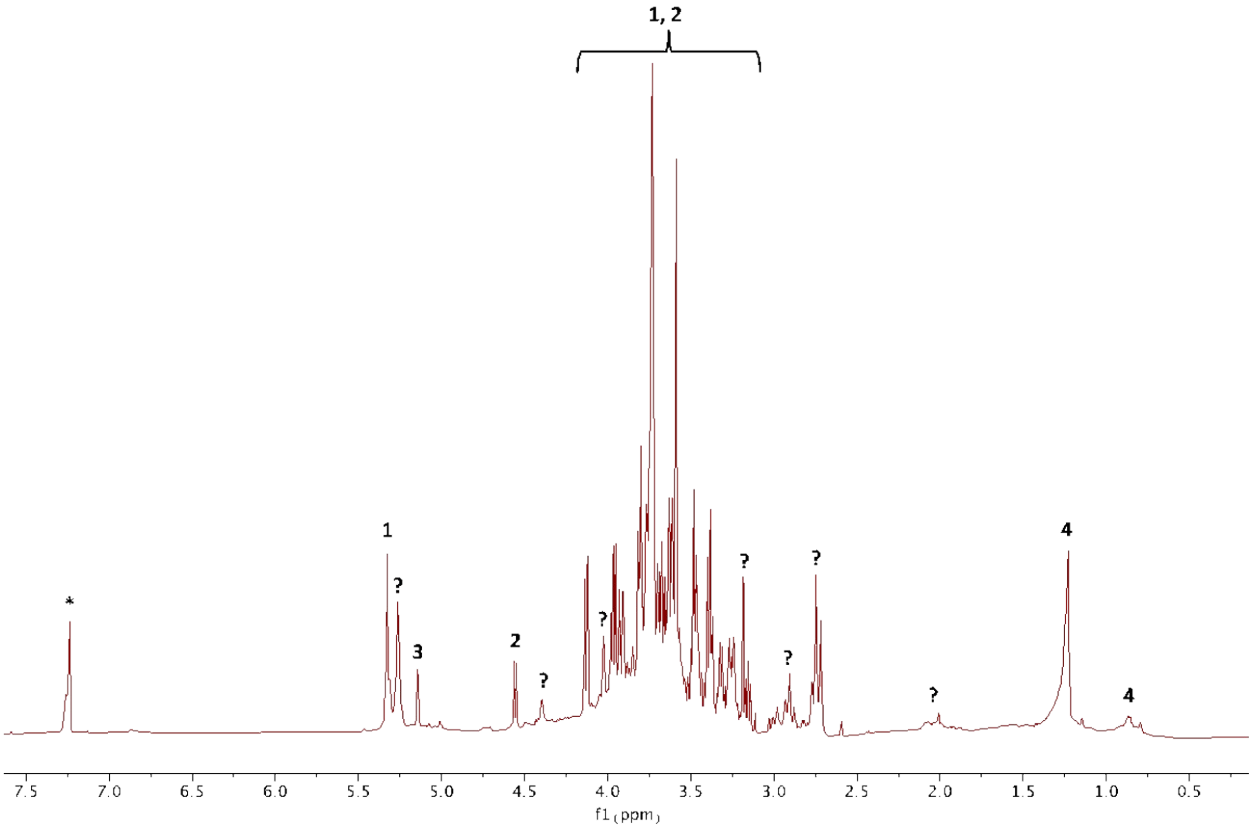

Fig. S4. ^{1}H spectrum of the CHT biphasic sample**.** (? indicates unidentified resonances.)

Table S3: Chemical shift assignment of CHT

Peak Label	Assignment	Chemical shift, δ (ppm)
1	Sucrose[1, 4]	5.34 (m), 4.15 (d), 3.82 (m), 3.76, 3.61
2	β-glucose[2]	4.58 (d), 3.18 (m)
3	α-glucose[1, 2]	5.17
4	Saturated fatty acid[5]	1.27 (s), 0.86 (m)

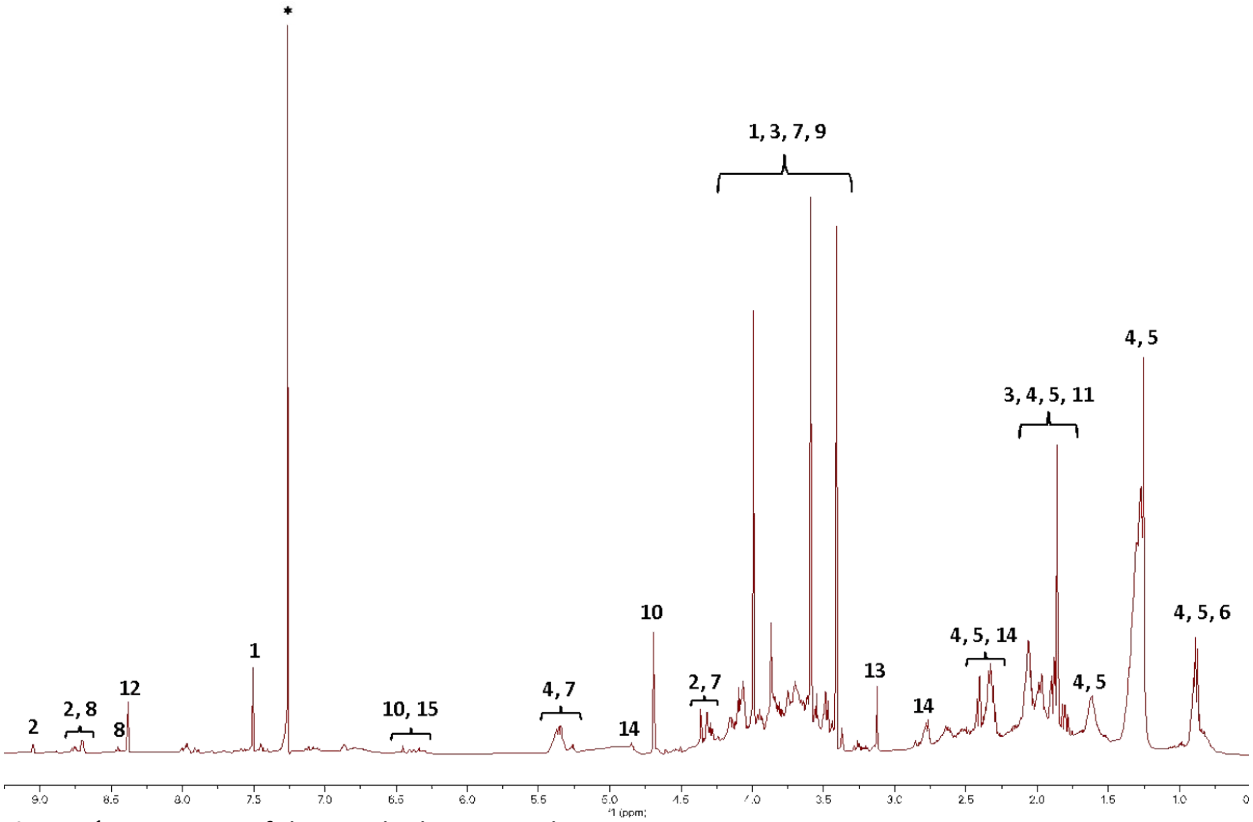

Fig. S5. ^{1}H spectrum of the HBC biphasic sample.

Table S4: Chemical shift assignment of HBC

Peak Label	Assignment	Chemical shift, δ (ppm)
1	Caffeine	7.50 (s), 3.99 (s), 3.59 (s), 3.41 (s)
2	Trigonelline[5]	9.05 (s), 8.76 (t), 4.36 (s)
3	Quinic acid[6]	4.07 (m), 3.95 (td), 3.48 (m)
4	Linoleic acid	5.35 (m), 2.77 (m), 2.33 (m), 2.06 (m), 1.60, 1.28, 0.88
5	Palmitic acid	2.33 (m), 1.60, 1.28, 0.88
6	Lactic acid[5]	4.07 (m)
7	Glycerol ester	4.29 (dd), 4.14 (m), 2.33 (m)
8	*N*-methylpyridinium[7]	8.70 (d), 8.45
9	*myo*-inositol[6]	3.44
10	2-furylmethanol[7]	6.41 (d), 4.69 (s)
11	Acetic acid[6]	1.86 (s)
12	Formic acid[5]	8.38 (s)
13	Choline[6]	3.13 (s)
14	*γ*-quinide[7]	4.84, 2.42 (d)
15	5-caffeoylquinic acid[7]	6.29

References

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