applied sciences

MDPI

Supplementary Materials: Non-Targeted NMR Method to Assess the Authenticity of Saffron and Trace the Agronomic Practices Applied for Its Production

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Summary

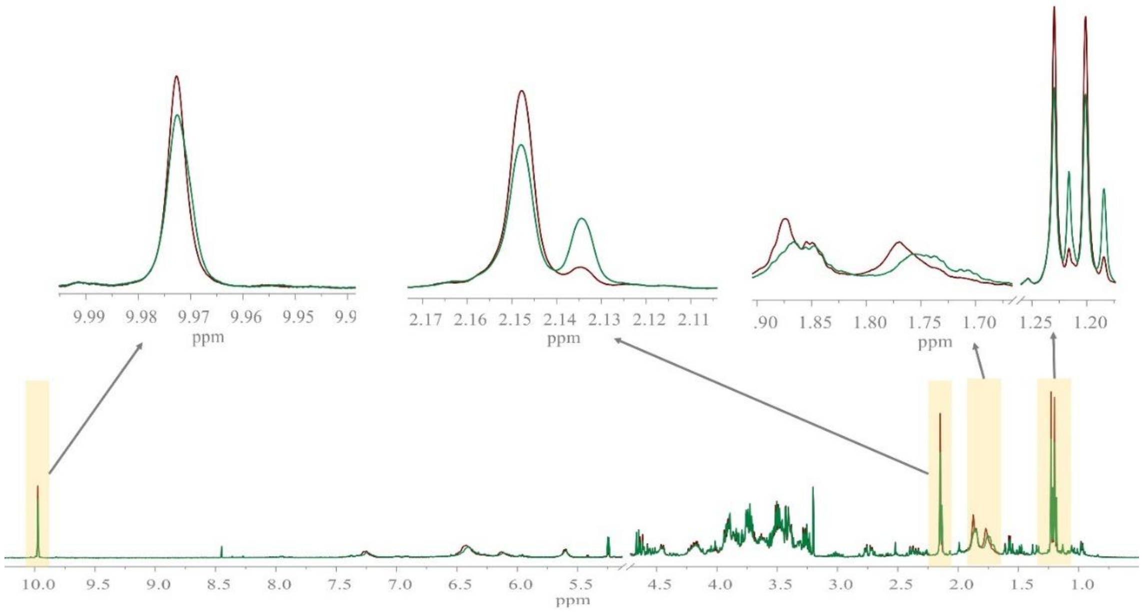

Figure 1. Effect of pasteurization step on the stability of aqueous extracts of pure saffron.

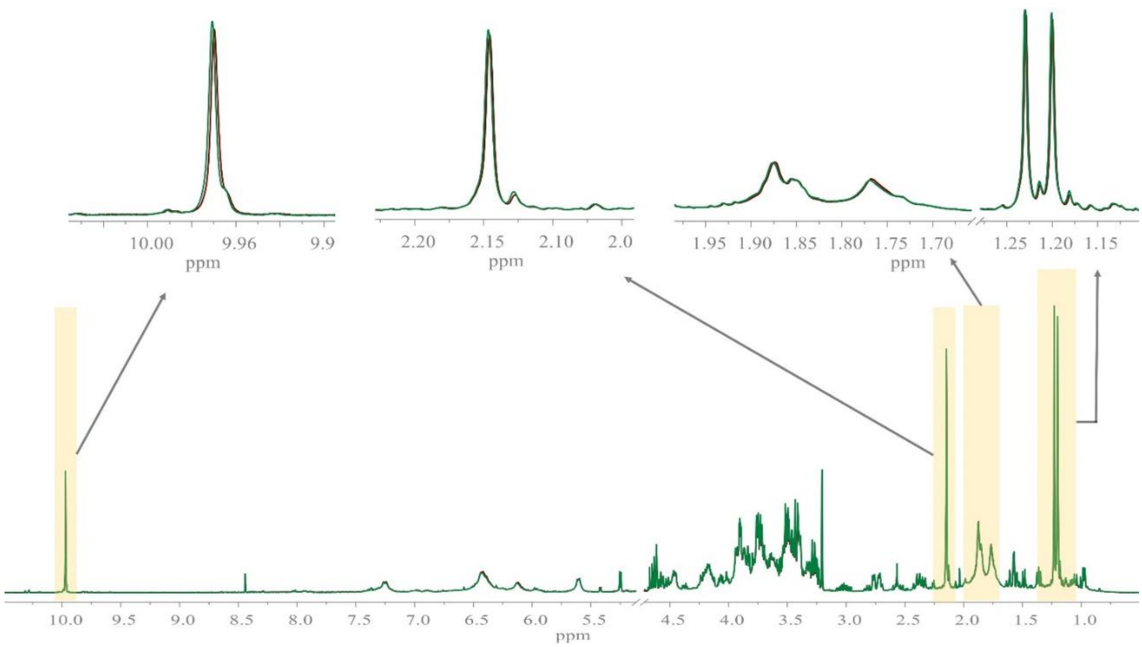

Figure S1 a. Comparison of typical 1D ^{1}H NOESY NMR spectra of aqueous extracts of pure saffron not subjected to pasteurization (80°C). The red spectrum was recorded on the freshly prepared sample; the green spectrum was recorded on the same sample after 24h. Selected regions of spectra (indicated with yellow rectangles and expanded for clarity) show noticeable variations in the metabolic composition over 24 h.

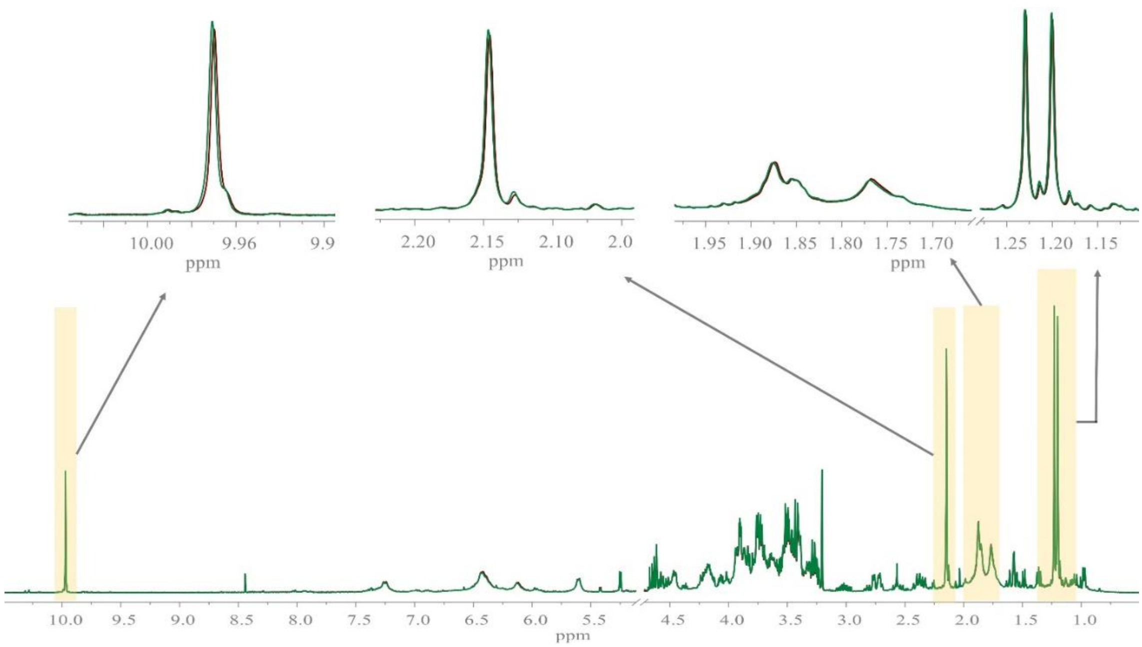

Figure S1 b. Comparison of typical 1D 1H NOESY NMR spectra of aqueous extracts of pure saffron subjected to pasteurization (80°C). Red spectrum recorded on the freshly prepared sample; green spectrum recorded on the same sample after 24h. Selected regions of spectra (indicated with yellow rectangles and expanded for clarity) that do not show noticeable variations in the metabolic composition over 24 h. The metabolic composition resulted stable over 24 h.

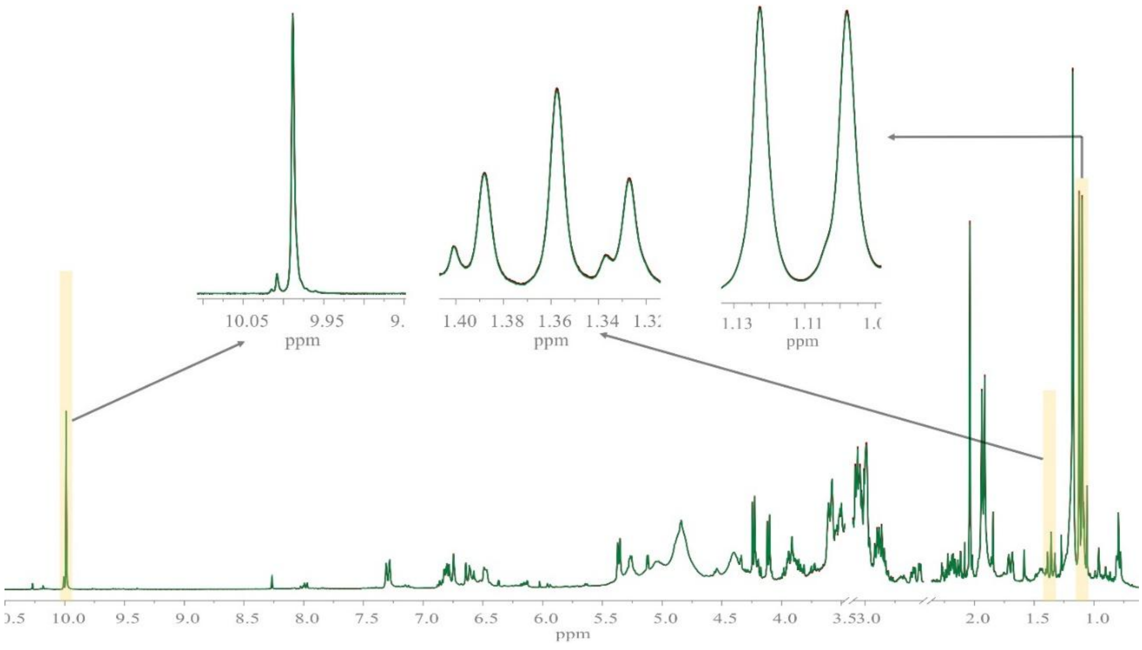

Figure S1 c. Comparison of typical ^{1}H NMR spectra of extracts of pure saffron in DMSO-d_6 not subjected to pasteurization (80°C). Red spectrum recorded on the freshly prepared sample; green spectrum recorded on the same sample after 24h. The metabolic composition resulted stable over 24 h.

Table S1. List of metabolites contained in the aqueous extracts of authentic saffron samples and identified by 1D ^{1}H NOESY NMR. Bruker Avance 400 MHz. The assignment of NMR signals has been obtained by comparison with standard compounds, except for the metabolites indicated with a star. For these metabolites the assignment was obtained referring to spectral data reported in the literature.[1]

Compound	^{1}H ppm	Multeplicity	J (Hz)
Monoterpenes			
Picrocrocin*	1.20	s	
	1.23	s	
	1.57	t	12.1
	1.85	brs	
	2.14	s	
	2.36	dd	18.8;9.4
	2.72	ddd overlapped	
	4.17	m	
	9.97	s	
Carotenoids			
***trans*-crocetin(β-D-gentibiosyl) ester**	1.77	brs	
	1.87	brs	
	6.12	brs	
	6.42	brs	
	5.60	d	7.7
	7.26	d	11.2
Organic acids			
Lactic acid	1.35	d	6.9
	4.15	q	6.9
Acetic acid*	2.03	s	
Malic acid	2.58	dd	16;8.5
	2.74	dd overlapped	16; 4
	4.37	dd	8.5; 4
Succinic acid	2.57	s	
Fumaric acid	6.58	s	
Formic acid*	8.44	s	
Flavonoids			
Kaempferol-3-sophoroside*	6.90	d	8.4
	7.94	d	8.7
Kaempferol-3-sophoroside-7-β-glucoside*	6.90	d	8.4
	6.97	d	8.6
	8.03	d	8.6
Carbohydrates			
D-glucose	3.25	dd	9.1; 7.9
	3.42	m	
	3.47	m	
	3.55	dd	9.8; 3.7
	3.74	m	
	3.84	m	
	3.90	dd overlapped	12.3; 2.1
	4.65	d	7.9
	5.24	dd	3.7
D-sucrose	3.48	t	9.2

	3.57	dd	9.9;3.7
	3.69	s	
	3.78	t	9
	3.83	m	
	3.87	m	
	3.91	dd overlapped	6.2; 3.5
	4.06	t	8.5
	4.22	d	8.7
	5.42	d	3.8
Fructose	3.57	m	
	3.72	m	
	3.81	m	
	3.91	dd	9.9; 3.4
	4.03	m	
	4.12	m	
Amino Acids			
Alanine	1.48	d	7.3
	3.79	q	7.3
Asparagine	3.02	m	
Glycine*	3.54	s	
Quaternary ammonium salts			
Choline	3.2	s	
	3.51	m	
	4.07	m	

Figure S2. Typical 1D [1]H NOESY NMR spectrum of aqueous extracts of authentic saffron. Bruker Avance 400 MHz.

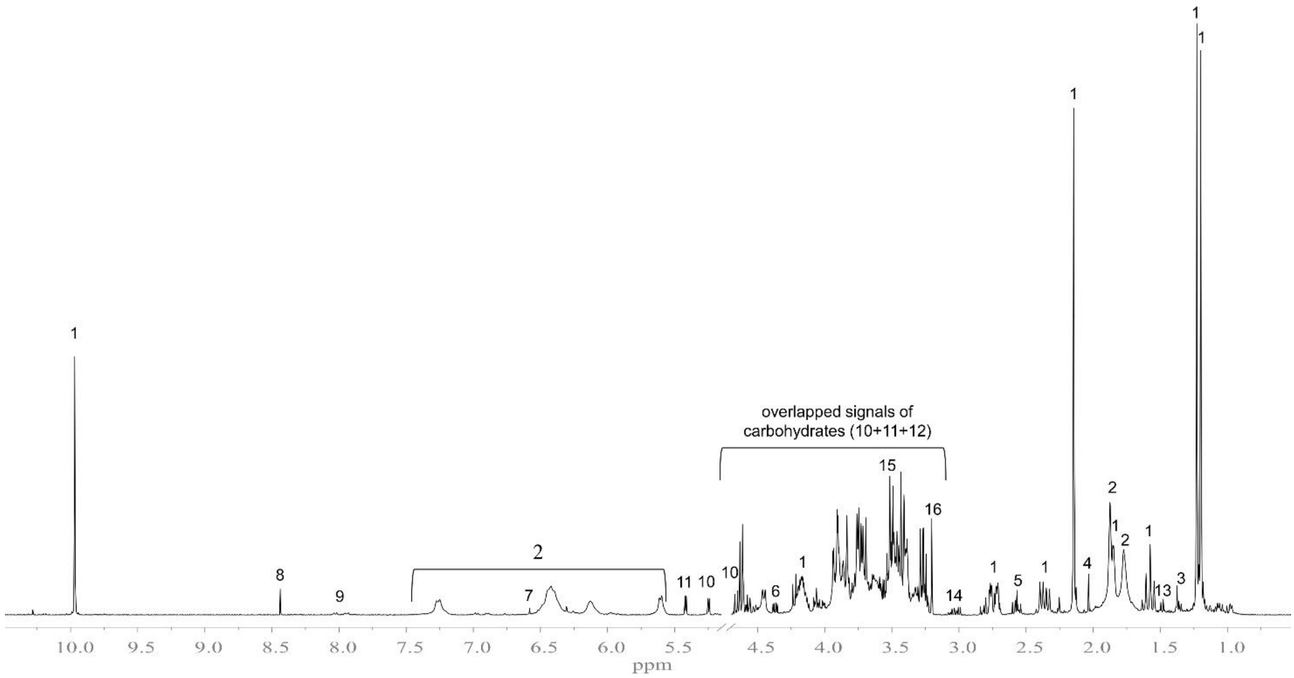

Figure S2 a. [1]H NOESY NMR spectrum. Residual water signal (4.78 ppm) is hidden. Signals are indicated as follow: 1, picrocrocin; 2, trans-crocetin (β-D-gentibiosyl) ester; 3, lactic acid; 4, acetic acid; 5, succinic acid; 6, malic acid; 7, fumaric acid; 8, formic acid; 9, glycosylated isoforms of kaempferol; 10, D-glucose; 11, D-sucrose; 12; Fructose; 13, alanine; 14, asparagine; 15, glycine; 16, choline.

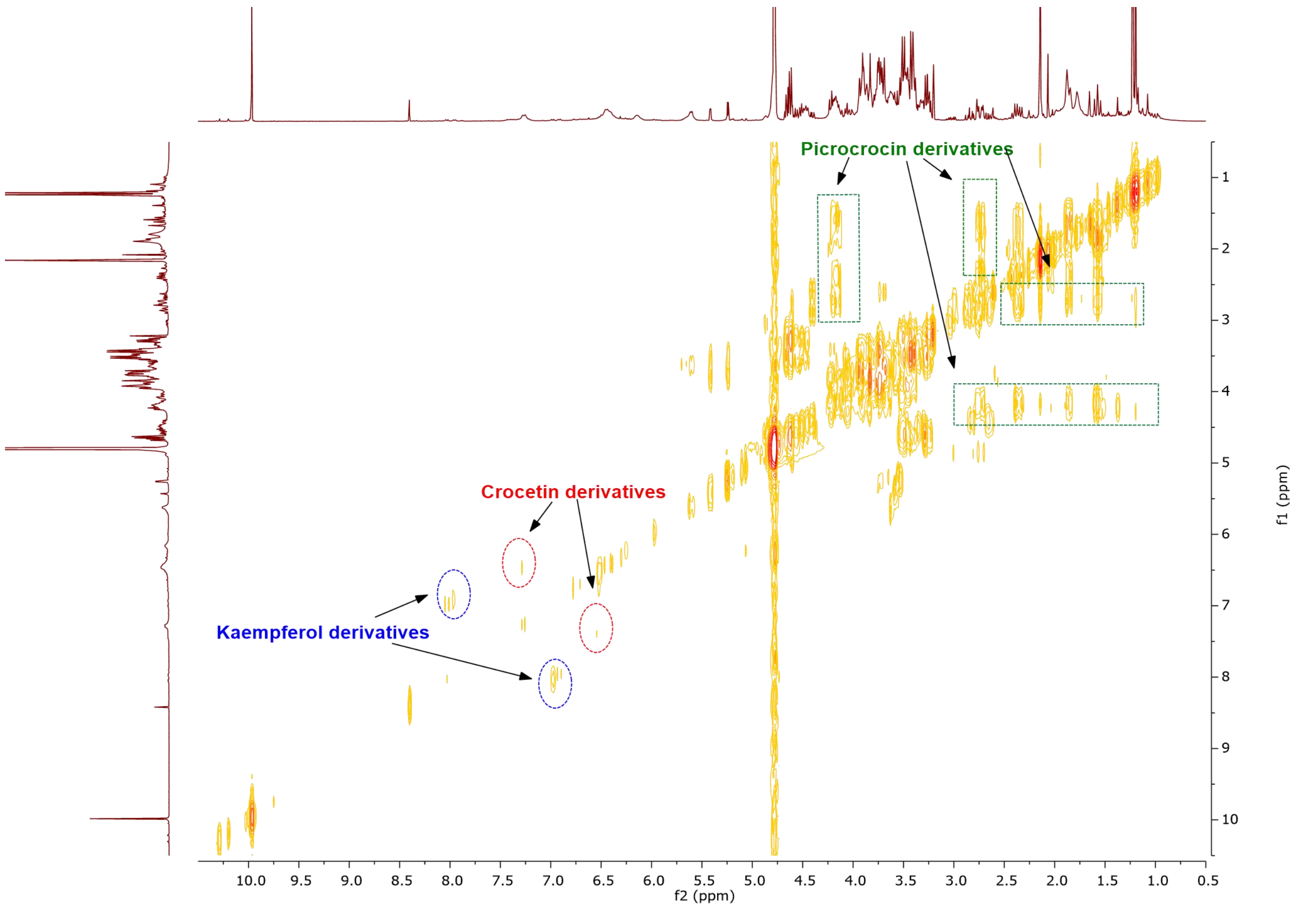

Figure S2. b. COSY spectrum.

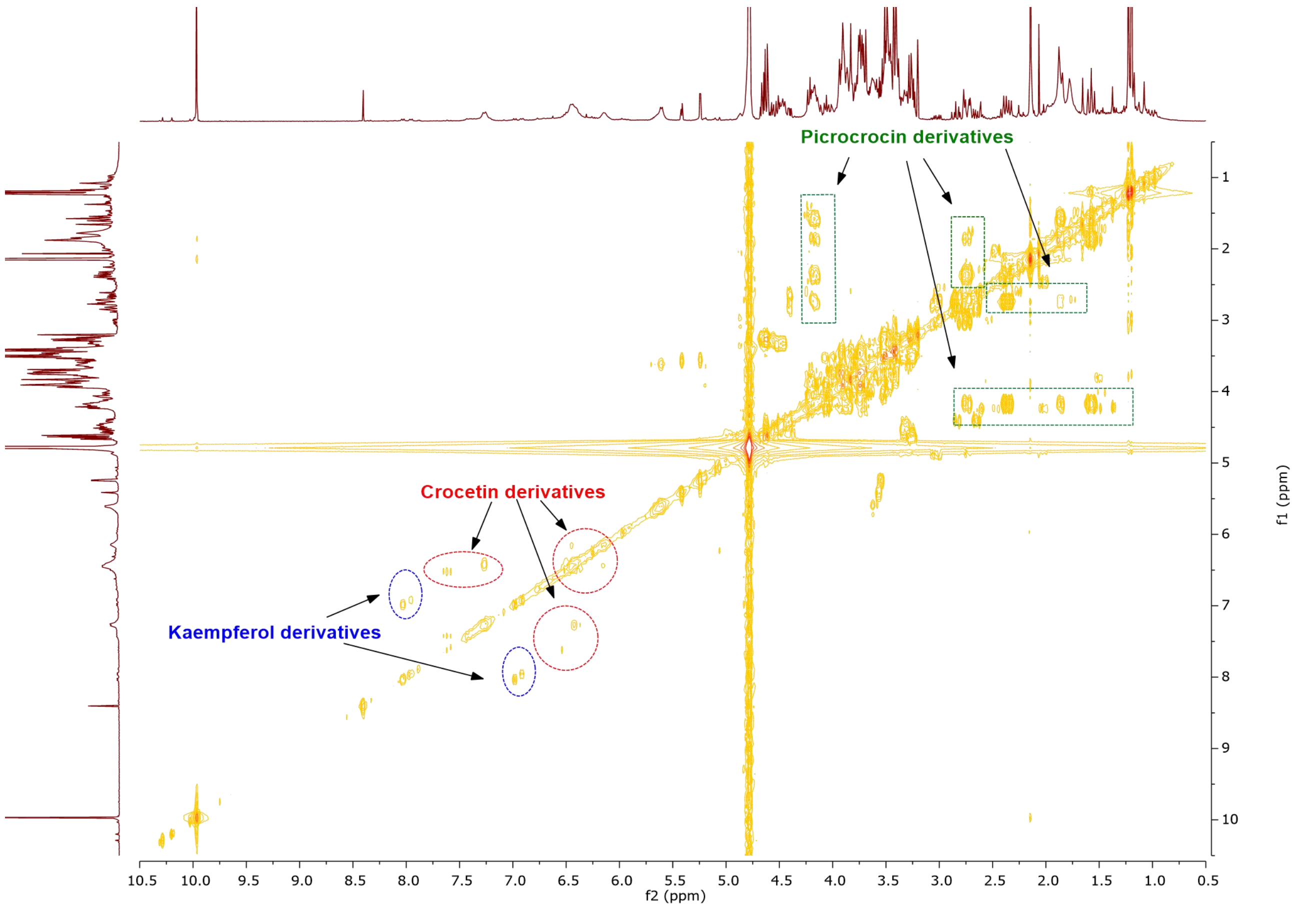

Figure S2. c. TOCSY spectrum.

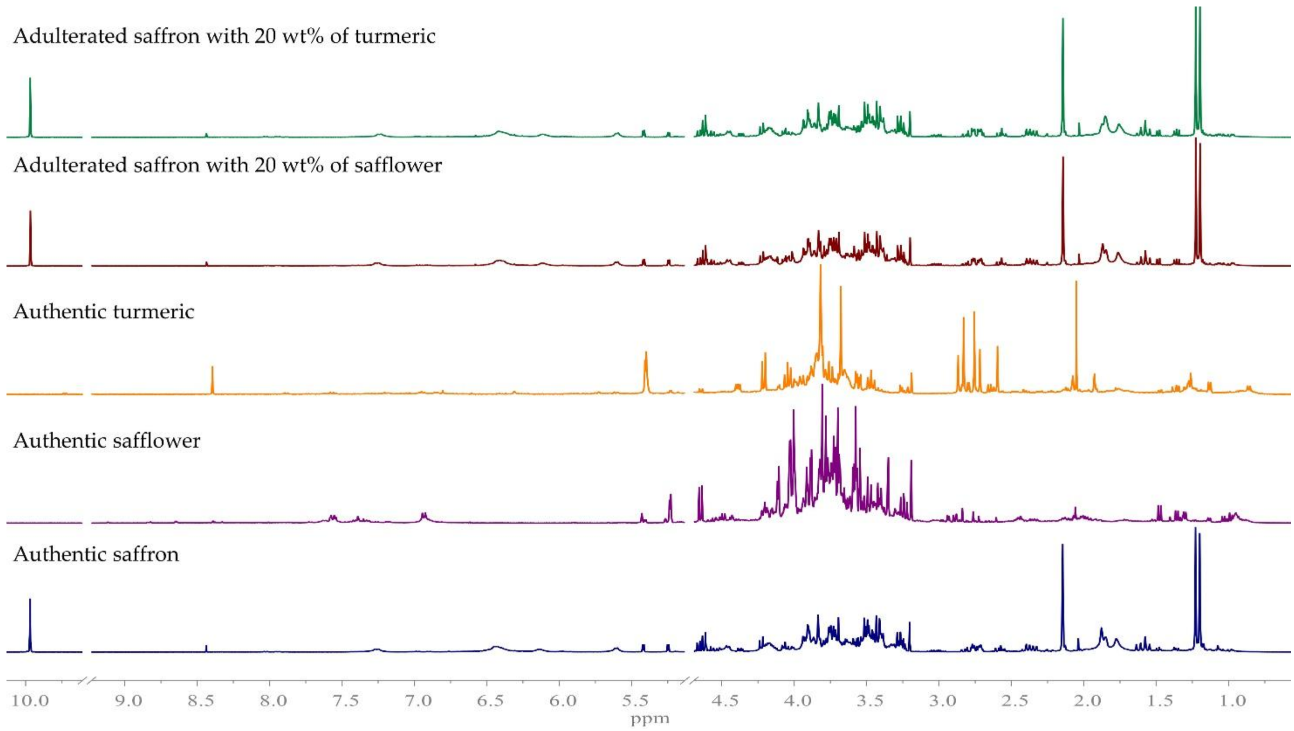

Figure S3. 1D ^{1}H NOESY NMR spectra of aqueous extracts of authentic saffron, authentic turmeric, authentic safflower, saffron adulterated with turmeric, and saffron adulterated with safflower. Comparison of typical 1D ^{1}H NOESY NMR spectra (Bruker Avance 400 MHz) of aqueous extracts (100 mg of the corresponding powder in 2.0 mL oxalate buffer pH 4.2) of authentic saffron (blue spectrum,), authentic safflower (purple spectrum), authentic turmeric (orange spectrum), adulterated saffron with 20 wt% of safflower (red spectrum), and adulterated saffron with 20 wt% of turmeric (green spectrum). Residual water signal (4.78 ppm) is hidden.

Table S2. List of the top 50 features identified by Wilcoxon rank-sum test applied to the aqueous extracts of authentic and adulterated saffron samples.

Range (ppm)	V	p.value	-log10(p)	FDR
1.77272 - 1.81272	0	4.4447e-15	14.352	5.0225e-13
6.13272 - 6.17272	0	4.4447e-15	14.352	5.0225e-13
6.45272 - 6.49272	2	1.7779e-14	13.75	1.3393e-12
5.61272 - 5.65272	28	8.0787e-11	10.093	4.5645e-09
3.65272 - 3.69272	688	1.8915e-10	9.7232	8.5498e-09
8.41272 - 8.45272	50	4.9928e-09	8.3017	1.8806e-07
5.37272 - 5.41272	631	9.6195e-07	6.0168	3.1057e-05
2.53272 - 2.57272	618	3.9254e-06	5.4061	0.00011089
9.49272 - 9.53272	616	4.8169e-06	5.3172	0.00012096
1.85272 - 1.89272	108	7.1909e-06	5.1432	0.00014774
8.77272 - 8.81272	612	7.1909e-06	5.1432	0.00014774
7.85272 - 7.89272	599	2.4547e-05	4.61	0.0004623
6.61272 - 6.65272	123	2.9371e-05	4.5321	0.00051061
6.93272 - 6.97272	596	3.2099e-05	4.4935	0.00051817
5.25272 - 5.29272	132	6.3974e-05	4.194	0.00096387
3.57272 - 3.61272	579	0.00013326	3.8753	0.0018822
4.17272 - 4.21272	577	0.00015596	3.807	0.0020054
3.41272 - 3.45272	144	0.0001686	3.7732	0.0020054
4.45272 - 4.49272	144	0.0001686	3.7732	0.0020054
7.17272 - 7.21272	575	0.00018216	3.7395	0.0020585
7.25272 - 7.29272	146	0.00019673	3.7061	0.0021172
1.01272 - 1.05272	572	0.0002291	3.64	0.0023535
5.77272 - 5.81272	149	0.00024706	3.6072	0.0024276
8.89272 - 8.93272	569	0.00028688	3.5423	0.0027015
9.09272 - 9.13272	567	0.00033249	3.4782	0.0029941
6.57272 - 6.61272	154	0.0003577	3.4465	0.0029941
7.53272 - 7.57272	566	0.0003577	3.4465	0.0029941
8.93272 - 8.97272	563	0.00044413	3.3525	0.0035848
6.33272 - 6.37272	557	0.00067642	3.1698	0.0052714
3.81272 - 3.85272	555	0.0007755	3.1104	0.0058421
6.41272 - 6.45272	166	0.00082982	3.081	0.0060496
2.21272 - 2.25272	168	0.00094892	3.0228	0.0067017
9.17272 - 9.21272	551	0.0010141	2.9939	0.006945
4.01272 - 4.05272	550	0.0010833	2.9653	0.0072006
6.65272 - 6.69272	172	0.0012346	2.9085	0.007972
5.65272 - 5.69272	173	0.0013172	2.8804	0.008269
10.0927 - 10.1327	546	0.0014047	2.8524	0.0085803
4.61272 - 4.65272	176	0.0015957	2.797	0.0092469
7.49272 - 7.53272	544	0.0015957	2.797	0.0092469
0.972723 - 1.01272	543	0.0016997	2.7696	0.009369
7.77272 - 7.81272	543	0.0016997	2.7696	0.009369
0.612723 - 0.652723	540	0.0020492	2.6884	0.01077
10.2527 - 10.2927	540	0.0020492	2.6884	0.01077
9.01272 - 9.05272	536	0.0026149	2.5825	0.013431

3.97272 - 4.01272	535	0.0027766	2.5565	0.013945
7.13272 - 7.17272	534	0.0029471	2.5306	0.014479
3.77272 - 3.81272	528	0.0041808	2.3787	0.019283
5.69272 - 5.73272	192	0.0041808	2.3787	0.019283
7.21272 - 7.25272	528	0.0041808	2.3787	0.019283
2.65272 - 2.69272	526	0.0046838	2.3294	0.020756
6.81272 - 6.85272	526	0.0046838	2.3294	0.020756
1.81272 - 1.85272	524	0.0052395	2.2807	0.022342
8.25272 - 8.29272	524	0.0052395	2.2807	0.022342
7.97272 - 8.01272	521	0.0061825	2.2088	0.024951
8.97272 - 9.01272	521	0.0061825	2.2088	0.024951
9.77272 - 9.81272	521	0.0061825	2.2088	0.024951
7.73272 - 7.77272	520	0.0065285	2.1852	0.025885
2.09272 - 2.13272	204	0.0080886	2.0921	0.030983
7.81272 - 7.85272	516	0.0080886	2.0921	0.030983
3.13272 - 3.17272	511	0.010491	1.9792	0.038241
3.33272 - 3.37272	511	0.010491	1.9792	0.038241
7.41272 - 7.45272	511	0.010491	1.9792	0.038241
9.29272 - 9.33272	510	0.01104	1.957	0.039602
10.3727 - 10.4127	508	0.012212	1.9132	0.043125
6.29272 - 6.33272	506	0.013492	1.8699	0.04691
6.97272 - 7.01272	505	0.014174	1.8485	0.048059
0.692723 - 0.732723	504	0.014886	1.8272	0.048059
5.33272 - 5.37272	504	0.014886	1.8272	0.048059
5.81272 - 5.85272	504	0.014886	1.8272	0.048059
5.89272 - 5.93272	504	0.014886	1.8272	0.048059
8.61272 - 8.65272	503	0.015628	1.8061	0.049054
8.81272 - 8.85272	503	0.015628	1.8061	0.049054

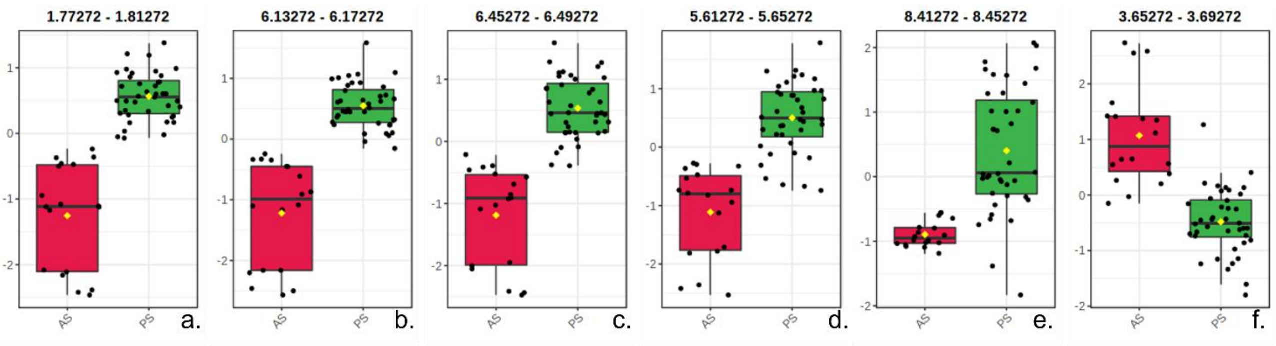

Figure S4. Trend of the top six most significant variables according to the Wilcoxon rank sum test applied to the aqueous extracts of authentic and adulterated saffron samples.Plots of the trend of the buckets containing the signals related to the following metabolites: a) crocin at 1.79 ppm; b) crocin at 6.13 ppm; c) crocin at 6.47 ppm; d) crocin at 5.63 ppm; e) formic acid at 8.43 ppm; f) glucose at 3.67 ppm.

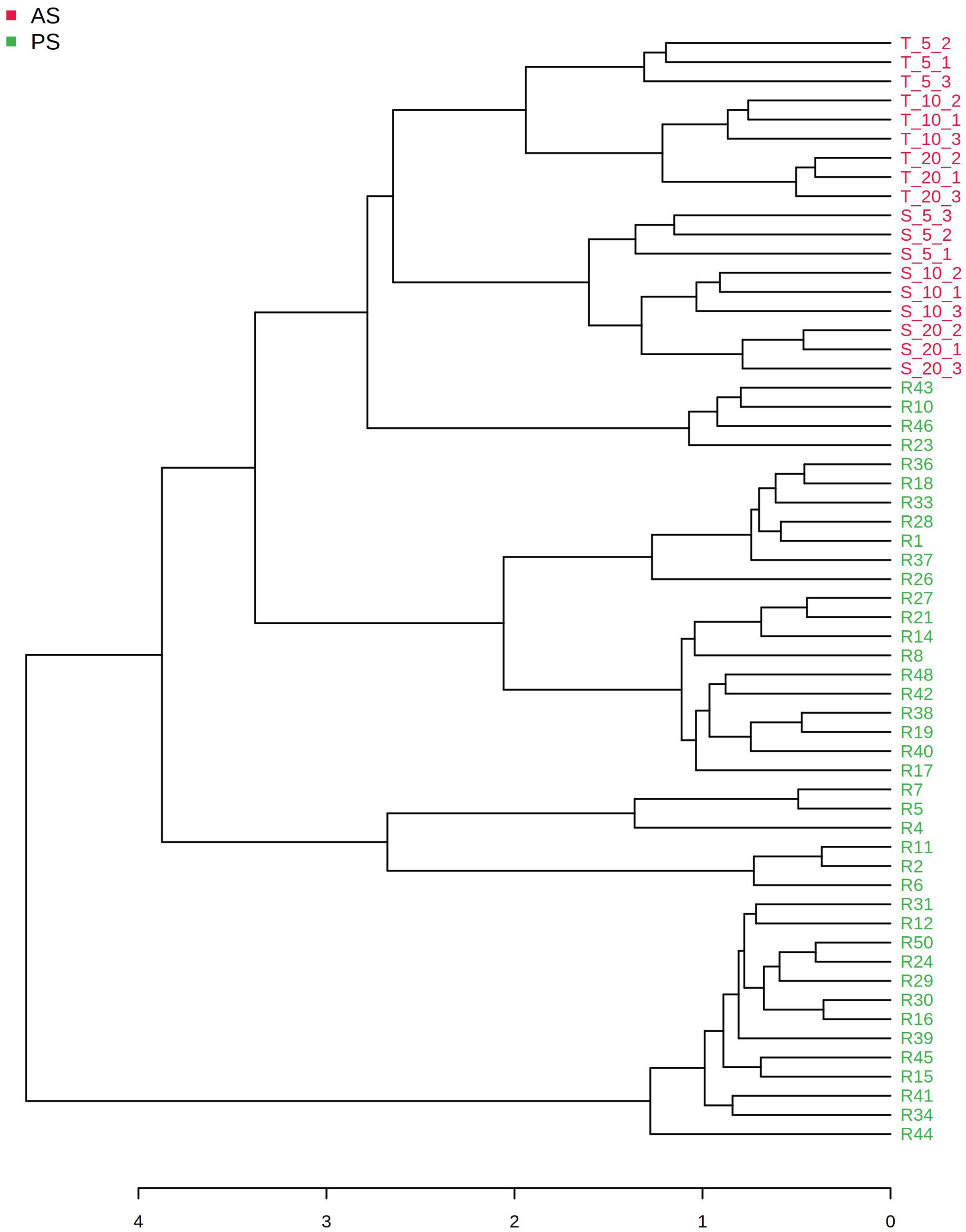

Figure S5. Results of the hierarchical analysis applied to the spectral data obtained from the aqueous extracts of authentic and adulterated saffron samples.Clustering uses the centroids of the observations. Distance measure: *Pearson*; clustering algorithm: average. The samples are indicated in red and green for adulterated samples (AS) and authentic ones (PS), respectively.

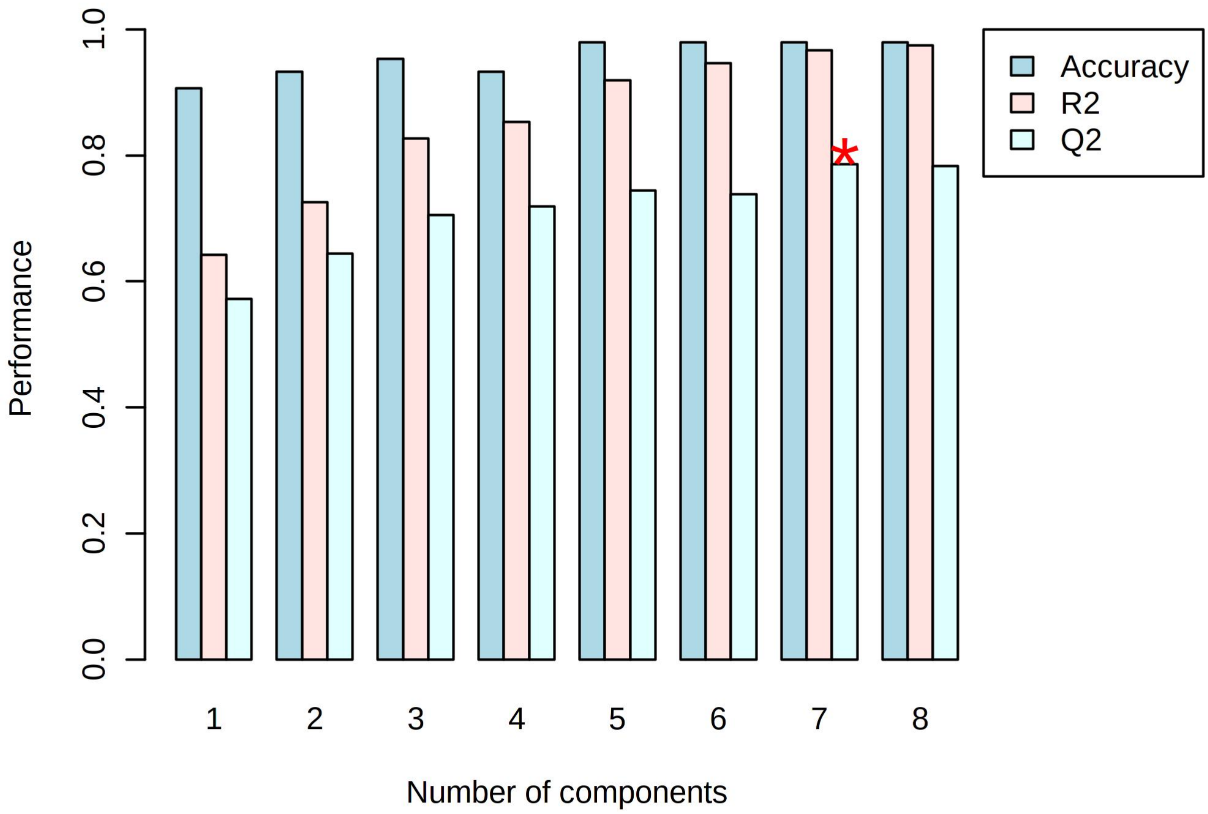

Figure S6. 10-fold cross-validation method applied to the PLS-DA model built on the spectral data obtained from the aqueous extracts for discriminating between authentic and adulterated saffron samples. Application of 10-fold cross-validation to PLS-DA model to select the optimal number of components for classification and estimate the predictive performance of the classification model. The red star indicates the best classifier.

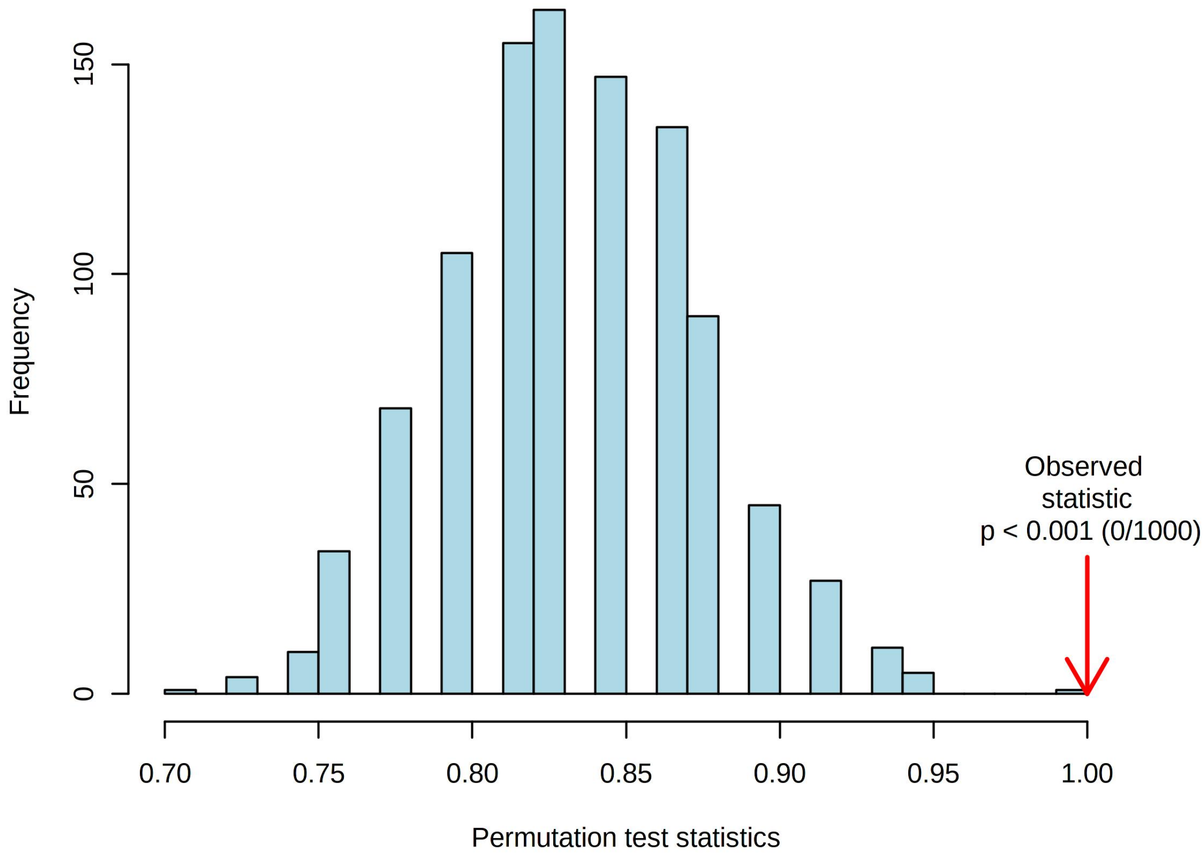

Figure S7. Permutation test applied to the PLS-DA model built on spectral data built on the spectral data obtained from the aqueous extracts for discriminating between authentic and adulterated saffron samples. The permutation test consists of a set of 1000 permutations and testing the prediction accuracy during training. The *p*-value based on permutation is $p < 0.001$ (0/1000).

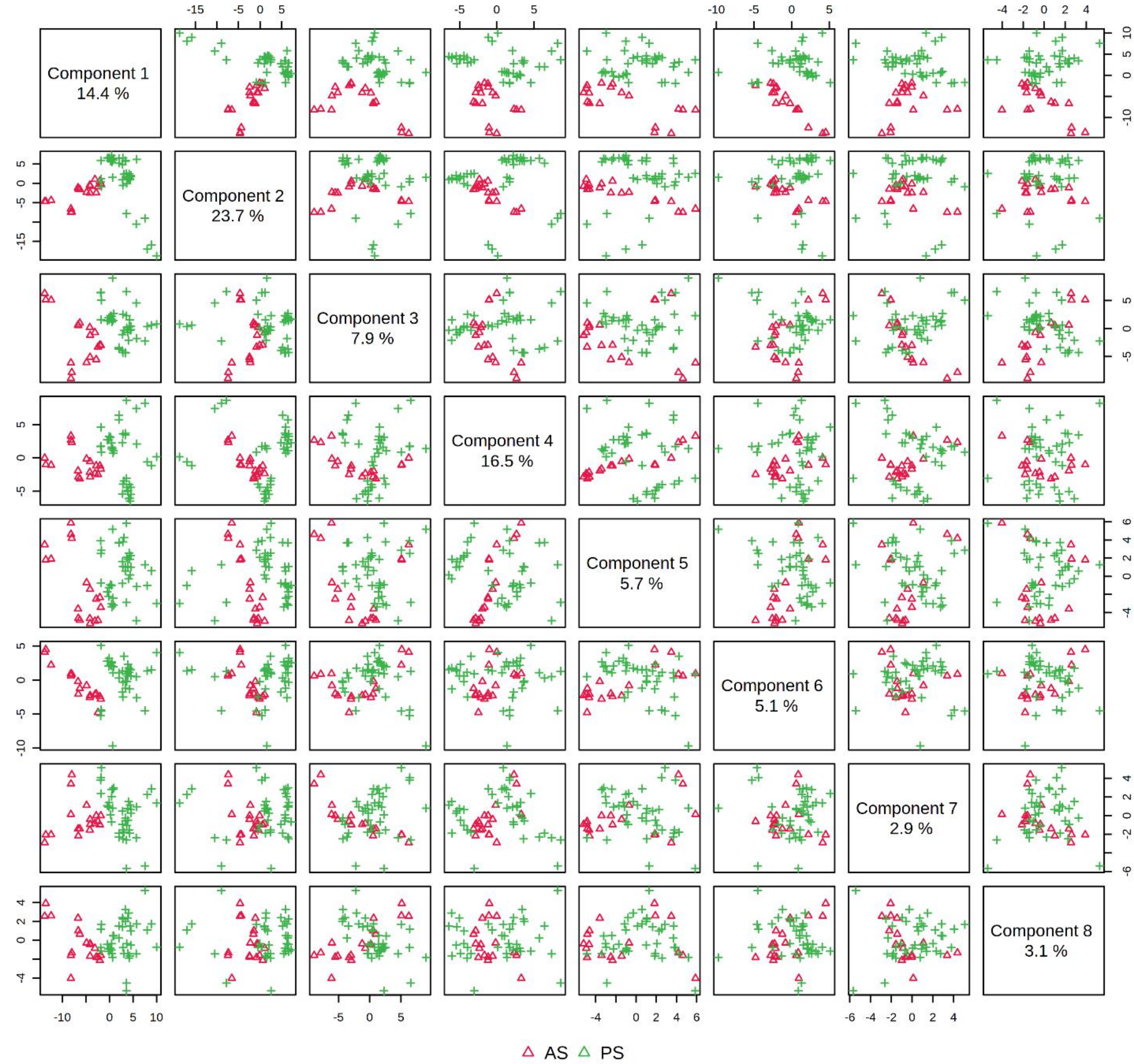

Figure S8. Overview of the PLS-DA model built on the spectral data obtained from the aqueous extracts for discriminating between authentic and adulterated saffron samples. Pairwise scores plots between the selected components, where the scores are indicated as follows: green crosses and red triangles for authentic samples (PS) and adulterated ones (AS), respectively. The explained variance of each component is shown in the corresponding diagonal cell.

Table S3. List of the top 66 variables importance in projection (VIPs) related to the PLS-DA model built on the spectral data obtained from the aqueous extracts for discriminating between authentic and adulterated saffron samples.List of the top 66 variables identified as VIP > 1 on Component 1.

Range (ppm)	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5
1.77272 - 1.81272	3.0151	2.8929	2.7209	2.6788	2.5834
6.13272 - 6.17272	2.9333	2.7799	2.6072	2.5658	2.482
6.45272 - 6.49272	2.8587	2.7254	2.5622	2.5215	2.4415
5.61272 - 5.65272	2.6757	2.5212	2.3627	2.3422	2.2601
3.65272 - 3.69272	2.576	2.4225	2.2789	2.2438	2.1683
5.37272 - 5.41272	2.158	2.0333	1.9231	1.914	1.8456
8.41272 - 8.45272	2.1505	2.0958	2.0563	2.0856	2.0631
1.85272 - 1.89272	2.1473	2.0798	1.951	1.9255	1.9084
4.01272 - 4.05272	1.9534	1.8372	1.7624	1.7475	1.6843
2.53272 - 2.57272	1.9475	1.9033	1.7889	1.7831	1.7525
3.57272 - 3.61272	1.8813	1.7692	1.68	1.6623	1.6128
7.25272 - 7.29272	1.8593	1.83	1.7234	1.6988	1.7087
6.41272 - 6.45272	1.8197	1.7489	1.6445	1.6205	1.6488
3.97272 - 4.01272	1.8121	1.7047	1.6611	1.6414	1.5863
1.81272 - 1.85272	1.7657	1.6813	1.6235	1.6693	1.6264
7.17272 - 7.21272	1.7632	1.762	1.7	1.7406	1.7025
3.77272 - 3.81272	1.7494	1.6452	1.5822	1.5753	1.5297
4.09272 - 4.13272	1.743	1.6481	1.6521	1.6331	1.5743
7.53272 - 7.57272	1.6824	1.5822	1.5491	1.5264	1.4714
4.17272 - 4.21272	1.6593	1.6282	1.5357	1.5175	1.4626
4.45272 - 4.49272	1.6569	1.5715	1.4991	1.501	1.4495
6.33272 - 6.37272	1.6453	1.5901	1.5203	1.5691	1.5136
7.85272 - 7.89272	1.6126	1.5447	1.4607	1.4421	1.3899
5.25272 - 5.29272	1.6085	1.5316	1.4369	1.4254	1.3892
4.61272 - 4.65272	1.6004	1.537	1.5111	1.4898	1.4746
9.05272 - 9.09272	1.5578	1.617	1.5943	1.5916	1.5563
3.41272 - 3.45272	1.5519	1.46	1.4231	1.4036	1.4129
8.61272 - 8.65272	1.5285	1.4636	1.4394	1.4221	1.3709
3.81272 - 3.85272	1.5057	1.4161	1.3776	1.358	1.3623
6.93272 - 6.97272	1.5014	1.4894	1.3985	1.3897	1.3568
5.65272 - 5.69272	1.4869	1.446	1.3641	1.345	1.3139
9.09272 - 9.13272	1.479	1.4477	1.5738	1.5579	1.5243
8.81272 - 8.85272	1.4688	1.4147	1.4385	1.4164	1.3811
1.93272 - 1.97272	1.451	1.3651	1.3092	1.296	1.2975
9.49272 - 9.53272	1.4393	1.3703	1.3022	1.2854	1.241
7.21272 - 7.25272	1.3818	1.3504	1.4218	1.4214	1.3746
3.73272 - 3.77272	1.3632	1.2963	1.2267	1.2393	1.2133
2.61272 - 2.65272	1.335	1.3057	1.2651	1.2451	1.2047
2.21272 - 2.25272	1.3279	1.2788	1.2141	1.2012	1.1612
8.97272 - 9.01272	1.3132	1.2349	1.2048	1.2181	1.1806
7.97272 - 8.01272	1.3129	1.2627	1.2192	1.2668	1.3242
7.57272 - 7.61272	1.304	1.2288	1.264	1.2444	1.2004
3.33272 - 3.37272	1.296	1.2194	1.178	1.1894	1.1586
1.61272 - 1.65272	1.2718	1.2428	1.1665	1.1487	1.2279

4.13272 - 4.17272	1.2605	1.2285	1.151	1.1364	1.1668
6.29272 - 6.33272	1.2574	1.2902	1.2263	1.2981	1.2575
2.57272 - 2.61272	1.2559	1.2223	1.1605	1.1422	1.1609
7.73272 - 7.77272	1.2366	1.1665	1.1783	1.1599	1.1206
7.41272 - 7.45272	1.2206	1.2463	1.1678	1.18	1.156
6.57272 - 6.61272	1.2189	1.206	1.1401	1.1222	1.123
7.01272 - 7.05272	1.1968	1.1405	1.1403	1.134	1.1334
9.77272 - 9.81272	1.1844	1.1693	1.1044	1.0873	1.1014
3.45272 - 3.49272	1.1765	1.1138	1.1029	1.1097	1.1066
3.01272 - 3.05272	1.1732	1.1033	1.0902	1.0734	1.1454
3.49272 - 3.53272	1.1694	1.1189	1.1327	1.1361	1.1575
2.09272 - 2.13272	1.1548	1.0901	1.0219	1.0078	0.99676
1.57272 - 1.61272	1.1075	1.0428	1.1431	1.1495	1.1165
6.61272 - 6.65272	1.091	1.1275	1.0644	1.0475	1.0335
1.25272 - 1.29272	1.0816	1.0181	1.0039	0.98803	1.0543
4.53272 - 4.57272	1.0679	1.0043	1.1189	1.1078	1.0688
4.05272 - 4.09272	1.0589	1.0119	0.97142	0.98594	0.99046
9.97272 - 10.0127	1.0447	0.98788	1.0181	1.0048	0.96931
9.57272 - 9.61272	1.0438	1.0596	1.0456	1.0291	0.99289
4.41272 - 4.45272	1.0418	1.0141	0.97588	1.001	1.0187
5.69272 - 5.73272	1.0044	1.0688	1.0261	1.01	0.97542
3.93272 - 3.97272	1.0033	0.94716	0.88779	0.9336	0.90987

Figure S9. NMR analysis of extracts of authentic saffron in DMSO-d_6.Bruker Avance 400 MHz.

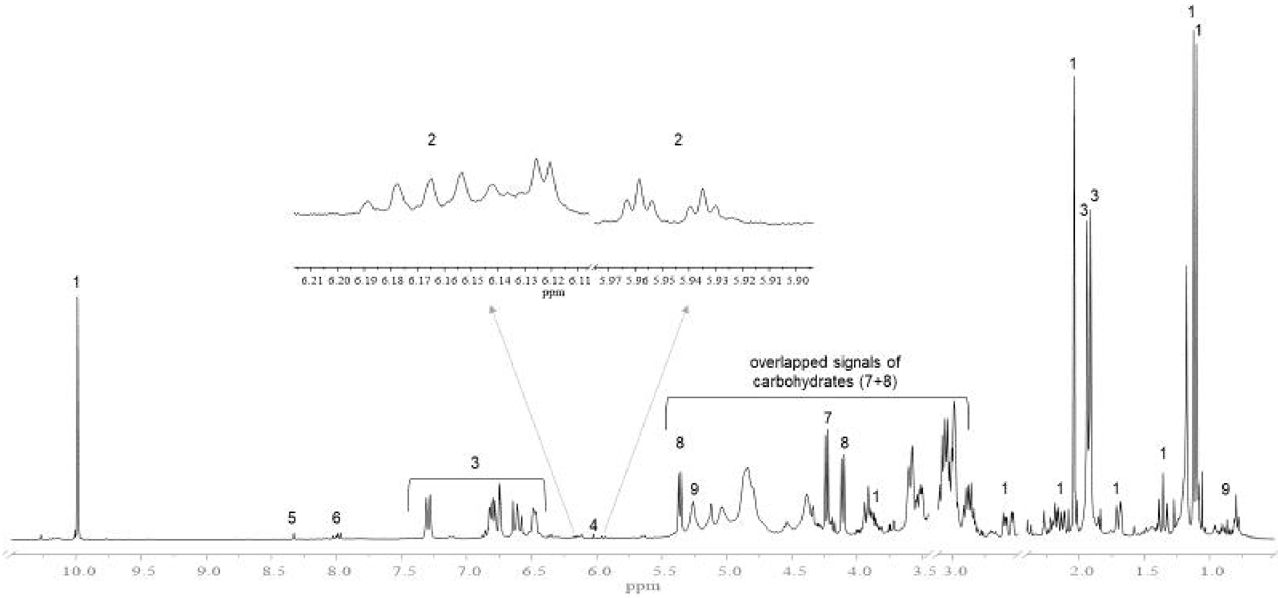

Figure S9. a. ^{1}H NMR. Residual solvent signals (2.44 and 3.27 ppm) are hidden. Signals are indicated as follow: 1, picrocrocin; 2,safranal; 3, trans-crocetin (β-D-gentibiosyl) ester ;4,fumaric acid; 5,formic acid; 6,glycosylated isoforms of kaempferol; 7,β-D-glucosyl of picrocrocin; 8,β-D-gentiobiosyl of trans-crocetin ester; 9,linoleic acid.

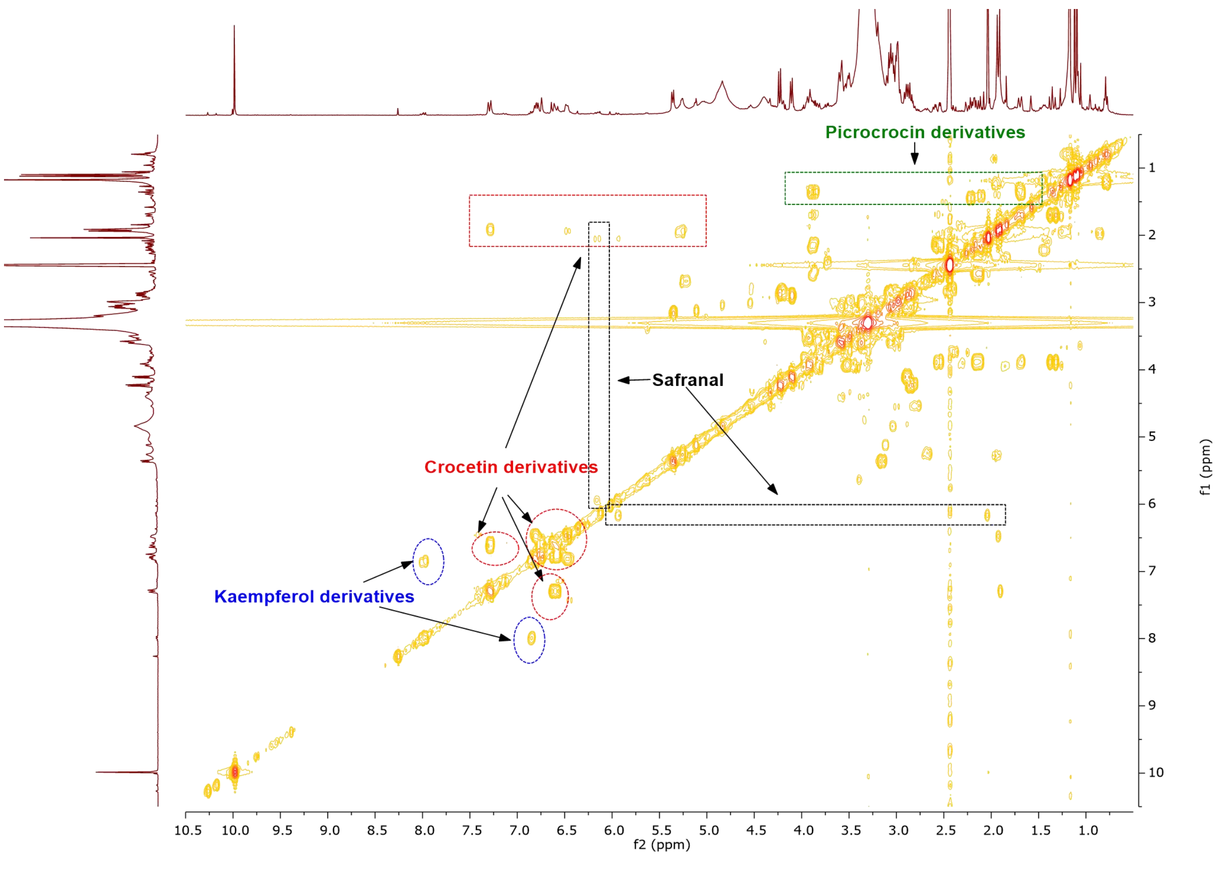

Figure S9. b. COSY spectrum.

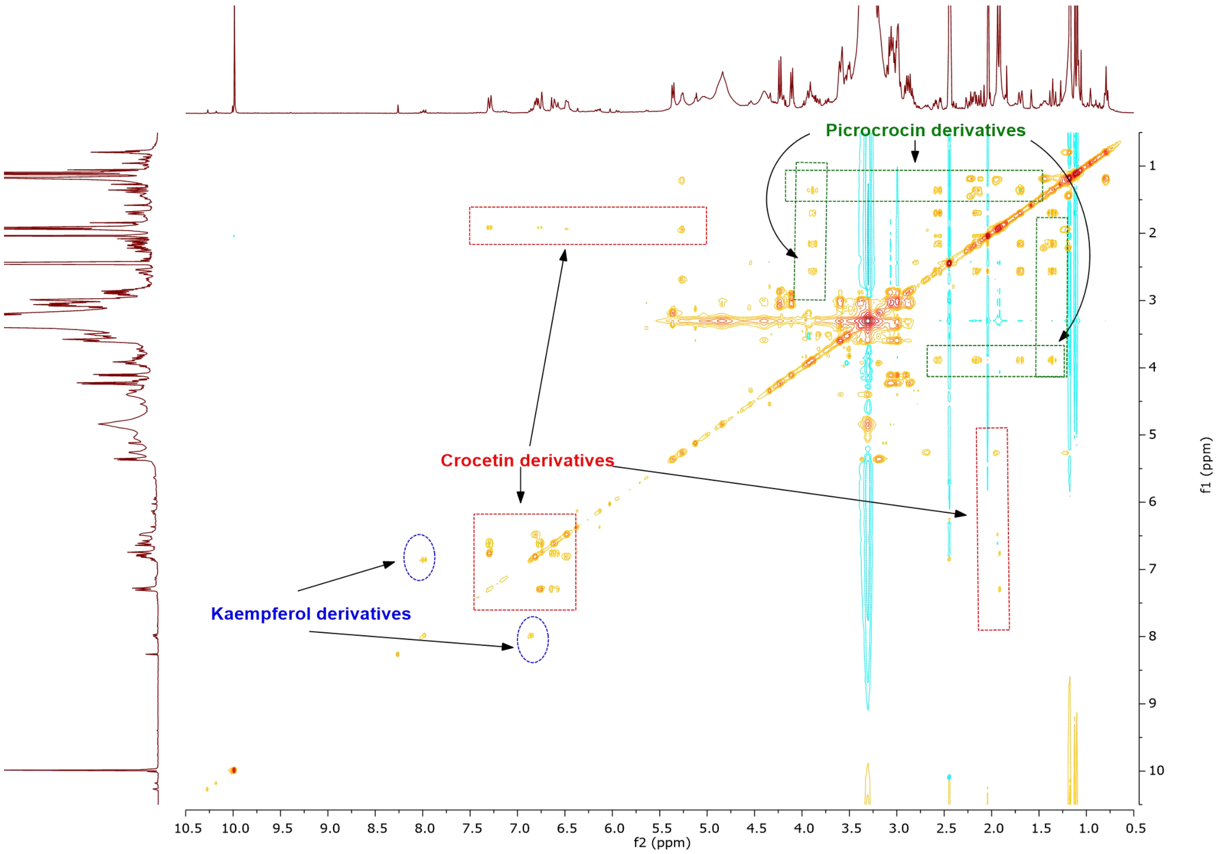

Figure S9. c. TOCSY spectrum.

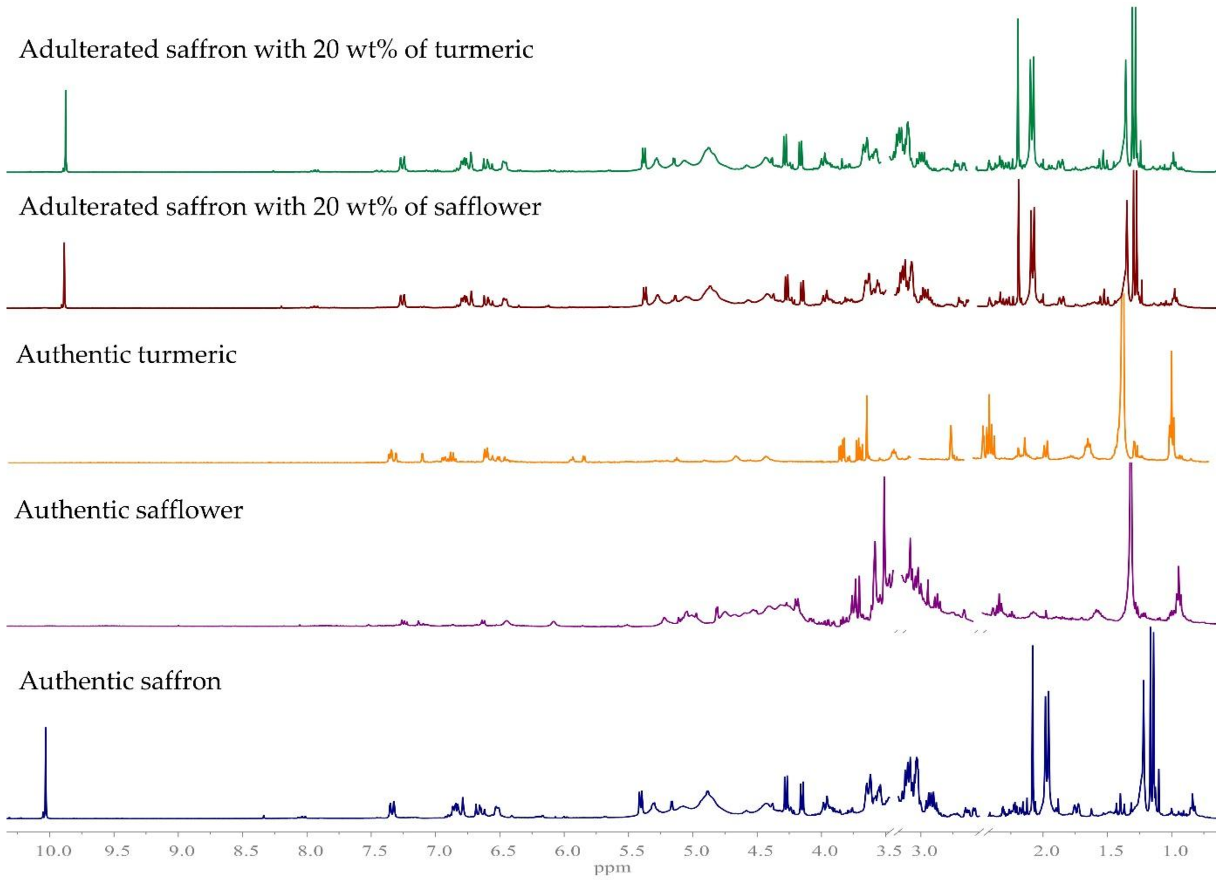

Figure S10. ^{1}H NMR spectra of extracts of authentic saffron, authentic turmeric, authentic safflower, saffron adulterated with turmeric, and saffron adulterated with safflower **in DMSO-*d*$_{6}$.** (Bruker Avance 400 MHz) Comparison of typical 1H NMR spectra of extracts in DMSO-d6 of authentic saffron (blue spectrum), authentic safflower (purple spectrum), authentic turmeric (orange spectrum), adulterated saffron with 20 wt% of safflower (red spectrum), and adulterated saffron with 20 wt% of turmeric (green spectrum). Residual solvent signals (2.44 and 3.27 ppm) are hidden.

Table S4. List of metabolites contained extracts of authentic saffron samples in DMSO-d_6 and identified by ^{1}H NMR. The assignment of NMR signals has been obtained by comparison with standard compounds, except for the metabolites indicated with a star. For these metabolites the assignment was obtained referring to spectral data reported in the literature. [2,3].

Compound	**^{1}H ppm**	**Multeplicity**	**J (Hz)**
Monoterpenes			
Picrocrocin*	1.10	s	
	1.12	s	
	1.36	t	12.2
	1.70	ddd	12.2; 2.5
	2.04	s	
	2.15	dd	18.8;9.2
	2.56	ddd	18.9; 5.3; 1.8
	3.88	m	
	9.99	s	
Safranal*	2.02	m	
	5.95	dt	9.4; 1.8
	6.16	dd	9.6; 5.1
Carotenoids			
trans-crocetin(β-D-gentibiosyl) ester *	1.91	s	
	1.94	s	
	6.48	dd	7.7;2.6
	6.61	dd	14.9; 11.5
	6.76	d	14.9
	6.81	dd	7.9;2.6
	7.29	d	11.2
Organic acids			
Fumaric acid	6.02	s	
Formic acid	8.33	s	
Flavonoids			
Kaempferol-3-sophoroside*	6.11	d	2.1
	6.37	d	2.1
	6.85	d	8.8
	7.98	d	8.9
Kaempferol-3-sophoroside-7-β-glucoside*	6.87	d	8.8
	8.01	d	8.9
Carbohydrates			
β-D-glucosyl of picrocrocin *	4.23	d	7.8
β-D-gentiobiosyl of trans-crocetin ester *	5.24	d	7.7
	4.11	d	7.7
Fatty acid			
Linoleic acid*	0.86	t	7.5

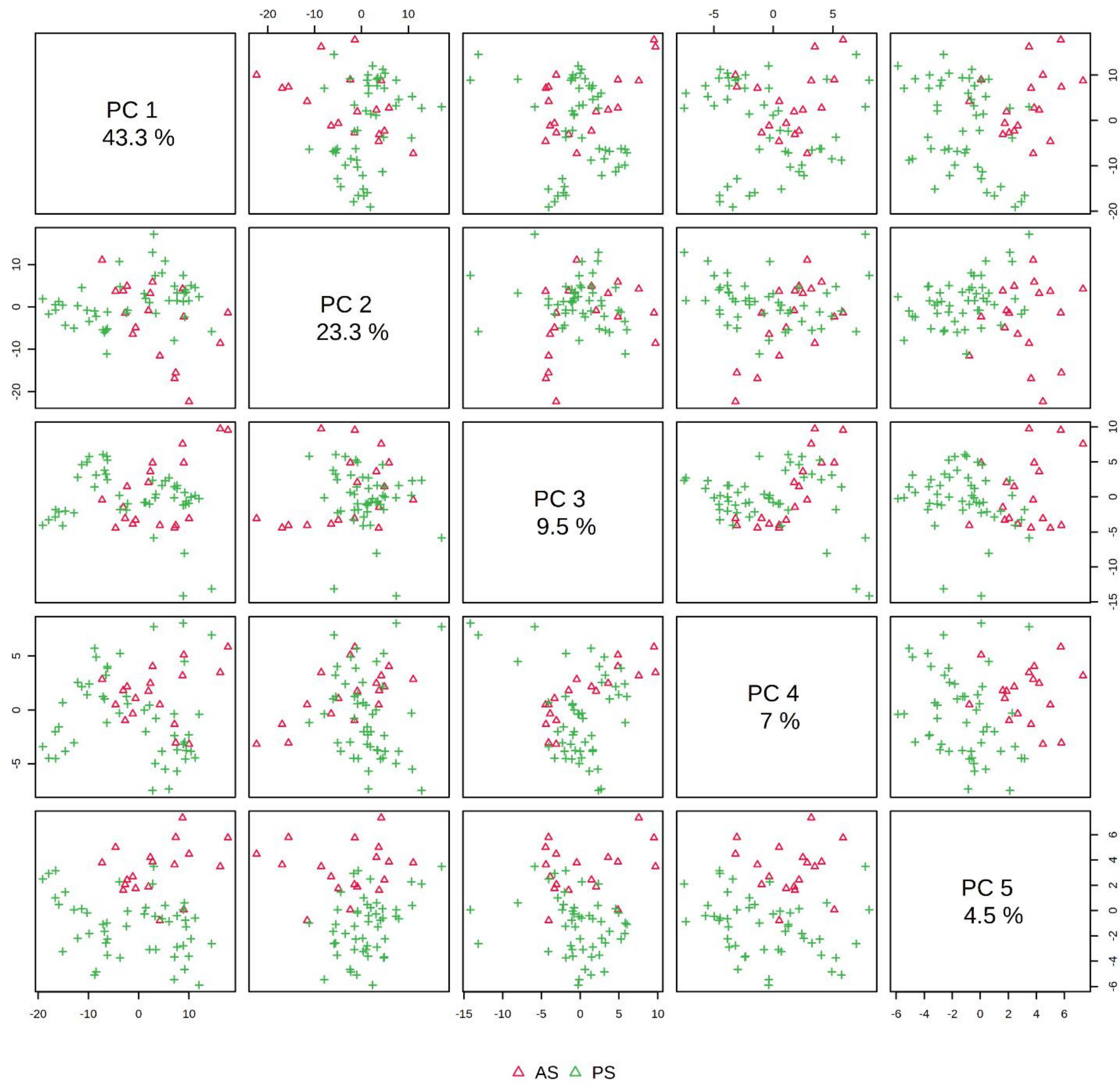

Figure S11. Results of PCA applied to the spectral data obtained from extracts of authentic saffron and adulterated saffron in DMSO-d_6. PCA was applied to the 67 spectra of authentic saffron and adulterated saffron extracted in DMSO-d_6 by using UV-scaled 0.04 ppm-sized bucketing. Pairwise scores plots between the selected components where the scores are indicated as follows: green crosses and red triangles for authentic samples and adulterated ones, respectively. The explained variance of each component is shown in the corresponding diagonal cell.

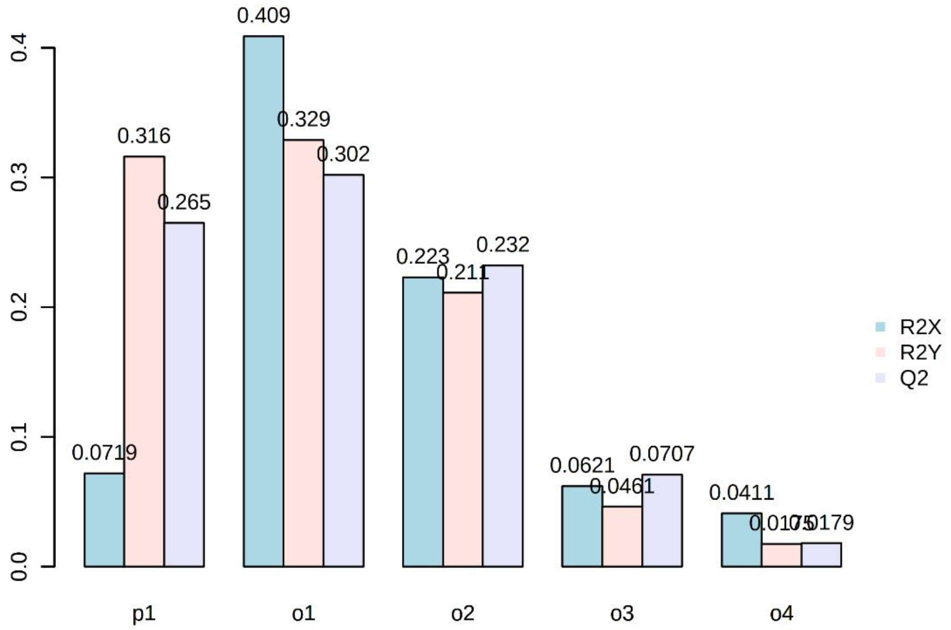

Figure S12. Overview of the OPLS-DA model built on the spectral data obtained from the extracts in DMSO-d_6 for discriminating between authentic and adulterated saffron samples.

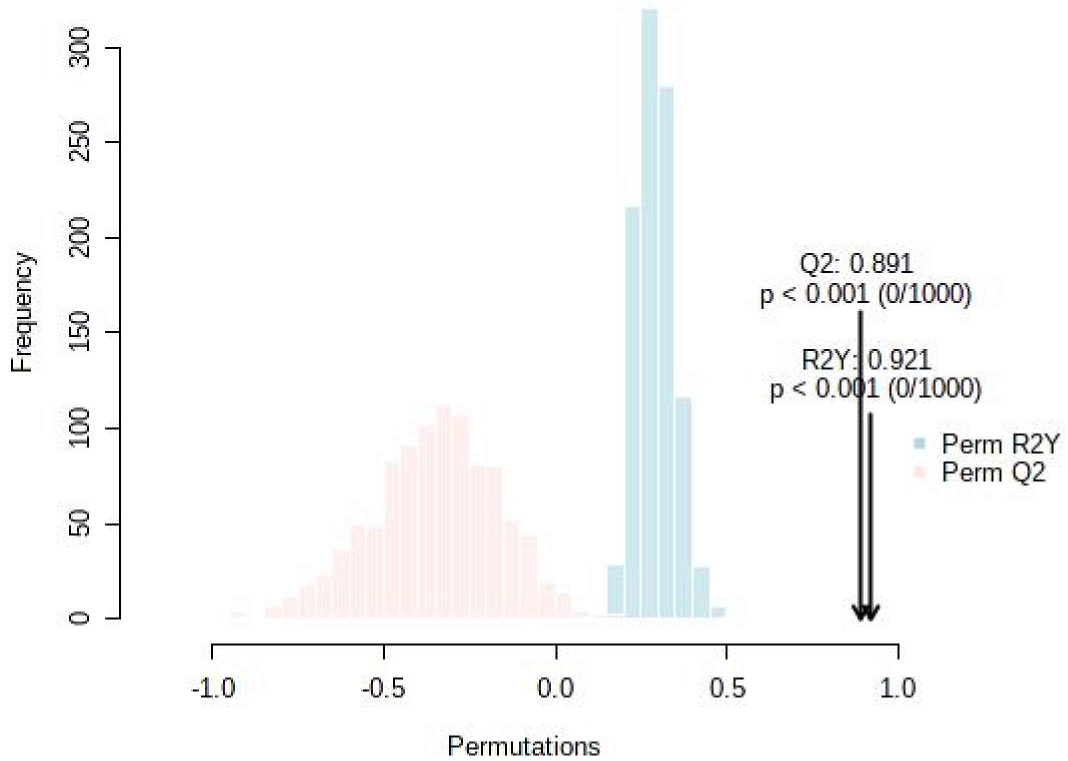

Figure S13. Permutation test applied to the OPLS-DA model built on the spectral data obtained from the extracts in DMSO-d_6 for discriminating between authentic and adulterated saffron samples. The permutation test consists of a set of 1000 permutations and testing the prediction accuracy during training. The *p*-value based on permutation is $p < 0.001$ (0/1000).

Table S5. List of features characteristic of each sample of authentic saffron under investigation.

Primary Id	Producer	Region	Town	Agronomic Practice
R1	Starace Filomena	Tuscany	GR	C
R2	Poggio al Sole	Tuscany	FI	B
R4	Starace Filomena	Tuscany	GR	C
R5	La saggezza della Terra	Veneto	PD	C
R6	Poggio al Sole	Tuscany	FI	B
R7	La saggezza della Terra	Veneto	PD	C
R8	La saggezza della Terra	Veneto	PD	C
R10	Alessandro Mazzuoli	Umbria	PG	B
R11	Poggio al Sole	Tuscany	FI	B
R12	Poggio al Sole	Tuscany	FI	B
R14	La saggezza della Terra	Veneto	PD	C
R15	Poggio al Sole	Tuscany	FI	B
R16	Poggio al Sole	Tuscany	FI	B
R17	La saggezza della Terra	Veneto	PD	C
R18	Starace Filomena	Tuscany	GR	C
R19	La saggezza della Terra	Veneto	PD	C
R21	La saggezza della Terra	Veneto	PD	C
R23	Alessandro Mazzuoli	Umbria	PG	B
R24	Poggio al Sole	Tuscany	FI	B
R26	Starace Filomena	Tuscany	GR	C
R27	La saggezza della Terra	Veneto	PD	C
R28	Starace Filomena	Tuscany	GR	C
R29	Poggio al Sole	Tuscany	FI	B
R30	Poggio al Sole	Tuscany	FI	B
R31	Poggio al Sole	Tuscany	FI	B
R33	Starace Filomena	Tuscany	GR	C
R34	Poggio al Sole	Tuscany	FI	B
R36	Starace Filomena	Tuscany	GR	C
R37	Starace Filomena	Tuscany	GR	C
R38	La saggezza della Terra	Veneto	PD	C
R39	Poggio al Sole	Tuscany	FI	B
R40	La saggezza della Terra	Veneto	PD	C
R41	Poggio al Sole	Tuscany	FI	B
R42	La saggezza della Terra	Veneto	PD	C
R43	Alessandro Mazzuoli	Umbria	PG	B
R44	Poggio al Sole	Tuscany	FI	B
R45	Poggio al Sole	Tuscany	FI	B
R46	Alessandro Mazzuoli	Umbria	PG	B
R48	La saggezza della Terra	Veneto	PD	C
R50	Poggio al Sole	Tuscany	FI	B

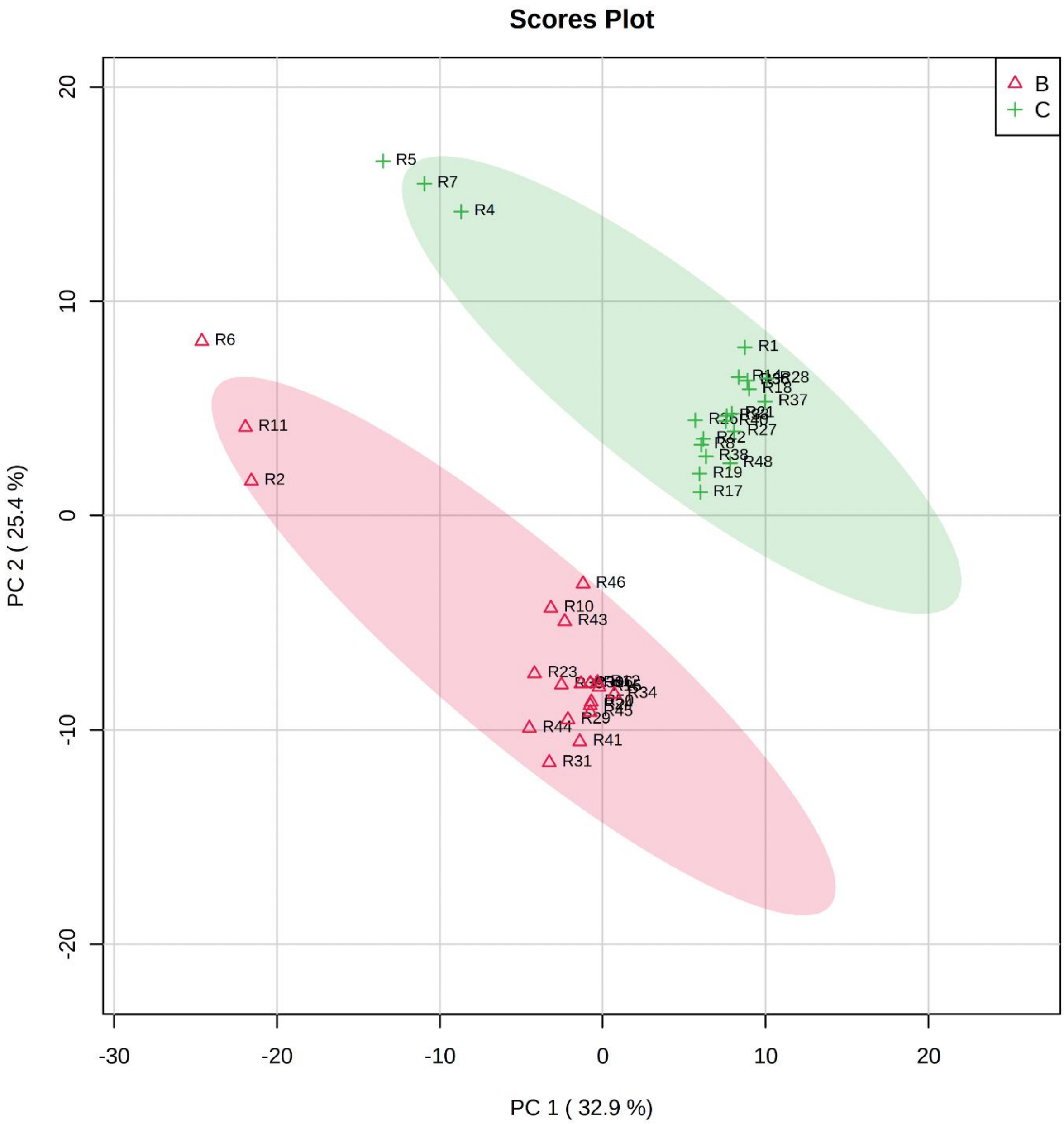

Figure S14. Results of PCA performed on the spectral data obtained from the aqueous extracts of authentic saffron**.** Scores plot between the selected PCs. The explained variances are shown in brackets. The samples are indicated according to the agronomic farming: conventional cultivation (C, represented as red crosses) and organic one (B, represented as red triangles).

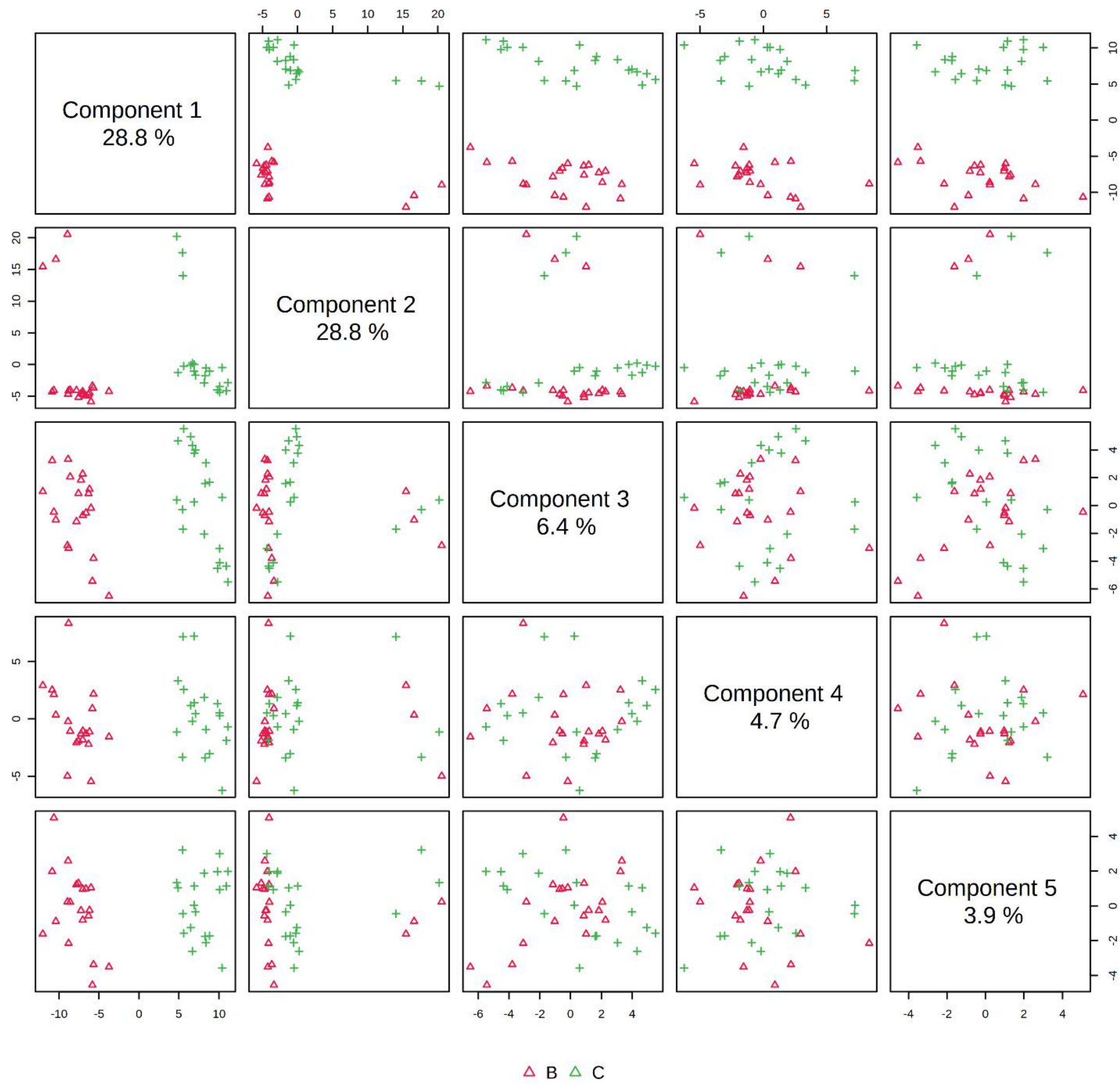

Figure S15. Overview of PLS-DA built on the spectral data of the aqueous extracts of authentic saffron samples for discriminating according to the agronomic practice: organic *vs* conventional. Pairwise scores plots between the selected components, where the scores are indicated as follows: green crosses and red triangles for authentic saffron cultivated through conventional cultivation (C), and organic one (B). The explained variance of each component is shown in the corresponding diagonal cell.

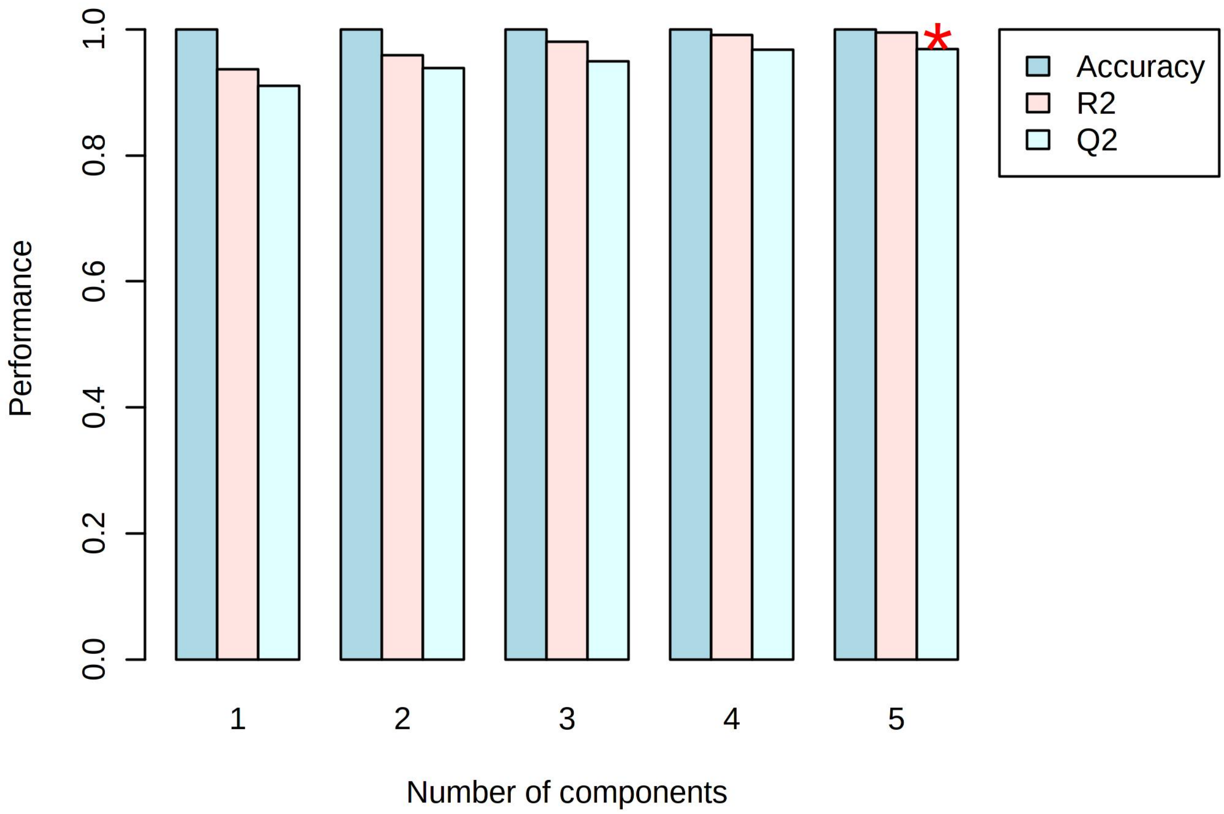

Figure S16. 10-fold cross-validation method applied to the PLS-DA model built on spectral data of the aqueous extract of authentic saffron samples for discriminating according to the agronomic practice: organic *vs* conventional. Application of 10-fold cross-validation to PLS-DA model to select the optimal number of components for classification according to the agronomic practice: organic (B) *vs* conventional (C), and estimate the predictive performance of the classification model. The red star indicates the best classifier.

Table S6. List of the top 78 Variables importance in projection (VIPs) related to the PLS-DA model built the spectral data of the aqueous extracts of authentic saffron samples for discriminating according to the agronomic practice: organic *vs* conventional. List of the top 78 variables identified as VIP > 1 on Component 1.

Range (ppm)	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5
2.69272 - 2.73272	2.0506	2.0195	1.9956	1.9827	1.9794
2.33272 - 2.37272	2.0137	1.9847	1.9613	1.9483	1.9452
2.13272 - 2.17272	1.942	1.912	1.8897	1.8775	1.8745
9.93272 - 9.97272	1.9379	1.9079	1.8859	1.8734	1.8706
4.21272 - 4.25272	1.935	1.9052	1.8824	1.8783	1.8772
7.25272 - 7.29272	1.9148	1.8854	1.8637	1.8534	1.8508
1.17272 - 1.21272	1.9107	1.8811	1.8624	1.8511	1.8486
6.41272 - 6.45272	1.8745	1.846	1.8292	1.8171	1.8141
1.21272 - 1.25272	1.8437	1.8154	1.7955	1.7848	1.7819
3.69272 - 3.73272	1.8359	1.8092	1.7932	1.7814	1.7787
2.57272 - 2.61272	1.8243	1.7971	1.7765	1.7651	1.7623
6.09272 - 6.13272	1.8145	1.7864	1.774	1.7632	1.7604
5.57272 - 5.61272	1.8057	1.7805	1.7673	1.756	1.7532
4.45272 - 4.49272	1.8056	1.7777	1.7613	1.7565	1.7536
1.85272 - 1.89272	1.7912	1.7663	1.7452	1.7343	1.7315
3.97272 - 4.01272	1.7908	1.7641	1.7512	1.7395	1.7367
2.05272 - 2.09272	1.7896	1.7621	1.7471	1.7361	1.7333
3.89272 - 3.93272	1.7847	1.7605	1.7445	1.7332	1.7306
3.49272 - 3.53272	1.7847	1.7604	1.7411	1.7304	1.7277
2.37272 - 2.41272	1.773	1.748	1.7304	1.7249	1.7236
4.01272 - 4.05272	1.7586	1.7317	1.7159	1.7052	1.7028
1.49272 - 1.53272	1.7464	1.7218	1.7045	1.6931	1.6906
5.21272 - 5.25272	1.7431	1.7161	1.6957	1.6852	1.6838
3.93272 - 3.97272	1.7191	1.6969	1.6766	1.6675	1.6669
6.37272 - 6.41272	1.7076	1.6829	1.6801	1.6694	1.6669
1.45272 - 1.49272	1.6995	1.6768	1.6626	1.6525	1.6503
3.73272 - 3.77272	1.6797	1.6612	1.6457	1.6347	1.6322
4.13272 - 4.17272	1.6692	1.6437	1.6242	1.6134	1.6117
1.89272 - 1.93272	1.6315	1.6062	1.5983	1.5981	1.5962
2.17272 - 2.21272	1.6189	1.5938	1.5878	1.5773	1.5771
3.61272 - 3.65272	1.6065	1.5824	1.5791	1.5686	1.567
4.65272 - 4.69272	1.6058	1.5816	1.5632	1.5534	1.555
2.29272 - 2.33272	1.6045	1.5935	1.5744	1.5665	1.5643
2.49272 - 2.53272	1.5825	1.562	1.5491	1.5389	1.5365
4.05272 - 4.09272	1.5615	1.5389	1.5274	1.5325	1.5304
1.77272 - 1.81272	1.5509	1.5297	1.5141	1.5042	1.5017
1.65272 - 1.69272	1.5488	1.5293	1.5561	1.5476	1.5453
1.57272 - 1.61272	1.548	1.5245	1.5063	1.5023	1.4998
5.25272 - 5.29272	1.5463	1.5251	1.5074	1.4987	1.4965
6.45272 - 6.49272	1.5417	1.5199	1.5073	1.4976	1.4952
3.81272 - 3.85272	1.5348	1.5112	1.4947	1.512	1.5135
4.41272 - 4.45272	1.5127	1.4893	1.4805	1.4724	1.47
5.65272 - 5.69272	1.5123	1.4984	1.4806	1.4707	1.4685
3.41272 - 3.45272	1.4844	1.4686	1.4551	1.4486	1.4466

9.61272 - 9.65272	1.4814	1.4744	1.4589	1.4514	1.4498
4.37272 - 4.41272	1.447	1.4292	1.4137	1.4046	1.4027
8.57272 - 8.61272	1.4345	1.4284	1.4479	1.4389	1.437
10.0127 - 10.0527	1.4113	1.4053	1.3885	1.3803	1.3781
6.05272 - 6.09272	1.3924	1.3709	1.3915	1.3824	1.3802
2.45272 - 2.49272	1.388	1.3758	1.3638	1.3548	1.3526
6.33272 - 6.37272	1.3855	1.3649	1.3809	1.3741	1.3718
0.972723 - 1.01272	1.3709	1.3535	1.3549	1.3541	1.352
4.61272 - 4.65272	1.3677	1.3468	1.3335	1.3365	1.3361
1.81272 - 1.85272	1.3558	1.3447	1.3313	1.3327	1.3306
5.41272 - 5.45272	1.3541	1.3359	1.3227	1.3387	1.3417
2.25272 - 2.29272	1.3425	1.3218	1.3241	1.3286	1.3279
10.1727 - 10.2127	1.3289	1.332	1.3164	1.3095	1.3077
0.892723 - 0.932723	1.3231	1.3102	1.2958	1.2885	1.2864
1.73272 - 1.77272	1.3118	1.3043	1.3115	1.3029	1.3014
1.37272 - 1.41272	1.2619	1.2484	1.2659	1.2789	1.278
9.81272 - 9.85272	1.2616	1.243	1.2348	1.2267	1.226
2.21272 - 2.25272	1.2451	1.2261	1.2349	1.2381	1.2361
3.17272 - 3.21272	1.2302	1.2117	1.1976	1.1916	1.1968
7.89272 - 7.93272	1.2178	1.2098	1.228	1.2199	1.2187
1.01272 - 1.05272	1.2162	1.1991	1.1979	1.1952	1.1933
7.21272 - 7.25272	1.2102	1.2028	1.2157	1.2169	1.2149
0.932723 - 0.972723	1.1751	1.1589	1.1657	1.1662	1.1643
6.13272 - 6.17272	1.1588	1.1472	1.1393	1.1323	1.1307
2.61272 - 2.65272	1.1588	1.1582	1.1455	1.1388	1.1376
9.73272 - 9.77272	1.1351	1.1237	1.1183	1.1119	1.1107
10.2527 - 10.2927	1.1329	1.1406	1.1359	1.1404	1.1396
3.01272 - 3.05272	1.1176	1.1267	1.1156	1.1092	1.1082
7.61272 - 7.65272	1.1082	1.1066	1.0948	1.0884	1.0923
5.61272 - 5.65272	1.1046	1.0997	1.1098	1.1025	1.1029
9.53272 - 9.57272	1.0579	1.0416	1.0453	1.039	1.0407
7.93272 - 7.97272	1.0428	1.0272	1.0178	1.0396	1.0416
9.69272 - 9.73272	1.0144	1.0073	1.0083	1.0066	1.005

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