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Stability of volatile compounds of Turkish saffron (*Crocus sativus*) after one-year storage

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Abstract

Despite the popularity of saffron (*Crocus sativus*), only a few studies have considered the alterations in the aroma profile of its shade-dried calyxes during the storage period. This study examined the influence of one-year storage at ambient temperature on the aroma compounds of saffron which is an important spice in terms of its nutritive and health benefits. It was found that storage period had significant effects on the aroma compositions of saffron samples. A total of 46 and 63 aroma compounds belonging different chemical groups mainly aldehydes, ketones, alcohols and esters were identified and quantified in shade dried and one-year stored saffron samples by the HS-SPME (Head Space–Solid Phase Micro Extraction) GC/MS (gas chromatography–mass spectrometry), respectively. The total amount of volatiles in directly shade-dried saffron sample was determined as 60824,1 µg/kg while one year stored saffron sample contained 145292,7 µg/kg. The degradation of crocin pigments during the one-year storage of saffron, resulted in the accumulation of some marker aroma compounds such as safranal and its isomers, while reduction of some others volatiles such as β-isophorone. Safranal, β-isophorone, isophorone, β-ionone and 4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-ene-1-carboxaldehyde (HTCC) were determined in the present study to be the dominant compounds in the volatile profile of the saffron samples. It was observed that the amount of safranal and isophorone sharply increased while β-isophorone, β-ionone and HTCC levels decreased as a result of one-year storage period..

Keywords: *Saffron, storage, volatile, GC/MS, SPME*

1. INTRODUCTION

Crocus belongs to Iridaceae family that contains nearly 100 taxa all over the world. *Crocus* species distribute in especially Mediterranean basin South west Asia, Europe and China. *C. sativus* is the highly popular species among the *crocus* species because of its economic values. This bulbous plant cultivated for its stigmas which is known as one of the most expensive spice in the world. This species mostly cultivated in Iran and other countries such as India, Morocco, Greece, Spain, and Turkey are the followers of the Iran for the saffron spice production (Ghorbani, 2008; Maggi et al., 2010; Guclu et al. 2020). In recent years, saffron production reached 30 kilos per year in Turkey (Cardone et al. 2020).

Saffron has known antitumoral and anticarcinogenic properties due to its bioactive components which has been used in the treatment of numerious medical indications since the acient time (Mousavi and Bathaie, 2011; Sanchez-Vioque et al. 2012). Cardone et al. 2020 called saffron as 'King of the Spice' and also 'red gold' because of its extraordinary medicinal and aromatic species (Leone et al., 2018). Additionally, saffron has been used as medicine at different traditions such as ancient Egypt, Crete, Rome during 4000 years (Shokrpour, 2019; Cardone et al. 2020). Recently it is used at both chronic disease cure and positive contribution to human health (Kyriakoudi et al., 2015; Cardone et al. 2020).

The dried red stigmas of C. sativus, saffron, are considered as the most valuable, infamous, and engaging spice all over the world. These main properties of saffron comes from its characteristic

colour, unique and characteristic aroma, and supplied health benefits when it is consumed (Guclu et al., 2020; Sharafzadeh 2012). The aroma structure of saffron is relatively complex, originating mainly from safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) compound. As a result of studies conducted so far in saffron spice, more than 160 volatile compounds have been identified (Carmona et al., 2007). The freshly picked saffron stigmas are nearly odourless, with characteristic saffron flavour being developed during the drying treatment. During food processing, especially in drying process, aroma components often change, degrade or disappear leading to an effect in the quality of the final product. Additionally, the storage of saffron can produce changes in composition, flavour and sensory properties. Specifically, safranal is synthesized through hydrolysis step of the bitter glycoside picrocrocin. Along with picrocrocin, there are many other glycosides that may undergo hydrolysis to yield a complex array of compounds that comprise the volatile pattern of saffron (Amanpour et al., 2015; Cadwallader, 2001). To the best knowledge of the authors, there are only two studies that examine the change in saffron aroma substances depending on storage time in the literature (Maggi et al., 2010; Sereshti et al. 2018). Different saffron samples volatiles belonging to three different storage time (<1 year, 3–4 and 8–9 years) were investigated using ultrasound assisted extraction and gas chromatography-mass spectrometry-olfactometry by Maggi et al. (2010). Authors reported that saffron with less than one year storage period had a higher proportion of saffron, flower and spicy odour notes, while the oldest saffron (3–4 and 8–9 years of storage) samples contained volatiles with vegetal, caramel and citrus notes. Sereshti et al. (2018) elucidated the effect of two year storage time on the saffron volatile profile and concluded that freshly dried samples had a higher level of the crocins, ß-isophorone, 4-hydroxy-3,5,5-trimethylcyclohex-2-enone and picrocrocin, while stored samples had low crocin and high safranal contents.

Composition and amounts of aroma compounds of saffron is affected by storage time in a significant extent. To the best knowledge of the authors, there is no detailed study on the differences of aroma compounds in freshly dried and one-year stored Turkish saffron stigmas in the literature. So, the current study was carried out to assess the influence of one-year storage period on the amounts and compositions of the aroma of dried saffron stigmas from Mersin province of Turkey by employing HS-SPME and GC/MS instruments.

2. MATERIALS AND METHODS

2.1. Saffron samples and chemicals

Saffron was harvested in December 2019 from Mersin province of Turkey. The samples are pure, and obtained directly from the producers. Saffron sample was transported from the growing area to the laboratory for drying. Samples were dried with traditional drying (in the shade), then stored during one year in a dark glass bottles away from light. Reference aroma compounds were used as a standard were purchased from Sigma-Aldrich (Steinheim, Germany). The Millipore-Q system (Millipore Corp., Saint-Quentin, France) was used to purify water.

2.2. Methods

2.2.1. HS-SPME-GC-MS analysis

Analysis of the volatile compounds in saffron samples was carried out by the combination of four analytical procedures: headspace, solid-phase microextraction, gas chromatography, and mass spectrometry (HS-SPME-GC-MS). Briefly, 0.5 g ground dried safrron sample and 5 µl internal standard (IS: 4-nonanol) were mixed in a 20-ml vial. The vial was then sealed using a screw cap with a PTFE-silicon septum. Subsequently, the vial was placed in a water bath at 50°C for 15 min to balance the headspace gas. Afterward, a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane 2 cm fiber (DVB/Car/PDMS; Supelco, Bellefonte, PA, USA) was inserted into the head space of the vial and the volatiles were absorbed for 30 min under 50°C water bath condition. The desorption volatiles from the fiber were achieved by injecting the fiber

into the GC injection port for 15 min at 250°C. An Agilent (KODU) gas chromatograph connected with Agilent GC-MS-KODU was used for the analysis of the volatile compounds in the samples. An DB-Wax capillary column (30m×0.25mm, 0.25 µm film thickness) was used for the separation of the volatile compounds. The carrier gas was helium with a flow rate of 1 ml/min. The oven temperature was programmed as follows: 40°C for 5 min; increasing to 100°C at a rate of 4°C/min, held for 5 min; and increasing to 250°C at a rate of 8°C/min, held for 6 min. The temperature of ion source and quadrupole was 220 and 200°C, respectively. The ion source was in an electron impact (EI) mode at 70 eV. Full scan mode with the m/z range of 35–400 was used. Ion source and transfer line temperature were maintained at 250 °C, respectively. Volatile compound identification was conducted by comparing their mass spectrum and retention indices (RIs) with the reference standards in the NIST11 and Wiley libraries and further confirmed with their external standard. The volatile compounds without the available external standards were tentatively identified by comparing their RIs and libraries database. For quantitation, the peak ratios of the target compound to the IS against the concentration were plotted to generate standard calibration curves. Volatile compounds that had no reference standards were approximately quantified by the standards with the same functional group and/or similar amounts of carbon atoms (Kesen et al., 2020).

2.2.2. Statistical Analysis

Statistical data analysis was conducted using SPSS 22.0 with One-way ANOVA (SPSS Inc). Duncan's test measured the variations in the content levels of the results. Means with p-values below 0.05 have been found to be statistically important.

3. RESULTS AND DISCUSSION

3.1. Volatile compounds of saffron samples

The aroma compounds extracted from the saffron samples by utilizing the headspace-solid phase micro extraction (HS-SPME) method are presented in Table 1. A total of 64 compounds were determined including aldehydes (19 compounds), ketones (19 compounds), esters (7 compounds), terpenes (6 compounds), 13-C-norisoprenoids (3 compounds), alcohols (2 compounds), phenols (2 compounds), lactone (1 compound), pyrrole (1 compound), benzene (1 compound), furan (1 compound), naphthalene (1 compound) and furanone (1 compound). Similar volatile compounds in various previous studies on different saffron samples were reported (Amanpour et al., 2015; D'Auria et al., 2004; Maggi et al., 2010; Urbani et al., 2015). Aldehydes, ketones, esters and terpenes were the major aroma groups in both of samples in the present work. The total amount of aroma compounds was quantified as 60824,1 µg/kg in the freshly dried sample and 145292,7 µg/kg in the one-year stored sample (Table 1). According to these results, it was concluded that the one-year stored sample had more aroma compounds as compared to the freshly dried sample. Similar findings were reported by Sereshti et al. (2018) stating that the concentration safranal, isophorone, 2-hydroxy- isophorone and eucarvone were higher in the stored saffron samples compared to the freshly dried ones.

Among all aroma compounds identified in both of saffron samples, aldehydes were determined to be the highest in concentration in the current study (Table 1). The total amount of aldehydes was higher in the one-year stored sample (91929.1 µg/kg) than in the freshly dried sample (22994 µg/kg). Among the aldehyde compounds, 2-methyl butanal, 3-methyl butanal, isocyclocitral, β-cyclocitral, 1,3,4-trimethyl 3-cyclohexene-1-carboxaldehyde and 3,4-dimethyl-3-cyclohexen-1-carboxaldehyde were only found in a one-year stored sample. It was determined that one-year storage period had significant effects on the amount of aldehyde compounds. Similar findings were reported in a study conducted by Sereshti et al. (2018) who stated that the total amounts of aldehydes were higher in the freshly dried sample than the two-year stored samples. Among the aldehydes, safranal was the major aroma compound in the

freshly dried (14093,9 µg/kg) and one-year stored (80394,4µg/kg) samples. As shown in Table 1, the one-year storage period increased the amount of safranal compound by five times compared to the freshly dried sample. Similarly, Maggi et al. (2010) reported that safranal were available in large amounts in first two years stored saffron samples. Safranal (2,6,6-trimethylcyclohexane-1,3-dien-1-carboxaldehyde) is the most abundant aroma compound and the most significant sensorial compound in the saffron aroma providing highly to saffron aroma due to its high impact properties (Amanpour et al., 2015; Bononi et al. 2015). During the process of dehydration and storage period of saffron, safranal is generated by the hydrolysis and dehydration of picrocrocin (José Bagur et al., 2018). Another important aldehyde in the samples was 4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-ene-1-carboxaldehyde (HTCC). The amount of freshly dried saffron sample (5777,2 µg/kg) was reduced in the sample stored for one year (2333,1 µg/kg). HTCC act as important sources of safranal creation during storage period of this spice, would decrease during a 1-4 years storage period reported by Carmona et al. (2006) and Maggi et al. (2010).

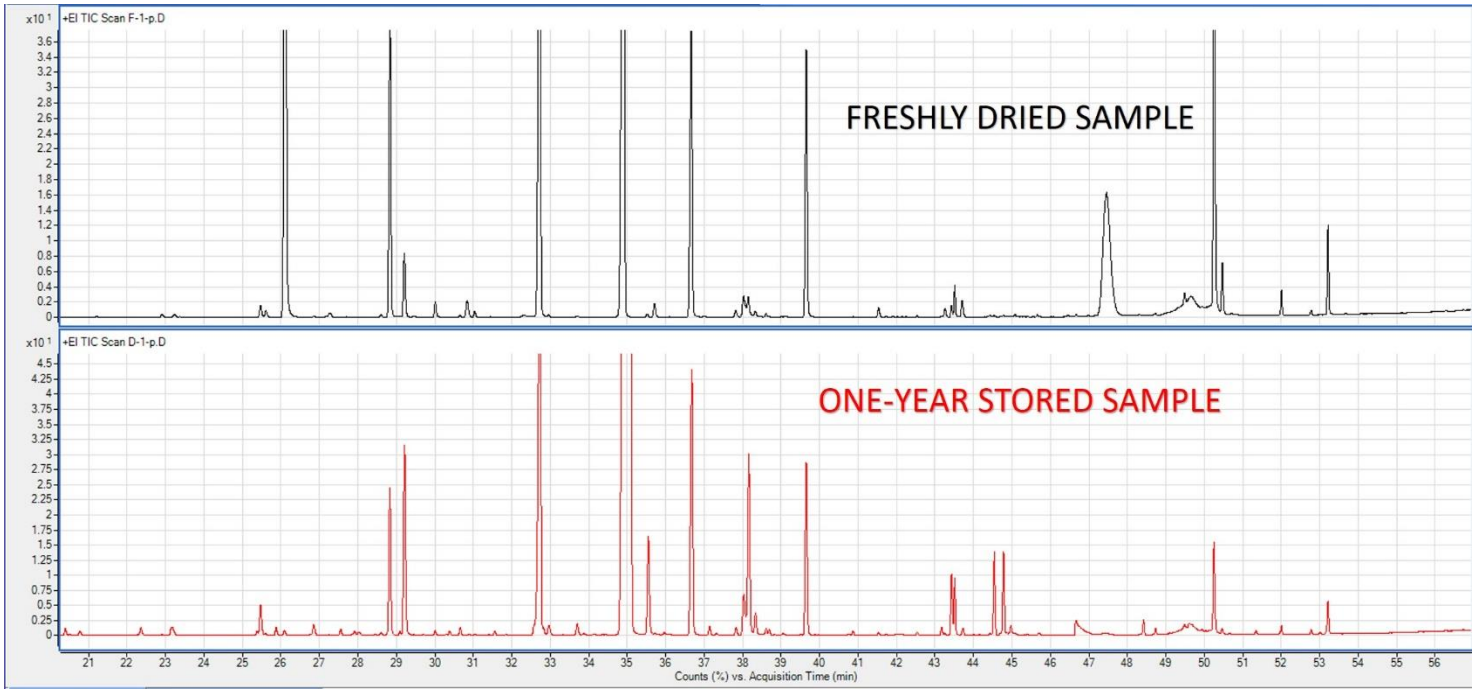

Figure 1. GC chromatograms of freshly dried and one-year stored saffron samples

Ketones were detected as the second main group of aroma compounds in the current study (Table 1). Among 19 ketone compounds, 2,2,5,5-tetramethyl-3-cyclopenten-1-one, 3,4,4-trimethyl-2-cyclohexen-1-one, 6-methyl-3,5-heptadien-2-one, (E)-3,6-dimethyl-3-(1-methylethyl)-4,6-heptadien-2-one were only available in one-year stored samples while 1-octen-3-one was available in only freshly dried samples. Besides 4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde (HTCC), isophorone-related compounds (isophorone, 4-oxoisophorone, β-isophorone are the major ketone compounds in both of the samples. Similarly, Urbani et al. (2015) found that isophorone-related compounds were the most dominant ketones in saffron from Central Italy and their amount in the samples increased with drying temperature and time and they could be characteristic of saffron produced in the selected geographical area. Isophorone derivatives are the main aroma compounds of the essential oil. These compounds are responsible for the characteristic of saffron aroma (Iborra et al., 1992). Among the ketones,

Table 1. Volatile compounds of saffron samples (µg/kg)

No	RT	LRI*	COMPOUND	FRESHLY DRIED	ONE-YEAR STORED	SIG.	IDEN.+
1	3,5	692	Acetaldehyde	22,0	21,7	*n.s.*	LRI, MS, Std
2	4,4	824	Butanal	6,9	40,3	*	LRI, MS, Std
3	4,6	832	Methyl acetate	ND	56,7	*	LRI, MS, Std
4	5,3	858	2-Methylfuran	12,1	15,6	*n.s.*	LRI, MS, tent
5	5,6	880	Ethyl acetate	47,5	24,9	**	LRI, MS, Std
6	6,2	916	2-Methyl-Butanal	ND	58,0	*	LRI, MS, Std
7	6,3	1032	3-Methyl-Butanal	ND	29,6	*	LRI, MS, Std
8	11,5	1105	Butyl acetate	ND	19,9	*	LRI, MS, Std
9	11,9	1111	Hexanal	52,4	19,8	*	LRI, MS, Std
10	16,4	1180	Heptanal	40,3	6,5	*	LRI, MS, Std
11	18,8	1239	Ethyl hexanoate	48,3	6,0	*	LRI, MS, Std
12	20,8	1275	1,2,4-Trimethyl benzene	ND	169,8	*	LRI, MS, tent
13	21,2	1291	Octanal	18,9	16,8	*n.s.*	LRI, MS, Std
14	21,7	1308	1-Octen-3-one	10,0	ND	*	LRI, MS, Std
15	22,4	1322	2,2,5,5-tetramethyl-3-cyclopenten-1-one	ND	265,8	*	LRI, MS, tent
16	22,9	1346	4-Nonanone	47,9	36,5	*n.s.*	LRI, MS, Std
17	23,2	1358	6-Methyl-5-heptene-2-one	53,0	443,9	*	LRI, MS, Std
18	25,6	1396	Nonanal	113,0	988,3	*	LRI, MS, Std
19	26,1	1416	β-Isophorone	11222,2	245,3	*	LRI, MS, Std
20	26,8	1431	Isocyclocitral	ND	164,0	*	LRI, MS, Std
21	26,9	1434	(E)-2-Octenal	19,8	352,4	*	LRI, MS, Std
22	27,3	1441	Ethyl octanoate	82,1	36,7	**	LRI, MS, Std
23	27,5	1521	3,4,4-trimethyl-2-cyclohexen-1-one	ND	176,9	*	LRI, MS, tent
24	27,9	1525	1,3,4-trimethyl 3-cyclohexene-1-carboxaldehyde	ND	128,0	*	LRI, MS, tent
25	28,4	1528	3,4-dimethyl-3-cyclohexen-1-carboxaldehyde	ND	33,1	*	LRI, MS, tent
26	28,6	1533	(*Z*)-Theaspirane	40,8	83,8	**	LRI, MS, Std
27	29,2	1536	2,6,6-Trimethylcyclohexa-1,4-dienecarbaldehyde	894,5	5687,1	*	LRI, MS, Std
28	30,0	1538	Benzaldehyde	201,5	135,5	**	LRI, MS, Std
29	30,6	1564	Phorone	27,8	225,8	*	LRI, MS, Std
30	30,8	1571	Ethyl nonanoate	304,8	38,1	*	LRI, MS, Std
31	31,0	1580	Linalool	73,2	50,0	**	LRI, MS, Std
32	32,6	1582	6-Methyl-3,5-heptadien-2-one	ND	247,0	*	LRI, MS, tent

33	32,7	1595	Isophorone	8761,9	15983,9	*	LRI, MS, Std
34	32,9	1597	Isophorol	44,7	365,6	*	LRI, MS, Std
35	33,6	1600	2-Methyl-benzaldehyde	20,0	398,4	*	LRI, MS, tent
36	33,9	1619	β-Cyclocitral	ND	398,4	*	LRI, MS, Std
37	34,9	1625	Safranal	14093,9	80349,4	*	LRI, MS, Std
38	35,5	1636	2-Hydroxyisophorone	47,2	3057,3	*	LRI, MS, tent
39	35,7	1638	2-Hydroxy-4-oxoisophorone	209,3	70,1	*	LRI, MS, Std
40	36,0	1642	Terpinen-4-ol	ND	84,5	*	LRI, MS, Std
41	36,7	1677	4-Oxoisophorone	4675,4	8946,5	*	LRI, MS, Std
42	37,1	1681	(*E*)-3,6-Dimethyl-3-(1-methylethyl)-4,6-heptadien-2-one	ND	254,2	*	LRI, MS, tent
43	37,8	1704	Carvenone	101,2	256,3	*	LRI, MS, tent
44	38,0	1707	2-Hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone	426,8	1643,5	*	LRI, MS, tent
45	38,1	1708	Eucarvone	375,2	7509,4	*	LRI, MS, tent
46	38,6	1732	2(5H)-Furanone	45,3	181,2	*	LRI, MS, Std
47	38,7	1758	Butylhydroquinone	ND	163,9	*	LRI, MS, tent
48	39,7	1787	2,2,6-Trimethyl-1,4-cyclohexanedione	3764,2	4878,0	*	LRI, MS, Std
49	40,8	1822	2-Phenylethylacetate	ND	118,1	*	LRI, MS, Std
50	41,5	1890	Dihydro-β-Ionone	137,4	90,3	**	LRI, MS, Std
51	43,3	1894	β-Thujaplicine	122,7	72,9	**	LRI, MS, tent
52	43,4	1898	Decahydro-2,6-dimethyl-naphthalene	132,9	2863,9	*	LRI, MS, Std
53	43,5	1902	Phenethyl alcohol	347,2	130,6	*	LRI, MS, Std
54	43,7	1904	2,6-Di-tert-butyl *p*-cresol	229,0	214,8	*n.s.*	LRI, MS, Std
55	44,7	1910	α-Ionone	22,9	2035,0	*	LRI, MS, Std
56	45,0	1948	2-Acetylpyrrole	ND	292,1	*	LRI, MS, Std
57	45,7	1949	Isomenthone	29,7	92,6	**	LRI, MS, Std
58	46,7	1951	4-Methylene isophorone	32,4	1319,8	*	LRI, MS, Std
59	47,5	1953	β-Ionone	6863,6	160,7	*	LRI, MS, Std
60	50,3	1962	4-Hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde	5777,2	2333,1	*	LRI, MS, Std
61	52,0	1978	4-Hydroxy-3,5,5-trimethylcyclohex-2-enone	247,8	216,2	*n.s.*	LRI, MS, Std
62	52,8	2233	Omega-Pentadecalactone	54,5	131,5	*	LRI, MS, tent
63	53,0	2291	Dihydroactinidiolide	ND	88,0	*	LRI, MS, tent
64	53,2	2302	4-Hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde	922,7	768,7	**	LRI, MS, tent
			TOTAL	**60824,1**	**145292,7**		

2,2,5,5-tetramethyl-3-cyclopenten-1-one, 6-methyl-5-heptene-2-one, 3,4,4-trimethyl-2-cyclohexen-1-one, phorone, 6-methyl-3,5-heptadien-2-one, isophorone, 2-hydroxyisophorone, 4-oxoisophorone, (E)-3,6-dimethyl-3-(1-methylethyl)-4,6-heptadien-2-one, carvenone, 2-hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone, eucarvone, 2,2,6-trimethyl-1,4-cyclohexanedione and 4-methylene isophorone were found to be in higher amounts in the samples dried with one-year. Similarly, Sereshti et al. (2018) reported that the amount of some ketone compounds increased as a result of storage period of the saffron.

In the present study, another aroma group determined in saffron samples was esters. Methyl acetate, ethyl acetate, butyl acetate, ethyl hexanoate, ethyl octanoate, ethyl nonanoate and 2-phenylethylacetate were detected ester compounds in the samples. Some of these compounds (methyl acetate butyl acetate and 2-phenylethylacetate) formed during the one-storage time while amount of the other esters were sharply decreased as a result of the storage. D'Archivio et al. (2018) found that the diethyl succinate was only ester compound in saffron samples. Interestingly, ester compounds have been identified mostly in tepals and anthers of saffron flowers (*Crocus sativus* L.) in previous studies (Tirillini et al., 2006).

(Z)-Theaspirane, linalool, terpinen-4-ol, β-thujaplicine, isomenthone and dihydroactinidiolide were detected as terpene compound in the samples (Table 1). Some of these compounds (terpinen-4-ol and dihydroactinidiolide) were not found in the freshly dried sample. As previously stated, linalool was detected in Spanish "Mancha Superior" by Cadwallader et al. (1997) and Iranian saffron samples by Amanpour et al. (2015) as an aroma-active compound with floral and honeysuckle aroma notes.

CONCLUSIONS

In this study, it was found that the one-year storage period had a significant effect on the aroma compounds of Turkish dried saffron sample. It was observed that the most of the volatile amounts in the one-year stored samples changed significantly as compared to the fresh dried samples. When the aroma compounds were examined, it was determined that aldehydes and ketones were very dominant in the both saffron samples. In one-year stored samples, safranal, isophorone, 2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde, 4-oxoisophorone and eucarvone were the most dominant volatiles and their amounts increased with the one-year storage while β-isophorone, β-ionone and 4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-en-1-carboxaldehyde (HTCC) were better protected in the samples freshly dried saffron samples.

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