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SCHMUTZER Gabriella Ramona

DETERMINATION OF MOLECULAR COMPOUNDS FROM COMPLEX MATRICES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY COUPLED SYSTEM

PhD thesis summary

Scientific supervisor:
Prof. Univ. Dr. Leontin Ioan DAVID

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Content

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Orange juice

Introduction

The quality of fruit juice is an important criterion for the consumer and it is a decisive factor for marketing. Consumer preferences are orientated especially to juices with similar characteristics of fresh single strength fruit juices and juices that preserve nutritional properties of the fruits. One of the most important characteristics of the quality of fruits and processed products is the *flavour/aroma*. The variety and amount of volatile organic compounds responsible for the flavour of fruit juices have effect on the product quality.

The aim of the present work was to determinate the volatile organic compounds responsible for the flavour of apple and orange juices by headspace SPME-GC/MS analytical method, in order to realize a comparative study of different type of fruit juices and to propose a new index for juices assessment and quality evaluation.

A significant number of volatile organic compounds were determined in commercial apple and orange juices, respectively in fresh single strength juices by headspace SPME-GC/MS analytical method. Differentiation characteristics of various types of juices are based on relative concentration differences of the major classes of compounds: esters, alcohols, ketones and terpenes. A new index needs to be introduced for the quality evaluation of different type of juices, along with distinctive characteristics of the amount of total esters, alcohols, ketones, terpenes, mono- and sesquiterpenes, respectively. The fresh single strength orange juice was differentiated by the country of origin based on the concentration of *markers*, selected from the detected volatile organic compounds responsible for flavour.

1. Methods for the determination of molecular compounds from complex matrices

Advances in physics, chemistry and technology induced an exceptional development of modern structural analysis methods and apparatus. Investigation methods of molecular compounds – characterized by speed and finesse incomprehensible a few decades ago – have increased exponentially the identification efficiency of active compounds from complex matrices. The spectroscopic methods can be used to identify structural aspects of organic compounds by interpretation of information obtained from the registration of electromagnetic radiation absorption spectra, capable for transferring quantifiable energy to organic compounds [1]. Mass spectrometry [2, 3, 4, 5], nuclear magnetic resonance, electron spin resonance [6, 7, 8, 9, 10], IR spectroscopy, UV-Vis spectroscopy are spectral methods which can be used for the determination of molecular compounds from complex matrices.

Gas chromatography – mass spectrometry (GC/MS) coupled system is a modern technique for qualitative and quantitative analysis, with a high sensitivity and selectivity providing valuable information about natural or synthetic mixtures. GC/MS has numerous applications in physics, chemistry, environment protection, food control, biology and medicine [11, 12]. Using this coupled system concentrations of organic compounds from complex matrices can be determined in concentrations of μg/L level (trace analysis) and even lower [13, 14].

Gas chromatography is today the most important analytical method used for the separation/determination of organic molecular compounds from complex matrices with many applications [15]. Mass spectrometry is an analytical technique used for the identification of analytes and quantitative determination of molecular compounds. The mass spectrum of a molecular compound is unique for a given compound, it is like a “fingerprint” of the compound, with a few exception, in particular in the case of isomers. Therefore using the coupled GC/MS system the separation, identification and quantitative determination of molecular compounds from complex matrices can be acomplished.

2. General aspects related to fruit juices

Fruit juices and derived products are an important segment of the fruit processing industry [16]. Fruit juice industry has become one of the largest agricultural area and business in the world [17].

Apple and orange juices are the most popular fruit juices. The popularity and high demand for fruit juices is the result of sensory properties and nutritional benefits – partly due to the fact that some products are enriched with vitamins and minerals [18].

Fruit juices are complex mixtures of sugars, organic acids, volatile compounds responsible for flavour, fatty acids, sterols, amino acids, flavonoids, pigments and others in water. Each juice is characterized by a unique composition of these ingredients. Water, sugar, organic acids, flavours, colorants, preservatives and other compounds are added in soft drink formula obtained from fruit juices [19].

The quality of fruit juices depends on many factors such as the quality of fruits (geographic area, climate, and cultivation practices), maturity on harvest (sugar concentration, acidity, color, and flavour), transport and storage conditions (humidity, temperature) and production processes [20].

The organoleptic quality of fruit juice can be decisive for consumers in the purchase/consumption of products. The flavour has a special contribution on the organoleptic quality of food products among many compounds [21]. The flavour is a resulting effect of two sensations: taste and odour, perceived on tasting of food products detected by chemical receptors from oral-naso-pharynges area [22]. The presence, content and composition of volatile compounds responsible for the flavour of fruit juices have an important influence on the quality of these products [21].

3. Determination of molecular compounds from apple juice

The most important apple product is apple juice, considered to be one of the most popular fruit juices and appreciated for its nutritional properties and specific flavour [23].

Apple juice quality is influenced by many factors: apple varieties, growing region and climate, cultivator practices, fruit's maturity on harvest, storage and transport conditions and technological processing [20, 23]. The quality of apple juice concentrate depends on the flavour recovery technology efficiency, applied during the concentration process [24]. Qualitative and quantitative identification of volatile organic compounds, responsible for the flavour is an important issue for characterizing the quality of fresh single strength and commercial apple juices [25].

3.1. Volatile organic compounds of apple juice

The volatile organic compounds responsible for the flavour of fresh single strength apple juices and processed apple juices are esters, alcohols, aldehydes, ketones, terpenes and others in low concentrations, whose levels never exceed mg/L [26]. The most common analytical technique for the determination of volatile flavour compounds from fruits and fruit juices is gas chromatography, and particularly gas chromatography coupled with mass spectrometry [27]. Due to the complexity of sample matrices, isolation and preconcentration of the analytes is a indispensable step, prior to introduction into the GC/MS system [21].

3.2. SPME extraction of volatile organic compounds from fruit juices

Volatile organic compounds responsible for flavour of fruit juices are present in low concentration, therefore an efficient extraction method - compatible with GC/MS analysis - is required in order to be used for the identification and quantification of the compounds [28, 29, 30]. The extraction and preconcentration of volatile flavour compounds from the fruit juices were performed using solid phase microextraction (SPME) technique, a relatively recent extraction method, which is compatible with the GC/MS analytical method [31, 32].

The volatile flavour compounds were extracted by SPME technique, using a 65 μm polydimethyl-siloxane /divinyl-benzene (PDMS/DVB) fiber. 10 ml of apple juice sample spiked with 54 μL internal standard solution (0.925 μg/mL of 2,4-disec-buthyl-phenol solution in ethanol) were put into 20 ml amber glass vials with septum, were equilibrated and extracted at 45°C under continuous stirring (1400 rpm) for 30 min. The detected quantity of internal standard was a measure of process reproducibility. The extraction was carried out without dilution of the juice and no salt was added, in order to avoid inducing modifications in the studied juice composition. The desorbtion of the analytes was carried out at 250°C – injector temperature – for 2 minutes. All samples were prepared in duplicate.

3.3. Experimental conditions for study of volatile flavour compounds from apple juices by headspace SPME-GC/MS

Analysis was performed on a GC/MS system (Trace GC Ultra – Polaris Q MS, Thermo Finnigan). The GC was equipped with a HP-5MS (crossbond 5% diphenyl and 95% dimethyl polysiloxane; 30m x 0.25mm x 0.25μm). Different oven temperature programs were tested, but the best separation was obtained for: initial temperature 40°C (2 min) then a rate of 10°C/min up to 300°C held for 10 min. The mass spectrometer was set at the following conditions: ion source temperature at 250°C, electron impact (EI) ionization mode (70 eV), current emission at 300 μA, and mass range of 50 – 650 Dalton.

Quantification method used in the comparative study of different types of apple juices was selected expressing the amount of detected volatile organic compounds as relative concentration. The relative concentration was calculated as the ratio of the base peak area of identified compound to the total area of all detected compounds. For convenience the ratios were multiplied by 10^4.

The relative standard deviations for all samples were below of 12.7% (maximum standard deviation was calculated for 3,5-bis(tert-butyl)4-hydroxy-propiophenon in the direct pressed apple juice sample).

3.4. Comparative study of various commercial apple juice by headspace SPME-GC/MS

Apple juices made in Romania were purchased from local markets and immediately analyzed by SPME-GC/MS after opening:

- **Sample 1** and **Sample 2** 100% apple juices made from concentrate, produced by different companies;
- **Sample 3** unclarified, processed 100% natural apple juice, named as direct pressed apple juice without preservatives and coloring added;
- **Sample 4** non-carbonated soft drink with green apple juice and puree, labeled green apple juice with pulp (**Figure 1**);

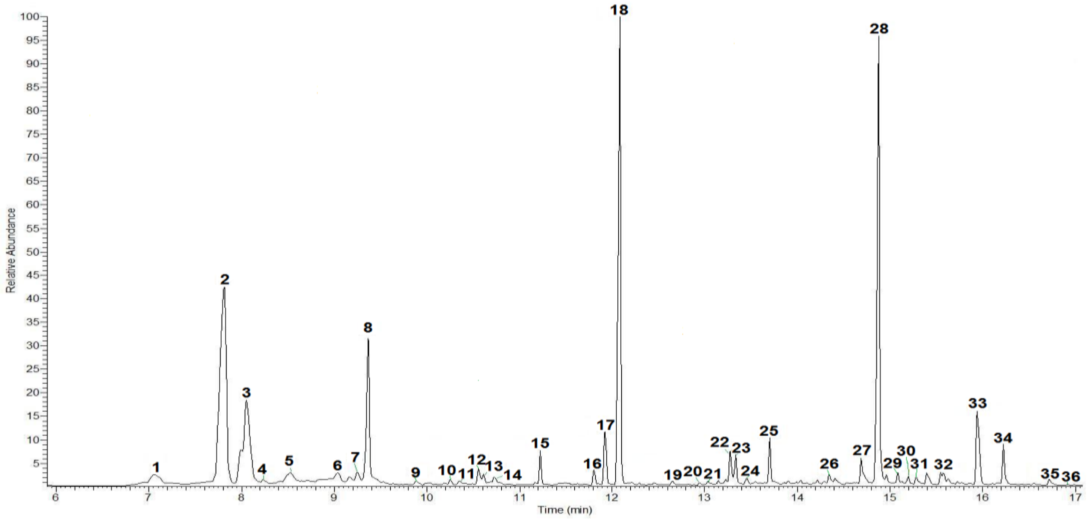

Figure 1*: The Total Ion Chromatogram (TIC) of determined volatile organic compounds from green apple juice with pulp (Sample 4), where: 2-Pentanol propanoate (***1***); n-Hexyl acetate (***2***); Limonene (***3***); Propyl pivalatea (***4***); γ-Terpinene (***5***); Diethylcyclooctane (***6***); Isopentyl-2-methylbutanoate (***7***); 4-Methylhexyl acetate (***8***); p-Propyl-benzaldehyde (***9***); Unknown alcohol 1 (***10***); Unknown alcohol 2 (***11***); Propyl hexanoate (***12***); α-Terpineol (***13***); Estragole (***14***); Hexyl-2-methylbutanoate (***15***); Citronellol (***16***); Ionone (***17***); cis-p-Menthan-7-ol (***18***); Ethyl safranate (***19***); Eugenol (***20***); α-Damascenone (***21***); Hexyl hexanoate (***22***); β-Damascenone (***23***); Propenyl guaiacol (***24***); Guaiene (***25***); Unknown terpene 7 (M=204) (***26***); trans-α-Bergamotene (***27***); α-Farnesene (***28***); Unknown terpene 8 (M=204) (***29***); Methyl-ethyl-cyclohexanecarboxylatea (***30***); Germacrene (***31***); Humulene (***32***); Unknown terpene 9 (M=204) (***33***); α-Pachoulene (***34***); Aristolene (***35***); Methyl-β-ionone (***36***);*

Esters

The commercial apple juices are characterized by high amount of total esters. The relative concentration of total esters obtained for juice made from concentrate (3860.7 a.u. - arbitrary unit - in Sample 1; 6195.8 a.u. in Sample 2) suggest - according to Heil and Nikfardjam - a sufficient re-aromatization process [33, 34]. In the case of commercial direct pressed apple juice, considered high quality apple juice according to the labeling of the manufacturer (2691.1 a.u. in Sample 3), the total esters does not reach half of the maximum amount of total esters detected for the juice made from concentrate (Sample 2). The green apple juice with pulp (4441.3 a.u. in Sample 4) contains a high concentration of total esters.

The results obtained for total esters are not unequivocal, therefore the purposal of Heil and Nikfardjam regarding of use of total esters for quality evaluation of apple juices are not proper [34]. The relative concentrations of commercial apple juices are listed in **Table 1**.

Table 1: *Identified esters in commercial apple juices by headspace SPME-GC/MS method*

Nr	Compound	t_R (min)	Apple juice made from concentrate 1 **Sample 1**	Apple juice made from concentrate 2 **Sample 2**	Commercial direct pressed apple juice **Sample 3**	Green apple juice with pulp **Sample 4**
1	2/3-Methylbutyl-acetate[a]	5.48	1338.0±22.5	2093.4±76.9	845.4±1.8	799.2±51.8
2	Propyl-2-methylbutanoate[a]	6.62	10.2±0.4	-	27.9±1.1	-
3	2-Pentanol propanoate[a]	7.07	-	-	-	3140.9±5.3
4	Butyl-butanoate[a]	7.49	-	-	-	162.1±1.4
5	n-Hexyl acetate[a]	7.71	656.2±2.0	343.9±5.0	483.6±14.3	1556.8±91.4
6	Propyl pivalate[a]	8.17	32.0±0.3	-	88.3±4.8	12.3±0.4
7	Unknown ester 1	9.06	-	-	-	-
8	Isopentyl-2-methylbutanoate[a]	9.25	-	1781.7±58.1	-	72.8±6.8
9	4-Methylhexyl acetate[a]	9.44	-	-	-	1004.2±70.8
10	Butyl-2-methylbutanoate[a]	9.70	-	-	-	-
11	Ethyl benzoate[a]	10.23	-	26.8±2.0	45.9±3.4	-
12	Propyl hexanoate[a]	10.50	-	-	-	49.1±5.9
13	Methyl-3-methyl-benzoate[a]	10.89	-	-	-	0.4±0.03
14	Hexyl-2-methylbutanoate[a]	11.15	16.4±0.9	9.9±0.6	50.6±2.6	118.5±5.5
15	Pentyl hexanoate[a]	11.37	-	-	-	1.9±0.1
16	Butyl benzoate[a]	11.69	-	-	-	-
17	Unknown ester 2	12.46	-	-	-	-
18	Ethyl safranate[a]	12.69	8.7±0.5	-	8.0±0.4	0.9±0.1
19	Hexyl hexanoate[a]	13.27	-	-	-	70.6±5.5
20	5-Isopropyl-methylphenethyl acetate[a]	13.37	-	-	-	-
21	Methyl-ethyl-cyclohexanecarboxylate[a]	15.11	14.2±1.0	-	16.4±0.9	63.9±2.0
22	4-Terpineol acetate[a]	15.20	2.8±0.1	-	8.4±0.4	28.1±0.2
23	Pentanoic acid 2,2,4-trimethyl-3-carboxyisopropyl isobutyl ester[a]	15.88	1782.2±123.2	1940.1±95.2	1116.8±58.3	185.5±15.9

[a] Identification by comparison of mass spectra and retention times with published data and mass spectra from the NIST ; ± standard deviation; Relative concentration calculated as the ratio of the base peak area of identified compound to the total area of all compounds. For convenience the ratios were multiplied by 10^4.

Alcohols

Alcohols are formed in thermal processes of fruit juices; therefore they are present in significant amounts in processed products [23]. High relative concentration of total alcohols was found in commercial apple juices, especially in Sample 1, apple juice made from concentrate (2494.3 a.u.). Compared the concentration of total alhohols of Sample 1 with Sample 2 - other juice made from concentrate – a lower concentration of total alcohols was found (890.8 a.u.). Surprisingly, in the Sample 3 was detected high amount of total alcohols (1613.6 a.u.), although the technological process of direct pressed juice is relatively short comparative to the processing of juice made from concentrate. A very low amount of total alcohol was detected in the green apple juice with pulp (81.0 a.u.). The alcohols determined in the commercial apple juices are listed in **Table 2**.

Table 2: *Identified alcohols in commercial apple juices by headspace SPME-GC/MS method*

Nr	**Compound**	**t_R (min)**	Apple juice made from concentrate 1 **Sample 1**	Apple juice made from concentrate 2 **Sample 2**	Commercial direct pressed apple juice **Sample 3**	Green apple juice with pulp **Sample 4**
1	cis-3-Hexenol	4.24	59.5±0.7	31.1±3.1	17.4±0.2	4.2±0.4
2	1-Octanol[a]	8.68	104.5±7.7	-	120.1±6.4	-
3	cis-6-Nonenol[a]	9.18	76.0±2.3	-	111.8±10.6	5.6±0.5
4	Unknown alcohol 1	10.18	2075.9±191.4	737.4±14.5	1019.6±10.0	28.1±3.4
5	Unknown alcohol 2	10.34	-	11.5±1.0	-	29.7±3.1
6	cis-4-Decen-1-ol[a]	10.71	111.0±4.9	77.5±1.9	288.2±32.7	11.8±0.5
7	Unknown alcohol 3	10.82	50.5±2.1	28.1±2.4	32.4±0.6	1.7±0.1
8	2-Nonyloxy-ethanol[a]	11.43	10.6±0.2	-	8.3±0.7	-
9	2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one[a]	12.23	6.3±0.1	5.2±0.1	15.7±1.0	-

[a] Identification by comparison of mass spectra and retention times with published data and mass spectra from the NIST ; ± standard deviation; Relative concentration calculated as the ratio of the base peak area of identified compound to the total area of all compounds. For convenience the ratios were multiplied by 10^4.

Ketones

Ketones are released in thermal treatment processing of fruit juices [35], therefore the high concentration of total ketones in the commercial apple juices can be an indicator of processing technology. The highest amount of total ketones was determined in the Sample 2, apple juice made from concentrate (1435.6 a.u.), and Sample 3, direct pressed apple juice (1039.0 a.u.). Sample 1, apple juice reconstituted from concentrate, is characterized by a lower concentration of total ketones (697.0 a.u.), whereas Sample 4, green apple juice with pulp is characterized by the smallest amount of total ketones (200.0 a.u.) detected for the studied commercial apple juices. β-damascenone was determined in significant concentration in the analyzed samples (**Table 3)**.

Table 3: *Identified ketones/aldehydes in commercial apple juices by headspace SPME-GC/MS method*

Nr	**Compound**	**t_R (min)**	Apple juice made from concentrate 1 **Sample 1**	Apple juice made from concentrate 2 **Sample 2**	Commercial direct pressed apple juice **Sample 3**	Green apple juice with pulp **Sample 4**
1	p-Propyl-benzaldehyde[a]	9.92	-	-	-	8.4±0.7
2	Ionone[a]	11.85	280.4±21.0	45.5±5.7	321.1±32.3	97.6±9.0
3	α-Damascenone[a]	12.95	24.1±1.3	77.9±2.2	45.9±1.6	6.7±0.2
4	2-Butyl-2-octenal[a]	13.15	-	-	-	4.6±0.04
5	β-Damascenone[a]	13.33	363.9±2.9	1305.2±31.1	655.3±30.4	82.6±1.7
6	Methyl-β-ionone[a]	16.72	28.6±1.9	7.1±0.4	16.7±1.1	-

[a] Identification by comparison of mass spectra and retention times with published data and mass spectra from the NIST ; ± standard deviation; Relative concentration calculated as the ratio of the base peak area of identified compound to the total area of all compounds. For convenience the ratios were multiplied by 10^4.

Terpenes

Terpenes are naturally occurring organic compounds, produced by a variety of plants, often with a strong smelling [36]. The least concentrations of total terpenes were determined for the juices reconstituted from concentrate (2349.2 a.u. for Sample 1; 1056.1 a.u. for Sample 2). A much higher amount of total terpenes was found in the direct pressed apple juice sample (4388.5 a.u.), but the highest concentration of total terpenes was established for the green apple juice with pulp (5202.1 a.u.) (**Figure 2**).

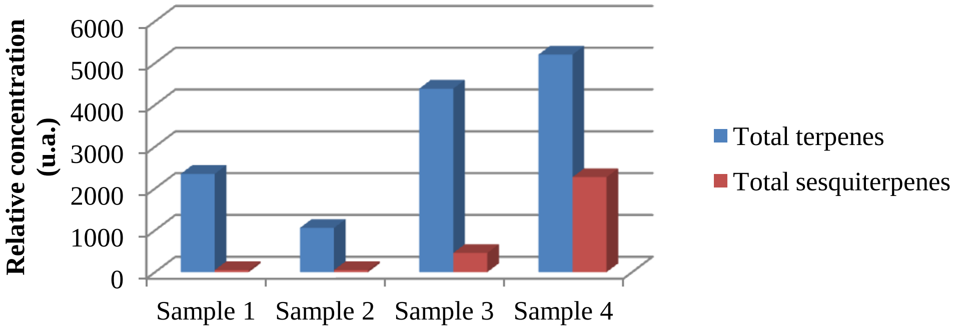

Figure 2: *Relative concentration of total terpenes and total sesquiterpenes in the studied commercial apple juices*

The relative concentration of total sesquiterpenes does not follow the tendency of total terpenes. The total amount of sesquiterpenes in the Sample 1, juice made from concentrate was determined as 47.8 a.u., representing only 2.0% of total terpenes (**Figure 2**). For Sample 2, juice from concentrate, the amount of total sesquiterpenes represent 4.6% of total terpenes. Direct pressed apple juice was characterized by a relative ratio of total sesquiterpene and total terpene of 10.4%. The highest concentration of total sesquiterpene was detected in the green apple juice with pulp (2270.8 a.u. in Sample 4). This amount of total sesquiterpenes represents 43.7% of total terpenes. The comparison of the relative ratios of terpenes show high values for authentic products, comparative to juice made from concentrate. The results suggest that the apple juices assessment can be performed based on the relative ratio of total sesquiterpenes and total terpenes. For the studied juices made from concentrate the relative ratio of sesquiterpenes and terpenes was under 10%, while for juices not from concentrate this value was over 10%. The relative concentrations of identified terpenes are listed in **Table 4**.

Table 4: *Identified terpenes in commercial apple juices by headspace SPME-GC/MS method*

Nr	**Compound**	**t_R (min)**	Apple juice made from concentrate 1 **Sample 1**	Apple juice made from concentrate 2 **Sample 2**	Commercial direct pressed apple juice **Sample 3**	Green apple juice with pulp **Sample 4**
1	α-Pinene[b]	6.40	-	-	-	1.0±0.01
2	Champhene[b]	7.40	1.9±0.03	62.6±7.3	58.2±0.6	14.7±1.2
3	Limonene[b]	7.99	431.1±32.6	309.1±28.7	1975.6±52.2	736.2±28.6
4	Unknown terpene 1	8.32	-	9.0±0.8	-	3.7±0.4
5	γ-Terpinene[b]	8.47	996.0±49.0	332.0±10.1	297.9±26.1	156.1±3.1
6	Terpinolene[b]	8.84	11.9±1.1	33.4±3.7	54.6±6.8	39.9±1.3
7	Linalool[b]	9.12	218.8±11.3	117.0±13.1	68.2±6.6	23.5±1.6
8	cis-p-2-Menthenol[a]	9.14	-	-	-	37.0±1.7
9	Unknown terpene 2	9.37	-	-	-	-
10	cis-Pinen-3-ol[a]	9.40	32.3±1.1	-	315.6±16.7	4.9±0.2
11	Unknown terpene 3	9.46	-	-	1.4±0.1	2.3±0.05
12	cis-β-Terpineol[a]	9.72	-	-	-	1.2±0.05
13	cis-Verbenol[b]	9.82	69.9±2.9	-	54.2±0.9	-
14	1-isopulegole[b]	9.86	-	-	-	-
15	p-Vinyl-anisole[a]	9.97	29.2±1.2	-	-	-

Table 4: *Identified terpenes in commercial apple juices by headspace SPME-GC/MS method - continuation*

Nr	Compound	t_R (min)	Apple juice made from concentrate 1 **Sample 1**	Apple juice made from concentrate 2 **Sample 2**	Commercial direct pressed apple juice **Sample 3**	Green apple juice with pulp **Sample 4**
16	1-Borneol[b]	10.04	-	-	1.5±0.1	-
17	Unknown terpene 4	10.13	9.9±1.1	-	8.6±0.1	-
18	p-Menth-2-en-1-ol[a]	10.42	-	5.0±0.3	-	3.5±0.1
19	α-Terpineol[b]	10.54	104.3±6.4	41.5±2.2	289.1±10.0	46.8±1.4
20	Estragole[a]	10.65	334.5±8.8	43.3±3.4	684.7±35.8	46.0±5.4
21	β-Cyclocitral[a]	10.99	30.9±1.0	15.9±0.03	16.1±0.7	3.1±0.4
22	2-Bornene[a]	11.10	30.7±0.6	38.3±1.7	56.8±5.6	1.8±0.1
23	Citronellol[b]	11.81	-	-	-	41.1±4.3
24	Anethole[a]	11.91	-	-	48.0±1.9	-
25	cis-p-Menthan-7-ol[a,c]	12.09	-	-	-	1751.2±102.1
26	Unknown terpene 5 (M=204)	12.60	-	-	16.0±1.5	-
27	Unknown terpene 6 (M=204)	12.76	-	-	-	-
28	Eugenol[a]	12.93	-	-	-	11.6±0.04
29	α-Cubebene (M=204)[a]	13.17	-	-	-	9.7±0.8
30	Dehydro-aromadendrene[a]	13.35	-	-	-	1.4±0.03
31	Propenyl guaiacol[a]	13.47	-	-	-	4.1±0.2
32	Guaiene (M=204)[a]	13.62	14.1±1.1	17.3±1.0	93.5±0.9	97.7±10.5
33	β-Caryophyllene (M=204)[b]	13.78	-	-	1.7±0.1	2.7±0.1
34	Aromadendrene (M=204)[b]	14.02	11.6±0.6	-	-	-
35	Unknown terpene 7 (M=204)	14.26	-	-	-	30.5±1.4
36	γ-Maaliene (M=204)[a]	14.46	-	-	4.4±0.5	3.2±0.3
37	trans-α-Bergamotene (M=204)[a,c]	14.60	-	-	-	96.3±9.6
38	Valencene (M=204)[b]	14.70	14.9±1.1	-	6.7±0.3	-
39	α-Farnesene (M=204)[a]	14.77	-	-	278.1±3.1	1447.9±99.3
40	Unknown terpene 8 (M=204)	15.00	-	-	-	41.6±1.1
41	Lilal (M=204)[a]	15.03	7.1±0.2	3.7±0.3	5.5±0.6	-
42	Germacrene (M=204)[a,c]	15.19	-	-	-	35.1±0.4
43	Humulene (M=204)[a,c]	15.53	-	-	-	21.4±2.3
44	Unknown terpene 9 (M=204)	15.85	-	-	-	287.9±13.3
45	α-Pachoulene (M=204)[a,c]	16.14	-	-	31.0±2.0	178.6±1.3
46	Unknown terpene 10 (M=204)	16.44	-	27.9±2.3	-	4.7±0.4
47	Aristolene (M=204)[a]	16.64	-	-	-	11.7±1.4
48	Cedrene (M=204)[a]	16.90	-	-	21.0±1.7	2.0±0.05

[a] Identification by comparison of mass spectra and retention times with published data and mass spectra from the NIST; [b] Identification by comparison of mass spectra and retention times with separate injection of aroma standards, respectively by comparison of mass spectra from the NIST; [c] Tentatively identified; ± standard deviation; Relative concentration calculated as the ratio of the base peak area of identified compound to the total area of all compounds. For convenience the ratios were multiplied by 10^4.

3.5. Comparative study of various single strength apple juice by headspace SPME-GC/MS

The Golden Delicious and Red Delicious apple (*Malus domestica*) fresh fruits were obtained from a famous Romanian growing region, the orchard situated near Reghin (46°46′33″N 24°42′30″E) and the Jonathan apple from an area near Cluj-Napoca, Gheorgheni village (46°42′55″N 23°41′11″E). Fresh single strength juice from apple fruits – produced in the year 2011 - were immediately extracted for analysis

- **Sample 5** fresh single strength apple juice from Golden Delicious;
- **Sample 6** fresh single strength apple juice from Red Delicious;
- **Sample 7** fresh single strength apple juice from Jonathan.

Esters

The fresh single strength juice obtained from different varieties of apple fruits are not characterized by high amount of total esters. For juice from Golden Delicious (Sample 5) was obtained the highest concentration of total esters determined for this set of samples

(1958.8 a.u.). Juices from Red Delicious (1811.4 a.u. for Sample 6) and from Jonathan (829.1 a.u. for Sample 7) are characterized by very low concentration of total esters, comparative to the studied commercial apple juices (2600 – 6200 u.a.). The low amount of total esters for fresh single strength apple juices are in agreement with the Wolter researches [38], sustaining the idea that some apple varieties do not contain or contain only small amount of esters. The low concentration of esters denoted that the total esters aroma index alone is not suitable for juice’s quality assessment.

Alcohols

The alcohols content of the studied single strength apple juices is relatively low compared to commercial juices - except green apple juice with pulp. In the fresh single strength Golden Delicious apple juice the lowest concentration of total alcohols (78.5 a.u.) was detected. The total alcohol concentration for Red Delicious juice and Jonathan juice are slightly higher (115.7 a.u. for Sample 6, 314.0 a.u. for Sample 7, respectively). It is important to note that the analyses were performed on May of 2012 from the apple production of 2011, therefore a few storage months have to be considered. According to data published by Woler the alcohol composition of apple juices increases significantly with the period of fruit storage [38]. Therefore the long storage period (about 8 months) may cause a significant increase of total alcohols from apple fruits.

Ketones

In all single strength samples only very small concentrations of natural ketones were found, as compared to the results obtained for commercial juices (200-1400 a.u.). The smallest quantity of total ketones was detected in Jonathan apple juice (1.6 a.u.). The total ketones concentration in Red Delicious juice was 7.0 a.u., respectively 10.6 a.u. in Golden Delicious apple juice, significantly lower than in commercial apple juices.

Terpene

The relative concentration of total terpenes was almost double in the fresh single strength juices versus to the commercial apple juices (1000-4400 a.u.). The highest amount of total terpene was detected in the fresh single strength Jonathan apple juice (8834.1 a.u.), comparable with those obtained for Golden Delicious (7946.5 a.u.) and Red Delicious (8063.0 a.u.) (**Figure 3**).

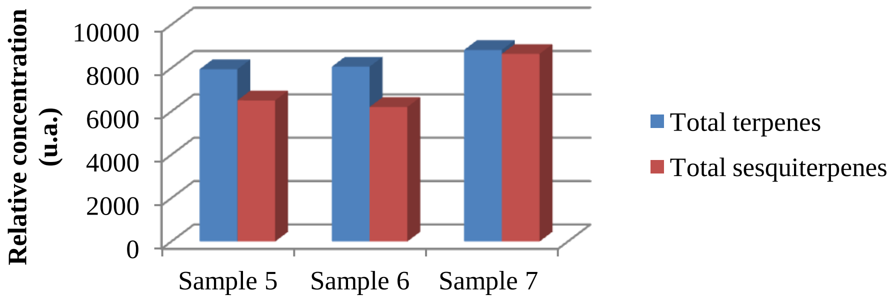

Figure 3: *Relative concentration of total terpenes and total sesquiterpenes in the fresh single strength juices*

The very high amount of total terpenes present in single strength fresh apple juice is due to the high concentration of total sesquiterpene. In comparison to commercial juices (47-2200 a.u.) the total sesquiterpenes content of single strength apple juices is very high, therefore the relative ratio of total sesquiterpene and total terpene is very high, too, of about 76% (**Figure 3**). Consequently, the relative ratio of total sesquiterpene and total terpene is 81.9% for Golden Delicious, 76.9% for Red Delicious and 97.9% for Jonathan. Considering the relative ratio of sesquiterpenes and terpenes the values obtained for the single strength fresh apple juice are much higher than those reported for commercial juices (in the range 2 – 43.7%). According to the results the relative ratio of total sesquiterpenes and total terpenes is a proper new index for apple juice quality evaluation and assessment.

Conclusions

In the studied commercial apple juices and fresh single strength juices from different varieties of apple fruits a number of 90 volatile organic compounds were determined using headspace SPME-GC/MS.

Differentiation characteristics of various type of apple juices are based on relative concentration differences of the major classes of compounds: total esters, alcohols, ketones and terpenes. A new index was proposed for the quality evaluation of different type of apple juices, the relative ratio of total sesquiterpenes and total terpenes. Using the proposed differentiation characteristics, quality evaluation of apple juices can be done in the relatively simple manner.

The presented data may be used for the qualitative and quantitative description of volatile organic compounds responsible for flavour of authentic apple fruits grown in different geographical areas, possibly being helpful in the creating of a data base.

4. Determination of molecular compounds from orange juices

Global production of orange juice in 2012/2013 was 1.9 million of tons [38]. This high production of orange juices reflects consumer preferences.

The commercial pasteurized and packed orange juices standard quality is the flavour of unpasteurized, fresh single strength orange juice. Volatile organic compounds responsible for the flavour are sensitive compounds and the use of thermal treatment initiates the degradation process of these compounds. Therefore, determination of volatile organic compounds responsible for the flavours in final products is very important for quality assurance of processed orange juices, especially for juice made from concentrate.

4.1. Volatile organic compounds of orange juice

Sensory quality of orange juice is not due only to basic components such as sugars or organic acids, but to volatile organic compounds responsible for flavour, also. The flavour of the orange juices, which gives the taste and odour of the product, is the result of a complex combination of volatile compounds from the class of esters, alcohols, ketones, aldeydes, terpenes and other compounds in specific proportions [39].

4.2. SPME optimization for extraction of volatile organic compounds responsible for flavour from orange juice

The SPME parameter optimization was performed on orange juice using the PDMS/DVB mix stationary phase. The SPME extraction was realized on the samples subjected to continuous stirring (1300 rpm) after 60 min equilibration. The fiber was removed from the sample vial and inserted into the injection port of the GC, where thermal desorption of the analytes was carried out at 250°C during 5 min. For the extraction optimization procedure 5 volatile and 5 semivolatile compounds were selected.

The volatile compounds selected for optimization were: limonene, terpinolene, linaool, 1-isopulegole, α-terpineol; and the semivolatile compounds were: pulegone, α-cubebene, caryophyllene, valencene and 2,4-disec-buthyl-phenol. The relative standard deviation (RSD) of the compounds selected for SPME optimization varied in the range of 0.08 – 10.55%.

The amount of volatile compounds extracted by SPME fiber from the orange juice depends on the ***extraction temperature*** [40]. In order to test the influence of temperature modification on the SPME extraction, the extraction temperature was varied from 25 to 55°C. The optimum temperature was found to be 45°C for the SPME extraction of compounds responsible for the flavour from orange juice

The amount of analyte extracted on the stationary phase is maximum when partition equilibrium is reached. The partition equilibrium can be considered as attained, when compound's area no more increase while increasing the ***extraction time***. The extraction time variation effects were assessed for 5, 10, 15, 30, 45, 60 and 75 min. extraction. The optimum extraction time was set at 30 min for the volatile and semivolatile compounds, too.

In order to optimize the ***NaCl amount*** for the SPME extraction of volatile organic compounds responsible for flavour from orange juice, the mineral salt concentration was varied between 0 to 36%. GC chromatograms obtained for 0%, 12%, 24% and 36% NaCl concentration are presented in the **Figure 4**. The results suggest that NaCl addition determined alteration of volatile flavour compounds profile, therefore further extractions were performed without mineral salt addition.

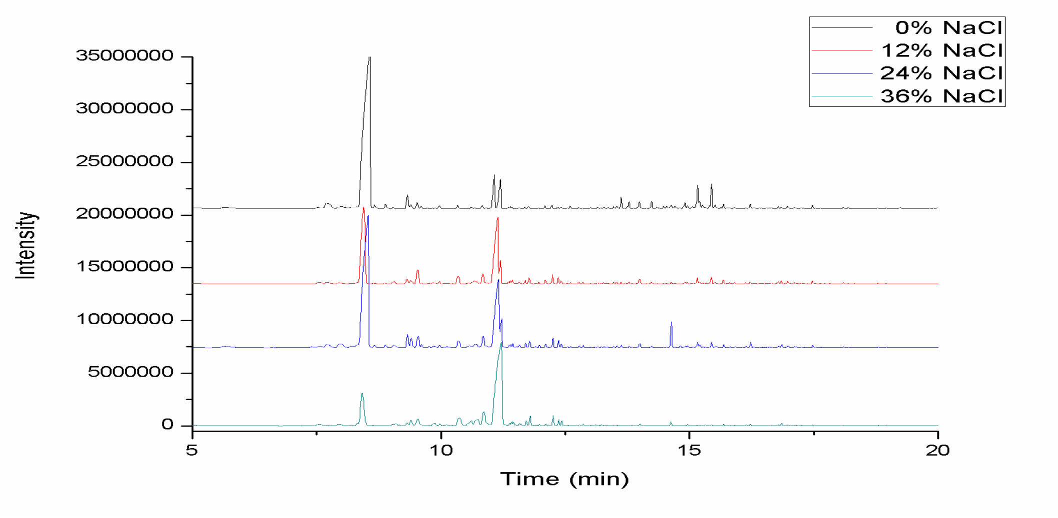

Figure 4: The GC chromatograms obtained for different concentration of NaCl (sample equilibrated for 60 min. and extract for 30 min. at 45°C, in continuous stirring (1300 rpm); using PDMS/DVB fiber)

4.3.Headspace SPME-GC/MS method validation for the volatile compounds determination from orange juice

The headspace SPME-GC/MS method validation was performed considering 17 standard compounds: α-pinene, β-pinene, ethyl hexanoate, d-limonene, γ-terpinene, terpinolene, fenchone, II-linalool, 1-isopulegole, 1-borneol, α-terpineol, citronellol, pulegone, geraniol, (-)-trans-caryophyllene, (+)-aromadendrene and (+)-valencene. Standard solutions were prepared in ethanol. Dilutions of the standard solutions were prepared using a model juice solution: HPLC water with 49.4 g/L D(+)-glucose-monohydrate, 50.6 g/L fructose, 8.5 g/L citric acid, 2.1 g/L L-(-)-malic acid and 0.4 g/L L-(+)-ascorbic acid. The parameters used in the headspace SPME-GC/MS method validation study are presented in the **Table 5**.

Table 5: *Validation parameters for headspace SPME-GC/MS method: linearity range, regression coefficient (R), limit of linearity (LOL), limit of detection (LOD), limit of quantification (LOQ), relative standard deviation (RSD)*

Nr	Standards	t_R (min)	Characteristic ions m/z (relative intensity, %)	Linearity range (μg/L)	RSD (%)	R	LOL (%)	LOD (μg/L)	LOQ (μg/L)
1	α-Pinene	6.73	91(100)77(40)105(5)121(3)136(0.5)	20-100	5.71	0.98	94.29	0.05	0.16
2	β-Pinene	7.07	91(100)93(74)67(25)121(22)107(14)	100-750	1.60	0.95	98.40	0.09	0.31
3	Ethyl hexanoate	7.81	55(100)61(46)99(13)145(11)115(6)	20-100	0.64	0.95	99.36	0.08	0.27
4	d-Limonene	8.35	67(100)91(94)107(17)121(12)136(6)	1000-1750	0.09	0.98	99.91	6.19	20.64
5	γ-Terpinene	8.83	91(100)65(13)121(10)105(8)136(6)	20-100	0.21	0.99	99.80	0.50	1.66
6	Terpinolene	9.28	91(100)121(32)136(16)154(0.5)	20-100	0.12	0.99	99.88	0.20	0.68
7	Fenchone	9.36	81(100)69(6)109(2)152(1)	2.5-15	0.34	0.97	99.66	0.01	0.04
8	II-Linalool	9.48	91(100)93(52)121(14)139,154(0.5)	20-100	1.07	0.99	98.93	0.05	0.18
9	1-Isopulegole	10.30	67(100)91(72)107(15)139(12)154(0.5)	20-100	0.57	0.99	99.43	0.01	0.04
10	1-Borneol	10.68	95(100)67(49)121(6)136(2)139(1)	2.5-15	0.62	0.99	99.38	0.01	0.05
11	α-Terpineol	11.00	91(100)121(44)136(19)105(12)139(2)	20-100	2.52	0.99	97.48	0.19	0.63
12	Citronellol	11.39	67(100)81(55)95(28)123(6)138(2)	20-100	0.59	0.99	99.41	0.02	0.08
13	Pulegone	11.65	67(100)81(77)152(28)109(25)91(14)	20-100	0.26	0.99	99.74	0.05	0.17
14	Geraniol	11.85	91(100)119(82)77(37)105(25)152(23)	2.5-15	2.80	0.98	97.20	0.02	0.07
15	(-)-trans-Caryophyllene	14.21	91(100)105(58)119(23)189(8)204(1)	20-100	0.25	0.98	99.75	0.25	0.84
16	(+)-Aromadendrene	14.44	91(100)119(38)147,189(15)204(6)	2.5-15	0.81	0.95	99.19	0.06	0.20
17	(+)-Valencene	15.13	91(100)105(90)189(28)204(24)	100-750	7.13	0.95	92.87	0.88	2.93

t_R- retention time;

Experimental conditions: SPME: equilibrate at 45°C, for 60 min., under continuous agitation (1300 rpm), extraction at 45°C, for 30 min, NaCl concentration 0%; GC/MS: desorption at 250°C for 5 min, He 1.5 mL/min, temperature program for BD-5MS (30 m x 0.25 mm x 0.25 μm) column: 40°C (maintain 2 min), raised to 300°C (5 min) with 10°C/min, transfer line 300°C, ionization source EI set at 250°C.

The low values obtained for the limit of detection (LOD) and the limit of quantification (LOQ) confirm that the headspace SPME-GC/MS method is a sensitive analytical method suitable for quantitative analysis of volatile flavour compounds of orange juice.

4.4. Experimental conditions for volatile flavour compounds study from orange juices by headspace SPME-GC/MS

Analysis was performed on GC-MS system (Trace GC Ultra–Polaris Q MS, Thermo Finnigan). The GC was equipped with a capillary column DB-5MS (30m × 0.25mm) with 0.25μm film thickness. The oven was programmed from 40°C (2 min) to 300°C with 10°C/min rate and maintained for 5 min. The mass spectrometer ion source temperature was 250°C, electron impact (EI) ionization at 70 eV, current emission 300 μA. The mass spectrometer was operated in the full scan mode, in the 50-650 Dalton mass range.

The relative concentrations of compounds were determined comparing the base peak area of identified compound with the base peak area of internal standard (m/z 67). The internal standard used for quantification of the compounds was pulegone, added to each sample in 600 ng/sample amount. Relative deviation standards for the studied compounds were in the range of 0.01 – 10.46%.

4.5. Study of volatile organic compounds from different type of orange juice by headspace SPME-GC/MS

The objective of the present study was the determination of volatile organic compounds responsible for the flavour of different orange juices and nectars by headspace SPME-GC/MS method, in order to propose a new index for assessment and quality evaluation of juices made from concentrate. This study was realized on a total of 19 orange juices (S1-S19), one of which was direct pressed orange juice (S9) and the others reconstituted from concentrate. In addition to this orange juices four orange nectars, obtained from concentrate (N1-N4) were subjected to headspace SPME-GC/MS analysis, along with seven fresh single strength juice from orange fruits (F1-F7).

A number of 113 volatile organic compounds responsible for the flavour were determined from the studied orange juices, included in four chemical families: esters, alcohols, ketones and terpenes – subdivided into monoterpenes and sesquiterpenes [41, 42]. In the present study were identified 8 esters, 5 alcohols, 7 ketones, 41 monoterpenes and 52 sesquiterpenes.

Esters

A number of 8 esters – generally characterized by fruity notes – were identified in the analyzed orange juices and nectars [43, 44]. The total esters concentration for the studied juices and nectars are shown in the **Figure 5**. It can be observed that the total esters concentration is relatively low in the direct pressed commercial juice (S9), which was considered - according to producers - high quality juice. The studied fresh single strength orange juice is characterized by the same low level of total esters.

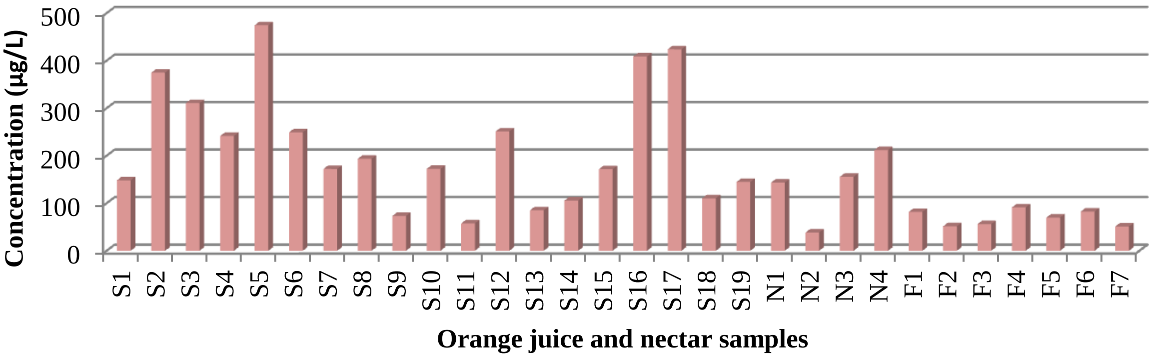

Figure 5: The total esters concentration in the: commercial orange juices (S1-S19), orange nectars (N1-N4) and fresh single strength orange juices (F1-F7)

Alcohols

In the orange juices and nectars 5 different alcohols were identified. In the **Figure 6** the total alcohols concentration is presented and a relatively high concentration of total alcohols determined in the commercial apple orange juices and in the orange nectars, too can be observed. An obvious characteristic of the fresh single strength orange juices is the low concentration of total alcohols.

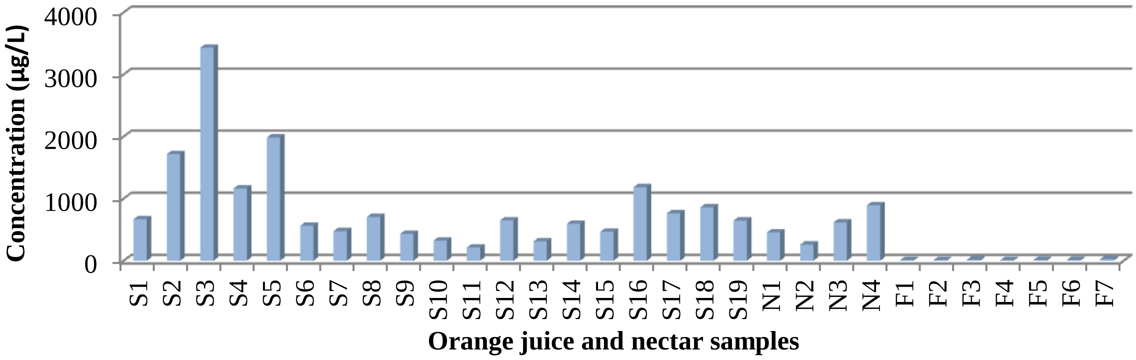

Figure 6: The total alcohols concentration in the: commercial orange juices (S1-S19), orange nectars (N1-N4) and fresh single strength orange juices (F1-F7)

Ketones

Seven volatile compounds identified in the orange juices were included in the ketones class. The total ketones high concentration in commercial juices is an indication of thermal treatment application [45]. The total ketones amount determined in the processed orange juices and nectars, varied in large intervals (**Figure 7**). According to the reported data, the unprocessed fresh single strength orange juices the total ketones concentration is low.

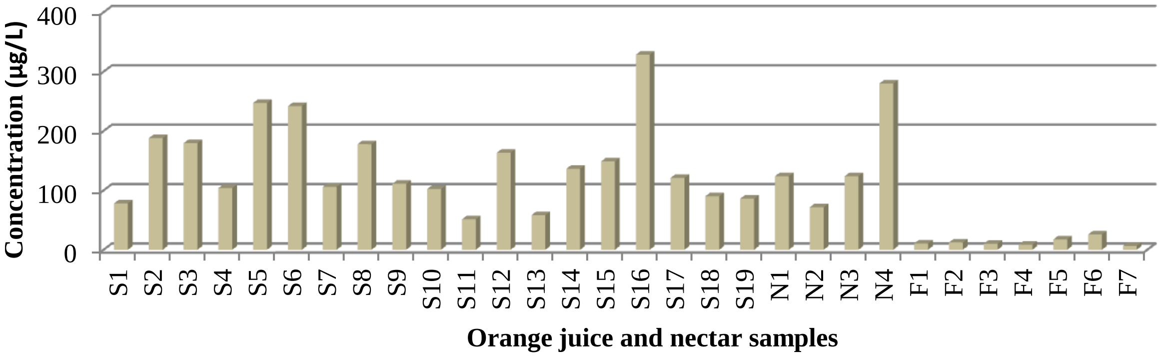

Figure 7: The total ketones concentrations in the: commercial orange juices (S1-S19), orange nectars (N1-N4) and fresh single strength orange juices (F1-F7)

Terpenes

The qualitatively and quantitatively dominant classes of compounds are terpenes. A number of 93 terpenes were determined in the present work. The class of terpenes was divided on two subclasses: monoterpenes and sesquiterpenes.

Monoterpenes

In the analyzed orange juices and nectars 41 monoterpenes and monoterpenes derivatives were identified. Limonene is the quantitatively dominant monoterpene of the orange juices and nectars. The quantitative ratio of limonene relative to the total of 113 compounds identified in the analyzed samples, are in the range of 50.0 – 71.4% for all samples (**Error! Reference source not found.**). The differentiation and quality evaluation of orange juices cannot be performed considering the ratio of limonen to all volatile compounds.

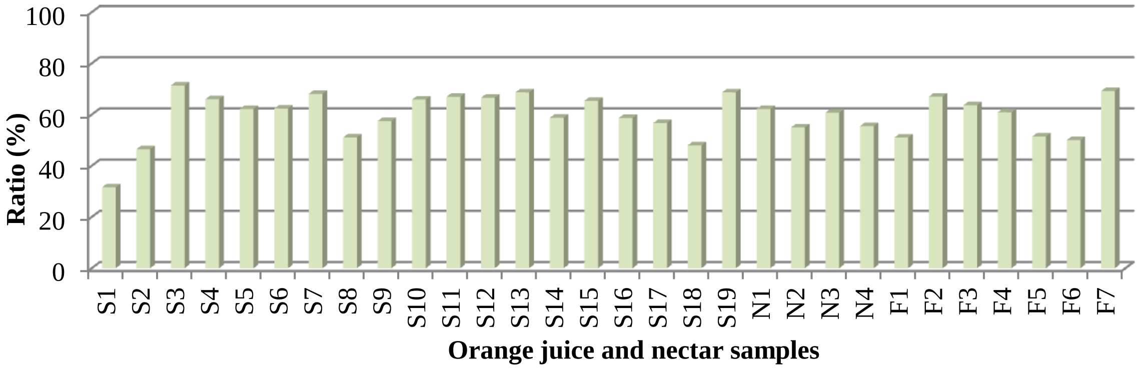

Figure 8: Ratio of limonen and total identified volatile compounds in the commercial orange juices (S1-S19), orange nectars (N1-N4) and fresh single strength orange juices (F1-F7)

The amount of total monoterpenes determined in the orange juices and nectars is presented in **Error! Reference source not found.**. The concentration of total monoterpenes in the analyzed *commercial orange juices* varied in large limits. In the *orange nectars* and *fresh single strength orange juice* the total amount of total monoterpenes are relatively low.

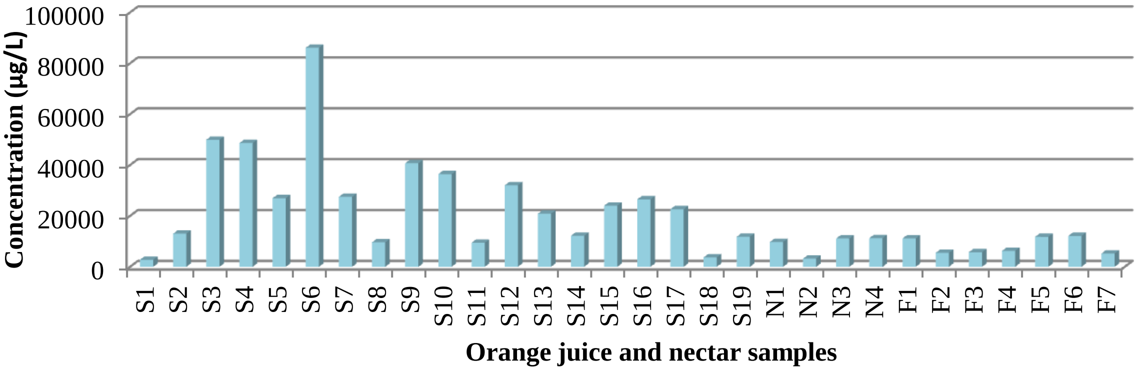

Figure 9: The total monoterpenes concentrations in the: commercial orange juices (S1-S19), orange nectars (N1-N4) and fresh single strength orange juices (F1-F7)

Sesquiterpenes

In the analyzed orange products 52 sesquiterpenes were determined, however only 20 were structurally identified, due to the high degree of mass spectra similarity. Moreover the structural identification of individual volatile compounds is not the main purpose of the present study.

In **Figure 10** the total sesquiterpenes content of the different type of analyzed orange product in this work is presented. It can be concluded that the total sesquiterpene concentration in the studied juices does not follow a specific behavior and based on this, a juices differentiation and quality evaluation could be performed.

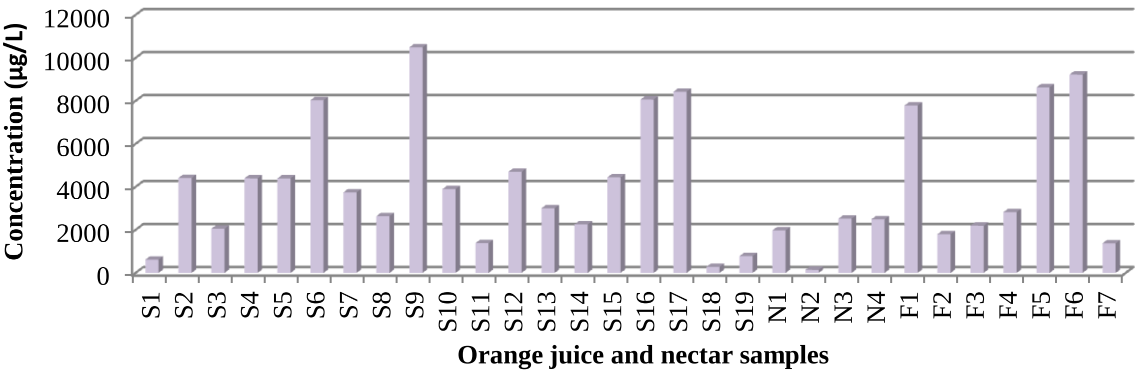

Figure 10: The total ketones concentrations in the: commercial orange juices (S1-S19), orange nectars (N1-N4) and fresh single strength orange juices (F1-F7)

In order to evaluate the quality of different type of orange juices it is necessary to introduce an index for quality evaluation. Considering the concentration of total terpenes without the major constituent of orange juices – limonene, and based on the relative ratio of sesquiterpenes and terpenes, the quality index proposed for the apple juice and the quality index for orange juice can be introduced. The quality index for orange juice is the relative ratio of total sesquiterpene and total terpenes without limonene.

In the **Figure 11** high values of quality index relative ratio of sesquiterpenes to terpenes without limonen can be observed for the *fresh single strength orange juices*. Based on the results a threshold value of minimum 40.0% for quality index was determined in the assessment of processed juice reconstituted from concentrate. The orange juices from concentrate characterized by a higher values for quality index than the threshold are considered high quality juices. Juices made from concentrate characterized by lower values for relative ration of sesquiterpenes and terpenes without limonen than 40.0% are categorized as low quality juice.

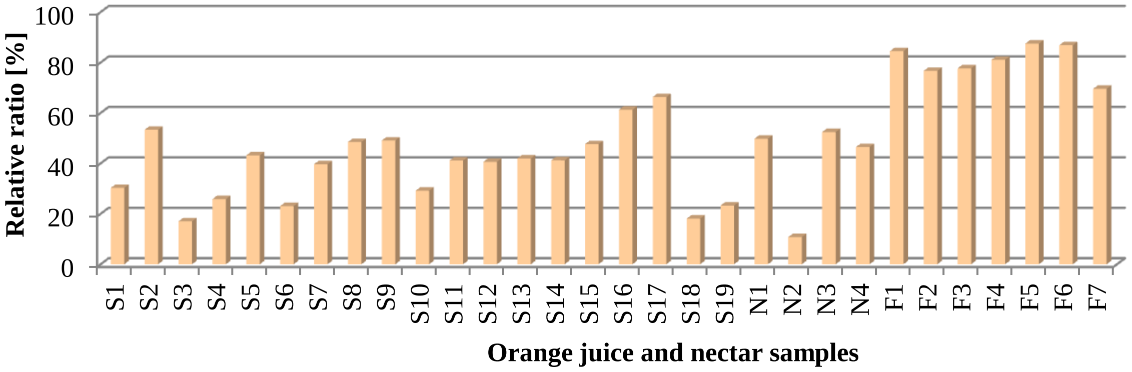

Figure 11: Relative ratio of total sesquiterpene/(total terpene without limonen) in the: commercial orange juices (S1-S19), orange nectars (N1-N4) and fresh single strength orange juices (F1-F7)

Conclusions

Headspace SPME-GC/MS analysis made possible the identification of 113 volatile organic compounds from different orange juices. The main volatile compounds identified were from the monoterpene class. Limonen content relative to the total identified volatile compounds for all samples were higher than 46.5%, therefore limonen is the major volatile constituent of orange juices.

Quality evaluation of orange juices and nectars can be performed considering threshold values for concentration of total alcohols (<1000 μg/L) and total ketones (<200 μg/L) and the relative ratio of total sesquiterpene/terpenes without limonen minimum of 40.0%.

4.6. Fresh single strength juices differentiation based on orange fruit origin

In order to differentiate orange fruits based on their origin the headspace SPME-GC/MS analysis was performed on eight samples from the following countries: South Africa, Greece, Turkey and Spain. Due to the high number of volatile compounds responsible for flavour of fresh single strength orange juice a selection of orange juice characteristic compounds was necessary, which may reflect specific properties of samples. For that purpose limonene, α-terpineol, valencene, terpinolene and α-cubebene were considered.

Limonene is the major constituent of volatile compounds responsible for the flavour. Valencene is a characteristic sesquiterpene of orange juice and the second major constituent of volatiles responsible for flavour (**Figure 12**).

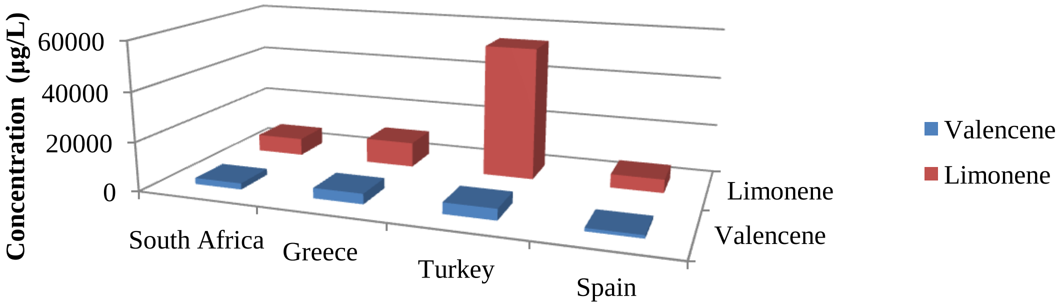

Figure 12: *Limonene and valencene concentrations based on orange fruit origin*

In order to try to make a differentiation of orange fruits based on origin, the ratio of limonene and valencene was considered. The values obtained for the limonene/valencene ratio are specific, allowing the assessment of fresh single strength juice based on the orange fruits origin (**Figure 13**). The values obtained for limonene/valencene ratio for South Africa and Greece were rather close but different enough to allow a differentiation based on origin. The limonene/valencene ratio is very high for orange fruit from Turkey. A specific value of limonene/valencene ratio was obtained for orange from Spain, therefore this ratio can be used for origin differentiation.

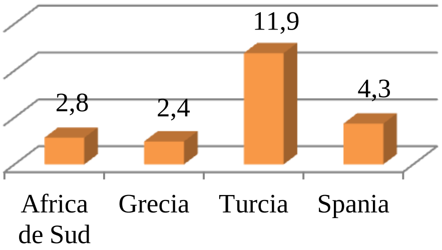

Figure 13: *Limonene/valencene concentration ratio based on the orange fruit origin*

The concentration of α-terpineol for the fresh single strength juice obtained from orange fruits originated from South Africa is 1.4 μg/L, from Greece is 2.9 μg/L, from Spain is 3.3 μg/L and a very high amount was detected in the orange from Turkey (48.8 μg/L). Terpinolene was determined at different concentrations: 11.2 μg/L for Spain, 29.2 μg/L for Greece, and 36.5 μg/L for South Africa, however Turkish origin orange juices contain higher concentration of terpinolene (1025.5 μg/L). The concentration of α-cubebene detected for orange from Spain is 59.2 μg/L, from South Africa is 124.7 μg/L, from Greece is 195.4 μg/L and higher from Turkey (454.9 μg/L) (**Figure 14**).

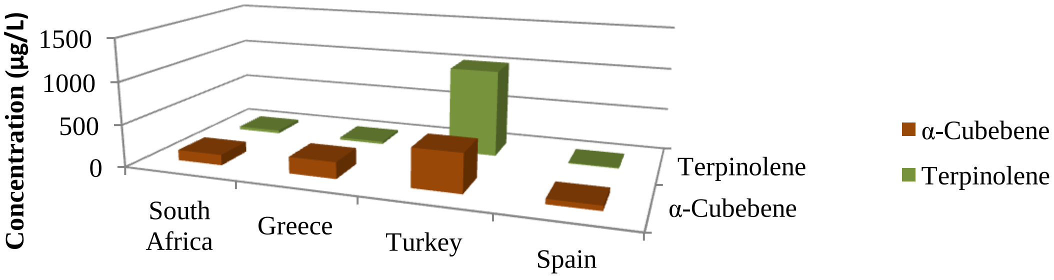

Figure 14: *α-Cubebene and terpinolene concentrations based on the orange fruits origin*

Using headspace SPME-GC/MS analytical method and considering the selected *markers* of orange juice: limonene, valencene, α-terpineol, terpinolene and α-cubebene and the limonene/valencene ratio respectively, the orange fruits can be differentiated based on origin.

Conclusions

The flavour is one of the most important characteristics associated with fruits quality and the most significant parameter of natural food and processed products, also. The qualitative and quantitative determination of volatile compounds responsible for the flavour from apple and orange juices/nectars was performed using headspace SPME-GC/MS analytical method.

In commercial apple juices and in fresh single strength juice obtained from different varieties of apple fruits 90 volatile organic compounds were determined. Significant qualitative and quantitative differences of volatile compounds responsible for flavour can be observed between the two types of juices: commercial and single strength. However differentiation criterions and quality evaluation based on variety and quantity of individual volatile organic compound is a lengthy process that proved not to be quite helpful in establishing a quality index. Differentiation characteristics of various type of apple juices are based on relative concentration differences of the major classes of compounds: total esters, alcohols, ketones and terpenes. A new index was proposed for the quality evaluation of different type of apple juices, the relative ratio of total sesquiterpene and total terpene. Using the proposed differentiation characteristics, the quality evaluation of apple juices can be done in the relatively simple manner.

Distinctive characteristics of *commercial apple juices* are: high concentration of total esters, ketones and alcohols; low concentration of total terpenes and total sesquiterpenes; relative ratio of total sesquiterpene and total terpene lower than 50%.

Distinctive characteristics of *single strength fresh apple juices* are: low concentration of total esters, ketones and alcohols; high concentration of total terpenes and total sesquiterpenes; relative ratio of total sesquiterpene and total terpene higher than 75%.

The flavour of the orange juices is the result of a complex combination of volatile compounds from the class of esters, alcohols, ketones, aldeydes, terpenes and other compounds in specific proportions. The headspace SPME-GC/MS analysis made possible the identification of 113 volatile organic compounds responsible for flavour from the studied orange juices. The main volatile compounds identified were monoterpenes and limonene was detected as the major constituent of orange juices.

The results highlight that only the content of total esters cannot be used for quality assessment of orange juice, because it does not provide specific information on the juice quality. Considering the concentration of total alcohols and total ketones a differentiation of various type of orange juice can be carried out. High quality orange juice is characterized by low relative concentration of total alcohols (<1000 μg/L) and total ketones (<200 μg/L).

The concentration of total monoterpenes and total sesquiterpenes does not provide univoque information that might be applied to quality evaluation of orange juice. In order to perform juice assessment and quality evaluation it is necessary to introduce a new index, defined as relative ratio of total terpenes and total terpenes without limonene. The threshold value of the quality index for processed juice reconstituted from concentrate is minimum 40.0%.

The quality evaluation of orange juices and nectars can be performed considering threshold values for concentrations of total alcohols (<1000 μg/L) and total ketones (<200 μg/L) and a relative ratio of total sesquiterpenes/terpenes without limonene of minimum 40.0%.

The orange fruit differentiation based on their origin it possible considering the concentration of volatile organic compounds responsible for flavour characteristic to fresh single strength juices (markers). The orange fruits differentiation with respect to the country origin can be performed based on the concentration of selected markers: limonene, valencene, α-terpineol, terpinolene and α-cubebene, and based on the concentration ratio of limonene/valencene, considering specific values obtained for each samples analyzed by headspace SPME-GC/MS analytical method.

Appendix

Appendix 1: Scientific papers published in journals

1. **Gabriella R. Schmutzer**, Alina D. Magdas, Leontin I. David, Zaharie Moldovan, “Determination of the Volatile Components of Apple Juice using Solid Phase Microextraction and Gas Chromatography – Mass Spectrometry”, accepted for publication in *Analytical Letters*; DOI:10.1080/00032719.2014.886694
2. **G. Schmutzer**, V. Avram, F. Covaciu, I. Feher, A. Magdas, L. David, Z. Moldovan, “Study of flavour compounds from orange juices by HS-SPME and GC-MS”, *AIP Conf. Proc*. 1565, 79, pp. 79-84 (2013); doi: 10.1063/1.4833701;
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5. **G. Schmutzer**, V. Avram, V. Coman, L. David, Z. Moldovan, “Determination of phenolic compounds from wine samples by GC/MS system”, *Rev. Chim. (Bucharest)*, 63, 9, pp. 855- 858, (2012);
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14. **Gabriella Schmutzer**, G. Damian, O. Cozar, V. Miclaus, Silvia Imre, Dina Petrisor, „EPR investigation of illuminated and UV irradiated nifedipine“, *Analele Universitatii de Vest Timisoara, Fizică,* 46/2005, pp 68-71; (2005);
15. V. Miclaus, G. Damian , **Gabriella Schmutzer**, Dina Petrisor, Claudia Cimpoiu, “EPR Study of Antioxidant Characteristics of some Wines“, *Studia Universitatis Babes-Bolyai, Physica*, I, 4b, 2005, pp 595-598; (2005);
16. G. Damian, **Gabriella Schmutzer**, Dina Petrisor, V. Miclaus, S. Simon, “Electron Spin Resonance Studies of γ-Irradiated B_3 Vitamin”, *Romanian J. of Biophysics*, Vol. 15, Nos. 1-4, pp 23-28; (2005);

Appendix 2: Scientific conferences and congresses participations

1. International Conference "Processes in Isotopes and Molecules" PIM2013, Cluj-Napoca, Romania, 25-27.09.2013., "Study of Flavour Compounds from Orange Juices by HS-SPME and GC/MS", **G. Schmutzer**, I. Feher, O Marincas, V. Avram, M. H. Kovacs, A Magdas, F. Covaciu, L. David, Z. Moldovan;
2. International Conference "Processes in Isotopes and Molecules" PIM2013, Cluj-Napoca, Romania, 25-27.09.2013., "Determination of formaldehyde in Romanian cosmetic products using coupled GC/MS system after SPME extraction", I. Feher, **G. Schmutzer**, C. Voica, Z. Moldovan
3. International Conference "Processes in Isotopes and Molecules" PIM2013, Cluj-Napoca, Romania, 25-27.09.2013., "Characterization of some Romanian white wines by volatile compounds composition", V Avram, **G. Schmutzer,** O Marincas, A Hosu, C. Cimpoiu, Z. Moldovan, C. Marutioiu
4. 9th GCxGC Symposium and 36th International Symposium on Capillary Cromatography, Riva del Garda, Italy, 27.05-01.06.2012., „Analysis of aroma compounds from fruit juice by HS-SPME and GC-MS", **G. Schmutzer**, A. Magdas, L. David, Z. Moldovan;
5. 9th GCxGC Symposium and 36th International Symposium on Capillary Cromatography,Riva del Garda, Italy, 27.05-01.06.2012., „Non-target pollutants detected in Prut river aqueous phase using GC/MS system", , Z. Moldovan, C.V. Avram, O. Marincas, C. Voica, F. Tusa, IC. Feher, **G. Schmutzer**, M.H. Kovacs, A. Alder;
6. 17th International Symposium on Separation Sciences, Cluj-Napoca, Romania, 05-09.09.2011., "Characterization of fruit juice flavour compounds by SPME-GC/MS", **G. Schmutzer**, A. Magdas, Z. Moldovan;
7. International Conference "Processes in Isotopes and Molecules" PIM2011, Cluj-Napoca, Romania, 29.09.-01.10.2011., "Determination of some Volatile Compounds in Alcoholic Beverage by Headspace Solid-phase Microextraction Gas Chromatography – Mass Spectrometry", **G. Schmutzer**, V. Avram, I. Feher, L. David, Z. Moldovan;
8. International Conference "Processes in Isotopes and Molecules" PIM2011, Cluj-Napoca, Romania, 29.09.-01.10.2011., "Determination of anionic surfactants in water samples using GC/MS system", Z. Moldovan, O. Marincas, V. Avram, **G. Schmutzer**
9. International Conference "Processes in Isotopes and Molecules" PIM2011, Cluj-Napoca, Romania, 29.09.-01.10.2011., "The use of Mass Spectrometry in the artwork studies", Z. Moldovan, I. Bratu, V. Avram, **G. Schmutzer,** O. Marincas, I. Feher, C. Marutoiu
10. International Conference "Processes in Isotopes and Molecules" PIM2011, Cluj-Napoca, Romania, 29.09.-01.10.2011., "Analysis Of Flavour Compounds By GC/MS After Liquid-Liquid Extraction From Fruit Juices", F. D. Tusa, Z. Moldovan, **G. Schmutzer**, D.A. Magdas, A. Dehelean, M. Vlassa
11. International Conference "Processes in Isotopes and Molecules" PIM2011, Cluj-Napoca, Romania, 29.09.-01.10.2011., "Multielement and stable isotope determinations in some Transylvanian fruit juices", A. Dehelean, D.A. Magdas F. D. Tusa, **G. Schmutzer**
12. *4th International Workshop on Liquid Chromatography-Tandem Mass Spectrometry for Screening and trace level Quantitation in Environmental and Food Samples, Barcelona, Spain, 7-8.02.2008., "*The formation of the characteristic ions used for determination of the antibiotics in water samples by LC/MS methods", Z. Moldovan and **Gabriella Schmutzer**

13. *4th NORMAN workshop: Integrated chemical and bio-monitoring strategies for risk assessment of emerging substances, Lyon, France, 17-18.03.2008., “*Environmental Exposure of Pharmaceuticals in the Somes Valley Watershed in Romania”, Z. Moldovan, **Gabriella Schmutzer**, A. C. Alder and R. Chira
14. 5th Conference on Isotopic and Molecular Processes, PIM - 2007, Cluj-Napoca, Romania, 20-22.09.2007., “Photodegradation of organic pollutants under UV irradiation in the presence of TiO_2”, **G. Schmutzer,** Z. Moldovan, V. Danciu, F. Vasiliu;
15. 5th Conference on Isotopic and Molecular Processes, PIM - 2007, Cluj-Napoca, Romania, 20-22.09.2007., “Determination of removal rate of the pharmaceuticals and personal care products (PPCPs) in the wastewater treatment plant from Cluj-Napoca, by *GC/MS* after *SPE* enrichment” Z. Moldovan, **G. Schmutzer**, R. Chira, A. Alder
16. 11th International Conference on Chemistry and the Environment, Torun, Poland, 09-12.09.2007., “Experimental Method for Determination of Some Antioxidants From Wines”, **G. Schmutzer**, Z. Moldovan, N. Palibroda, V. Coman;
17. 25th Informal Meeting on Mass Spectrometry, Nyiregyhaza-Sosto, Hungary, 06-10.05.2007., "The Study of the Thermal Decomposition of Some Compounds by Mass Spectrometry ", Z. Moldovan, **G. Schmutzer,** A. Bende, L. David;
18. *Water Status Monitoring under the WFD Conference, Lille, France, 12-14.03.2007., “*Study of occurrence and distribution of pharmaceutical and personal care products as micropollutants in surface water from Romania”, Z. Moldovan, **Gabriella Schmutzer**, Florina Tusa, Roxana Calin and Alfredo C. Alder
19. Al XIII-lea Congres National de Farmacie, Cluj-Napoca, Romania, 28-30.09.2006., „Detection Of Traces Of Pharmaceuticals Products In Romanian Surface Waters By GC/MS System”, Z. Moldovan**, G. Schmutzer,** N. Palibroda, A. Alder, R. Calin;
20. 2nd Advanced Spectroscopies on Biomedical and Nanostructured Systems, Cluj-Napoca, Romania, 03-06.09.2006, “Mass spectrometric study of Pharmaceuticals thermal decomposition”, Z. Moldovan, **G. Schmutzer**, A. Alder;
21. 1st European Chemistry Congress, Budapest, Hungary, 27-31.08.2006., “Characterization of Pharmaceutical and Personal Care Products as Micropollutants in Wasterwater Treatment Plant”, Z. Moldovan, **G. Schmutzer,** F. Tusa, N. Palibroda, A. Alder, L. Mic, R. Chira;
22. 17th International Conference on Mass Spectrometry, Praga, Czech Republic, 27.08-01.09.2006., “Determination of Pharmaceutical Compounds in Aquatic Environment by GC/ITMS”, Z. Moldovan, **G. Schmutzer,** A. Alder;
23. International Conference on Automation, Quality and Testing, Robotics, Cluj-Napoca, Romania, 25-28.05.2006., „Assurance of Quality Control in Analytical Procedures Using Mass Spectrometry”, Z. Moldovan, **G. Schmutzer**, A. Alder;
24. Conferinţa Ştiinţifică din Transilvania, Ediţia a VIII-a, Cluj-Napoca, Romania, 10-11.12.2005., “Studiul ESR a radicalilor liberi prezenti in nifedipin”, **G. Schmutzer;**
25. „Physics Conference TIM-05”, Timisoara, Romania, 25-27.11.2005., “EPR investigation of illuminated and UV irradiated nifedipine”, **G. Schmutzer**, G. Damian, O. Cozar, V. Miclaus, S. Imre, D. Petrisor;
26. 4th Conference “Isotopic and Molecular Processes”, Cluj-Napoca, Romania, 22–24.09.2005., “EPR study of antioxidant characteristics of some wines“, V. Miclaus, G. Damian, D. Petrisor, **G. Schmutzer**, C. Cimpoiu;
27. „8th Romanian Biophysics Conference”, Iaşi, Romania, 26-28.05.2005., **“**Electron Spin Resonance Studies of γ-Irradiated B_3 Vitamin”, G. Damian, **G. Schmutzer,** D. Petrisor, V. Miclaus, S. Simon;
28. Conferinţa Ştiinţifică din Transilvania, Ediţia a VII-a, Cluj-Nacoca, Romania, 04-05.12.2004., „Utilizarea ultrasunetelor şi a ecografului în diagnosticarea medicală”, **G. Schmutzer;**

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