Orange juice with pulp: impact of pasteurization and storage on flavour, polyphenols, ascorbic acid and antioxidant activity

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Summary

Effect of storage on volatile aroma compounds of orange juice with pulp of Brazilian origin processed in Slovakia was investigated during 4 months of its expiration period. Headspace – solid phase microextraction (HS-SPME) and gas chromatography – mass spectrometry (GC-MS) detection was involved to isolate and analyse the composition of the volatile fractions. Parallel gas chromatography – olfactometry (GC-O) analysis revealed forty-four key odorants, and their changes within the expiration period were characterized. D-Limonene, α-phellandrene, nonanal, decanal, linalool, L-limonene, ethyl octanoate, ethyl butanoate, nonanol and α-copaene were found to be principal aroma-active volatiles from the viewpoint of high and practically stable odour intensity during the shelf-life of the juice. Besides that, the results proved that aroma composition and overall flavour of orange juice changed during the storage. In addition, complementary measurements of ascorbic acid concentration, antioxidant activity, total polyphenols concentration and colour changes in the stored juice were carried out proving gradual worsening of all these parameters during the storage.

Keywords

orange juice; volatiles; storage; pasteurization; antioxidant properties; ascorbic acid; polyphenols

Sweet orange (*Citrus sinensis* L.) of the family *Rutaceae* is considered the most popular fruit of typical sweet to sour taste. It is also well known as a rich source of antioxidants including ascorbic acid (vitamin C), but pulp is also a source of fibre, minerals (mainly potassium, calcium and magnesium) and other phytonutrients, concentration of which varies with orange variety, and is generally higher in peel than in the pulp [1–7]. A large portion of the citrus fruit produced worldwide is used in processed juices and other beverages, from among which orange juice is the most appreciated.

Looking on composition of juice and its changes, the presence of organic acids (e.g. citric, malic and ascorbic acid), saccharides (dominantly saccharose, glucose and fructose) and a variety of phenolic compounds, including hydroxybenzoic acids, hydroxycinnamic acids and flavanones, was proven. Hesperidin, narirutin and ferulic acid were the most abundant phenolic compounds in orange juice [2, 6, 7]. In addition, some authors previously pointed that hesperidin, narirutin but also ascorbic acid were the key constituents of orange juices responsible for their antioxidant activity [8]. Composition of juice affects also the predisposition of ascorbic acid to partial oxidation, revealing a moderate protecting ability of phenolic compounds present in orange juices [9].

Orange juice composition and quality may be affected by several factors, including varietal differences, pasteurization and storage conditions, but also by production parameters [10]. Thermal processing as well as storage conditions can significantly influence physical properties as well as composition of the final juice product, but effects of cultivar on compositional changes cannot be un-

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derestimated in this context. Pasteurization itself can cause the concentration decrease of acetaldehyde and ethyl acetate, but also of α-pinene, β-myrcene, limonene, α-terpineol, 1-hexanol, 3-hexen-1-ol and last but not least, ascorbic acid [11, 12].

Esteve et al. [10] confirmed that the increase of storage temperature by 6 °C influenced the concentration of essential oils, ascorbic acid and hydroxymethylfurfural, but also several parameters including viscosity of Spanish orange juices, while some other colour characteristics were practically non-affected. Martinéz et al. [13] pointed at differences in composition between the traditionally produced orange juices and those produced from concentrates. Fortification of fresh juices by ascorbic acid or other compounds may result in negative effects on deterioration of carotenoids, which subsequently may influence also colour characteristics of the juice. Klimczak et al. [7] proved that ascorbic acid as well as free and conjugated hydroxycinnamic acids were the most affected components by both temperature and storage duration, thus affecting the total antioxidant capacity.

The stability of polyphenols and ascorbic acid in terms of antioxidant capacity of minimally processed cold-stored orange juices and segments was the subject of the study of Del Caro et al. [14]. In dependence on variety, a significant increase in total flavonoids (mainly hesperidin) was found in the segments, while the juices showed a diminution of flavonoid concentration. The antioxidant capacity was clearly correlated with the ascorbic acid concentration rather than with the presence of flavanone glycosides.

The flavour of orange has been studied more extensively than that of any other type of citrus fruit [8, 15–24]. Organoleptic quality is a significant factor of the consumer's acceptability of the product. Some studies showed that aroma composition of orange juices changed during the storage [15]. However, it is widely accepted that there are not one or two flavour-impacting compounds primarily responsible for orange flavour, but the overall flavour is the result of a combination of several volatile compounds in specific mutual ratios [22, 25]. Additional factors for consideration are differences in orange flavour in dependence on a way of juice form/way of preparation (fresh, reconstituted frozen concentrate, chilled juice, orange-flavoured drinks) or character of package (e.g. cans, aseptic packaging). All these aspects contribute to flavour differences between freshly squeezed orange juices and commercially processed orange drinks [19, 20, 24, 25]. Concerning the flavour or fragrance of citrus peel oils and citrus juice extracts, these consist of dominant amounts of limonene accompanied by monoterpenes such as myrcene, γ-terpinene, α- and β-pinene or sabinene, and sesquiterpenes such as caryophyllene, bisabolenes, bergamotenes or valencene, and fatty aldehydes such as octanal and decanal [26]. In spite of this common general profile, each single citrus species can be distinguished by an individual characteristic organoleptic "fingerprint", which is developed by complex and well-balanced mixtures of components occurring in trace concentrations.

Recently, considerable effort is noticeable towards the identification of significant key compounds of extremely low abundance and extraordinarily low odour thresholds in citrus fruits [26]. Solid–phase microextraction (SPME) is a method of choice as a robust isolation technique for the rapid, solvent-less extraction or pre-concentration of volatile and semi-volatile organic compounds [27]. Headspace solid-phase microextraction (HS-SPME) sampling method combined with gas chromatography – mass spectrometry (GC-MS) was effectively used for qualitative and quantitative analysis of volatiles from the headspace of apple juice, orange juice and of different lemon variety juices [16, 28–30]. This technique is suitable to detect very low levels of non-polar volatiles, which are present in fruits at contents close to 0.04 $mg \cdot kg^{-1}$ [27], and it was also successfully used in several previously published gas chromatography – olfactometry (GC-O) studies on aroma-active components of various food products, e.g. of brandy beverages or bryndza cheese [31, 32].

The present study is a result of a complex research focused on the characterization of the volatile composition of orange juice. The influence of a modified "slight" pasteurization practice and chosen storage conditions on alteration of particular volatile compounds, as constituents of orange juice flavour, was evaluated. Furthermore, an established GC-O method with the procedure of frequency odour intensity analysis, previously described by Blank [33], was used to compile a list of principal aroma compounds of orange juice with pulp.

To the best of our knowledge, the study aimed at complex evaluation of fruit juices from organoleptic point of view is still missing. Besides the aroma-active components identification, our attention in this study was focussed on monitoring of the effects of pasteurization and subsequent storage on selected phytonutrients, i.e. ascorbic acid, total phenolic compounds, hesperidin, but also on antioxidant activity and colour stability of orange juice with pulp.

MATERIALS AND METHODS

Sample and storage conditions characterization

Samples of orange juice enriched with pulp were provided by McCarter (Dunajská Streda, Slovakia), a company that imports orange juice in frozen state from suppliers in Brazil. After defreezing, juice was enriched with pulp, mixed and immediately thermally treated by so-called "slight" pasteurization at up to 95 °C during 20 s. Afterwards, the product was filled aseptically into 200 ml polyethylene terephthalate (PET) bottles with oxygen scavengers. Residual concentration of oxygen in headspace above the juice in bottles was (2.7 ± 0.4) mg·l^{-1}.

Bottled samples of the same batch were delivered immediately after their filling into the laboratory in a number corresponding to the chosen experimental setup multiplied by 2 (as due to replications). Samples were stored at (7 ± 1) °C in darkness during 21 weeks, i.e. 3 weeks longer than the expiration period declared by the producer. For the purposes of GC analysis, samples were stored at (7 ± 1) °C in the showcase refrigerator under the conditions simulating the daylight exposure, i.e. under typical conditions in a retail chain, within 4 months of the expiration period. The temperature of 7 °C was chosen in order to simulate the storage conditions recommended by the producer. Moreover, samples of orange juice directly taken from stirring tank and filled into glass bottles before pasteurization and after pasteurization were also delivered for the research purposes. These samples were analysed within 24 h after their delivery in the laboratory.

Chemicals

GC analysis

All chemicals used as reference standards for identification purposes of volatiles (listed in Tab. 1 and Tab. 2) were gifts donated from Bedoukian Research (Danbury, Connecticut, USA).

Electron paramagnetic resonance (EPR) and UV-VIS analysis

In EPR and UV-VIS experiments, 2,2'-azino-bis(3-ethylbenthiazoline-6-sulfonic acid) salt (ABTS; Polysciences, Warrington, Pennsylvania, USA); 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (Tempol), Folin-Ciocalteu's phenol reagent, gallic acid of analytical grade purity (Sigma-Aldrich, Steinheim, Germany); $K_2S_2O_8$ (Merck, Darmstadt, Germany), ascorbic acid and sodium carbonate (both Lachema, Brno, Czech Republic) were used. In all experiments, water of HPLC purity grade freshly prepared in the laboratory (Rodem 6, Ecotest, Topoľčany, Slovakia) with an average resistance of 18.5 mΩ was utilized.

HPLC analysis

Following chemicals and solvents were used: ascorbic acid 99% (Fluka Chemie, Sigma-Aldrich, Steinheim, Germany), hesperidin 97% (Sigma-Aldrich), methanol Chromasolv, gradient grade purity ≥99.9% (Sigma-Aldrich) and ortho-phosphoric acid 85% (Lachema).

Methods

GC-MS and GC-O

Conditions of HS-SPME GC analysis

To carry out the SPME procedure, four types of fibre stationary phases were tested: 65 μm PDMS/DVB, 75 μm Carboxen/PDMS, 70 μm Carbowax/DVB StableFlex and 50/30 μm DVB/Carboxen/PDMS StableFlex (Agilent Technologies, Waldbron, Germany). Extracts were separated on GC columns with different stationary phases, namely, non-polar Ultra 1, semi-polar DB-5, and polar DB-WAX (all Agilent Technologies). Recovery of volatile compounds and ability of GC to separate the acquired volatile fraction of the investigated matrix were decisive parameters for method selection. Best results were obtained with DVB/Carboxen/PDMS SPME fibre and Ultra 1 GC column. This setup was chosen for further analyses.

Extraction of volatiles by HS-SPME

Sample of orange juice (5.0 ml) was incubated statically in a 40 ml glass vial in a metallic block thermostat (Liebisch, Bielefeld, Germany) at 35 °C for 30 min, with an SPME fibre (2 cm) placed in the headspace. The SPME fibre DVB/Carboxen/PDMS "For odours", film thickness 50/30 μm (Cat. no. 57328-U; Supelco, Bellefonte, Pennsylvania, USA) was used. The fibre was initially conditioned by heating in the GC injector block at 270 °C for 1 h. HS-SPME samples were desorbed at 250 °C in the GC injector block during the entire GC analysis.

GC-MS analysis

Compounds extracted by HS-SPME were analysed by GC-MS using the gas chromatograph Agilent 6890N (Agilent Technologies, Palo Alto, California, USA) coupled to the mass spectrometric detector 5973 inert (Agilent Technologies) equipped with fused silica capillary column Ultra 1 (50 m × 0.32 mm × 0.52 μm; Agilent Technologies) operating with a temperature programme 35 °C (2 min), 4 °C·min^{-1}, 200 °C.

Tab. 1. Effects of the pasteurization procedure and packaging material on changes in profile of orange juice volatiles, as determined by HS-SPME coupled to GC-MS.

No.	*LRI* U1	RT [min]	Compound	A	B	C
				Area [%]	Area [%]	Area [%]
1	600	4.387	Ethyl acetate	0.3	0.3	0.3
2	772.4	8.963	Hexanal	0.3	0.2	0.2
3	782.9	9.241	Ethyl butanoate	0.8	0.6	0.6
4	926.6	15.204	α-Pinene	0.7	0.7	0.8
5	979.1	17.319	Octanal	1.26	1.23	1.27
6	–	17.418	α-Thujene	7.7	8.6	8.8
7	993.1	18.044	α-Phellandrene	0.3	0.3	0.3
8	1001.3	18.396	δ-3-Carene	0.3	0.4	0.5
9	1005.9	18.562	α-Terpinene	0.2	0.3	0.5
10	1018.1	19.231	D-Limonene	52.1	59.1	57.2
11	1018.5	19.792	*cis*-β-Ocimene	0.2	0.2	0.3
12	–	20.301	γ-Terpinene	0.4	0.5	0.5
13	1054.3	20.392	1-Octanol	0.8	0.5	0.5
14	–	21.270	*p*-Cymenene^t	0.1	0.1	0.1
15	–	21.537	4-Carene	0.4	0.6	0.6
16	1081.4	21.622	Nonanal	0.3	0.4	0.3
17	1083	21.729	Linalool	3.8	2.9	2.9
18	1102.2	22.456	Ethyl 3-hydroxyhexanoate	0.1	0.1	0.1
19	–	22.580	*trans*-4,8-Dimethyl-1,3,7-nonatriene^t	0.2	0.2	0.2
20	–	23.052	*p*-Menth-2,8-dien-1-ol (unknown isomer)	traces	traces	traces
21	–	23.465	Unknown	0.2	0.1	0.1
22	1155	24.535	Nonanol	traces	traces	traces
23	1157.1	24.930	Terpinen-4-ol	1.3	1	1
24	1167.9	25.340	α-Terpineol	1.7	1.2	1.2
25	1180.3	25.521	Ethyl octanoate	0.3	0.3	0.3
26	1183.4	25.756	Decanal	1.8	1.6	1.6
27	1193.9	26.051	Octyl acetate	0.4	0.3	0.3
28	1204.8	26.325	*cis-Carveol*	0.1	0.1	0.1
29	1207.9	26.751	Nerol	0.3	0.2	0.2
30	1209.7	27.019	L-Carvone	0.5	0.3	0.3
31	–	28.205	Perillaldehyde	0.4	0.2	0.3
32	1285.3	29.652	Undecanal	0.1	0.1	0.1
33	1329.5	30.738	α-Terpinyl acetate	0.2	0.1	0.1
34	1342	31.659	Neryl acetate	0.3	0.1	0.1
35	1360	32.280	Geranyl acetate	0.2	0.1	0.1
36	1370.8	33.075	α-Copaene	0.4	0.3	0.3
37	–	33.329	*cis*-9-Octadecen-1-ol^t	0.3	0.2	0.2
38	–	33.454	β-Elemene	0.5	0.4	0.4
39	1400.3	33.723	*p*-Menth-1-en-9-ol (unknown isomer)	0.1	0.1	0.1
40	1411	34.591	β-Caryophyllene	0.4	0.3	0.4
41	–	34.875	β-Cubebene^t	0.1	0.1	0.1
42	–	35.529	Alloaromadendrene	0.3	0.2	0.3
43	–	35.706	α-Humulene	0.2	0.2	0.1
44	–	36.365	β-Maaliene	0.5	0.4	0.4
45	–	36.628	4,11-Selinadiene^t	0.9	0.6	0.7
46	–	36.744	β-Chamigrene^t	0.7	0.5	0.5
47	1482.7	37.030	Valencene	13.3	9.6	10.1
48	–	37.106	α-Selinene	1.1	0.8	0.8
49	–	37.324	Eremophylene^t	0.4	0.2	0.3
50	–	37.802	α-Panasinsen^t + unknown compound	2.2	1.3	1.4
51	–	39.664	Caryophyllene oxide	traces	traces	traces

LRI U1 – linear retention index measured on Ultra 1 GC column; A – raw orange juice from the stirring tank (in glass bottles for up to 24 h max.); B – orange juice after pasteurization (in glass bottles for up to 24 h max.); C – orange juice after pasteurization (in PET bottles for up to 24 h max.); *t* – tentative identification (only on the basis of GC-MS spectra).

Tab. 2. Key aroma-active compounds of pasteurized Brazil orange juice.

No.	LRI U1	Odour intensity during storage					Compound	Odour description	References
		0 month	1 month	2 months	3 months	4 months			
1	600	–	–	0.5	0.5	0.5	Ethyl acetate	fruity, stone fruit, pear-like	LRI, MS, ST, OD, LIT
2	–	–	–	–	0.5	0.5	Unknown^{o}	ester-like, weak fruity	–
3	–	–	–	0.5	1	1	Unknown^{o}	caramel-like, baked, roasted odour	–
4	772.4	–	–	1	1	1	Hexanal	green leafy, grassy, tallowy, slight citrus-like	LRI, MS, ST, OD, LIT
5	782.9	2	2	3	3	3	Ethyl butanoate	fruity, apple-like, sweet	LRI, MS, ST, OD, LIT
6	–	–	–	1	1	1.5	Unknown^{o}	bittery, roasted odour	–
7	–	1	1	1	1	1.5	Unknown^{o}	fresh, citrus peel-like, bittery	–
8	–	0.5	1	1	2	2	Unknown^{o}	sweet, fruity, pear-like, esteric	–
9	926.6	0.5	0.5	1	1.5	1.5	α-Pinene	sharp, pine, terpeny	LRI, MS, ST, OD, LIT
10	–	0.5	0.5	1	1	1	Unknown^{o}	herbaceous, fresh, pleasant fresh yeast	–
11	979.1	–	–	1	1	1	Octanal	soapy, herbaceous, oily, fatty, citrus-like	LRI, MS, ST, OD, LIT
12	–	–	–	1	1	1	α-Thujene	fresh, terpene-like, essential oil-like	MS, OD, LIT
13	993.1	3	3	3	3	3	α-Phellandrene	balsamic, mint, herbaceous	LRI, MS, ST, OD, LIT
14	1 001.3	3	3	3	2	2	δ-3-Carene	turpentine-like, varnish-like, sharp, sweet	LRI, MS, ST, OD, LIT
15	1 005.9	3	3	2	2	2	α-Terpinene	balsamic, herbaceous, marjoram-like	LRI, MS, ST, OD, LIT
16	1 018.1	3	3	3	3	3	D-Limonene	citrus, terpenic, intensive citrus-peel odour	LRI, MS, ST, OD, LIT
17	1 018.5	2	2	2	2	2	*cis*-β-Ocimene	lime, green,sweet, lemon, orange	MS, ST, OD, LIT
18	1 054.3	1	1	2	2	2	1-Octanol	herbaceous, earthy, waxy	LRI, MS, ST, OD, LIT
19	–	2	2.5	3	2	2	4-Carene	weak citrus-like, fuel-like, dill, terpenic	MS, OD, LIT
20	1 081.4	3	3	2.5	3	3	Nonanal	soapy-fruity, waxy, tallowy	LRI, MS, ST, OD, LIT
21	1 083	3	3	3	3	3	Linalool	refreshing, floral, fragrant, citrus-like	LRI, MS, ST, OD, LIT
22	–	–	–	2	2	2	Unknown^{o}	floral, fragrant, soap, softener-like	–
23	1 102.2	1	1.5	2	2	2	Ethyl 3-hydroxyhexanoate	smoky, leather, tobacco	LRI, MS, ST, OD, LIT
24	–	2.5	2.5	3	3	3	Unknown^{o}	intensive floral, rose, sweet, linden, honey	–
25	–	1.5	1.5	2	2	2	Unknown^{o}	liquorice, fresh, sweet, cinnamon	–
26	1 155	2	2	2	2	2	Nonanol	citrus, terpenic, intensive citrus-peel odour	LRI, MS, ST, OD, LIT
27	1 157.1	–	–	1.5	2	2	Terpinen-4-ol	earthy, woody, waxy	LRI, MS, ST, OD, LIT
28	1 167.9	1	1	1	0.5	0.5	α-Terpineol	pleasant, sweet, fruity	LRI, MS, ST, OD, LIT
29	1 180.3	2.5	2.5	2.5	2.5	2.5	Ethyl octanoate	banana, pineapple, pear, sweet, floral, soap	LRI, MS, ST, OD, LIT
30	1 183.4	3	2	2	3	3	Decanal	orange peel-like, waxy	LRI, MS, ST, OD, LIT
31	1 209.7	1	1	1	–	–	*l*-Carvone	spicy, peppermint, fresh	LRI, MS, ST, OD, LIT
32	–	1	1	1	1	1	Perillaldehyde	fresh, herbal, cumin, spicy	MS, OD, LIT

Tab. 2. continued

No.	LRI U1	Odour intensity during storage					Compound	Odour description	References
		0 month	1 month	2 months	3 months	4 months			
33	1 285.3	1	1	1	1	1	Undecanal	fatty with orange and rose undertone, waxy	LRI, MS, ST, OD, LIT
34	–	0.5	0.5	0.5	1	1	Unknown^o	herbal, balsamic, pleasant	–
35	1 329.5	1	1	1	1	1	α-Terpinyl acetate	herbal, bergamot, lime	LRI, MS, ST, OD, LIT
36	1 342	1	1	1	1	–	Neryl acetate	sweet, fruity, dried plum - like	LRI, MS, ST, OD, LIT
37	1 360	1	1	1	1	–	Geranyl acetate	fresh, green, lavender, soap-like	LRI, MS, ST, OD, LIT
38	1 370.8	2	2	2	2	2	α-Copaene	spicy, woody, dill-like, earthy	LRI, MS, ST, OD, LIT
39	1 411	0.5	0.5	0.5	0.5	0.5	β-Caryophyllene	terpenic, spicy, woody	LRI, MS, ST, OD, LIT
40	1 444	1.5	1.5	1.5	1.5	1.5	α-Humulene	terpenic, woody, spicy, hop oil	LRI, MS, ST, OD, LIT
41	1 451.3	2.5	2.5	2	2	–	Alloaromadendrene	woody, pleasant, soap-like, waxy, green	LRI, MS, ST, OD, LIT
42	–	0.5	0.5	0.5	1	2	β-Maaliene	essential oil-like, terpenic, herbal	MS, OD, LIT
43	1 482.7	1	1	1	1	2	Valencene	woody, orange - citrus peel	LRI, MS, ST, OD, LIT
44	–	0.5	0.5	1	1	1	Caryophyllene oxide	woody, herbaceous	MS, OD, LIT

Compounds were identified on the basis of following criteria: *LRI* - linear retention index, MS - mass spectrum, ST - comparison with a standard compound, OD - odour quality, LIT - literature reference. *o* – detected only by olfactometry.

The linear velocity of carrier gas helium was 33 cm·s^{-1} (measured at 143 °C). Splitless injection mode was used at an injector temperature of 250 °C. Ionization voltage (EI) was set to 70 eV.

GC-O analysis

In parallel with GC-MS, samples were analysed by GC-O using the detection frequency concept of posterior evaluation of odour intensity according to the modified procedure of Janáčová et al. [31]. Briefly, a sniffing procedure panel of 5 judges (2 men, 3 women, age 29–61 years) was formed from the laboratory staff at VÚP Food Research Institute (Bratislava, Slovakia), created from 11 assessors trained in sensory evaluation. Results of GC-O analyses were expressed as the average values of odour intensities in a scale from 0 to 3 with increments of 0.5, obtained from 5 independent measurements. Each sensory perception was based on at least 4 citations. The value of ± 0.5 was considered as measurement deviation. The gas chromatograph Agilent 7890A (Agilent Technologies) was coupled to flame ionization detector and an olfactory detector port ODP3 (Gerstel, Mülheim an der Ruhr, Germany). The capillary column Ultra 1 (50 m × 0.32 mm × 0.52 μm; Agilent Technologies) operated with the temperature programme 35 °C (2 min), 4 °C·min^{-1}, 200 °C. Hydrogen was used as a carrier gas at a linear velocity of 44.6 cm·s^{-1} (measured at a column temperature of 143 °C). Splitless injection mode was used at an injector temperature of 250 °C. The olfactory detector port (ODP) operated at a temperature of 180 °C, interface temperature was 230 °C and the flow of added nitrogen in ODP humidifier was 12 ml·min^{-1}. The sniffing time of each judge did not exceed 30 min.

Identification and semi-quantitative analysis of volatile compounds

The volatiles were identified on the basis of comparison of their linear retention indices, mass spectra, GC analysis of standards, and by the comparison of data on occurrence and odour description with literature [16, 18, 22, 25, 30, 34, 35]. The linear retention indices (*LRI*) were calculated using the equation of Van den Dool and Kratz [36]. Standard mixture of *n*-alkanes C_6–C_{15} was used as a reference (Bedoukian Research, Danbury, Connecticut, USA). *LRI* data were compared and confirmed with *LRI* data obtained by measurement of standard volatile compounds. For this

purpose, our in-house database of *LRI* data was used. Identification of compounds was performed by comparison of mass spectra with available mass spectral libraries Wiley and NIST MS (National Institute of Standards and Technology, Gaithersburg, Maryland, USA). Relative proportions of individual volatile compounds as semi-quantitative parameters were calculated by the method of internal normalization and expressed as a percentage; given values are the averages of three replicates.

EPR and UV-VIS measurements

Sample preparation

Immediately before the experiments, solid matters of the analysed orange juices were removed using a centrifuge (SciQuip, Wem, United Kingdom) at 166.67 Hz and a temperature of 5 °C (EPR measurements) and 20 °C (UV-VIS measurements) during 10 min. Supernatants were stored at (7 ± 1) °C in darkness between the experiments. All the measurements with a respective sample were performed within 8 h after its preparation.

EPR measurements

The entire EPR experiments were performed in duplicates using a portable X-band EPR spectrometer e-scan (Bruker Biospin, Karlsruhe, Germany) with accessories. The ability of orange juices to terminate $ABTS^{\bullet+}$ and Tempol free radicals was examined as previously described by Polovka et al. [37]. The $ABTS^{\bullet+}$ radical-scavenging activities were expressed as Trolox equivalents ($TEAC_{ABTS\bullet+}$), in case of Tempol as ascorbic acid equivalents (*AAE*, in milligrams per litre) using the calibration curve constructed from standard solutions of ascorbic acid as previously described by Tobolková et al. [38].

UV-VIS measurements

The entire UV-VIS experiments were carried out using UV-VIS-NIR spectrophotometer Shimadzu 3600 (Shimadzu, Tokyo, Japan) with accessories. The experiments were performed in duplicates. Total phenolic compounds concentration was estimated by a modified Folin-Ciocalteu's method, using standard solution of gallic acid for calibration curve construction. Results were expressed as gallic acid equivalents (*GAE*, in milligrams per litre).

To assess the colour changes of orange juices, transmittance measurements were performed in a spectral range from 380 nm to 780 nm in a quartz cell 100-QS-Suprasil ($d = 1$ cm; Hellma, Müllheim, Germany) against distilled water as a blank, with a sampling interval of 2 nm and a slit width of 0.1 nm. Colour model *CIE L*a*b** was used for objective colour evaluation and the changes were evaluated by the procedure previously described by Tobolková et al. [38].

HPLC analysis

Sample preparation

Juice samples were diluted ten-fold and twenty-fold and filtered through a syringe filter (pore size 0.45 μm).

HPLC measurements

Ascorbic acid and hesperidin were determined on a liquid chromatograph Agilent Technologies 1100 Series (Agilent Technologies) equipped with diode array detector (DAD), quaternary pump, degasser, column thermostat and autosampler. The separation was performed using analytical column Zorbax SB-C18, 250 mm × 4.6 mm, with the sorbent particle size 5 μm (Agilent Technologies), and the pre-column Zorbax SB-C18, 12.5 mm ×4.6 mm with the same particle size (Agilent Technologies).

HPLC setup for ascorbic acid determination

Mobile phase was 0.01 mol·l^{-1} H_3PO_4 at a flow rate of 0.7 ml·min^{-1}; DAD detection at 240 nm; column temperature 25 °C; injection volume 50 μl. Limit of detection was 1.4 mg·l^{-1}, limit of quantification was 1.8 mg·l^{-1}, recovery was 84–90%, coefficient of determination $R^2 = 0.9998$.

HPLC setup for hesperidin determination

Mobile phase consisted of A: 0.01 mol·l^{-1} H_3PO_4:methanol, 80:20, v/v) and B: 100% methanol, was proportioned by the following gradient programme: 0–1 min 100%A, 1–23 min 43%A linear, 23–28 min 100%A linear, 28–33 min 100%A (post-run); flow rate 1.0 ml·min^{-1}; DAD detection at 285 nm; column temperature 25 °C; injection volume 50 μl. Limit of detection was 1.2 mg·l^{-1},·limit of quantification was 2.0 mg·l^{-1}, recovery was 92–94% and coefficient of determination $R^2 = 0.9996$.

Statistical evaluation

Statistical calculations were performed by the software package Unistat 6.0 (Unistat, London, United Kingdom). ANOVA and multivariate statistics were used to compare, explore and discriminate the complex dataset of experimental characteristics, with particular focus on GC-MS data. Multiple comparisons were performed by ANOVA Tukey's HSD (honest significant difference) test at a level of significance of $p \leq 0.05$. The differences

in means of individual compared characteristics were recognized as highly significant at $p < 0.001$. Principal component analysis (PCA) and canonical discriminant analysis (CDA) were used in order to define, interpret and visualize the differences between the compared orange juice samples as well as to assess the effects of pasteurization. Using CDA, the recognition ability was calculated as the percentage of correctly classified samples in the original data set in which all the samples were of known properties for the classification model. The software MicroMath Scientist (MicroMath, St. Louis, Missouri, USA) was used to design the first-order kinetic model for ascorbic acid and hesperidin degradation, using the equation:

$$c_t = c_0 \cdot e^{-kt} \quad (1)$$

in which c_0 and c_t represented the original concentration of ascorbic acid/hesperidin at $t = 0$ and the concentration at particular time t of storage, respectively; k was the first-order rate constant in week^{-1}.

RESULTS AND DISCUSSION

GC study of volatiles

The profiles of individual components separated from orange juice volatile fraction were obtained by HS-SPME coupled to GC-MS. Volatiles in orange juice represented a complex multi-compositional matrix, as obvious from typical chromatographic records depicted in Fig. 1. Fifty volatile organic compounds of different chemical nature were separated (Fig. 1, Tab. 1), in good accordance with previously published data [18]. Volatiles were identified by a combination of several independent methods as indicated in the experimental part. In certain cases, when only partial information was available, only tentative identification was possible.

Analysed volatiles belonged mostly to esters, aldehydes, alcohols and terpenes. As regards terpenes, D-limonene (52.1%) was dominant in the volatile fraction of raw orange juice, followed by valencene (13.3%), α-thujene (7.7%) and terpene alcohols such as linalool (3.8%), α-terpineol (1.7%) and terpinen-4-ol (1.3%). In addition, some aldehydes, alcohols and esters were also present in higher amounts, e.g. decanal (1.8%), 1-octanol (0.8%) or ethyl butanoate (0.8%).

Effect of pasteurization on the content of volatiles

In the next steps of the study, the effects of pasteurization on the components of orange juice volatile fraction were investigated. As follows from the obtained results, pasteurization (at 95 °C for 20 s) caused some alterations within volatiles. A decrease ($p < 0.05$) in the content of the majority of terpene alcohols, esters, aldehydes, ketones and sesquiterpenes was observed. These comprised terpinen-4-ol, α-terpineol, ethyl octanoate, ethyl butanoate, linalool, 1-octanol, L-limonene, decanal, carveol, nerol, L-carvone, α-terpinyl acetate, neryl acetate, geranyl acetate, α-copaene, β-elemene, β-caryophyllene, alloaromadendrene, β-maaliene, valencene, and tentatively identified volatiles such as cis-9-octadecen-1-ol, β-cubebene, 4,11-selinadiene, β-chamigrene, eremophylene and α-panasinsene (Tab. 1.). The observed decrease in the content of some volatiles was a consequence of their thermolability, which regarded in particular sesquiterpenes with sensitive thermolabile double bonds in their molecules.

On the other hand, pasteurization imposed a significant increase ($p < 0.05$) of some volatile monoterpenes, e.g. of α-pinene, α-thujene, α-phellandrene, δ-3-carene, α-terpinene, D-limonene, cis-β-ocimene, γ-terpinene, 4-carene, but also of aldehyde nonanal. These compounds were recognized as the most volatile components of the analysed orange juice aroma.

The observed growing trend of the content of monoterpenic fraction, which was influenced by the temperature is in good agreement with the study of TINGEY et al. [39], proving that the effect of light on five dominant monoterpenes (α-pinene, β-pinene, myrcene, limonene and β-phellandrene) present in the vapours of slash pine plants was negligible, whereas their contents increased exponentially with temperature. We suppose that the changes resulted from the formation of thermal isomerization products of some terpenes, as was shown previously [40–42].

Statistical processing of the experimental data obtained for juice samples packed into different material exhibited that the kind of packing material (glass or PET) used after pasteurization did not have such significant impact on changes in the content of individual volatile compounds, as the pasteurization procedure itself. The difference in the contents of particular components between the raw sample from the stirring tank and the bottled samples after pasteurization (irrespective of whether bottled to glass or plastic material) was statistically significant ($p < 0.05$) in a majority of cases. The differences between samples stored in glass versus plastic bottles were not so evident. It is also important to mention that the analyses were carried out until 24 h after pasteurization and thus, the contact of orange juice with the packing materials (glass or PET) was very short.

The results of ANOVA Tukey's HSD analysis also supported the processing of the experimental dataset by PCA to visualize the inner mutual relationships within the dataset of experimental characteristics of individual samples. As regards the results of PCA, the first principal component described almost 88% of the total dataset variability and the second principal component described 100% of the total variability. The table of eigenvalues indicated that practically all experimental characteristics contributed to the description of inner variability by the same value (eigenvalues of ~0.15), except for the contents of ethylacetate, trans-4,8-dimethyl-1,3,7-nonatriene and α-humulene with very low eigenvalues. However, only the last three mentioned components played a domi-

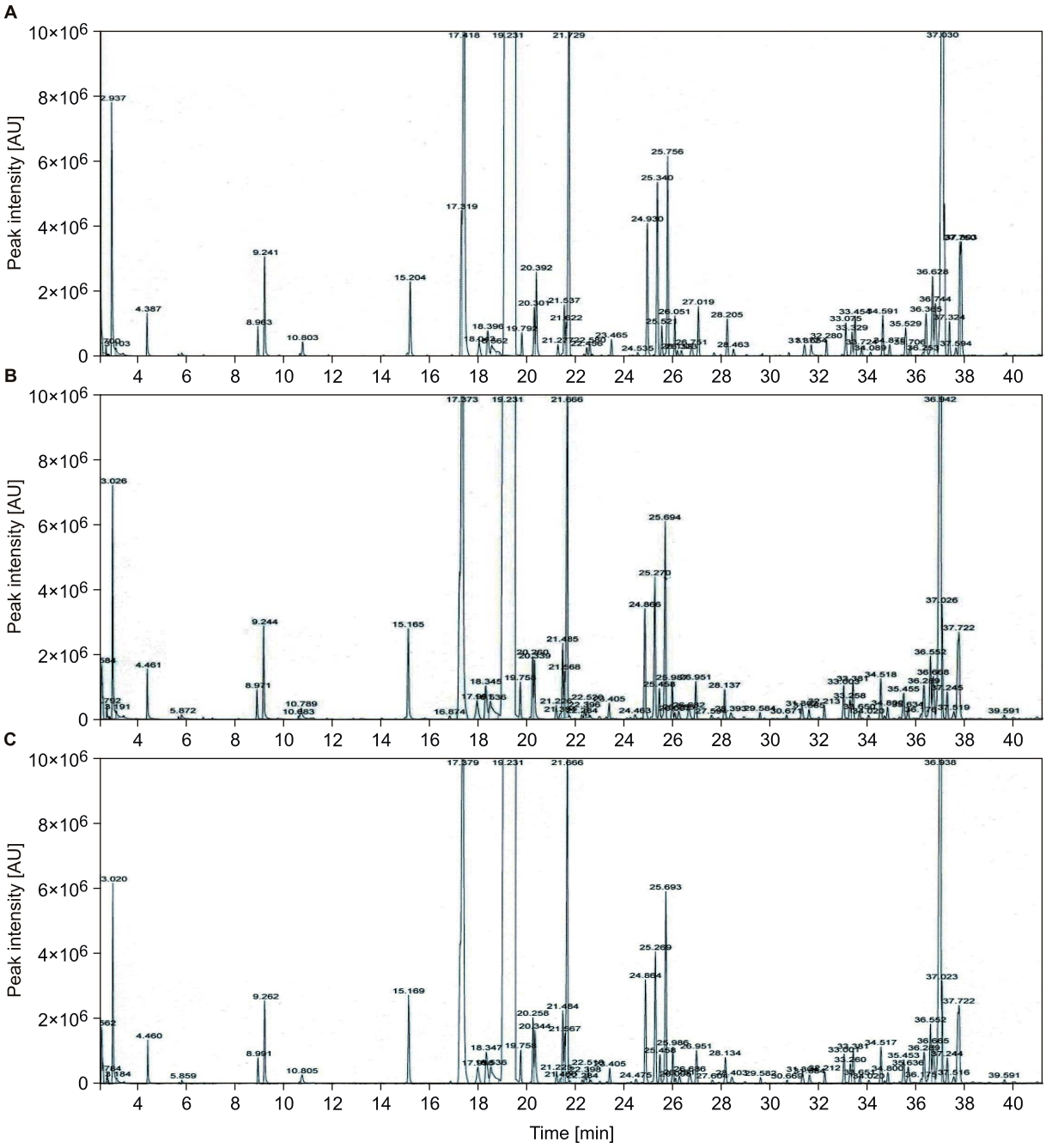

Fig. 1. Typical chromatograms of orange juice of Brazil origin obtained by HS-SPME coupled to GC-MS.

A – raw orange juice from the stirring tank bottled into glass bottles prior to pasteurization; **B** – orange juice bottled immediately after the pasteurization to PET bottles; **C** – orange juice bottled immediately after the pasteurization to glass bottles. Analyses were performed within 24 h after production of the juice using Ultra 1 GC column.

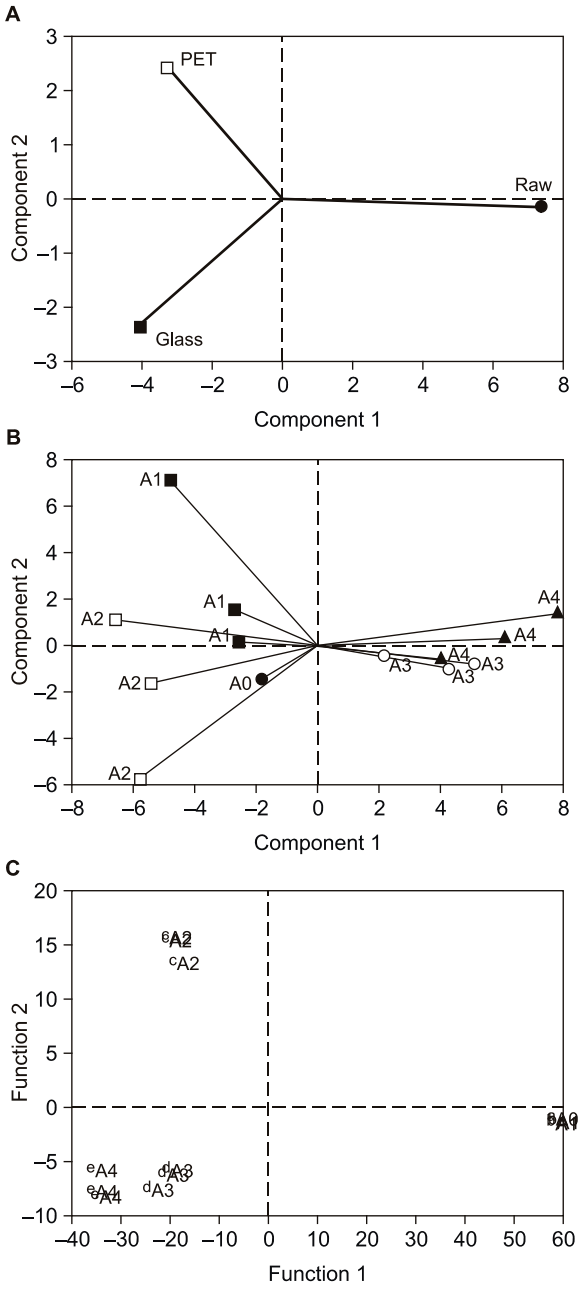

Fig. 2. Evaluation of the dataset of volatiles identified in orange juice with pulp by the methods of principal component analysis and canonical discrimination analysis.

A – plot of principal components demonstrating the differentiation of raw orange juice sample from the stirring tank, and pasteurized juice samples packed into glass and PET, constructed on the basis of the content of individual volatile compounds; **B** – plot of principal components constructed on the basis of contents of individual volatile compounds of orange juice with pulp stored for 0 (fresh, A0) and up to 4 months after its production (A1–A4); **C** – discrimination of orange juice samples with pulp using the time of storage as the discriminating criterion. The entire dataset of the contents of volatile compounds recorded in samples stored for 0 (fresh, A0) and up to 4 months after its production (A1–A4). Samples were stored at (7 ± 1) °C for 4 months.

nant role in the second principal component construction. The plot of principal components (Fig. 2A) clearly demonstrates the differentiation of eigenvectors belonging to the sample from stirring tank (raw), and the samples after pasteurization, stored in glass (Pasteur-glass) and PET (Pasteur-PET) containers.

Effect of storage on the content of volatiles and on the odour intensity of aroma-active compounds

Generally, flavour-impacting compounds as the key volatiles are the major contributors to the unique aroma of orange juice. These compounds do not exhibit high stability in the presence of microorganisms and high temperatures, which lead to their deterioration [43]. The content of individual volatiles in orange juice with pulp was monitored in one-month intervals within the declared expiration period by HS-SPME coupled to GC-MS. The effect of storage on the relative content of selected flavour-impact volatiles throughout the whole storage period is depicted in Fig. 3A and Fig. 3B. In accord with expectations, the content of some volatile compounds had an increasing tendency, e.g. monoterpenes α-phellandrene (weak continuous growth), *cis*-β-ocimene (mild growth for up to 3 months), α-copaene (weak growth only), and sesquiterpene β-maaliene (obvious increase). An increasing trend was also shown for other volatiles, such as monoterpene α-pinene, aldehyde nonanal, esters α-terpinyl acetate and neryl acetate, alcohols 9-octadecen-1-ol and *p*-menth-1-en-9-ol, as well as for a group of sesquiterpenes, such as β-elemene, β-caryophyllene, β-cubebene, alloaromadendrene, α-humulene, and five tentatively identified sesquiterpenes 4,11-selinadiene, β-chamigrene, α-selinene and eremophylene.

On the contrary, contents of linalool, α-terpineol and δ-3-carene showed a decrease during storage (Fig. 3B). α-Terpineol even after the first month, linalool clearly after the third month, and δ-3-carene showed a weak degradation tendency after the second month of the storage. A decrease was observed also for the content esters ethyl acetate and ethyl butanoate, aldehyde hexanal, monoterpenes α-thujene, α-terpinene, γ-terpinene, 4-carene, sesquiterpene valencene, and terpene alcohol terpinen-4-ol. Some other recognized volatiles, such as D-limonene, nonanol as well as tentatively identified perillaldehyde, undecanal and ethyl octanoate did not show any monotonic trend during the storage.

ANOVA Tukey's HSD analysis confirmed the effects of storage on compositional changes of orange juices. As regards concentration changes, the differences between fresh sample and samples

stored for three and four months were found to be statistically significant ($p < 0.05$) for hexanal, ethyl butanoate, α-terpinene, γ-terpinene linalool, terpinen-4-ol, undecanal, α-terpinyl acetate, *p*-menth-1-en-9-ol, valencene and α-panasinsen. In accord with expectations, differences between the first and the fourth month of storage were mostly more significant than between the first and the third month. It can be also noted here that there was practically no significant variance between the fresh sample and samples stored for one and two months, except for nonanol, *cis*-9-octadecen-1-ol, β-cubebene and alloaromadendrene. Contents of the last mentioned compounds significantly changed during the whole monitored period. Results of PCA, as well as subsequent differentiation of samples according to the storage time, were coherent with the above-described general changes in the composition of orange juice samples. The plot of principal components (Fig. 2B) clearly indicates the existence of 2/3 partially differentiated groups of eigenvectors, belonging to samples stored in period from zero (fresh sample) to the second month and from the third to the fourth month, respectively. Thus, it can be concluded that the changes induced by the storage of orange juice of the same quality became meaningful even during the second month of storage. Similar trend was proven by the canonical discriminant analysis (Fig. 2C), which classified the samples into groups on the basis of compositional analysis with 100% correctness. As can be also followed from the plot of discriminant functions, reference (fresh juice sample) was closer by its properties to the samples stored for one month, while the properties of samples stored for three and four months (and partially also for two months) were considerably different. The discriminant scores of individual groups were different and, as a result, the existence of four/five groups of points belonging to the juice samples stored for four months is obvious from the plot of discriminant functions. The opposite position of individual groups – distinctively for zero/one and three/four month stored samples, fully confirmed the above-discussed trends.

In order to characterize the organoleptic quality of the "slightly" pasteurized orange juice, volatile compounds isolated by HS-SPME were investigated by means of GC/FID-O, to identify sensorially significant compounds as well as to describe the dynamics of their changes during the storage. The measurements were performed simultaneously with GC-MS, described above. In GC-O analysis, altogether 44 olfactometric responses were recorded (Tab. 2). In certain cases, the compounds remained unidentified at this stage as certain they were identified only by olfactometry. In the future research, the unknown odour-active compounds should be further investigated by determination of their *LRI* on a GC column with a stationary phase of different polarity.

The data on dynamics of odour intensity of individual aroma-active compounds of orange juice and their corresponding retention within the storage period are presented in Tab. 2. The results of GC-O clearly demonstrated that the principal

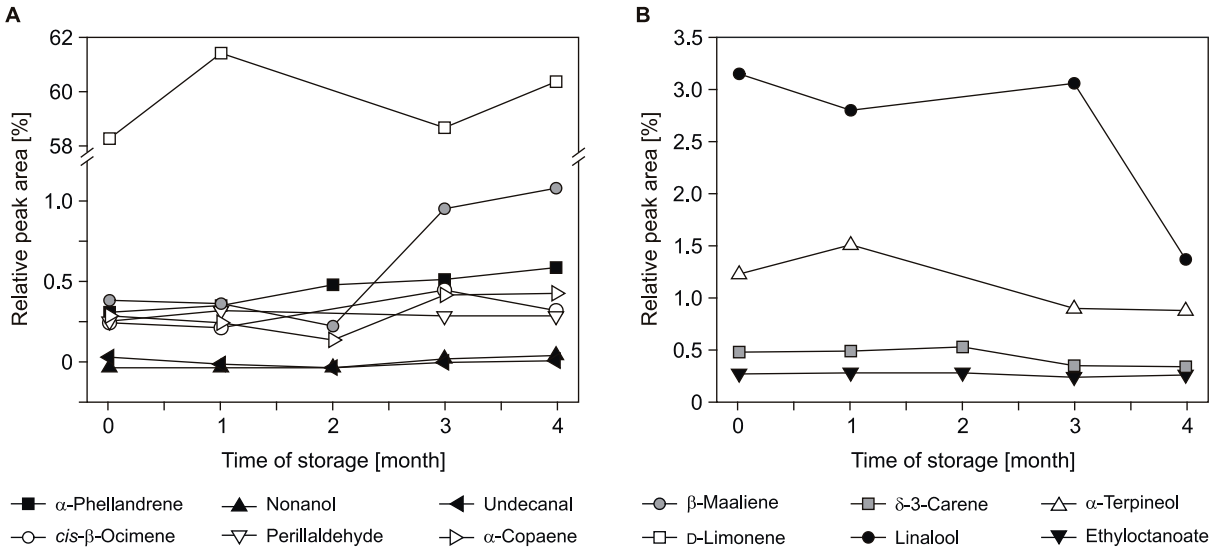

Fig. 3. Impact of storage of orange juice with pulp at (7 ± 1) °C in a showcase at daylight conditions on the contents of selected flavour-impacting volatiles.

A – increase, B – decrease.

aroma-active compounds creating the aroma of orange juice were D-limonene, α-phellandrene, nonanal, decanal, linalool, L-limonene, ethyl octanoate, ethyl butanoate, nonanol and α-copaene. All of them contributed with their odour intensity to the overall aroma of the studied orange juice to a decisive degree, and were with their individual odour descriptions the most characteristic components of the aroma. As evident from Tab. 2, other flavour-impacting compounds were α-terpinene, *cis*-β-ocimene, 1-octanol, ethyl 3-hydroxyhexanoate, nonanol, perillaldehyde, undecanal, α-pinene, α-humulene and valencene.

Concerning the influence of the storage on the changes of odour intensity of the key odourants, it is evident that the sensorially most stable compounds, without significant change, were α-phellandrene (with balsamic, mint, herbaceous odour), D-limonene (with terpenic, intensive citrus-peel note), nonanal (with fatty, bitter, citrus, waxy odour), linalool (with refreshing, floral, fragrant, citrus-like notes), nonanol (with intensive citrus-peel odour), ethyl octanoate (with banana, pineapple, pear, sweet, floral notes), α-copaene (spicy, woody, dill-like, earthy odour), α-humulene (with terpenic, woody, spicy, hop oil-like notes) and β-caryophyllene (with terpenic, spicy, woody odour). The intensity of their odour remained principally unchanged during the entire storage period.

Regarding ethyl butanoate (fruity, apple-like, sweet notes), α-pinene (sharp, pine, terpeny odour), α-terpinene (balsamic, herbaceous, marjoram-like notes), 1-octanol (herbaceous, earthy, waxy), ethyl 3-hydroxyhexanoate (smoky, leather, tobacco odour), β-maaliene (essential oil-like, terpenic, herbal notes), valencene (woody, orange-citrus peel odour), caryophyllene oxide (woody, herbaceous odour) and unknown odourants Nos. 7, 8, 10, 25, 34, a progressive increase in their odour intensity was noticed. On the other hand, some identified odourants, such as ethyl acetate (weak stone fruit, pear-like notes), hexanal (with green leafy, grassy, tallowy, slight citrus-like odour), octanal (soapy, herbaceous, oily, fatty, citrus-like notes), α-thujene (fresh, terpene-like, essential oil-like notes), terpinen-4-ol (earthy, woody, waxy) and unknown compounds Nos. 2, 3, 6, 22 revealed the odour intensity after certain time during the storage when they achieved the odour threshold concentration of sensory perception. In contrast, odourants with significantly reduced/lost odour intensity within the monitored period were also identified, e.g. α-terpineol (pleasant, sweet, fruity odour), L-carvone (spicy, peppermint, fresh notes), neryl acetate (sweet, fruity, dried plum-like notes), geranyl acetate (fresh, green, lavender, soap-like odour) and alloaromadendrene (woody, pleasant, soap-like, waxy, green notes).

Certain correlation can be found between the odour intensities of some compounds determined by GC-O (Tab. 2) and their contents determined by GC-MS (Fig. 3A, 3B), e.g. for α-phellandrene, δ-3-carene, D-limonene, nonanol, ethyl octanoate, perillaldehyde, undecanal or β-maaliene. For other components, e.g. for linalool with very low odour threshold concentration (in air only 0.6 $ng \cdot l^{-1}$ [44]), correlation between the GC-MS and GC-O results was less evident. These "apparent discrepancies" may result from specific features of GC-O, e.g. different threshold concentrations for sensory detection of various aroma-active compounds often varying through several orders of magnitude, and/or different psychophysical trend, and/or selectivity of human nose, and/or non-linear response for various kinds of aroma-active compounds.

Regarding comprehensive evaluation of the results, it can be stated that the overall juice aroma was the richest in overall harmonic flavour just after the juice production and after the first month of storage. Noticeable changes in odour intensity of same odourants were observed even during the second month of storage, with several emerging odourants such as ethyl acetate, unknown compounds Nos. 3, 6, 22, hexanal, octanal, α-thujene and terpinen-4-ol, contents of which exceeded the threshold values of sensory perception. These compounds altogether contributed to the increased bitterness of juice, and to certain loss of freshness and fruity sweetness. This trend continued also during the remaining two months of the storage period. It is obvious that the above-described changes followed predominantly from the continuous increase in the content of aldehydes during storage, mostly hexanal, which is a typical indicator of the "off-flavour" phenomenon for various foods. At the end of the storage period, sensorial changes were evident, accompanied also by colour and compositional changes as well as with changes in antioxidant compounds.

Effect of storage on antioxidant activity and colour characteristics

As expected, general gradual worsening of all characteristics monitored by UV-VIS and EPR spectroscopy was noticed. It is clearly indicated on Fig. 4A that, during the shelf-life period of the juice of 18 weeks of storage in darkness at 7 °C, a decrease in total polyphenols by approx. 11% was noticed. Subsequent storage for additional four weeks resulted in additional, approx. 1.5%

decrease in total polyphenols concentration. On the other hand, a decrease in radical-scavenging ability characterized by $ABTS^{\bullet+}$ assay (Fig. 4B) had a diverse character, as the most significant changes were perceived during the first 5 weeks of storage, with a decrease in *TEAC* values from 2.28 (reference – fresh sample) to approx. 1.35 (5th week of storage). Further storage for 18 weeks and 21 weeks led only to fluctuations in *TEAC* values around the steady-state value of 1.20, which was reached on the 11th week of storage under the defined conditions. As regards the results of Tempol assay reflecting the presence of low-molecular organic acids including ascorbic acid, the trend identical with total phenols concentration diminution described above was observed, indicating a decrease in organic acids concentration in orange juices during the storage.

All the changes described above, in particular the changes in total phenols concentration, directly influenced the colour of orange juice and its changes throughout the storage. For the purpose of objective evaluation of colour changes, 5 parameters were selected and examined, including *CIE L*a*b** trichromatic parameters, chromaticity and hue angle. The obtained results are presented in Tab. 3. From *CIE L*a*b** values, total colour difference was calculated, as indicated in the experimental part. In view of variability of the obtained data, significance of differences in means of all the monitored characteristics during the storage period was evaluated by means of ANOVA Tukey's HSD pair test. Special attention was paid to the significance of differences of values recorded for the reference sample and for samples stored for 18 and 21 weeks, i.e. at the end of orange juice shelf-life declared by the producer, and in the end of the monitored period. Minimal storage-induced changes in *CIE L*a*b** characteristics of orange juices were observed. In case of lightness, absolute values indicate oscillation within a very narrow interval, with a slight tendency toward lightening (increase in L^* during the storage). As regards a^* and b^* values, the generally observed decrease indicated a shift of colour towards green and blue, respectively. ANOVA Tukey's HSD test however proved that, for all the compared values, the results were significantly different ($p < 0.05$) for a majority of both a^* and b^* values. Nevertheless, in case of the last mentioned, some pair correlations indicated similarities of b^* values. The difference in b^* values for 18th and 21st week of storage was also recognized as non-significant. For chromaticity and hue angles, significantly different values were found only within first 5 weeks of storage. This was in good correlation with the

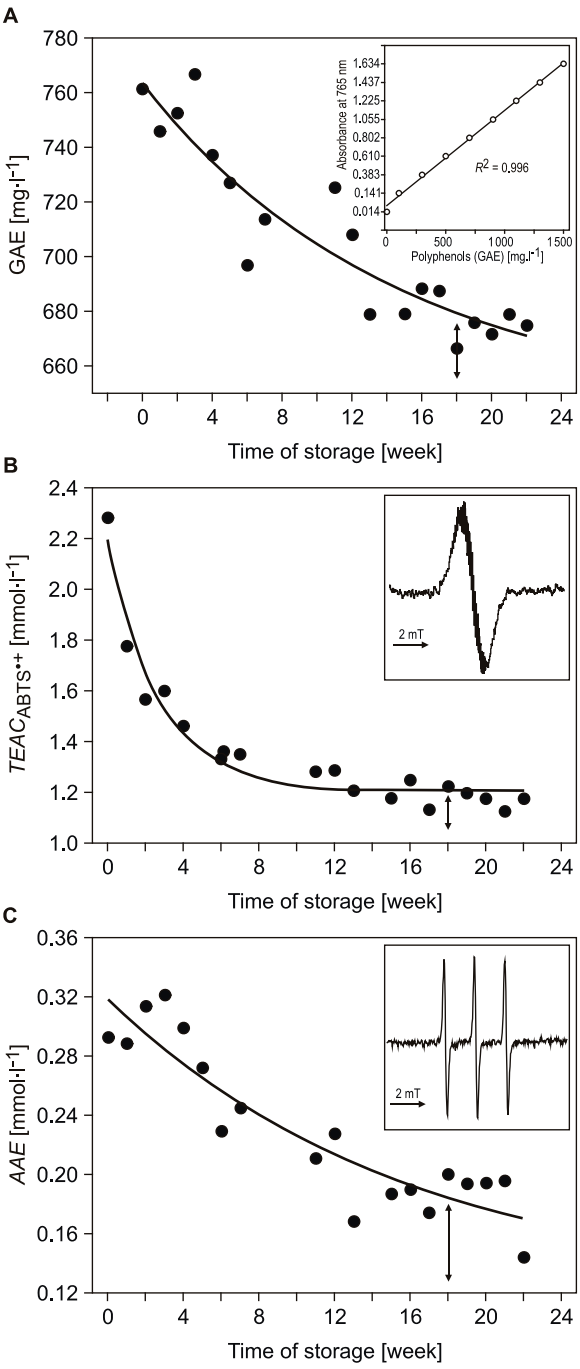

Fig. 4. Effect of storage on the concentration of total polyphenols and radical-scavending ability of orange juice with pulp.

A – dependence of polyphenols concentration on time of storage of orange juice with orange pulp in darkness at (7 ± 1) °C. Inset represents calibration curve of gallic acid solutions; **B** – dependence of antioxidant activity expressed in terms of Trolox-equivalent antioxidant activity toward $ABTS^{\bullet+}$ ($TEAC_{ABTS\bullet+}$) on time of storage of orange juice with orange pulp in darkness at (7 ± 1) °C. Inset represents typical spectrum of $ABTS^{\bullet+}$ cation-radical; **C** – dependence of ascorbic acid equivalents toward Tempol (*AAE*) on time of storage of orange juice with orange pulp in darkness at (7 ± 1) °C. Inset represents typical spectrum of Tempol free radical.

Tab. 3. Colour characteristics of orange juices with orange pulp and their changes resulting from long-term storage at (7 ± 1) °C.

Time of storage [week]	*L**	*a**	*b**	Chromaticity	Hue angle	Δ*E*
0	98.30 ± 0.03	0.18 ± 0.00	2.30 ± 0.01	2.31 ± 0.01	85.64 ± 0.02	–
1	98.74 ± 0.01	0.09 ± 0.00	1.82 ± 0.01	1.83 ± 0.01	87.05 ± 0.00	0.66
2	98.51 ± 0.00	0.15 ± 0.00	2.02 ± 0.01	2.03 ± 0.01	85.65 ± 0.00	0.35
3	98.82 ± 0.01	0.08 ± 0.00	1.76 ± 0.02	1.76 ± 0.02	87.53 ± 0.07	0.75
4	98.70 ± 0.00	0.10 ± 0.02	1.96 ± 0.03	1.97 ± 0.03	87.05 ± 0.41	0.53
10	98.75 ± 0.02	0.09 ± 0.00	1.87 ± 0.00	1.87 ± 0.00	87.39 ± 0.11	0.63
12	98.47 ± 0.03	0.12 ± 0.00	2.10 ± 0.10	2.10 ± 0.10	86.85 ± 0.25	0.27
14	98.53 ± 0.01	0.11 ± 0.00	2.12 ± 0.00	2.12 ± 0.00	87.08 ± 0.05	0.30
16	98.43 ± 0.00	0.12 ± 0.00	2.20 ± 0.01	2.20 ± 0.01	86.80 ± 0.05	0.18
18	98.41 ± 0.01	0.09 ± 0.01	2.14 ± 0.02	2.14 ± 0.02	87.53 ± 0.12	0.21
21	98.62 ± 0.05	0.08 ± 0.00	2.04 ± 0.01	2.04 ± 0.01	87.85 ± 0.00	0.43

Results are presented as mean ± standard deviations (n = 2).

above-presented results, particularly for $ABTS^{\bullet+}$ assay.

In order to compare the colour of two or several objects, total colour difference (TCD, Δ*E*), was proposed, expressing the magnitude of difference between the initial (reference) and stored orange juice samples [45]. According to the scale suggested by Cserhalmi et al. [45], the colour changes induced in orange juice samples with pulp were recognized as mostly not-noticeable (Δ*E* up to 0.5) to slightly noticeable (Δ*E* up to 1.5). To discuss properly all the observed changes, several factors should be taken into account, e.g. effect of pasteurization, as well as storage conditions, in particular temperature.

It is widely accepted, that the antioxidant activity of fruit juices is directly related to the concentration of ascorbic acid and total phenolics. According to some authors, storage at low temperature did not affect the concentration of polyphenols in some fruits and vegetables including apple, pears or onion [10, 46]. However, the observed decrease in polyphenols concentration was most probably a result of partial oxidation of a group of easily oxidized polyphenols and/or polymerization due to the oxidation reactions taking place during storage [3, 7, 47, 48]. Oxidation of polyphenols contributes also to changes in the quality of foods, particularly in colour and organoleptic characteristics [6, 7, 10, 11, 14, 49]. Such changes may be both positive and negative from the consumer's perception point of view, resulting in deterioration of taste, colour (browning) as well as other parameters. Moreover, the Folin-Ciocalteu's phenol reagent is a non-specific method, results of which being affected by interference with any reducing substances, reflecting thus the total reducing capacity of a sample, not just phenolic compounds [50–52].

Worsening of antioxidant and radical-scavenging properties of orange juices is in accord with previous observations of several authors not only in the case of fruit juices, but also wines, syrups and other natural products [4, 7, 10, 11, 14, 37, 48]. This phenomenon is tightly connected with oxidation-induced changes in the samples resulting in deterioration of its properties, accompanied by an increase in the concentration of some aldehydes and ketones that can be controlled, but not fully eliminated by the modification of the processing technology, e.g. via application of inert atmosphere, packaging into materials containing oxygen scavengers and/or minimizing the headspace volume and its contact with the atmosphere.

Effect of pasteurization and storage on ascorbic acid and hesperidin concentration

During the period of the storage of orange juice with pulp, changes in concentrations of ascorbic acid and hesperidin were investigated by HPLC. Concerning the changes following from juice processing, the obtained results revealed that the orange juice just before the thermal processing (pasteurization), yet without pulp, contained (346 ± 23) $mg \cdot l^{-1}$ of ascorbic acid and (120 ± 14) $mg \cdot l^{-1}$ of hesperidin. After the addition of pulp and subsequent pasteurization, i.e. in the sample herein reported as fresh juice sample, the concentration of ascorbic acid reached (303 ± 17) $mg \cdot l^{-1}$ and of hesperidin

(222 ± 2) mg·l^{-1}. It should be pointed out, that the level of hesperidin increased after pasteurization most probably due to the addition of pulp, which is known to contain hesperidin. On the contrary, owing to the known thermal instability of ascorbic acid, its concentration decreased by 12% after the thermal treatment.

The retention of both investigated bioactive compounds decreased with time during the storage, in accord with expectations. This observation was fully in agreement with other studies confirming time-dependent degradation of ascorbic acid in citrus juices [53–55]. With respect to non-linear evolution of degradation of both constituents, it was found that their loss to the best followed a first-order kinetic model (Eq. 1, Fig. 5). The so-calculated formal reaction rate constants k revealed a notable distinction between decomposition of ascorbic acid and hesperidin during the storage ($k = 0.054 \pm 0.002$ and $k = 0.023 \pm 0.002$, for ascorbic acid and hesperidin, respectively), indicating thus much lower stability of ascorbic acid. At the end of juice expiration period, about 39% of ascorbic acid and 66% of hesperidin were retained in comparison to their concentrations in fresh juice sample. During additional 3 weeks of storage, the concentration of both constituents further declined only slightly and, at the end of the monitored period, the remaining concentration of ascorbic acid reached 36% and that of hesperidin 57%, related to fresh sample.

In order to examine the relative relationship between the ascorbic acid and hesperidin concentrations, linear regression analysis with two-tailed Spearman's correlation at 95% confidence level was applied. Concentrations of the compounds exhibited high positive correlation ($R^2 = 0.916$ at $p < 0.001$). In connection with this result, MIGUEL et al. described a direct correlation between the ascorbic acid concentration and antioxidant potential of orange juice determined by TEAC assay with high correlation ($R^2 = 0.837$), while the correlation between antioxidant activity and concentration of flavanone glycosides was not appreciable [56]. In another study, a decrease of ascorbic acid, polyphenols and antioxidant activity in citrus juice during the storage was found to follow the same downward trend [7].

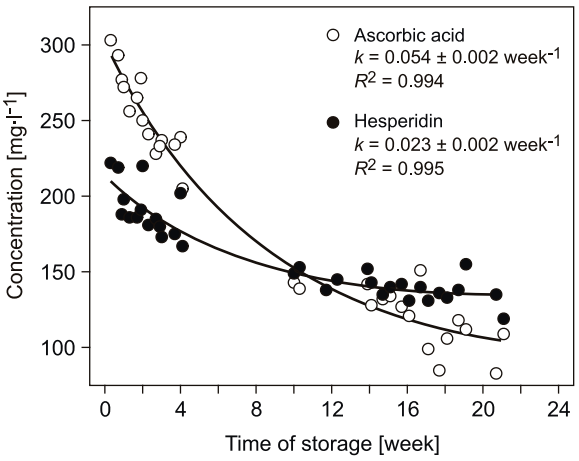

Fig. 5. The first-order kinetic model of ascorbic acid and hesperidin degradation in orange juice stored at (7 ± 1) °C in darkness for 21 weeks.

CONCLUSION

Comprehensive analysis of the effects of pasteurization together with storage conditions on composition of volatile components of orange juices with pulp proved the presence of 44 aroma-active components, including ethyl acetate, hexanal, ethyl butanoate, α-pinene, octanal, α-thujene, α-phellandrene, δ-3-carene, α-terpinene, D-limonene, *cis*-β-ocimene, 1-octanol, 4-carene, nonanal, linalool, ethyl 3-hydroxyhexanoate, nonanol, terpinen-4-ol, α-terpineol, ethyl octanoate, decanal, L-carvone, perillaldehyde, undecanal, α-terpinyl acetate, neryl acetate, geranyl acetate, α-copaene, β-caryophyllene, α-humulene, alloaromadendrene, β-maaliene, valencene, caryophellene oxide and some compounds, which were detected only by olfactometry and were not identified at this stage. These compounds were also recognized as principal constituents of orange juice of Brazil origin. Results also demonstrated that pasteurization had a more significant effect on the flavour than the packaging material, and that the organoleptic profile of pasteurized juice started to change meaningfully during the second month of storage.

Obtained results also proved the gradual colour changes towards brown and dark, as well as worsening of antioxidant properties of juice samples, most intensive during first 5 weeks of storage under the defined conditions. The decrease of concentration of total polyphenols, as well as flavonoid hesperidin and ascorbic acid indicates the complexity of the (not only oxidation) processes taking place in orange juice during storage. It should be noted here that besides the other factors, also the amount of pulp/solid particles can influence the monitored characteristics, being the source of polyphenols, and non-flavonoid antioxidants, but also compounds directly influencing the aroma profile.

With an aim of effective maintenance of juice stability as well as minimization of the deterioration of its properties, the continued research will be oriented on the effects of applications of inert gases in technology of fruit juices production. Topic of interest would be also the study of effects of orange pulp on the monitored characteristics, including the volatile compounds content profile and the distribution of volatiles between the liquid and solid phase of orange juice.

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REFERENCES

1. Economos, C. – Clay, W. D.: Nutritional and health benefits of citrus fruits. In: Lupien, J. R. – Randell, A. – Malek, Z. – Cotier, J. P. – Clay, W. D.: Food, Nutrition and Agriculture. Rome: Food and Agriculture Organization, 1999. pp. 11–18. ISSN 1014-806X. Available at <ftp://ftp.fao.org/docrep/fao/X2650t/X2650t03.pdf>.
2. Manach, C. – Scalbert, A. – Morand, C. – Rémésy, C. – Jiménez, L.: Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition, *79,* 2004, pp. 727–747.
3. Robards, K. – Prenzler, P. D. – Tucker, G. – Swatsitang, P. – Glover, W.: Phenolic compounds and their role in oxidative processes in fruits. Food Chemistry, *66,* 1999, pp. 401–436. DOI: 10.1016/S0308-8146(99)00093-X.
4. Rodriguez-Amaya, D. B. – Kimura, M. – Godoy, H. T. – Amaya-Farfan, J.: Updated Brazilian database on food carotenoids: Factors affecting carotenoid composition. Journal of Food Composition and Analysis, *21,* 2008, pp. 445–463. DOI: 10.1016/j.jfca.2008.04.00.
5. de Moraes Barros, H. R. – de Castro-Ferreira, T. A. P. – Genovese, M. I.: Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. Food Chemistry, *134,* 2012, pp. 1892–1898. DOI: 10.1016/j.foodchem.2012.03.090.
6. Kelebek, H. – Selli, S. – Canbas, A. – Cabaroglu, T.: HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. Microchemical Journal, *91,* 2009, pp. 187–192. DOI: 10.1016/j.microc.2008.10.008.
7. Klimczak, I. – Małecka, M. – Szlachta, M. – Gliszczyńska-Świgło, A.: Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. Journal of Food Composition and Analysis, *20,* 2007, pp. 313–322. DOI: 10.1016/j.jfca.2006.02.012.
8. Rekha, C. – Poornima, G. – Manasa, M. – Abhipsa, V. – Pavithradevi, J. – Vijay Kumar, H. T. – Prashith Kekuda, T. R.: Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. Chemical Science Transactions, *1,* 2012, pp. 303–310. DOI: 10.7598/cst2012.182.
9. Miller, N. J. – Rice-Evans, C. A.: The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food Chemistry, *60,* 1997, pp. 331–337. DOI: 10.1016/S0308-8146(96)00339-1.
10. Esteve, M. J. – Frígola, A. – Rodrigo, C. – Rodrigo, D.: Effect of storage period under variable conditions on the chemical and physical composition and colour of Spanish refrigerated orange juices. Food and Chemical Toxicology, *43,* 2005, pp. 1413–1422. DOI: 10.1016/j.fct.2005.03.016.
11. Farnworth, E. R. – Lagacé, M. – Couture, R. – Yaylayan, V. – Stewart, B.: Thermal processing, storage conditions, and the composition and physical properties of orange juice. Food Research International, *34,* 2001, pp. 25–30. DOI: 10.1016/S0963-9969(00)00124-1.
12. Jordán, M. J. – Goodner, K. L. – Laencina, J.: Deaeration and pasteurization effects on the orange juice aromatic fraction. LWT – Food Science and Technology, *36,* 2003, pp. 391–396. DOI: 10.1016/S0023-6438(03)00041-0.
13. Meléndez-Martínez, A. J. – Escudero-Gilete, M. L. – Vicario, I. M. – Heredia, F. R.: Effect of increased acidity on the carotenoid pattern and colour of orange juice. European Food Research and Technology, *230,* 2010, pp. 527–532. DOI: 10.1007/s00217-009-1190-1.
14. Del Caro, A. – Piga, A. – Vacca, V. – Agabbio, M.: Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. Food Chemistry, *84,* 2004, pp. 99–105. DOI: 10.1016/S0308-8146(03)00180-8.
15. Buettner, A. – Schieberle, P.: Evaluation of aroma differences between hand-squeezed juices from Valencia Late and Navel oranges by quantification of key odorants and flavor reconstitution experiments. Journal of Agricultural and Food Chemistry, *49,* 2001, pp. 2387–2394. DOI: 10.1021/jf001363l.
16. Cerdán-Calero, M. – Sendra, J. M. – Sentandreu, E.: Gas chromatography coupled to mass spectroscopy analysis of volatiles, sugars, organic acids and aminoacids in Valencia Late orange juice and reliability of the automated mass spectral deconvolution and identification system for their automatic identification and quantification. Journal of Chromatography A, *1241,* 2012, pp. 84–95. DOI: 10.1016/j.chroma.2012.04.014.
17. Kelebek, H. – Selli, S.: Determination of volatile, phenolic, organic acid and sugar components in a Turkish cv. Dortyol (*Citrus sinensis* L. Osbeck) orange juice. Journal of the Science of Food and

Agriculture, *91,* 2011, pp. 1855–1862. DOI: 10.1002/jsfa.4396.
18. Moshonas, M. G. – Shaw, P. E.: Quantitative analysis of orange juice flavor volatiles by direct-injection gas chromatography. Journal of Agricultural and Food Chemistry, *35,* 1987, pp. 161–165. DOI: 10.1021/jf00043a025.
19. Moshonas, M. G. – Shaw, P. E.: Quantitative determination of 46 volatile constituents in fresh, unpasteurized orange juices using dynamic headspace gas chromatography. Journal of Agricultural and Food Chemistry, *42,* 1994, pp. 1525–1528. DOI: 10.1021/jf00043a025.
20. Nisperos-Carriedo, M. O. – Shaw, P. E.: Comparison of volatile flavor components in fresh and processed orange juice. Journal of Agricultural and Food Chemistry, *38,* 1990, pp. 1048–1052. DOI: 10.1021/jf00094a029.
21. Selli, S. – Cabaroglu, T. – Canbas, A.: Volatile flavour components of orange juice obtained from the cv. Kozan of Turkey. Journal of Food Composition and Analysis, *17,* 2004, pp. 789–796. DOI: 10.1016/j.jfca.2003.10.005.
22. Shaw, P. E.: Fruits II. In: Maarse, H. S: Volatile compounds in foods and beverages. New York: Marcel Dekker, 1991, pp. 305–327. ISBN 9780824783907.
23. Shaw, P. E. – Buslig, B. S. – Moshonas, M. G.: Classification of commercial orange juice types by pattern recognition involving volatile constituents quantified by gas chromatography. Journal of Agricultural and Food Chemistry, *41,* 1993, pp. 809–813. DOI: 10.1021/jf00029a025.
24. Tonder, D. – Petersen, M. A. – Poll, L. – Olsen, C. E.: Discrimination between freshly made and stored reconstituted orange juice using GC odor profiling and aroma values. Food Chemistry, *61,* 1998, pp. 223–229. DOI: 10.1016/S0308-8146(97)00097-6.
25. De Winne, A. – Dirinck, P.: HS-SPME GC-MS analysis of fresh and reconstituted orange juices. In: Blank, I. – Wüst, M. – Yeretzian, C.: Expression of multidisciplinary flavour science. Proceedings of the 12th Weuerman Flavour Research Symposium, Interlaken, Switzerland 2008. Zurich: Zürcher Hochschule für Angewandte Wissenschaften, 2010, pp. 607. ISBN 9783905745191.
26. Naef, R.: Minor components in extract of citrus fruits. In: Dugo, G. – Mondello, L.: Citrus oils: Composition, advanced analytical techniques, contaminants, and biological activity. Florida: CRC Press, 2011, pp. 463. ISBN 9781439800287.
27. Harmon, A. D.: Solid-phase microextraction for the analysis of aromas and flavors. In: Marsili, R.: Flavor, fragrance, and odor analysis. New York : Marcel Dekker, 2002, pp. 75–106. ISBN 9780824706272.
28. Allegrone, G. – Belliardo, F. – Cabella, P.: Comparison of volatile concentrations in hand-squeezed juices of four different lemon varieties. Journal of Agricultural and Food Chemistry, *52,* 2006, pp. 1844–1848. DOI: 10.1021/jf051206s.
29. Rega, B. – Fourier, N. – Nicklaus, S. – Guichard, E.: Role of pulp in flavour release and sensory perception in orange juice. Journal of Agricultural and Food Chemistry, *52,* 2004, pp. 4204–4212. DOI: 10.1021/jf035361n.
30. Šnebergrová, J. – Čížková, H. – Rajchl, A. – Ševčík, R. – Voldřich, M.: Evaluation of aroma restoration of apple and orange juices from concentrates in the Czech Republic. Journal of Food and Nutrition Research, *51,* 2012, pp. 156–163.
31. Janáčová, A. – Sádecká, J. – Kohajdová, Z. – Špánik, I.: The identification of aroma-active compounds in Slovak brandies using GC-sniffing, GC-MS and sensory evaluation. Chromatographia, *67,* 2008, pp. S113–S121. DOI: 10.1365/s10337-008-0635-1.
32. Sádecká, J. – Kolek, E. – Pangallo, D. – Valík, L. – Kuchta, T.: Principal volatile odorants and dynamics of their formation during the production of May Bryndza cheese. Food Chemistry, *150,* 2014, pp. 301–306. DOI: 10.1016/j.foodchem.2013.10.163.
33. Blank, I.: Gas chromatography-olfactometry in food aroma analysis. In: Marsili, R.: Flavor, fragrance, and odor analysis. New York : Marcel Dekker, 2002, pp. 297–331. ISBN 9780824706272.
34. Arnoldi, A.: The Maillard reaction as a source of off-flavours. In: Baigrie, B.: Taints and off-flavour in food. Cambridge : Woodhead Publishing and CRC Press, 2003, pp. 170. ISBN 9781855734494.
35. Rychlik, M. – Schieberle, P. – Grosch, W.: Compilation of odor thresholds, odor qualities and retention indices of key food odorants. Garching : Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität München, 1998. 63 pp. ISBN 9783980342650.
36. Van den Dool, H. – Kratz, P.: A generalization of the retention index system including gas-liquid partition chromatography. Journal of Chromatography A, *11,* 1963, pp. 463–471. DOI: 10.1016/S0021-9673(01)80947-X.
37. Polovka, M. – Šťavíková, L. – Hohnová, B. – Karásek, P. – Roth, M.: Offline combination of pressurized fluid extraction and electron paramagnetic resonance spectroscopy for antioxidant activity of grape skin extracts assessment. Journal of Chromatography A, *1217,* 2010, pp. 7990–8000. DOI: 10.1016/j.chroma.2010.08.003.
38. Tobolková, B. – Durec, J. – Belajová, E. – Mihalíková, M. – Polovka, M. – Suhaj, M. – Daško, Ľ. – Šimko, P.: Effect of storage on physico-chemical properties of pineapple juice with addition of small pineapple pieces. Journal of Food and Nutrition Research, *52,* 2013, pp. 181–190.
39. Tingey, D. T. – Manning, M. – Grothaus, L. C. – Burns, W. F.: Influence of light and temperature on monoterpene emission rates from slash pine. Plant Physiology, *65,* 1980, pp. 797–801. DOI: 10.1104/pp.65.5.797.
40. Farag-Zaied, S. E. A. – Aziz, N. H. – Ali, A. M.: Comparing effects of washing, thermal treatments and gamma irradiation on quality of spices. Nahrung, *40,* 1996, pp. 32–36. DOI: 10.1002/food.19960400109.
41. Richard, H. M. – Jennings, W. G.: Volatile composition of black pepper. Journal of Food Sciences, *36,*

1971, pp. 584–588. DOI: 10.1111/j.1365-2621.1971.tb15135.x.
42. Sádecká, J.: Influence of two sterilisation ways, gamma-irradiation and heat treatment, on the volatiles of black pepper (*Piper nigrum* L.). Czech Journal of Food Sciences, *28,* 2010, pp. 44–52.
43. Leizerson, S. – Shimoni, E.: Stability and sensory shelf-life of orange juice pasteurized by continuous ohmic heating. Journal of Agricultural and Food Chemistry, *53,* 2005, pp. 4012–4018. DOI: 10.1021/jf047857q.
44. Blank, I. – Fischer, K. H. – Grosch, W.: Intensive neutral odourants of linden honey. Differences from honeys of other botanical origin. Zeitschrift für Lebensmittel Untersuchung und Forschung, *189,* 1989, pp. 426–433.
45. Cserhalmi, Z. – Sass-Kiss, Á. – Tóth-Markus, M. – Lechner, N.: Study of pulsed electric field-treated citrus juices. Innovative Food Science and Emerging Technologies, *7,* 2006, pp. 49–54. DOI: 10.1016/j.ifset.2005.07.001.
46. Pandey, K. B. – Rizvi, S. I.: Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity, *2,* 2009, pp. 270–278. DOI: 10.4161/oxim.2.5.9498.
47. Cheynier, V.: Polyphenols in foods are more complex than often thought. The American Journal of Clinical Nutrition, *81,* 2005, pp. 223S–229S.
48. Krifi, B. – Metche, M.: Degradation of anthocyanins from blood orange juices. International Journal of Food Science and Technology, *35,* 2000, pp. 275–283. DOI: DOI: 10.1046/j.1365-2621.2000.00330.x.
49. Caminiti, I. M. – Noci, F. – Morgan, D. J. – Cronin, D. A. – Lyng, J. G.: The effect of pulsed electric fields, ultraviolet light or high intensity light pulses in combination with manothermosonication on selected physico-chemical and sensory attributes of an orange and carrot juice blend. Food and Bioproducts Processing, *90,* 2012, pp. 442–448. DOI: 10.1016/j.fbp.2011.11.006.
50. Bueno, F. G. – Machareth, M. A. D. – Panizzon, G. P. – Lopes, G. C. – Leite-Mello, E. V. S. – Mello, J. C. P.: Development of a UV/VIS spectrophotometric method for analysis of total polyphenols from *Caesalpinia peltophoroides* Benth. Química Nova, *35,* 2012, pp. 822–826. DOI: 10.1590/S0100-40422012000400031.
51. Cicco, N. – Lanorte, M. T. – Paraggio, M. – Viggiano, M. – Lattanzio, V.: A reproducible, rapid and inexpensive Folin-Ciocalteu micro-method in determining phenolics of plant methanol extracts. Microchemical Journal, *91,* 2009, pp. 107–110. DOI: 10.1016/j.microc.2008.08.011.
52. Everette, J. D. – Bryant, Q. M. – Green, A. M. – Abbey, Y. A. – Wangila, G. W. – Walker, R. B.: Thorough study of reactivity of various compound classes toward the Folin-Ciocalteu reagent. Journal of Agricultural and Food Chemistry, *58,* 2010, pp. 8139–8144. DOI: 10.1021/jf1005935.
53. Burdurlu, H. S. – Koca, N. – Karadeniz, F.: Degradation of vitamin C in citrus juice concentrates during storage. Journal of Food Engineering, *74,* 2005, pp. 211–216. DOI: 10.1016/j.jfoodeng.2005.03.026.
54. Kabasakalis, V. – Siopidou, D. – Moshatou, E.: Ascorbic acid content of commercial fruit juices and its rate of loss upon storage. Food Chemistry, *70,* 2000, pp. 325–328. DOI: 10.1016/S0308-8146(00)00093-5.
55. Nagy, S. – Smoot, J. M.: Temperature and storage effects on percent retention and percent U.S. recommended dietary allowance of vitamin C in canned single-strength orange juice. Journal of Agriculture and Food Chemistry, *25,* 1977, pp. 135–138. DOI: 10.1021/jf60209a031.
56. Miguel, M. G. – Duarte, A. – Nunes, S. – Sustelo, V. – Martins, D. – Dandlen, S. A.: Ascorbic acid and flavanone glycosides in citrus: Relationship with antioxidant activity. Journal of Food, Agriculture and Environment, *7,* 2009, pp. 222–227.

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