Electronic Supplementary Material (ESI) for Food & Function.

Supplementary Information:

Metabolomics reveals the toxicological effects of polar compounds from frying palm oil

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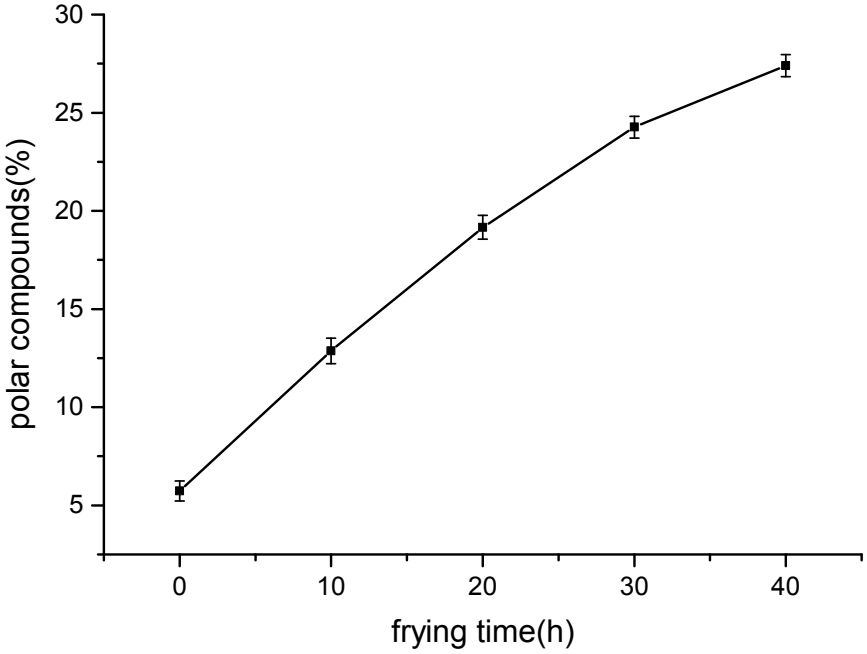

Figure S1. The evolution of polar compounds content during frying process

Table S1. Fatty acid composition of frying palm oil during frying process

FFA (%)	0 h	10 h	20 h	30 h	40 h
C10:0	0.00±0.00	0.00±0.00	0.03±0.00	0.03±0.00	0.03±0.00
C12:0	0.16±0.01	0.24±0.01	0.26±0.01	0.31±0.02	0.31±0.01
C14:0	1.14±0.01	1.62±0.02	1.13±0.02	1.67±0.01	1.67±0.02
C15:0	0.04±0.00	0.06±0.00	0.05±0.00	0.06±0.00	0.06±0.00
trans C16:1	0.11±0.01	0.37±0.01	0.43±0.02	0.79±0.02	0.96±0.02
C16:0	51.42±0.21	51.36±0.20	50.84±0.29	55.13±0.36	61.64±0.39
C17:0	0.08±0.01	0.08±0.01	0.09±0.01	0.09±0.01	0.09±0.01
C18:2	7.22±0.37	6.70±0.32	6.98±0.51	6.01±0.26	6.18±0.25
C18:1	33.85±0.25	32.56±0.27	31.91±0.22	28.08±0.19	21.26±0.13
trans C18:1	0.37±0.01	0.53±0.02	0.64±0.03	0.68±0.02	0.65±0.03
trans C18:2	0.09±0.01	0.06±0.01	0.06±0.01	0.04±0.01	0.05±0.01
C18:0	4.84±0.05	5.52±0.04	5.97±0.09	5.62±0.03	5.49±0.03
C20:1	0.10±0.01	0.17±0.01	0.22±0.02	0.23±0.01	0.26±0.02
C20:0	0.43±0.03	0.49±0.04	0.49±0.02	0.44±0.03	0.42±0.02
C22:0	0.07±0.01	0.09±0.02	0.09±0.01	0.08±0.01	0.07±0.01
C23:0	0.04±0.01	0.08±0.01	0.06±0.01	0.07±0.01	0.07±0.01
C24:0	0.06±0.01	0.07±0.01	0.06±0.01	0.06±0.01	0.05±0.01
SFAs	58.27±0.35	59.60±0.36	59.06±0.51	63.56±0.49	69.91±0.51
UFAs	41.73±0.66	40.40±0.64	40.23±0.87	35.82±0.51	29.36±0.46

SFAs, Saturated fatty acids; UFAs, unsaturated fatty acid

Table S2.The formula list of KM mice diets(%)

Component	ND	FEO	FYO	PC
maize starch	54.45	49.45	49.45	49.45
casein	20.29	20.29	20.29	20.29
maltodextrin	5.07	5.07	5.07	5.07
cellulose	5.07	5.07	5.07	5.07
lard	10.00	10.00	10.00	10.00
fresh palm oil	0.00	5.00	0.00	0.00
heated palm oil	0.00	0.00	5.00	0,00
polar compounds	0.00	0.00	0.00	5,00
calcium carbonate	1.32	1.32	1.32	1.32
calcium bicarbonate	1.01	1.01	1.01	1.01
cholesterol	1.00	1.00	1.00	1.00
potassium citrate	1.01	1.01	1.01	1.01
DL-methionine	0.30	0.30	0.30	0.30
sodium chlori	0.20	0.20	0.20	0.20
sucrose	0.10	0.10	0.10	0.10
choline bitartrate	0.10	0.10	0.10	0.10
mineral mixture	0.06	0.06	0.06	0.06
vitamin mixture	0.02	0.02	0.02	0.02
total polar compounds	0	0.25	1.25	5

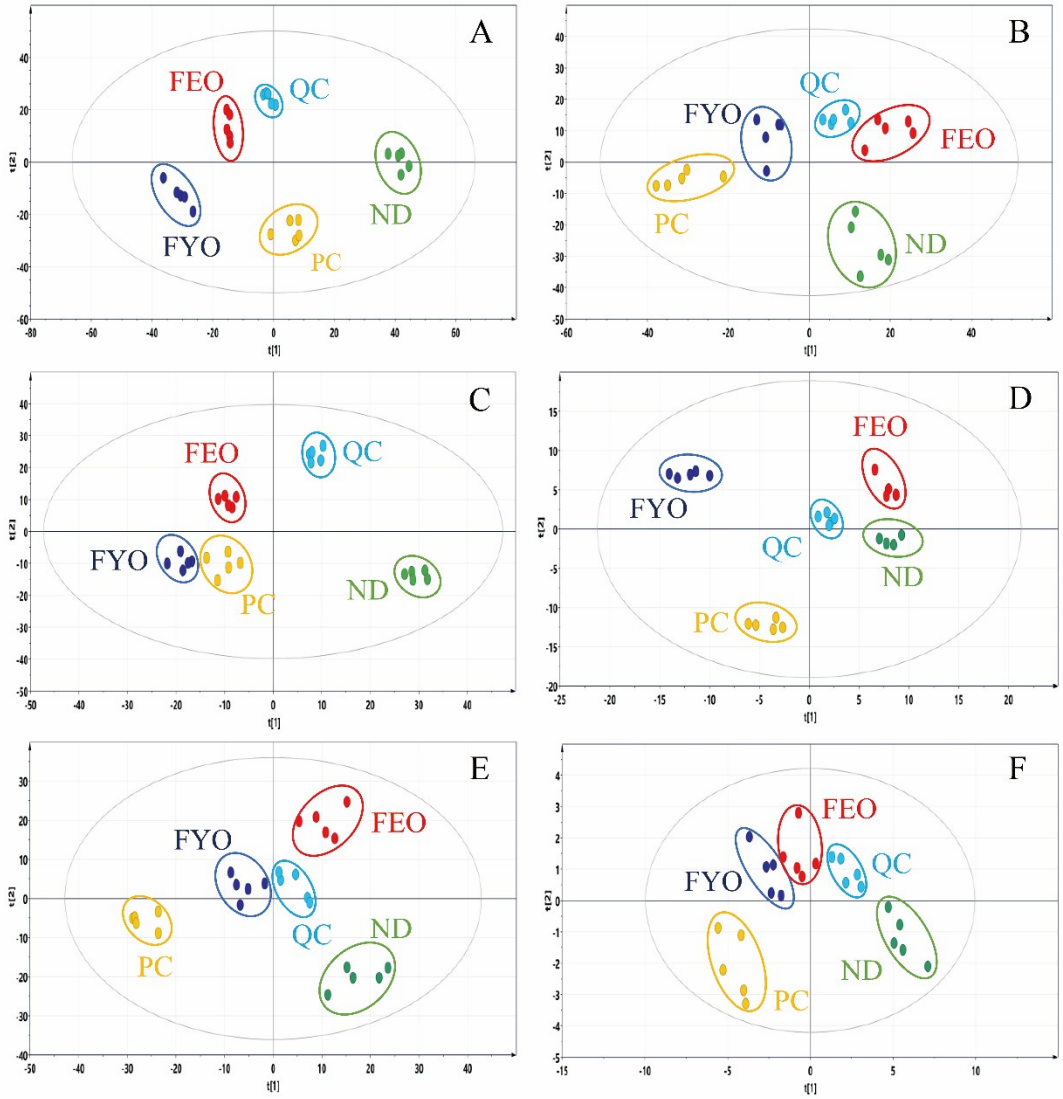

Figure S2. Principal components analysis (PCA) score plots obtained from GC-MS data of serum(A) and liver (B). PCA score plots obtained from LC-MS data of serum metabolites profile in positive (C) and negative (E) ion mode, and liver in positive (D) and negative (F) ion mode. QC, quality control samples; ND, normal diet group; FEO, fresh palm oil group; FYO, frying palm oil group; PC, polar compounds group.

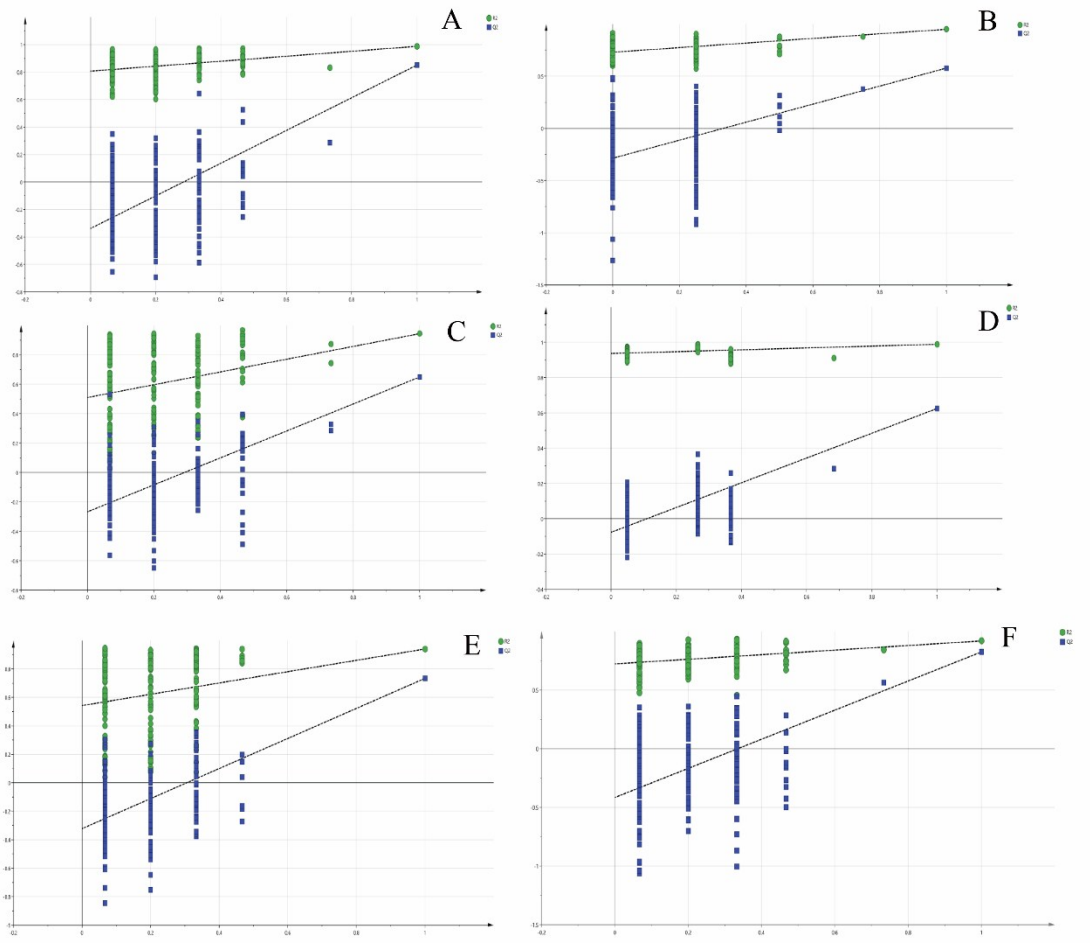

Figure S3. Cross validation of OPLS-DA models with a permutation test (n = 200) from GC-MS data of serum(A) and liver (B). Cross validation from LC-MS data of serum metabolites in positive (C) and negative (E) ion mode, and liver metabolites in positive (D) and negative (F) ion mode.

Table S3. Tests of normality of variables in Pearson correlation

	Shapiro-Wilk		
	Statistic	Df	Sig
Serum MDA	0.942	4	0.669
Serum SOD	0.92	4	0.535
Hepatic MDA	0.962	4	0.792
Hepatic SOD	0.963	4	0.796
LysoPC(16:0)	0.664	4	0.054
LysoPC(18:1)	0.978	4	0.89
LysoPC(20:1)	0.961	4	0.782
LysoPC(20:4)	0.818	4	0.138
LysoPC(22:6)	0.859	4	0.255
LysoPE(20:4)	0.78	4	0.071
PC(16:0/16:0)	0.724	4	0.061
PC(14:0/20:1)	0.959	4	0.774
PC(22:6/16:0)	0.997	4	0.99
PE(16:1/16:0)	0.924	4	0.561
Bovinic acid	0.844	4	0.207
Oleic acid	0.989	4	0.954
Stearic acid	0.908	4	0.474
Palmitic acid	0.956	4	0.752
Octadec-9-enoic acid	0.993	4	0.971
Arachidonic acid	0.942	4	0.664
Elaidic acid	0.95	4	0.714
Trans-palmitoleic acid	0.952	4	0.73
Cholic acid	0.726	4	0.053
Prostaglandin G2	0.971	4	0.847
Prostaglandin F2a	0.961	4	0.788
Varanic acid	0.921	4	0.542
Glycocholate	0.831	4	0.169
Glycochenodeoxycholate	0.829	4	0.165
Corticosterone	0.897	4	0.417
Phenylalanine	0.796	4	0.095
Tryptophan	0.939	4	0.647
Tyrosine	0.997	4	0.991
Isoleucine	0.8	4	0.103
Leucine	0.831	4	0.17
Valine	0.786	4	0.08
Arginine	0.951	4	0.72
Aspartate	0.951	4	0.725

Serine	0.831	4	0.171
Ornithine	0.905	4	0.454
Uric acid	0.957	4	0.758
Lactic acid	0.991	4	0.965
LysoPC(17:0)	0.993	4	0.973
LysoPC(18:0)	0.886	4	0.366
LysoPC(18:3)	0.913	4	0.501
Cholesterol	0.951	4	0.724
Cholesterol ester (20:3)	0.907	4	0.466
Choline	0.891	4	0.386
Progesterone	0.72	4	0.059
L-Acetylcarnitine	0.971	4	0.85
Succinate	0.884	4	0.358
Ethyl 9-hexadecenoate	0.869	4	0.294
Octadecadienoate	0.869	4	0.294
Elaidic acid	0.966	4	0.817
Trimethyltridecanoic acid	0.879	4	0.334
Betaine	0.995	4	0.983
Ethanolamine	0.956	4	0.753
D-Glucose	0.807	4	0.116
D-Maltose	0.929	4	0.591
β-Mannobiose	0.903	4	0.444

Df, degrees of freedom; Sig, significance

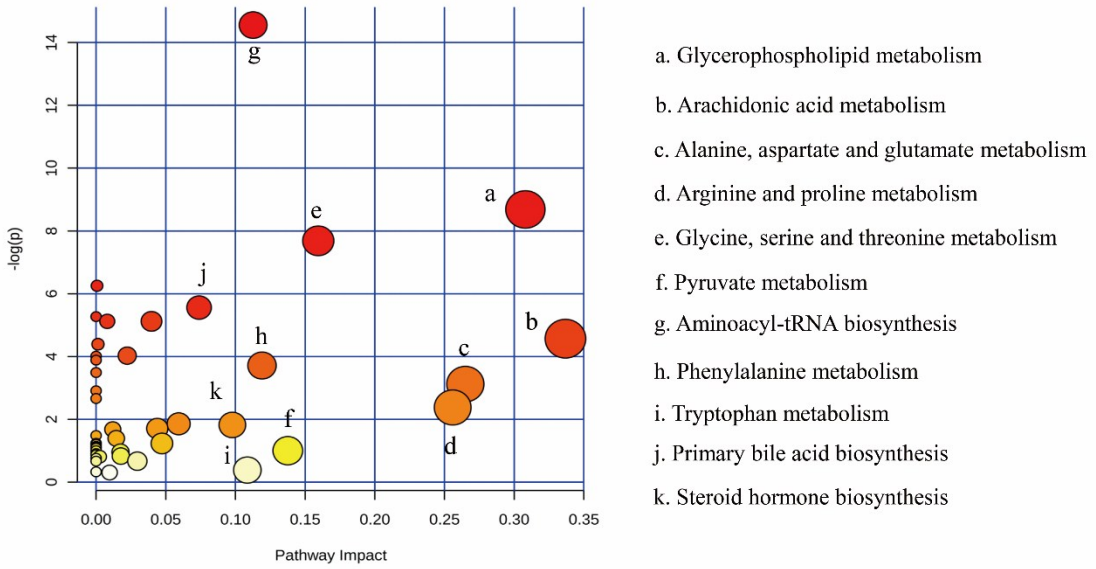

Figure S4. Overview of differential metabolites pathway analysis. The metabolome view shows all matched pathways according to the p values from the pathway enrichment analysis and pathway impact values from the pathway topology analysis. The size and color (varying from yellow to red) of bubbles means the different levels of significance of pathways.

Table S4. Parameters of differential metabolites pathway analysis

Pathway Name	Match	p	-log(p)	Holm p	FDR	Impact
Aminoacyl-tRNA biosynthesis	9/75	4.7754E-7	14.555	3.8203E-	3.8203E-5	0.11268
Glycerophospholipid metabolism	5/39	1.6937E-4	8.6834	0.01338	0.0067748	0.30805
Glycine, serine and threonine	5/48	4.5981E-4	7.6847	0.035865	0.012262	0.15941
Nitrogen metabolism	4/39	0.0019252	6.2527	0.14824	0.038505	6.7E-4
Primary bile acid biosynthesis	4/47	0.003854	5.5586	0.29291	0.059769	0.07389
D-Arginine and D-ornithine	2/8	0.0051429	5.2701	0.38572	0.059769	0.0
Phenylalanine, tyrosine and tryptophan	3/27	0.0059769	5.1198	0.44229	0.059769	0.008
Valine, leucine and isoleucine	3/27	0.0059769	5.1198	0.44229	0.059769	0.03975
Arachidonic acid metabolism	4/62	0.010378	4.5681	0.74718	0.092245	0.33678
Propanoate metabolism	3/35	0.012383	4.3914	0.87919	0.099064	0.00134
Valine, leucine and isoleucine	3/40	0.017817	4.0276	1.0	0.12085	0.02232
Linoleic acid metabolism	2/15	0.018128	4.0103	1.0	0.12085	0.0
Cyanoamino acid metabolism	2/16	0.020536	3.8856	1.0	0.12638	0.0

Phenylalanine metabolism	3/45	0.024392	3.7135	1.0	0.13938	0.11906
Fatty acid biosynthesis	3/49	0.030482	3.4906	1.0	0.16257	0.0
Alanine, aspartate and glutamate	2/24	0.04403	3.1229	1.0	0.22015	0.26496
Pantothenate and CoA biosynthesis	2/27	0.054546	2.9087	1.0	0.25669	0.0
Glycolysis or Gluconeogenesis	2/31	0.069788	2.6623	1.0	0.31017	0.0
Arginine and proline metabolism	3/77	0.092778	2.3775	1.0	0.39064	0.25588
Starch and sucrose metabolism	2/50	0.1561	1.8573	1.0	0.61665	0.05925
Steroid hormone biosynthesis	3/99	0.16187	1.821	1.0	0.61665	0.09777
Glycosylphosphatidylinositol(GPI)-	1/14	0.18103	1.7091	1.0	0.64859	0.0439
Cysteine and methionine metabolism	2/56	0.18647	1.6795	1.0	0.64859	0.01197
Sulfur metabolism	1/18	0.22662	1.4845	1.0	0.7554	0.0
Citrate cycle (TCA cycle)	1/20	0.24848	1.3924	1.0	0.79514	0.01446
Thiamine metabolism	1/24	0.29042	1.2364	1.0	0.8587	0.0
Tyrosine metabolism	2/76	0.29178	1.2317	1.0	0.8587	0.04724
Sphingolipid metabolism	1/25	0.30054	1.2022	1.0	0.8587	0.0
Fatty acid elongation in mitochondria	1/27	0.32037	1.1383	1.0	0.86492	0.0
beta-Alanine metabolism	1/28	0.33008	1.1084	1.0	0.86492	0.0
alpha-Linolenic acid metabolism	1/29	0.33966	1.0798	1.0	0.86492	0.0
Pentose phosphate pathway	1/32	0.36759	1.0008	1.0	0.86492	0.0
Lysine biosynthesis	1/32	0.36759	1.0008	1.0	0.86492	0.0
Pyruvate metabolism	1/32	0.36759	1.0008	1.0	0.86492	0.13756
Methane metabolism	1/34	0.38557	0.95303	1.0	0.8813	0.01751
Ubiquinone and other terpenoid-	1/36	0.40305	0.90868	1.0	0.89568	0.0
Glutathione metabolism	1/38	0.42006	0.86737	1.0	0.90823	0.0
Butanoate metabolism	1/40	0.43659	0.82877	1.0	0.91216	0.01774
Galactose metabolism	1/41	0.44468	0.8104	1.0	0.91216	0.00276
Histidine metabolism	1/44	0.46829	0.75867	1.0	0.91373	0.0
Nicotinate and nicotinamide	1/44	0.46829	0.75867	1.0	0.91373	0.0
Glyoxylate and dicarboxylate	1/50	0.51262	0.66823	1.0	0.9537	0.0
Fatty acid metabolism	1/50	0.51262	0.66823	1.0	0.9537	0.02959
Tryptophan metabolism	1/79	0.68102	0.38417	1.0	1.0	0.10853
Amino sugar and nucleotide sugar	1/88	0.72064	0.32761	1.0	1.0	0.0
Purine metabolism	3/92	0.73668	0.3056	1.0	1.0	0.00969