Evaluation of aroma restoration of apple and orange juices from concentrates in the Czech Republic

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Summary

According to the requirements of the European legislation, fruit juice from concentrate must display organoleptic and analytical characteristics equivalent to those of an average direct juice. The evaporation during the concentration process, the thermal treatment and aroma restoration step are critical factors that may contribute to aroma loss or deterioration. The aim of the work was to evaluate the Czech juice market from the point of proper aroma restoration. The results of the solid-phase microextraction/gas chromatography-mass spectrometry measurements of the impact of apple and orange aroma components, their sums and aroma indexes, were correlated with basic quantitative parameters (soluble solids, relative density, colour) and sensory evaluations. For the apple juices, the sum of esters, carbon 6-aldehydes and alcohols, and the index calculated thereof correlated well with the hedonic sensory evaluation. In contrast for the orange juices, any direct correlation of the hedonic scores or the olfaction intensity to the concentrations of any individual aroma compound or their sums was not observed. To summarize the situation on the market, orange and apple juices have in about 50% cases lower values of typical esters, aldehydes, alcohols and terpenes than is mentioned in the literature or would correspond to complete aroma restoration.

Keywords

aroma restoration; apple; orange; juice; SPME/GC/MS; volatiles

Flavour assessment is important for quality and authenticity controls of fruit juices. In central Europe, the most often consumed juices are apple and orange juices made from concentrates. According to EC Regulation (Council Directive 2001/112/EC), fruit juice from a concentrate is defined as the product obtained by replacing in the concentrated fruit juice water extracted from that juice during concentration, and restoring the aromas, but recovered during the process of producing the fruit juice [1]. The product thus obtained must display organoleptic and analytical characteristics at least equivalent to those of an average type of juice obtained from the fruit of the same kind. The manufacturer is therefore obliged to restore all reconstituted juice aroma separated from the juice on evaporators during the stage of pre-concentration. However, with regard to the cost of aromas and their possible application in other industrial fields, some unscrupulous or unaware manufacturers of final products do not return this fraction at all or fail to perform the restoration properly, not reaching the proper recombination of the water phase of aromas and the water–insoluble (essential oils) phase of aromas.

In order to ensure and control adequate restoration of fruit juices from concentrate regarding flavours, numerous analytical approaches focusing on aroma-active compounds have been developed in addition to the already established sensorial test. Various models for quantitative evaluation of fruit juice aromas have been suggested. All of them are based on quantification of several aroma components typical of fruit juice or the indexes calculated thereof [2, 3].

In the case of apple juice, organic acid esters, as well as carbon-6-aldehydes and alcohols are considered to contribute most to the apple aroma.

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Tab. 1. The most abundant flavour-active volatiles in apple juice.

Compounds	Flavour	Concentration range [$\mu g \cdot l^{-1}$]				Odour threshold value in water [$\mu g \cdot l^{-1}$] (Wolter et al.) [3]	Standardization factor for Aroma index (Wolter et al.) [3]
		Wolter et al. [3] (n = 59)	Nikfardjam and Maier [6] (n = 85)	Gasperi et al. [7] (n = 4)	Komthong et al. [8] (n = 2)		
2-Methyl-1-butanol	roasted, wine, fruity	158 – 4591	538 – 3398	n.d.	n.d.	500	0.033
Ethyl butanoate	ethereal, fruity, buttery, ripe fruit notes	2 – 183	24 – 403	n.d.	170 – 190	0.76	0.500
Hexanal	strong, penetrating, fatty-green, grassy, unripe fruit	2 – 356	94 – 781	54 – 86	70 – 270	2.4	0.100
Butyl acetate	fruity-banana, ethereal, yeasty	1 – 683	41 – 1144	18 – 31	1370 – 1420	66	0.100
Ethyl-2-methylbutanoate	strong, green, fruity, apple-strawberry	1 – 119	18 – 195	n.d.	n.d.	0.13	2.000
E-2-Hexenal	green, fruity, fresh, apple, woody, leafy, grassy	0 – 2968	111 – 1520	12 – 52	160 – 800	110	0.025
E-2-Hexenol	fruity, green, leafy	5 – 2968	n.d.	6 – 8	n.d.	75	0.050
Hexan-1-ol	chemical, winey, slight fatty-fruity	233 – 9374	303 – 4289	60 – 65	1850 – 2100	500	0.017
2-Methyl butylacetate	ethereal, rum-like, fermented, fruity, banana, juicy fruit	0 – 432	30 – 469	0 – 1	170 – 180	5	0.250
Hexyl acetate	sweet-fruity	0 – 163	37 – 851	6 – 21	4040 – 4340	2	0.333

Standardization factor for Aroma index is generated by dividing the weighting (w_i) in percent by the reference value (rv_i). In the case of 2-methyl-1-butanol weighting is 10%, referenced value is 300 $\mu g \cdot l^{-1}$, and standardization factor is 0.033.
n.d. – not detected

Tab. 2. The most abundant flavour-active volatiles in orange juice.

Compounds	Flavour	Concentration range [$\mu g \cdot l^{-1}$]			Odour threshold value in water [$\mu g \cdot l^{-1}$] (Averbeck and Schieberle) [10]	FD factor (Averbeck and Schieberle) [10]
		Averbeck and Schieberle [10] (n = 4)	Averbeck [11] (n = 15)	Seideneck and Schieberle [12] (n = 6)		
Ethyl butanoate	key driver of orange flavour; sweet, fruity	220 – 351	147 – 283	237 – 680	1	2048
R-Limonene	citrus-like, main component of orange oils; little flavour, adds lift	108000 – 163000	90800 – 137000	86400 – 375000	34	32
Linalool	most abundant oxygenate in orange oils; sweet, floral	1360 – 1830	179 – 1590	132 – 1890	0.22	2048
Octanal	adds fresh character; fatty, green, melon-like	211 – 567	33 – 373	54 – 373	0.8	512

FD factor – flavour dilution factor (represents the last dilution of aroma etract dilution in which the odorant was still detectable).

To set some limits for evaluation of a properly restored apple aroma, HEIL et al. [2] suggested the minimum concentration of the sum of esters, which are important for the typical fruity aroma of apple juice, of 150 $\mu g \cdot l^{-1}$ (excluding butyl acetate). The authors also discussed monitoring the concentration of carbon-6-aldehydes and alcohols, which are important contributors to the green flavour. A disadvantage of these two sum parameters is that some substances, which occur in low concentrations but contribute significantly to the overall aroma, may be discriminated [4]. This fact was considered while developing the so-called Aroma index by WOLTER et al. [5], which is a model based on reference values for ten key aroma substances obtained on the basis of a comprehensive data analysis (627 apple juices collected in 2007). The Aroma index of a particular apple juice is composed of the sum of each aroma compound with its standardization factor (Tab. 1), which is derived from reference concentrations. The index value of at least 100 has to be reached for an appropriately re-aromatized juice made from a concentrate.

The aroma compounds that are believed to make a positive contribution to the orange aroma include methyl and ethyl butanoates, myrcene, octanal, decenal and linalool. In the case of orange juice made from a concentrate, mainly two fractions are considered: a) a group of esters (ethyl butanoate as the main component) and alcohols that are contained both in the orange oil and the water phase, and b) terpenes (most of all limonene) present in the orange oil phase.

So far, there has been no indication as to the optimum levels of concentrations for these compounds, except for limonene (150 000–220 000 $\mu g \cdot l^{-1}$, minimum 30 000 $\mu g \cdot l^{-1}$ according to JORDÁN [9]).

In the following tables (Tab. 1, 2), the most flavour-active volatile components, their typical concentrations and organoleptic characteristics are presented. However, in order to control adequate aroma restoration, not only natural variety and geographical variability has to be taken into consideration, but also some critical factors that may contribute to aroma loss or deterioration, such as thermal treatment during the juice concentration process, thermal treatment in the pasteurizing process and storage of the final product [10].

The aim of this work was to evaluate the products from the Czech juice market regarding proper aroma restoration. The results of chromatographic measurements of the impact aroma components, their sums and Aroma indexes, were correlated with the basic quantitative parameters and results of sensory evaluations.

MATERIALS AND METHODS

Materials

Fifteen different apple juices and 15 different orange juices were collected from various local stores. All samples were declared to have 100% fruit content and to be made from concentrates.

The standard compounds of 2-methyl-1-butanol (purity ≥ 99%), ethyl butanoate (≥ 98%), hexanal (≥ 98%), butyl acetate (≥ 98%), ethyl-2-methylbutanoate (≥ 99%), *E*-2-hexenal (≥ 98%), *E*-2-hexenol (≥ 96%), hexan-1-ol (≥ 99%), 2-methyl butylacetate (≥ 95%), hexyl acetate (≥ 99%), *R*-(+)-limonene (≥ 99%), linalool (≥ 95%), octanal (≥ 99%) and 3-octanol (≥ 98%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Sodium chloride p.a. was obtained from Penta (Prague, Czech Republic). Water purified by Milli-Q (Millipore, Milford, Massachusetts, USA) was used throughout the analyses.

Basic qualitative parameters

Soluble solids (°Brix) were determined using indirect method by refractometry [13]. Relative density was determined using a pycnometer method [14]. Colour properties (CIE $L^*a^*b^*$) were determined by the tristimulus reflectance measurement; the samples were poured into 25 ml ceramic crucibles and colour parameters were determined using Minolta CM-2600 d spectrophotometer (Konica Minolta, Tokyo, Japan).

Sensory evaluation

Sensory evaluation was performed by the total of 13 panellists from the Department of Food Preservation (Institute of Chemical Technology, Prague, Czech Republic). The assessors were selected, trained and monitored according to the relevant standard [15]. The performance was in compliance with the relevant international standard [16]. The assessors evaluated the overall quality of taste, appearance and odour using a 5-point system, where 1 was the best and 5 the worst rating (hedonic scoring). Evaluation of odour intensity of the characteristic aromas was performed on the following scale: 1 = high intensity, 2 = middle intensity, 3 = no characteristic aroma present.

Determination of volatile compounds

Solid phase microextraction (SPME) procedure was used. A polydimethylsiloxane fibre (100 μm; Supelco, Bellefonte, Pennsylvania, USA) was inserted into the headspace of a 10 ml vial filled with 1 ml of a sample, 4 ml of water, 3 g of NaCl and 10 μl of internal standard (0.1 mg of

3-octanol per litre in ethanol), which was agitated at 15 Hz. The optimized extraction conditions were 30 min at 25 °C.

Gas chromatography conditions: Sample analyses were performed using a 6890N gas chromatograph, equipped with a mass detector (MS 5973) and the DB-5MS column (30 m × 0.25 mm i.d. × 0.25 μm film thickness), all from Agilent Technologies (Santa Clara, California, USA). The split (1:10) GC inlet was maintained at 250 °C and desorption time of 2 min was used. Temperature programme was: initial temperature held at 60 °C for 2 min, then ramped at 10 °C·min^{-1} to 250 °C and held at 250 °C for 3 min. The detector (EI 70 eV, mass range 33–550 Da) temperature was 280 °C. The carrier gas (He) flow was 1.2 ml·min^{-1}. Internal calibration method was used for quantification.

Statistical analysis

The tests were done in triplicate for each sample and the mean values are reported. All statistical analysis were performed using Excel 2010 (Microsoft, Redmond, Washington, USA).

RESULTS AND DISCUSSION

SPME/GC/MS method parameters

The characteristics of the chromatographic method (linear range, coefficient of determination, limit of detection, limit of quantification, recovery, repeatability) are presented in Tab. 3. The validation parameters were determined on real samples (with a low concentration of natural volatiles) spiked by *R*-limonene at 50 000 μg·l^{-1} concentration level, and other standards at 500 μg·l^{-1} concentration level. *E*-2-hexenol and hexan-1-ol showed co-elution in the total ion chromatogram and complete peak separation was not achieved even using their respective qualitative masses (m/z 41, 57 and 82 for *E*-2-hexenol and m/z 43, 56 and 89 for hexan-1-ol). Because of a much higher concentration of hexan-1-ol in the apple juices, only its concentration was taken into consideration.

Basic characterization of samples

Basic physicochemical and sensory parameters of the 15 apple and the 15 orange juices from concentrates were determined (Tab. 4). The results showed that the total soluble solids ranged from 11.0 °Brix to 11.9 °Brix and the relative density from 1.045 g·cm^{-3} to 1.050 g·cm^{-3}, being within a proper range for authentic juices. CIE tristimulus values for the L^*, a^* and b^* values of the apple juices were 27.2 ± 1.3, 2.5 ± 0.4 and 2.7 ± 1.6, respectively, and for the orange juices 49.8 ± 4.7, –0.7 ± 1.1 and 19.6 ± 3.9, respectively.

The hedonic scores ranged from 1.8 (good) to 5.0 (poor). The odour intensity of the characteristic fruit aroma ranged from 1.0 (high) to 2.0 (middle). The apple juices showed greater variance in hedonic values than the orange juices.

Volatile components

More than 30 volatiles were identified in the apple juices in agreement with literature [6, 8], from which, according to recent research [5], ten key aroma compounds of apple juice were used

Tab. 3. Working characteristics of the HS-SPME/GC/MS method.

Matrix	Compounds	Linear range [μg·l^{-1}]	R^2	*LOD* [μg·l^{-1}]	*LOQ* [μg·l^{-1}]	Recovery [%]	Repeatability *RSD* [%]
Apple juice	Ethyl butanoate	10.0 – 2500	0.993	3.2	10.3	88.2	2.4
	Butyl acetate	11.0 – 2600	0.999	4.2	12.4	92.3	3.2
	Hexanal	15.0 – 2600	0.994	7.5	21.3	92.3	4.3
	2-Methyl butylacetate	12.0 – 2400	0.997	5.3	15.4	94.5	5.5
	Hexyl acetate	13.0 – 2400	0.996	5.1	14.9	96.7	2.3
	2-Methyl-1-butanol	15.0 – 2500	0.998	5.0	16.2	91.3	3.2
	Ethyl-2-methylbutanoate	10.0 – 2200	0.992	3.7	9.8	98.9	2.8
	E-2-Hexenal	15.0 – 2500	0.997	5.1	17.3	98.3	3.6
	Hexan-1-ol	8.0 – 2300	0.989	3.4	9.5	91.3	2.9
Orange juice	Octanal	10.0 – 2200	0.999	3.2	10.3	89.3	3.5
	R-Limonen	5000 – 200000	0.991	1670	5010	99.6	2.9
	Linalool	15.0 – 2500	0.994	5.2	17.8	97.6	3.1

R^2 – coefficient of determination, *LOD* – limit of detection, *LOQ* – limit of quantification, *RSD* – relative standard deviation.

Tab. 4. Basic physicochemical and sensory parameters of apple and orange juices.

		Soluble solids [°Brix]	Relative density [g·cm-3]	*L**	*a**	*b**	Price per litre [EUR]	Hedonic scoring	Intensity of characteristic aroma
Apple juice	Average	11.5	1.048	27.2	2.5	2.7	1.1	3.3	1.6
	Min.	11.1	1.046	25.0	1.7	0.7	0.6	1.8	1.0
	Max.	11.9	1.050	28.6	3.0	5.2	1.6	5.0	1.9
Orange juice	Average	11.6	1.047	49.8	–0.7	19.6	1.2	3.3	1.6
	Min.	11.4	1.045	41.8	–1.9	12.4	0.6	2.0	1.1
	Max.	11.9	1.048	54.3	1.0	23.6	1.6	4.0	2.0

for evaluating the restored aroma. Concentrations of these compounds were highly variable (Tab. 5). Hexanal, butyl acetate, *E*-2-hexenal and 2-methyl butylacetate were found to be the most abundant components in almost all apple juice samples. Contrary to that, two samples did not contain any volatile compounds (level under the detection limit of the monitored key volatile compounds). Further, the Aroma index of two samples was slightly above the proposed limit of 100 (139 and 143), the mean value of Aroma index being 251 and the maximum value 494.

The obtained results were compared with the critical limits and indexes of certain components of the apple aroma given in literature. The correctness and sufficiency of aroma restoration was assessed according to a) concentration of the sum of esters, ethyl butanoate, ethyl-2-methylbutanoate, 2-methyl butylacetate and hexyl acetate for which HEIL and ARA [2] suggested the minimal concen-

Tab. 5. Descriptive statistics of the analyses of volatile components of apple juices ($n = 15$).

Compound [µg·l-1]	Mean	Median	Minimum	Maximum
2-Methyl-1-butanol	50	n.d.	n.d.	200
Etyl butanoate	127	129	n.d.	368
Hexanal	239	186	n.d.	648
Butyl acetate	599	681	n.d.	1 444
Ethyl-2-methylbutanoate	9	10	n.d.	20
E-2-Hexenal	316	320	n.d.	580
Hexan-1-ol + *E*-2-Hexenol	144	150	n.d.	325
2-Methyl butylacetate	165	178	n.d.	336
Hexyl acetate	102	89	n.d.	223
Sum of esters	403	433	n.d.	832
Sum of carbon 6-aldehydes	699	569	0	1 493
Aroma index	251	266	0	494
Sum of key aroma compounds	1 751	1 590	0	3 233

n.d. – not detected.

Tab. 6. Descriptive statistics of the analyses of volatile components of the orange juices ($n = 15$).

Compound [µg·l-1]	Mean	Median	Minimum	Maximum
Etyl butanoate	95	n.d.	n.d.	473
R-Limonene	96 732	97 815	59 517	126 681
Linalool	658	431	130	1 581
Octanal	146	47	0	455
Sum of key aroma compounds	899	609	170	1 878

n.d. – not detected

Tab. 7. Correlation between the results of the sensory evaluation and the qualitative parameters of the apple juices ($n = 15$).

	Soluble solids	Relative density	*L**	*a**	*b**	Price	Hedonic scoring	Intensity of characteristic aroma	Sum of esters	Sum of carbon 6-aldehydes and alcohols	Aroma index	Sum of key aroma compounds
Soluble solids	1.00											
Relative density	0.80[a]	1.00										
*L**	–0.47	–0.55[a]	1.00									
*a**	–0.72[a]	–0.65[a]	0.44	1.00								
*b**	–0.66[a]	–0.50[a]	0.75[a]	0.56[a]	1.00							
Price	–0.73[a]	–0.47	0.16	0.36	0.23	1.00						
Hedonic scoring	0.67[a]	0.58[a]	–0.03	–0.78[a]	–0.36	–0.51[a]	1.00					
Intensity of characteristic aroma	0.03	0.10	–0.66[a]	0.28	–0.38	0.13	–0.65[a]	1.00				
Sum of esters	–0.47	–0.22	–0.13	0.48	0.18	0.58[a]	–0.74[a]	0.63[a]	1.00			
Sum of carbon 6- aldehydes and alcohols	–0.76[a]	–0.62[a]	0.07	0.64[a]	0.33	0.74[a]	–0.88[a]	0.49[a]	0.88[a]	1.00		
Aroma index	–0.48	–0.23	–0.18	0.48	0.17	0.60[a]	–0.77[a]	0.66[a]	0.99[a]	0.89[a]	1.00	
Sum of key aroma compounds	–0.60[a]	–0.42	–0.08	0.55[a]	0.25	0.69[a]	–0.83[a]	0.59[a]	0.95[a]	0.95[a]	0.97[a]	1.00

a – correlation significant on a level of $\alpha = 0.05$.

Tab. 8. Correlation between the results of the sensory evaluation and the qualitative parameters of the orange juices ($n = 15$).

	Soluble solids	Relative density	*L**	*a**	*b**	Price	Hedonic scoring	Odour intensity of characteristic aroma	*R*-Limonene concentration	Sum of key aroma compounds
Soluble solids	1.00									
Relative density	0.70[a]	1.00								
*L**	–0.28	–0.56[a]	1.00							
*a**	0.26	0.60[a]	–0.82[a]	1.00						
*b**	–0.36	–0.49[a]	0.91[a]	–0.70[a]	1.00					
Price	–0.23	–0.61[a]	0.44	–0.70[a]	0.17	1.00				
Hedonic scoring	0.41	0.59[a]	–0.90[a]	0.86[a]	–0.80[a]	–0.66[a]	1.00			
Odour intensity of characteristic aroma	–0.16	–0.23	0.80[a]	–0.46	0.66[a]	0.17	–0.65[a]	1.00		
R-Limonene concentration	0.31	0.25	–0.49[a]	0.27	–0.48	0.22	0.19	–0.47	1.00	
Sum of key aroma compounds	–0.19	–0.62[a]	0.52[a]	–0.64[a]	0.38	0.76[a]	–0.68[a]	0.29	0.30	1.00

a – correlation significant on a level of $\alpha = 0.05$.

tration of 150 $\mu g \cdot l^{-1}$, b) the Aroma index. Both approaches showed that in two samples from 15, the aroma had not been restored, and two other samples had values above the critical limit but low in comparison with "average literature levels" [4].

From 5 to 20 volatiles were identified in each orange juice, which is in agreement with literature data [11]. The main component of orange juices was *R*-limonene (Tab. 6), the concentration of which ranged from 60 000 $\mu g \cdot l^{-1}$ to 126 000 $\mu g \cdot l^{-1}$. This compound is the major constituent of the so called essence oil of the orange aroma characterized by a juicy and sweet fruity flavour. The second part of the recovered orange aroma, i.e. the aqueous phase (water phase), which is characterized by an orange pulp note, juicy and fruity flavour given by water-soluble alcohols, aldehydes and low organic acid esters, was almost missing in the majority of the samples. Ethyl butanoate, linalool and octanal, which were marked by AVERBECK [10] as the constituents with the higher flavour dilution factor in freshly reconstituted orange juice from a concentrate, were quantified as the key odorants.

Based on the above, all orange samples complied with requirements regarding the concentration of orange oil, but the water phase of the aroma was properly restored only in three samples from 15.

Correlation of the quantitative parameters

The results of the basic composition of the juices and the profiles of volatile compounds were correlated with the results of the sensory evaluation (Tab. 7 and 8). A question arose of how the composition pre-determines sensory acceptance of the product. From the results, it was evident that the hedonic scoring was influenced by a set of different parameters. Only parameters for which the correlation coefficient was higher than the critical value ($r = 0.49$ for $\alpha = 0.05$), were taken into account.

The apple juice sensorial preferences were positively affected by the soluble solids content and the concentration of all key aroma components, from which the sum of carbon 6-aldehydes and alcohols had the highest correlation coefficient ($r = 0.88$). The sensorially evaluated intensity of the characteristic aroma strongly correlated with the Aroma index.

The orange juice sensorial preferences were mostly affected by colour. A direct correlation of hedonic scores or the olfaction intensity of the characteristic aroma to the concentration of any individual aroma compound of orange juice was not observed. We suggest, contrary to the literature, that the orange juice aroma is created by a complex mixture of components where no individual component can be identified to have a prevalent impact on the aroma. Another reason for these results may be the difference in time-temperature loadings of the analysed samples. Thermal processing and storage are known to profoundly affect the aroma composition, heating reducing the levels of reactive aroma impact compounds such as ethyl butanoate, linalool, neral and geranial, creating e.g. 4-vinylguaiacol, *p*-cymene and carvone, which are off-flavour precursors [17]. On the other hand, juices with good hedonic values predominantly contained ethyl butanoate and octanal at rather high concentrations.

CONCLUSIONS

The determination of volatile components by SPME/GC/MS was found to be an appropriate tool for evaluation of proper aroma restoration of orange and apple juices made from concentrates. The study has also shown that in the case of the apple juice, the results of the instrumental method, and the sums and indexes calculated according to the suggestions from the literature, all correlated well with, to a certain degree subjective, sensory hedonic evaluation. And because the survey of the market of apple juice shows that some, in particular low-priced, products do not contain any characteristic volatile components, producers should realize that proper aroma restoration is recognizable and appreciable by consumers.

Results for the orange juices were ambiguous. *R*-limonene, the indicator of restoration of the orange oil phase, was found to be present in all analysed samples at relevant concentrations. However, although the group of water-soluble esters and alcohols facilitated discrimination between the correctly and the incorrectly produced products, the contribution of these compounds to the typical orange aroma was not proven within our set of samples by hedonic evaluation.

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