Supporting Information

Integration of Untargeted and Pseudotargeted Metabolomics for Authentication of Three Shrimp Species using UHPLC-Q-Orbitrap

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Figure S1. The two extraction methods and three extracts.

4	dd Settings		R	4	General		
	Minimum AGC ta	8.00e3	D .		Runtime	0 to 21 mi	
	Intensity thresh	1.6e5			Polarity	positive	
	Apex trigger	-			In-source CID	0.0 eV	
	Charge exclusio				Default charge :	1	
	Peptide match	-			Inclusion	on	
	Exclude isotope:	on		4	MS ²		
	Dynamic exclus	10.0 s			Microscans	1	
4	dd-MS2 / dd-S	IM			Resolution	17,500	
	Microscans	1			AGC target	2e5	
	Resolution	17,500			Maximum IT	100 ms	
	AGC target	2e5			Loop count	26	
	Maximum IT	50 ms			MSX count	1	
	Loop count	10			MSX isochronou	on	
	MSX count	1			Isolation windov	0.5 m/z	
	TopN	10			Isolation offset	0.0 m/z	
	Isolation windov	2.0 m/z			Fixed first mass	_	
	Isolation offset	0.0 m/z			(N)CE / stepped	nce: 25	
	Fixed first mass	-			Spectrum data t	Profile	
	(N)CE / stepped	nce: 40					
	Spectrum data t	Profile					
	Full MS						
	Microscans	1					
	Resolution	70,000					
	AGC target	2e5					
	Maximum IT	50 ms					
	Number of scan	1					
	Scan range	80 to 1200 m/z					
	Spectrum data t	Profile					
4	General						
	Runtime	0 to 30 min					
	Polarity	positive					
	In-source CID	0.0 eV					
	Default charge s	1					
	Inclusion	-					
	Exclusion	-					
	Tage		-				

A.

Figure S2. Parameters for the (A) full ms/dd-MS² and (B) PRM mode.



Figure S3. Compound Discoverer workflow.



Figure S4. Base peak ion chromatograms of the three shrimp extracts from different extraction methods



Figure S5. PLS-DA score plots in positive ionization mode



Figure S6. PLS-DA score plots in negative ionization mode



Figure S7 Permutation test of PLS-DA models



Figure S8 Accuracy-Top N VIPs' variables



Figure S9 DD-SIMCA model

Sample set	Sample size	Collection data	Species (code)
Sample set 1	10	20191212	Litopenaeus vanmamei (Lv)
Sample set 1	10	20200729	Litopenaeus vanmamei (Lv)
Sample set 1	10	20190926	Penaeus japonicus (Pj)
Sample set 1	10	20200729	Penaeus japonicus (Pj)
Sample set 1	4	20191009	Penaeus monodon (Pm)
Sample set 1	16	20200729	Penaeus monodon (Pm)
Sample set 2	15	20191212	Litopenaeus vanmamei (Lv)
Sample set 2	15	20200729	Litopenaeus vanmamei (Lv)
Sample set 2	15	20190926	Penaeus japonicus (Pj)
Sample set 2	15	20200729	Penaeus japonicus (Pj)
Sample set 2	4	20191009	Penaeus monodon (Pm)
Sample set 2	26	20200729	Penaeus monodon (Pm)
Sample set 3	12	20191212	Litopenaeus vanmamei (Lv)
Sample set 3	12	20200729	Litopenaeus vanmamei (Lv)
Sample set 3	13	20190926	Penaeus japonicus (Pj)
Sample set 3	12	20200729	Penaeus japonicus (Pj)
Sample set 3	4	20191009	Penaeus monodon (Pm)
Sample set 3	21	20200729	Penaeus monodon (Pm)
Sample set 4	20	20201104	Litopenaeus vanmamei (Lv)
Sample set 4	20	20201104	Penaeus japonicus (Pj)
Sample set 4	20	20201104	Penaeus monodon (Pm)
Sample set 5	20	20191009	Macrobrachium rosenbergii (Mr)

 Table S1. Description of four sample sets.

ID	RT/min	Molecular Weight	Parent ion	Product ion 1	Product ion 2	NCE
M1	1.784	271.90755	272.9148	122.9244	136.9411	10
M2	1.786	289.83978	290.8471	122.9244	206.8858	10
M3	1.853	169.08511	170.0924	124.0868	109.0759	45
M4	1.869	197.1164	198.1238	154.1336	95.0603	25
M5	1.889	257.10269	258.11	104.1067	184.0732	30
M6	1.925	103.09965	104.1069	60.08102	58.0654	10
M7	2.006	115.06331	116.0706	70.06509	68.04962	10
M8	5.38	219.11057	220.1178	90.05482	202.1071	30
M9	13.126	451.26985	452.2771	311.2573	104.1068	20
M10	13.231	358.25053	359.2579	93.06974	67.05434	25
M11	14.22	302.22211	303.2293	257.2266	123.1165	30
M12	14.522	323.28223	324.2895	60.08117	86.06016	30
M13	14.803	371.2857	372.2932	268.2993	326.2871	30
M14	15.643	605.44237	606.4495	184.0732	104.1069	30
M15	16.285	632.4893	633.4963	492.4766	474.4669	20
M16	16.561	698.53388	699.541	640.4671	516.4741	20
M17	16.858	620.48051	621.4878	337.273	95.0854	25
M18	16.894	511.49653	512.5035	494.4922	228.232	20
M19	17.137	663.5202	664.5272	184.0731	523.5267	25
M20	17.705	797.5205	798.528	613.5181	327.2892	10
M21	17.884	761.59295	762.6004	168.0781	498.3518	25
M22	17.893	783.48361	784.491	643.4711	359.2578	15
M23	18.328	715.51551	716.5227	575.5025	306.2782	15
M24	18.387	809.4991	810.5066	669.4869	385.2735	15
M25	18.928	835.51447	836.522	695.5023	385.2736	15
M26	19.55	703.55153	704.5588	313.2729	392.2923	20

Table S2. PRM table.

Table S3. Description of acquired compounds in the untargeted metabolomics for comparison of

Description	ESI +			ESI -		
	BipA	BipO	Monop	BipA	BipO	Monop
Total compounds in Compound	7818			10104		
Discoverer						
Compounds after background subtraction	5405			8275		
Detected compounds ^a	709	1023	1270	1146	1144	1540
Detected compounds with good	413	878	1060	833	964	1301
repeatability ^b						

monophasic extraction and biphasic extraction.

a present in at least 50% of samples of one extract.

b with a CV $\leq 30\%$.