

Impact of inert gas atmosphere application on the complex profile of commercial orange juice volatiles during four-month storage

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Summary

The effectiveness of three processing atmospheres to preserve the volatiles of orange juice with pulp during four-month shelf life was investigated over two years. Application of conventional “air” (O₂) and nitrogen (N₂) was evaluated in 2014, use of N₂ and carbon dioxide (CO₂) in 2015. Headspace-solid phase microextraction, gas chromatography-mass spectrometry, as well as gas chromatography coupled to flame ionization detection and olfactometry were used for analysis of the volatiles. Twenty-four odour-active compounds were detected in the orange juice, of which D-limonene, (Z)- β -ocimene, δ -3-carene, α -terpinolene[†], linalool, L-limonene[†] and decanal were the most characteristic components. None of inert atmospheres was able to inhibit completely the changes in the composition of volatiles during storage. However, these changes were not significant to the extent to lead to worsening of the overall flavour of juices. Contrarily, negative sensory changes, mainly generation of aldehydes, such as hexanal, nonanal, perillaldehyde and decanal, were observed for juices processed in O₂ atmosphere as early as in the second month of the shelf life, and they were getting worse gradually over the storage. Thus, production of juices under inert atmosphere of N₂ or CO₂ can protect their organoleptic quality from undesirable changes caused by oxidative load or acid-catalysed reactions.

Keywords

orange juice; inert atmosphere; aroma analysis; volatiles; solid phase microextraction; gas chromatography-mass spectrometry; gas chromatography-olfactometry

The flavour of fresh hand-squeezed orange juice is generally considered to be the most attractive one, and it is used as a reference standard against which all other types of juices are judged. However, sensory perception evoked by commercial orange juice can be quite different because individual stages of industrial processing result in some alterations in original fresh juice aroma [1–4].

During mechanical squeezing of oranges, larger amount of peel oil passes into the juice. As a consequence, higher concentrations of some dominant peel oil components, such as α -pinene,

β -myrcene, limonene, linalool, octanal, nonanal and decanal, were revealed in commercial orange juice and their contribution to its more terpene, bitter, peel-like aroma was proven [1]. Furthermore, the process of freezing/thawing of unpasteurized juice was found to lead to a nearly complete degradation of (Z)-3-hexenal, which is known as a key odorant causing fresh, green note in hand-squeezed orange juice [1].

After squeezing of oranges, the pulp is separated and the juice is concentrated to reduce costs of storage and transportation [2–4]. BRAT et al. [2, 3] reported that the volatile compounds associated

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with pulp and cloud from fresh orange juice represent about 80% of total volatiles. Therefore, enormous amount of aroma compounds can be removed from the juice during depulping [2, 3]. Finally, in the distributing countries, juice is reconstituted by diluting the concentrate with water and adding certain oil- or water-based flavours, deaerated, pasteurized and packed [2–4]. AVERBECK and SCHIEBERLE [4] evaluated the composition of freshly reconstituted orange juice, which was obtained by mixing three fractions: concentrate, aqueous phase and essential oil. In reconstituted juice, they found higher odour activity values (*OAVs*) for linalool, octanal, decanal, limonene, α -pinene and β -myrcene and, on the other hand, significantly lower *OAVs* for acetaldehyde, ethyl-2-methylbutanoate, ethyl butanoate and (*Z*)-3-hexenal as compared to the hand-squeezed juice investigated by the same methodology. Moreover, (*E*)- β -damascenone, dimethyl sulphide and carvone, which are proposed to be formed by thermal treatment and/or oxidation, respectively, were found only in the reconstituted juice [4].

Deaeration and pasteurization are essential steps of the juice-processing technology, used to improve the shelf-life and safety of final products. In addition to the impact of these operations on the nutritional value of orange juices, several studies dealt also with their effects on the juice aromatic fraction [5–8]. According to JORDÁN et al. [5, 6], the biggest changes in the content of volatile components occurred during deaeration, while the pasteurization process did not influence the composition of deaerated orange juice in a significant way. The effect of the deaeration on the components of juice was reflected by statistically significant decrease in the concentrations of most of alcohols and aldehydes as well as some terpenic hydrocarbons [5, 6]. In similarly oriented study of SADECKÁ et al. [7], a decrease mainly in the contents of terpene alcohols, esters, aldehydes, ketones and sesquiterpenes, together with an increase in the content of some volatile monoterpenes were observed after deaeration and pasteurization of orange juice. These changes were assigned to thermolability of compounds with sensitive double bonds in their molecules as well as to the formation of thermal isomerization products of some terpenes.

FARNWORTH et al. [8] compared the content of volatiles as well as other parameters of pasteurized and frozen unpasteurised orange juice after 2 and 9 months of storage. Concerning the unpasteurized juice, they found higher concentrations of acetaldehyde, ethyl acetate, α -terpineol, 1-hexanol, 3-hexen-1-ol, α -pinene, sabinene, β -myrcene

and limonene but, on the contrary, lower concentration of valencene. In addition, pasteurization significantly reduced the content of ascorbic acid and increased juice density, cloud and fructose levels. The authors concluded that unpasteurized orange juice, which is quickly frozen and kept frozen until use, may be more acceptable by the consumer, who is looking for a fresh squeezed juice rather than for pasteurized juice [8]. Therefore, alternatives to traditional thermal processing that do not involve direct heating were investigated in order to obtain safe products but with fresh-like quality attributes. Among these, high pressure (HP) and pulsed electric field (PEF) processing received attention. Nevertheless, the comparison of these novel methods with mild thermal treatment of orange juice demonstrated that when processing conditions are selected based on equivalent microbial safety, the impact of heat, HP and PEF pasteurization on the volatile profile of orange juice can be considered comparable [9].

Additional changes in juice composition can occur during its storage in retail chain. Numerous studies were focused on the influence of temperature, time, oxygen content, light exposure and packaging material on the organoleptic quality of orange juice [10–17]. At the same time, several compounds were found as important contributors to orange juice off-flavours formed during storage, which have been described as “aged” or “heated” [10–12].

Regarding the temperature, PETERSEN et al. [10] proved that storage at 5 °C, as compared with higher temperatures (20 °C), prevents both sensory changes and changes in concentration of aroma compounds during 9 months. Storage at ambient temperature led to an increase in concentrations of α - and β -terpineol, which are products of limonene breakdown and contribute to the oxidized aroma and bitterness of juice. In addition, the loss of linalool and octanal, positively contributing to the typical orange aroma, was observed at elevated temperature. Similarly, MOSHONAS et al. [11] observed a decrease in concentrations of 1-penten-3-one, hexanal, ethyl butyrate, octanal, neral and geranial, and an increase in concentrations of some undesirable components, such as furfural and α -terpineol, during storage of aseptically packed orange juice at 21 °C and 26 °C. The increase in contents of other well known off-flavour compounds, in particular dimethyl sulphide, 2-methoxy-4-vinylphenol, α -terpineol and 4-hydroxy-2,5-dimethyl-3(2H)-furanone, was revealed by AVERBECK and SCHIEBERLE [12] during forced (at 37 °C for 4 weeks) as well as normal (20 °C for 1 year) storage of orange juice reconstituted

from concentrate. Under both storage conditions, concentrations of α -terpineol and 4-hydroxy-2,5-dimethyl-3(2H)-furanone did not reach their odour perception thresholds, while the concentrations of dimethyl sulphide and 2-methoxy-4-vinylphenol clearly exceeded their odour perception thresholds, and so confirmed the crucial role of the latter odorants for the formation of undesirable “stale” off-flavour effect in stored orange juice from concentrate [12].

Some kinds of packaging materials can participate on the decrease of volatiles during storage through their absorption into the bulk of the packaging. MOSHONAS and SHAW [13] observed this phenomenon for laminated composite carton. BERLINET et al. [14] observed it also for plastic packaging materials (polyethylene terephthalate), although in their next study [15] they revealed that the larger permeation of aroma compounds took place through the cap of the bottle made from high-density polyethylene (HDPE), and the use of a multilayer cap (HDPE with internal barrier layer of low-density polyethylene) can considerably limit the permeation of aroma compounds, despite the use of a PET bottle [15]. Nevertheless, all these changes are mostly of minor importance compared to the modification of the juice composition that occurs due to the ongoing chemical reactions [14]. In majority of these reactions, oxygen can play an important role and thus barrier properties of the packaging material against oxygen diffusion from the surrounding environment is the next often studied parameter, which is supposed to affect the stability of juice during storage. However, in the study of BERLINET et al. [14], the same evolution in the content of volatiles was observed during the storage of orange juice, regardless of the type of packaging material (glass and three PET with different values of oxygen permeability). Used PET packaging materials and their related values of oxygen permeability showed no correlation with the loss of aroma compounds [14]. These findings were confirmed by BACIGALUPI et al. [16], who investigated sensitivity of orange juice to oxidation, when the standard PET or active PET bottles with oxygen scavengers were used. Contrary to ascorbic acid, degradation of which strongly correlated with the access of oxygen, the aroma changes were not relevant markers of oxygen ingress and permeability properties of the packaging, suggesting that the modification of the aroma profile during storage was caused mainly by acid-catalysed reactions and only to a lesser extent by oxidation. The role of acid-catalysed reactions in the degradation of juice during storage was supported also by another study [17],

in which the rise in pH from 3.2 up to 4.0 significantly reduced the concentrations of off-flavours furfural and α -terpineol during storage by 79 % and 65 %, respectively.

In view of all the above mentioned facts, it is obvious that thorough optimization of production and packaging process is necessary to maintain the high quality of the fruit juice during its shelf life, which can be up to several months. On the other hand, current market demands products with flavour as close as possible to the unpasteurized fresh hand-squeezed juices. This requirement justifies active development of new food technologies that would allow only minimal technological processing of raw juice but still warrant microbial safety of the product. However, a gentler treatment of raw juice can speed up oxidation of its components resulting in changes of organoleptic properties of juice or to formation of undesirable „off-flavour“ effects. One potential solution can be production and packaging of orange juices in inert atmosphere. Thus, the aim of this paper was to evaluate the influence of the application of nitrogen or carbon dioxide in the production of orange juice with pulp on its key aroma compounds during a 4-month shelf life.

MATERIALS AND METHODS

Samples and storage conditions

Samples of orange juice enriched with pulp were obtained from McCarter, Bratislava, Slovakia (production premises Dunajská Streda, Slovakia). This company imports raw unconcentrated juice in frozen state from several countries of origin, in this case from Costa Rica. After defreezing, juice was enriched with pulp, mixed, pasteurized at up to 95 °C during 20 s and filled aseptically into 200 ml polyethylene terephthalate (PET) bottles with oxygen scavengers. In the first year (2014), one series of samples from the same batch of raw juice was processed and packed under nitrogen atmosphere (N₂), and the second one was produced by usual technology in conventional “air” atmosphere (O₂, as a control). In the second year (2015), one series of samples was processed under nitrogen, and the second one under carbon dioxide (CO₂) atmosphere. Bottled samples were stored at (7 ± 1) °C in a 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. Analyses were performed within 24 h after delivery of samples to the laboratory and then on a monthly basis.

Tab. 1. Key odour-active compounds of orange juice with pulp revealed by HS-SPME coupled to GC-FID/O.

No.	LRI U1	Compound	Odour intensity during storage								Odour description	References		
			0 month		1 month		2 months		3 months				4 months	
			O ₂	N ₂	O ₂	N ₂	O ₂	N ₂	O ₂	N ₂			O ₂	N ₂
1	772.4	Hexanal	0.5	–	0.5	–	0.5	–	0.5	–	0.5	–	Nutty, slightly bitter	LRI, MS, ST, OD, LIT
2	782.9	Ethyl butanoate	1	1	1	1	1.5	1.5	1.5	1.5	1.5	2	Fruity, apple-like, sweet	LRI, MS, ST, OD, LIT
3	822.8	(E)-2-Hexenal	1	1	1	1	1	1	1	1	1	1	Green, leafy, apple pip-like, slightly bitter	LRI, MS, ST, OD, LIT
4	926.6	α-Pinene	1	1	–	–	0.5	0.5	–	–	0.5	–	Sharp, pine, terpenic	LRI, MS, ST, OD, LIT
5	979.1 + 981.8	Octanal + β-myrcene	1	–	1	–	1	0.5	1	0.5	1	1	Herbaceous, bitterish, terpenic, hop oil-like	LRI, MS, ST, OD, LIT
6	1001.3	δ-3-Carene	2	2	2	2	1	2	1	2	1	2	Turpentine-like, sweet citrus, sharp	LRI, MS, ST, OD, LIT
7	1005.9	α-Terpinene	0.5	–	0.5	–	0.5	0.5	0.5	0.5	0.5	0.5	Balsamic, herbaceous, marjoram-like	LRI, MS, ST, OD, LIT
8	1008.6	p-Cymene	1	–	1	–	1	1	1	1	1	1	Citrus-peel, fresh, weak fuel-like	LRI, MS, ST, OD, LIT
9	1018.1	D-Limonene	2	2	2	2	2	2	2	2	2	3	Citrus, terpenic, intensive citrus-peel odour	LRI, MS, ST, OD, LIT
10	1018.5	(Z)-β-Ocimene	2	2	2	2	2	2	2	2	2	3	Lime, green, sweet, lemon, orange	LRI, MS, ST, OD, LIT
11	1054.3	1-Octanol	1	1	1	1	1	1	1	1.5	1	1.5	Herbaceous, earthy, waxy	LRI, MS, ST, OD, LIT
12	–	α-Terpinolenet	2	2	2	2	2	2	2	2	2	2	Mushroom-like, plastic	MS, OD, LIT
13	1081.4	Nonanal	–	–	1	–	2	–	2	–	2	–	Soapy-fruity, waxy, tallowy	LRI, MS, ST, OD, LIT
14	1083	Linalool	2	2	2	2	2	2	2	2	2	2	Refreshing, floral, fragrant	LRI, MS, ST, OD, LIT
15	1102.2	Ethyl 3-hydroxyhexanoate	–	–	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	Smoky, leather, tobacco	LRI, MS, ST, OD, LIT
16	–	L-Limonenet	2	2	2	2	2	2	1	1	1	1	Intensive fresh floral, rose, sweet orange	OD, LIT
17	1157.1	Terpinen-4-ol	1	–	1	1.5	2	1.5	1	1	1	1	Earthy, woody, musty, waxy	LRI, MS, ST, OD, LIT
18	1180.3	Decanal	2	2	2	2	2	1	2	1	2	1	Orange peel-like, waxy	LRI, MS, ST, OD, LIT
19	–	Perillaldehydet	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5	Fresh, herbal, cumin, spicy	MS, OD, LIT
20	1285.3	Undecanal	0.5	0.5	0.5	0.5	1	1	1	1	1	1.5	Fatty with orange and rose undertone, waxy	LRI, MS, ST, OD, LIT
21	1360	Geranyl acetate	–	0.5	–	0.5	–	0.5	–	0.5	–	0.5	Fresh, green, lavender	LRI, MS, ST, OD, LIT
22	–	δ-Cadinenet	1	–	1	–	1	–	1	–	1	–	Thyme, slightly sweet, herbal, woody	LRI, MS, ST, OD, LIT
23	–	Unknowno	1	1	1	1	1	1	1	1	1	1	Pleasant, floral, slight fruit, conditioner-like	MS, OD, LIT
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Orange juice was produced under nitrogen (N₂) or in conventional “air” (O₂) atmosphere.

Compounds were identified on the basis of following criteria: LRI U1 – linear retention index measured on GC column Ultra 1, MS – mass spectrum, ST – comparison with the reference compound, OD – odour quality, LIT – literature reference. t – tentative identification (only on the basis of mass spectra), o – compound detected only by GC-FID/O.

Chemicals

All chemicals used as reference standards for identification purposes of volatiles (listed in Tab. 1) were gifts donated from Bedoukian Research (Danbury, Connecticut, USA), Graz University of Technology (Graz, Austria) or French National Institute for Agricultural Research (INRA) laboratories (Dijon, France).

Methods

Headspace-solid phase microextraction

The method was selected for the purpose of extraction of orange juice overall volatile fractions containing aroma-forming compounds. Each sample of 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 a solid phase microextraction (SPME) fibre placed in the headspace of the sample. The SPME fibre with divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/Carboxen/PDMS) film (2 cm), film thickness 50/30 µm, "For odours" (Supelco, Bellefonte, Pennsylvania, USA) was used. The fibre was initially conditioned by heating in the injector block of gas chromatograph at 270 °C for 1 h. Headspace-solid phase microextraction (HS-SPME) samples were desorbed at 250 °C in the injector block of the gas chromatograph during the entire GC analysis.

Gas chromatography-mass spectrometry

Complex mixtures of volatile compounds extracted by HS-SPME were analysed by gas chromatography-mass spectrometry (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. 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. Ion source operated at a temperature of 230 °C and quadrupole at a temperature of 150 °C. Ionization voltage (EI) was set to 70 eV.

Gas chromatography-olfactometry

In parallel with GC-MS, HS-SPME extracts of volatiles were separated and analysed by gas chromatography coupled to flame ionization detection and olfactometry (GC-FID/O) using the detection frequency concept of posterior evaluation of odour quality and odour intensity of individual odorants, according to the modified proce-

dures of JANÁČOVÁ et al. [18]. A sniffing procedure panel was formed from 5 judges (2 men, 3 women, aged 26–61 years), who were chosen from 11 assessors trained in sensory evaluation. Results of GC-FID/O analyses were expressed as average values of estimated 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 ± 0.5 was considered as measurement deviation.

For the performance of these analyses, the gas chromatograph Agilent 7890A (Agilent Technologies) was coupled to flame ionization detector (FID) and an olfactory detector port (ODP) ODP3 (Gerstel, Mülheim an der Ruhr, Germany). The capillary GC column was 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 the linear velocity of 44.6 cm·s⁻¹ (measured at column temperature 143 °C). Splitless injection mode was used at injector temperature of 250 °C. For GC-FID/O experiments, the effluent of the column was splitted with a split ratio of 1:1 to FID and the olfactory (sniffing) port ODP with addition of humidified air to protect the nose epithelium from dehydration. FID temperature was set to 250 °C. 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. The linear retention indices (LRI) were calculated using the equation of VAN DEN DOOL and KRATZ [19] and standard mixture of *n*-alkanes C₇–C₁₄ was used as reference. 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 additionally by comparison of measured 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 percentage. Given values are means of three replicates.

Statistical analysis

Statistical calculations were performed by means of Unistat v. 6.0 (Unistat, London, United Kingdom) statistical package. ANOVA and multivariate statistics were used to compare, explore and discriminate the GC-MS data on the relative content of individual volatiles. Multiple comparisons were performed by ANOVA Tukey's honestly significant difference (HSD) test at the 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 inert gas application. Using CDA, the recognition ability was calculated as the percentage of correctly classified samples in the original data set. For this purpose, the designed classification model was tested by applying it to the test data with known target values, which came from the same data set as records used to build the model and comparing the predicted values with the known values.

RESULTS AND DISCUSSION

GC-MS study of orange juices produced in 2014 and 2015

As described in Materials and methods section, two series of commercially produced orange juices with pulp were analysed in 2014 (processing under conventional „air“ atmosphere and nitrogen atmosphere) and two series in 2015 (processing under nitrogen atmosphere and carbon dioxide atmosphere). This approach was chosen because the production company introduced the new technology including inert gas application step by step during two years and, thus, it was not able to produce juices from the same batch of raw juice under three different atmospheres at the same time. Therefore, impact of the year of production on the quality of provided samples was significant, despite the fact that country of raw orange juice origin (Costa Rica) remained the same in both years.

Regardless of the used atmosphere, orange juice produced in 2015 had richer aroma. In total, 54 compounds of various chemical nature were separated and identified by GC-MS in the volatile fraction of the orange juice produced in 2015, in contrast to 35 volatiles identified in orange juice produced in 2014. Significant variability between the two production years is obvious from results

of PCA. Plot of principal components (Fig. 1) clearly indicates the existence of 2 differentiated groups of eigenvectors belonging to the samples from year 2014 and 2015. As regards the results of PCA, the first principal component described almost 69% of the total dataset variability and the first and second principal component described together 83% of the total variability.

Nevertheless, the compounds with the highest relative content were almost the same for both series of juices. From the most abundant terpene group, D-limonene was dominant (relative content being greater than 90 %), followed by β -myrcene, valencene, α -pinene, γ -terpinene, α -terpinolene, δ -3-carene and terpenic alcohols such as linalool, α -terpineol and terpinen-4-ol. Concerning the other chemical groups, decanal, octanal, nonanal, 1-octanol and ethyl butanoate were present in higher amounts in the juices produced in both years. Except for δ -cadinene and 4,11-selinadiene as well as trace amount of undecanal, all other volatiles identified in the juice from production year 2014 were present also in the profile of the juice produced in 2015. In addition to them, in the juice from 2015, compounds such as 6-methyl-5-heptenone, nerol, geraniol, dodecanal, β -caryophyllene, (*Z*)-carveol, 4-acetylanisole and

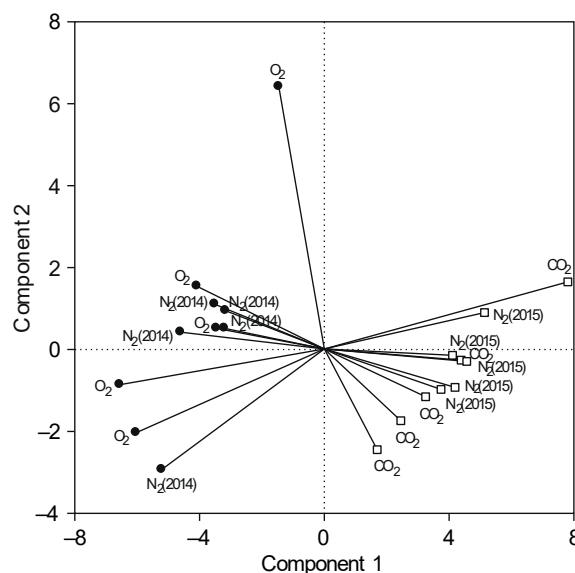


Fig. 1. Plot of principal components demonstrating differentiation of orange juices with pulp processed under different atmospheres over two years.

O₂ – conventional „air“ atmosphere; N₂(2014) – nitrogen atmosphere, year of production 2014; N₂(2015) – nitrogen atmosphere, year of production 2015; CO₂ – carbon dioxide atmosphere.

Plot of principal components was constructed on the basis of relative contents of individual volatile compounds determined by GC-MS.

aromadendrene were also significantly present. The above mentioned data on the presence of individual volatiles in orange juice obtained by GC-MS, were in good accordance with our previously published paper [7] that dealt with the effect of pasteurization and storage on the orange juice volatiles, as well as with other published data from different authors [20–22].

In the next step of study, the effect of four-month storage on the orange juice volatiles was investigated. During storage in 2014 as well as in 2015, continuous increase in contents of some monoterpenes, e.g. α -pinene, β -myrcene, α -phellandrene, and of sesquiterpene valencene in all three tested atmospheres was observed. A slight increase was observed also for δ -3-carene, γ -terpinene in “air” as well as nitrogen atmospheres, but only during storage in 2014, whereas in the next year, the contents of these compounds remained almost unchanged during the storage experiment. Several volatiles showed some increase in their contents only under conventional “air” processing atmosphere, mainly terpenic alcohols such as α - and β -terpineol, terpinen-4-ol and linalool, and from the other chemical groups 1-octanol and ethyl butanoate, and also some aldehydes such as hexanal, 2-furaldehyde and octanal. The increase of the relative contents of these compounds was the most significant after three months of storage. ANOVA Tukey’s HSD analysis of the GC-MS data confirmed the effects of storage on compositional changes of orange juices for all of the four sets of samples (three atmospheres). As regards concentration changes, the differences between fresh samples and samples stored for three and four months were found to be statistically significant ($p < 0.05$) for 14 compounds, namely, ethyl butanoate, α -pinene + benzaldehyde, octanal + β -myrcene, p -cymene, D-limonene, 1-octanol, p -cymenene, nonanal, decanal, octyl acetate, carvone and perillaldehyde. Subsequent differentiation of samples according to the storage duration by CDA classified samples into groups on the basis of composition with 100% correctness. As it can be seen from the plot of discriminant functions (Fig. 2), changes in orange juice composition occurred already in the first month of storage and continued during the entire monitored period. The discriminant scores of individual groups were different and, as a result, the existence of five groups of points belonging to the samples stored for four months is obvious from the plot of discriminant functions.

In the next step, differentiation of samples according to the used production atmosphere was proven. Once again, CDA showed strong relation-

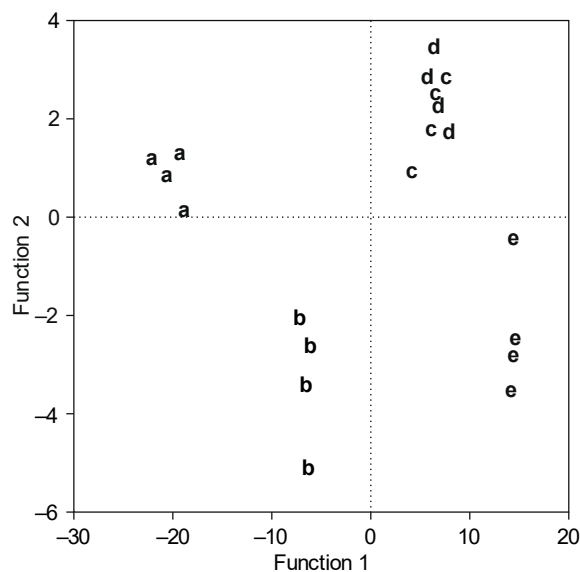


Fig. 2. Discrimination of orange juice samples, irrespective of used production atmosphere, using the time of storage as a discriminating criterion.

a – fresh samples (stored 0 month); b, c, d, e – samples stored for 1, 2, 3 and 4 months, respectively. Plot of discriminant functions was constructed on the basis of relative contents of individual volatile compounds determined by GC-MS.

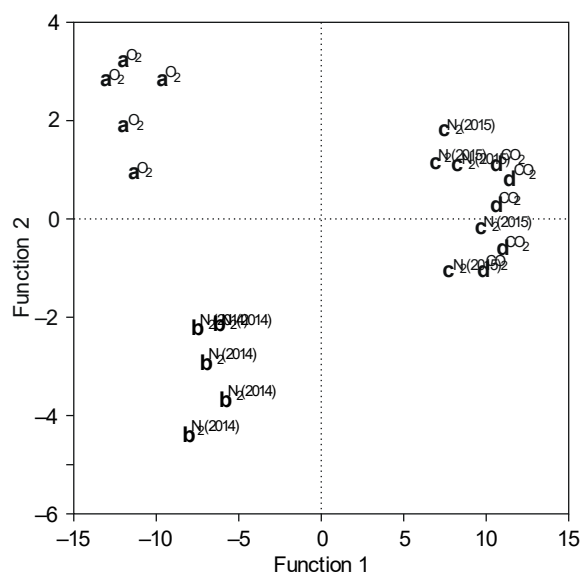


Fig. 3. Discrimination of orange juice samples using the production atmosphere as a discriminating criterion.

aO₂ – conventional “air” atmosphere; bN₂(2014) – nitrogen atmosphere, year of production 2014; cN₂(2015) – nitrogen atmosphere, year of production 2015; dCO₂ – carbon dioxide atmosphere.

Plot of discriminant functions was constructed on the basis of relative contents of individual volatile compounds determined by GC-MS.

ship between the composition of orange juice and year of its production. It is evident from the plot of discriminant functions (Fig. 3), where two opposite groups were formed belonging to the samples produced in 2014 and 2015. Nevertheless, within the samples from 2014, two smaller groups belonging to the samples produced under conventional or nitrogen atmosphere were separated. This indicated that the use of these two atmospheres had different effects on the composition of orange juice volatiles. On the contrary, samples produced under nitrogen and carbon dioxide atmosphere in 2015 were not separated, suggesting that juices produced under nitrogen were closer to the juices produced under carbon dioxide by their composition of volatiles. However, correctness of classification into groups on the basis of compositional analysis for samples produced under carbon dioxide atmosphere was only 60 %, while samples produced under other atmospheres were classified into groups with 100% correctness. Ultimately, the use of nitrogen or carbon dioxide as production atmospheres seems to have very similar effects on volatiles of orange juice during the storage.

The observed deterioration of organoleptic properties of juices produced in conventional atmosphere and, on the contrary, stable sensory character of juices produced under nitrogen or carbon dioxide during the entire monitored storage period, correlated with above mentioned results and indicated that application of inert gases could have protective effect on the aroma of orange juice with pulp.

GC-FID/O study of orange juices produced in 2014

In order to detect and identify volatiles that can be responsible for the sensory differences observed between juices processed in inert nitrogen and conventional „air“ atmosphere during the storage, as well as to reveal potential off-flavour compounds causing negative changes in the aroma of juices processed in conventional „air“ atmosphere, samples from the year 2014 were analysed also by GC-FID/O.

In general, 24 odour-active compounds were detected in the orange juice irrespective of used atmosphere (Tab. 1). However, only 23 olfactory responses were recorded, due to the overlap between odours in one case (octanal + β -myrcene). Nineteen compounds were identified by a combination of independent methods as indicated in Materials and Methods section. In four cases (α -terpinolene, L-limonene, perillaldehyde, δ -cadinene), only partial information was available and, thus, only tentative identification was possible. One compound remained unidentified

at this stage, because it was detected only by GC-olfactometry. In the future research, the unknown or tentatively identified odour-active compounds should be further investigated by determination of their LRI on a GC column with a stationary phase of different polarity.

The overall aroma of analysed samples of juice was found to be formed by the following volatile odour-active components: ten terpenes (α -pinene, β -myrcene, δ -carene, α -terpinene, *p*-cymene, D-limonene, (*Z*)- β -ocimene, α -terpinolene^t, L-limonene^t, δ -cadinene^t), seven aldehydes (hexanal, (*E*)-2-hexenal, octanal, nonanal, decanal, perillaldehyde, undecanal), three alcohols (1-octanol, linalool, terpinen-4-ol), three esters (ethyl butanoate, ethyl 3-hydroxyhexanoate, geranyl acetate), and one unknown compound.

From the above mentioned compounds, D-limonene, (*Z*)- β -ocimene, δ -3-carene, α -terpinolene^t, linalool, L-limonene^t and decanal were principal odour-active compounds in the volatile fraction of orange juice. They contributed with their high odour intensity (from 2 to 3) to the overall odour of orange juice to a decisive degree and, thus, they were the most characteristic components of its odour. Several studies were focused on the GC-O characterization of volatile fraction of orange juice, in which different compounds were identified as most sensorially important. TØNDER et al. [22] identified ethyl butanoate, β -pinene, limonene, octanal and linalool to be the most important odourants in fresh and stored orange juice by the calculation of aroma values, as well as GC-sniffing technique called GC Odour Profiling. AVERBECK and SCHIEBERLE [4] revealed linalool, limonene, ethyl 2-methylbutanoate, octanal, α -pinene, β -myrcene, acetaldehyde, decanal and β -damascenone as volatiles with the highest odour activity values in the aroma of freshly reconstituted orange juice from concentrate. In a comparative study of different orange varieties, ARENA et al. [21] observed the highest frequency of odour detection for ethyl butanoate, α -pinene and β -myrcene in juices from blond orange varieties. Comparison of our above mentioned findings and results presented in these studies showed some differences. However, these could be explained by different ways of orange juice production, fruit varieties and techniques used to collect and process GC-O data.

In our study, as regards the changes in odour intensity during the four-month storage, there were compounds with stable odour such as (*E*)-2-hexenal, D-limonene, (*Z*)- β -ocimene, α -terpinolene^t, linalool, perillaldehyde and the unknown compound No. 23, intensities of which

remained unchanged in both atmospheres. An increase in odour intensities during the storage was observed for ethyl butanoate (from 1 to 1.5 in conventional, and from 1 to 2 in nitrogen atmosphere) and undecanal (from 0.5 to 1.5 in both atmospheres). For 1-octanol and β -myrcene + octanal, an increase was noticed only in nitrogen atmosphere (from 1 to 1.5 for 1-octanol, and from 0 to 1 for β -myrcene + octanal), whereas concerning the conventional atmosphere, their odour intensities were stable with a value of 1 during the entire storage period. For nonanal, the increase of odour intensity was noticed only in conventional atmosphere (from 0 to 2), whereas in nitrogen atmosphere, this compound was not sensorially active. In contrast, a decreasing trend in odour intensities was observed for δ -3-carene (from 2 to 1) and decanal (from 2 to 1) in nitrogen atmosphere, and for L-limonene^t (from 2 to 1) in both processing atmospheres. Most of the above mentioned changes took place after the second month of storage, which is in good accordance with our previously published results concerning the effect of storage on orange juice with pulp packed in atmosphere with a content of oxygen [7]. In addition, the time when first significant changes in odour intensities took place correlated with the previously discussed statistically processed results of GC-MS analyses of these series of juices.

On the basis of GC-MS results, mainly terpenic alcohols α -terpineol and terpinen-4-ol were supposed to be potential off-flavours, because of the significant increase in their relative contents only in conventional atmosphere. These compounds are degradation products of limonene and linalool. An increase in α -terpineol concentration during storage of orange juice was previously reported also in other studies [10–12, 16], indicating that this compound can represent a typical off-flavour compound in stored orange juice. However, in our study, despite its higher relative content revealed by GC-MS, α -terpineol was not detected by GC-FID/O in the same samples of orange juices. It implies that its respective concentration level did not achieve nasal odour threshold, and its role as off-flavour in orange juice was not confirmed in our study. Similarly, BACIGALUPI et al. [16] in a GC-MS study observed a significant increase in the concentration of α -terpineol during a six-month storage irrespective of storage conditions or the packaging material and, despite that, its final concentration was not higher than the reported odour detection threshold. Similar observations for α -terpineol were reported also by BERLINET et al. [14] and AVERBECK and SCHIEBERLE [12].

Regarding terpinen-4-ol (with musty, waxy,

earthy, woody odour description), it was sensory active, but with the same odour intensity in all investigated atmospheres and no increasing trend was observed for this compound during the storage. Similarly, α -terpinolene (mushroom, plastic odour) was detected in all samples, but its odour intensity was stable during the storage time, irrespective of used atmosphere. The increase in the concentration levels revealed by GC-MS mainly for terpinen-4-ol and, to a lesser extent, for α -terpinolene in contrast to their stable odour intensities, can be explained by the specific features of GC-FID/O, e.g. different psychophysical trend, selectivity of human nose and non-linear responses for various kinds of odour-active compounds, which could cause that the observed concentration changes did not manifest also in a sensory way.

Concerning the other compounds considered as typical off-flavours, furaldehyde, which is created as a degradation product of ascorbic acid [16], was detected by GC-MS in our study, but its relative content was very low and did not exceed the concentration threshold, which is needed for detection of furaldehyde also by GC-FID/O method. The increase in the furaldehyde level was described previously by MOSHONAS et al. [11, as well as by BACIGALUPI et al. [16], but these authors also observed only low concentrations and, moreover, in the latter study, the initial increase of furaldehyde was followed by its slight decrease. These observations can be explained by the fact, that furaldehyde represents only an intermediate product, which can be also degraded as quickly as it is formed [16].

Despite the fact that results of our GC-FID/O analyses did not confirm the importance of the above mentioned typical orange juice off-flavour compounds, with regard to the evaluation of the efficiency of investigated atmospheres to protection of orange juice volatiles, it should be emphasized that generation of some aldehydes as typical oxidation products was observed predominately in juices processed/packed under the conventional “air” atmosphere. Above all, hexanal (green, nutty and bitter odour), which is a typical indicator of the off-flavour phenomenon in various foods, and also nonanal (soapy, waxy, tallow-like odour) were detected by GC-FID/O only in samples produced in conventional atmosphere. Perillaldehyde (smoked, cumin, spicy odour) was noticed in both atmospheres without any change during the storage, but in conventional atmosphere it showed higher odour intensity. Intensity of decanal (orange peel-like, waxy odour) remained considerably high for the entire storage period in conventional atmosphere, whereas in nitrogen

atmosphere, it dropped after two months. Only undecanal (fatty, citrus, aldehydic, waxy odour) showed an increasing trend in both atmospheres. Concerning the observed changes in odour intensities of some aldehydes, they can explain worsening in the organoleptic properties of orange juice processed/packed in conventional atmosphere that occurred during second month and continued until the end of the storage. Mentioned worsening of organoleptic properties manifested itself mainly in increased bitter and astringent taste of juice, a certain loss of freshness and fruity sweetness, and undesirable colour changes. In contrast, orange juice processed and packed in nitrogen atmosphere showed standard organoleptic properties comparable to the fresh product during the entire storage period.

CONCLUSIONS

Statistical processing of GC-MS data proved that use of nitrogen atmosphere in the production of orange juice had different effect on the composition of its volatile fraction during 4-month storage than technological processing of the identical juice in the conventional “air” atmosphere. Effectiveness of the application of carbon dioxide as inert atmosphere was comparable with application of nitrogen, but its acceptability by consumers has to be considered because of the sparkling character of final products. None of the investigated inert atmospheres was able to avoid all changes in the composition of volatiles during 4-month storage. However, GC-FID/O analysis carried out in parallel with GC-MS proved that the changes taking place in juices processed in any inert atmosphere were not sensorially significant, and they did not lead to noticeable deterioration of organoleptic properties of juices. On the contrary, negative sensory changes in flavour were observed for juices processed in conventional “air” atmosphere. GC-FID/O analyses revealed that generating some aldehydes, as a consequence of oxidative changes, could be responsible for the off-flavour phenomenon occurred during the storage time.

Acknowledgements

This study was supported by research projects “Quality and authenticity of fruit juices - study of relationships between the origin of feedstock, processing technology and quality of fruit juices” (APVV-15-0023) and “Improvement of nutritional and sensorial parameters of fruity and vegetable drinks via an inert gases application (ITMS 26220220175, Research and

Development Operational Programme funded by the European Regional Development Fund).

A part of the used instrumental equipment was obtained by implementing the project „Establishment of the hi-tech centre for research on formation, elimination and assessment of contaminants in foods” (ITMS 26240120041, Research and Development Operational Programme funded by the European Regional Development Fund).

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Received 3 February 2018; 1st revised 11 May 2018; accepted 1 June 2018; published online 20 September 2018.