Supplementary Table 1. Stastically significant differences in sinapine levels between accessions as determined by the Kruskal-Wallis test, $p < 0.01$.

	ID#	Groups
C. sativa	239	ac
C. sativa	252	acd
C. sativa	596	acde
C. sativa	605	acde
C. sativa	621	ac
C. sativa	1044	ac
C. sativa	1062	a
C. sativa	1063	acd
C. sativa	1662	ac
C. rumelica rumelica	245	be
C. rumelica rumelica	247	be
C. rumelica rumelica	609	be
C. rumelica rumelica	1022	bcde
C. rumelica rumelica	1034	b
C. rumelica rumelica	1255	be
C. microcarpa 2x	246	acde
C. microcarpa 4x	168	acde
C. microcarpa 4x	718	bde
C. microcarpa 4x	965	bcde
C. microcarpa 6x	198	bde
C. microcarpa 6x	818	bcde
C. laxa	612	a
C. hispida hispida	240	bde
C. hispida grandiflora	248	bde

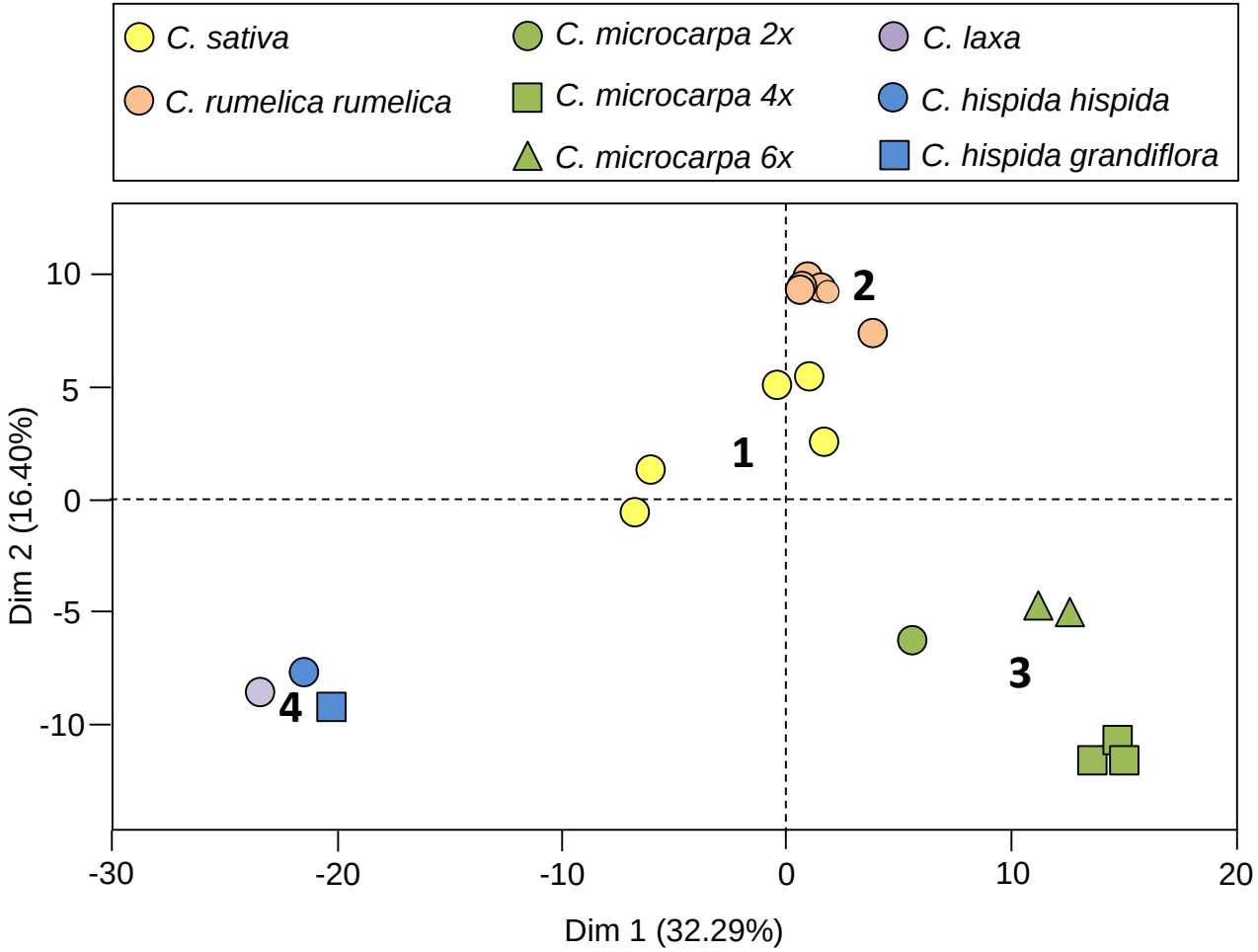
Supplementary Table 2. Additional sulfur containing compounds in Camelina seed extracts that showed group specific differences*

Formula	Identity	m/z (pos)	m/z (neg)	RT[a]	MS-MS[b]
$C_{12}H_{24}O_3S$	sulfur-containing aliphatic compound	249.15206		3.24	69, 81, 83, 93, 97, 107, 109, 133, 135, 189, **217**
$C_{13}H_{26}O_3S$	sulfur-containing aliphatic compound	263.16758		3.44	61, 69, 81, **83**, 93, 97, 107, 121, 1213, 147, 149, 203, **231**
$C_{14}H_{28}O_3S$	sulfur-containing aliphatic compound	277.18333		3.62	69, 81, 83, 95, 97, 107, 111, 121, 161, 163, 217, **245**
$C_{11}H_{20}N_2O_5S$	sulfur-containing aliphatic compound	293.11679		2.21	70, 84, 116, 118, 130, 136, **147**, 184
$C_{10}H_{18}N_2O_5S_2$	sulfur-containing aliphatic compound	311.07322		2.19	88, 134, 139, **149**, 151, 185
$C_{19}H_{37}NO_{11}S_3$	11-methylsulfonylundecyl GS? (GS11-like)		550.14459	2.70	
$C_{13}H_{27}NO_3S_2$	GS11-like (M-SO3)	472.20343		2.70	**69**, 81, **83**, **97**, 111, 163, 180, 212, 243, 244, 277, 310
$C_{19}H_{37}NO_8S_2$	GS11-like (M-SO3-glucose)	310.15048		2.70	**69**, 81, **83**, **97**, 117, 163, 180, 212
$C_{12}H_{25}NO_6S_2$	sulfate-containing compound		342.10452	2.62	**96.9**

***Additional sulfur-containing compounds identified in Camelina spp. seed meal.**

We were able to identify seven other sulfur-containing compounds with prominent m/z peaks that showed significant differences between groups. Three aliphatic metabolites with positive m/z's of 249.15206, 263.16758, and 277.18333, corresponding to $C_{12}H_{24}O_3S$, $C_{13}H_{26}O_3S$, and $C_{12}H_{26}N_3O_2S$, respectively, had a major daughter ion representing a loss of CH_4O (-32 m/z) and the differences in relative abundance between groups were consistent with the relative group differences observed for GS9, GS10, and GS11, respectively (compare Figures 6 and Supplementary Figure 4). Another sulfur-containing aliphatic compound that ionized in positive mode, with a putative formula of $C_{10}H_{18}N_2O_5S_2$ and a positive m/z of 311.07322, had a major daughter fragment (m/z 149) representing a loss of glucose. This compound was most abundant in *C. microcarpa* cytotypes but barely detectable in *C. laxa* and *C. hispida* accessions (Group 4). There were two sulfur-containing compounds identified in negative mode that showed differences in abundance between groups. One of these was of particular interest as it appears to be related to GS11, containing a methyl sulfonyl group instead of a methyl sulfinyl group. This is discussed in the manuscript.

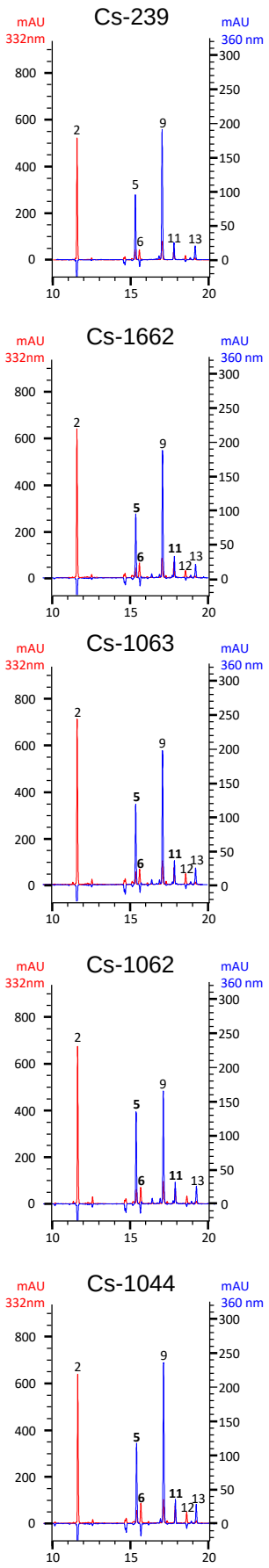
Supplementary Figure 1



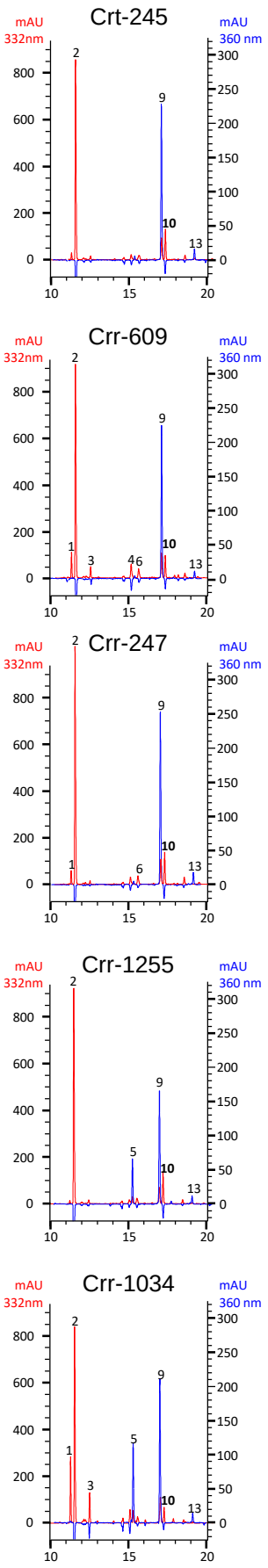
Supplementary Figure 1. Overlaid HPLC chromatographs from seed extracts used in the metabolomics study: **(a)** Group 1, *C. sativa* (Cs), **(b)** Group 2, *C. rumelica* subsp. *rumelica* (Crr), **(c)** Group 3, *C. microcarpa* (Cm cytotypes 6x, 4x, and 2x) and **(d)** Group 4, *C. laxa* (Cl), *C. hispida* subsp. *hispida* (Chh) and *C. hispida* subsp. *grandiflora* (Chg). Channels 332 nm and 360 nm are colour-coded red and blue, respectively and accession numbers are listed beside the species initials

Supplementary Figure 2

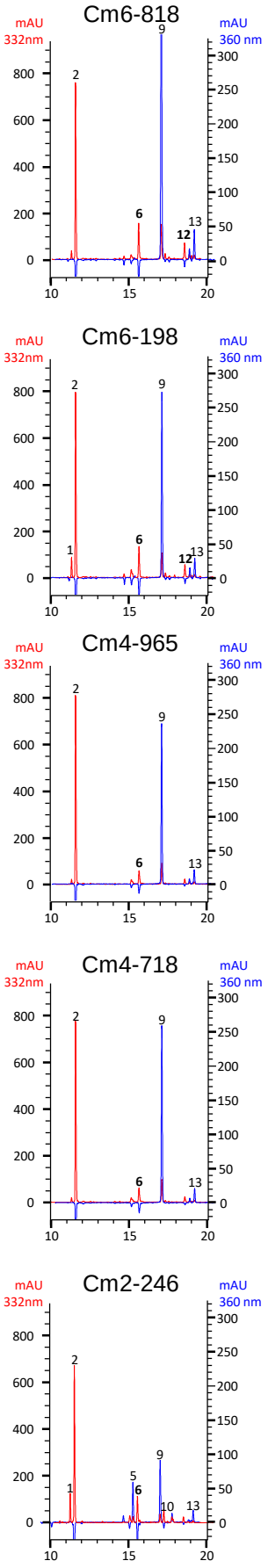
(a) Group 1, *C. sativa*



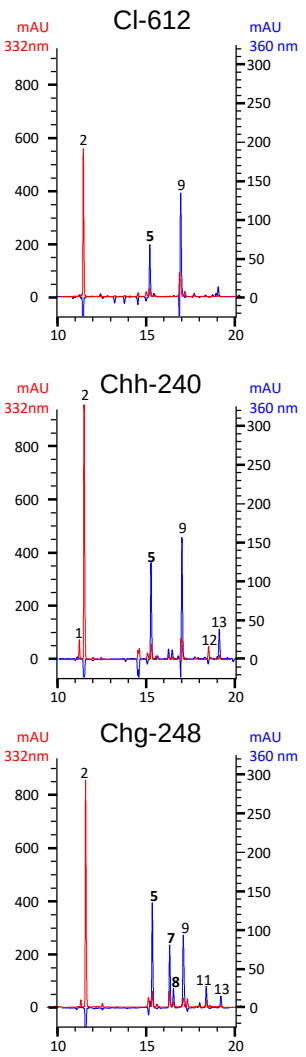
(b) Group 2, *C. rumelica*



(c) Group 3, *C. microcarpa*

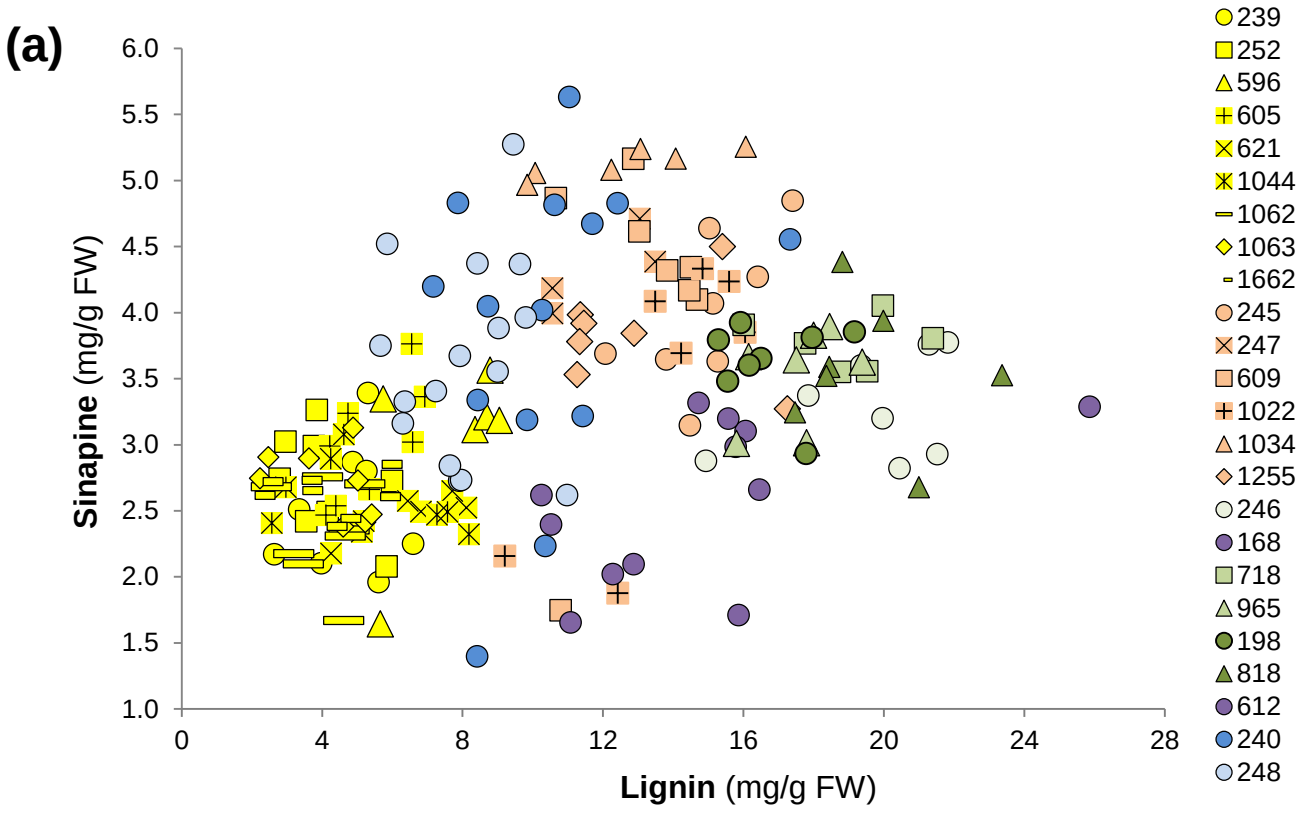


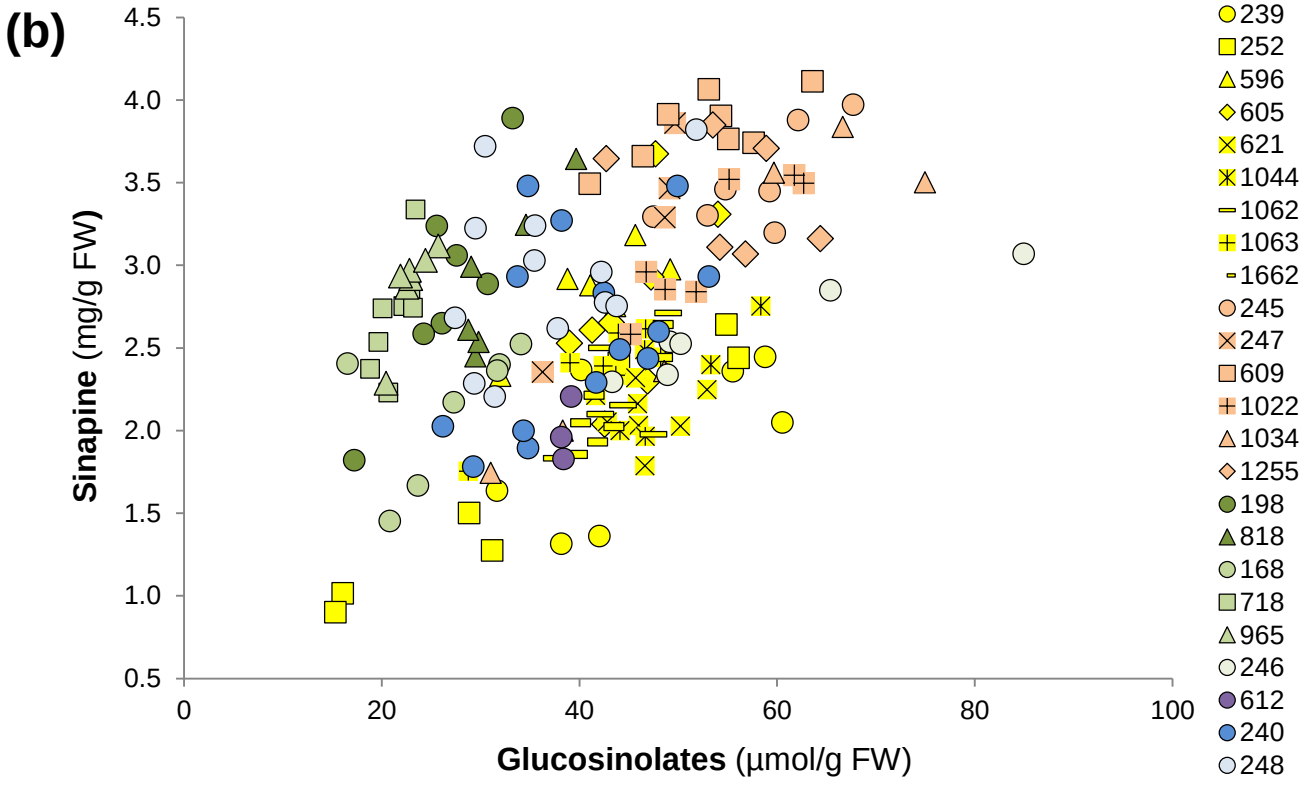
(d) Group 4, *C. laxa* and *C. hispida*



Supplementary Figure 2. Principal component analysis of 591 LC-MS negative mode features from 20 Camelina seed extracts. Note: Four *C. sativa* accessions were not included in this analysis (ID# 252, 596, 605, and 621).

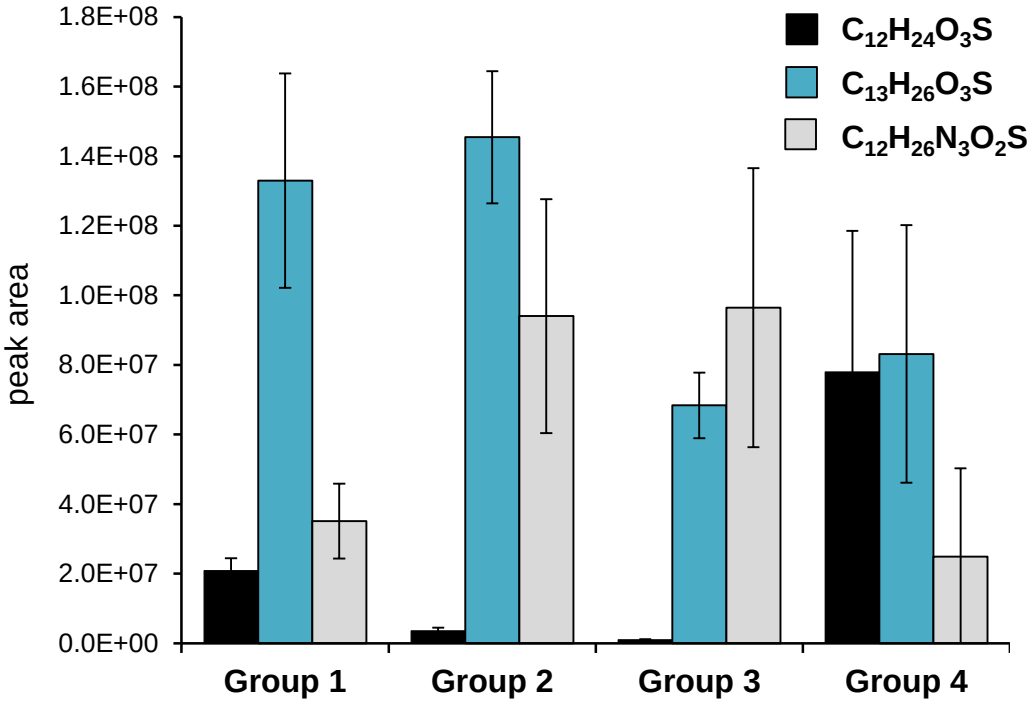
Supplementary Figure 3





Supplementary Figure 3. Correlation of anti-nutrients in Camelina seed meal of all biological replicates of the 24 accessions: **(a)** sinapine and lignin and **(b)** sinapine and total GS.

Supplementary Figure 4



Supplementary Figure 4. Relative abundance of additional sulfur-containing metabolites in Camelina seeds that have Group patterns similar to the GSs. **Group 1**: *C. sativa*; **Group 2**: *C. rumelica* subsp. *rumelica*, *C. microcarpa 2x*; **Group 3**: *C. microcarpa 4x* and *6x*; **Group 4**: *C. laxa*, *C. hispida* spp.