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EVALUATION OF ORANGE JUICES ON THE BASE OF SELECTED AUTHENTICITY MARKERS

S u m m a r y

Samples of orange juices were evaluated according to standard criteria in Code of Practice and compared with standard RSK values (Richtwerte und Schwankungsbreiten bestimmter Kennzahlen). In 15 orange juices, the content of chlorides, total acidity, volatile acids and ammonia did not exceed values in Code of Practice. It was found that samples F and K (66.30 g/dm^3 and 57.65 g/dm^3) contained more glucose than is allowed by Code of Practice. Juices G and K did not exceed RSK values for the D-isocitric acid content (103.68 g/dm^3 and 109.96 g/dm^3). Criterion according to citric and D-isocitric acid ratio corresponded to the normal values in 100% orange juice in samples G, K, L and also in 12% orange nectar P (69.3; 118.6; 51.4 and 72.3).

Key words: orange juices, adulteration, authentication, isotachophoresis

Introduction

Generally, adulterated foodstuff are products with changed appearance, taste, composition or another signs in the way of devalue and which are presented as genuine with accustomed appellation to the consumer [1].

Falsification of foodstuffs means [2]:

1. addition of cheaper or more available substance to the foodstuff which modify sensory properties at the least bit
2. total or partial replacement of some specific components of foodstuff (substitute of natural vitamins, aromas and dyes to synthetic which is frequent in fruit juices)

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3. addition of additives which are cover up the real quality or origin (for example the supplementary colour or aromatize foodstuffs with less quality)
4. mendacious trademark or information about composition, origin and age.

Orange juices and nectars represent the food commodity that is very often subjected to adulteration. The main deviations are: lowering of fruit content (addition of sugars, acids, artificial mixtures), unlabelled sugar addition (usually without lowering of fruit content), pulp wash addition (including pure pulp wash juices), lower refractive index, low quality of water used for reconstitution and others [3].

Criterions for quality of orange juices are specifying in Council Directive 2001/112/EC which is in force for member states in EU [4]. Association of the Industry of Juices and Nectars from Fruits and Vegetables issued Code of Practice (CP) that is generally standard in EU [5]. First standards for fruit juices were developed in Germany and they are known as RSK values (Richtwerte und Schwankungsbreiten bestimmter Kennzahlen) [6]. Slovak national criteria and other regulations for orange juices are laid down by Decree [7, 8].

Quality requirements such as minimal relative density and corresponding Brix values, isotopes, ethanol, arsenic and heavy metals and the essential parameters and their characteristic value or range for the evaluation of identity and authenticity as well as some recommended quality criteria for juices, e.g. acids, minerals, sugars, amino acids, flavonoids, etc. is used [3]. Simple adulteration of orange juices by addition of water, sugars and acids is possible to detect by determination ratio of citric acid to D-isocitric acid. Recently enzymatic methods, high-performance liquid chromatography [9], capillary isotachophoresis [3, 9, 10], electrophoresis [11] and gas chromatography [12] is using to measurement content of citric and D-isocitric acid.

The aim of this study was authentication of commercial orange juices by determination of selected authenticity markers (titrable acids, volatile acids, glucose, chlorides, ammonia, L-ascorbic acid, lactic acid, D-isocitric acid, ratio of citric and D-isocitric acid) and comparison of measured results with RSK [6, 13, 14] and Code of Practice standards [5, 14]. Finally it was applying of principal component analysis and selection analytical parameters with the best power of testimony for orange juices.

Material and methods

Orange juices analysed in experiment were randomly purchased from retail chain. List of analysed samples according to the respective labels such a producer, country of origin, packaging and description is in the Tab. 1.

The measurement of pH was performed by pH meter type OK – 104 (Radelkis, Budapest, Hungary). The titrable acidity was determined by the visual titration with NaOH using phenolphthalein indicator and expressed as tartaric acid [5, 15]. The determination of volatile acids was performed by titration with NaOH using phenolphthalein

T a b l e 1

List of analysed orange juices.
Wykaz analizowanych soków pomarańczowych.

Sample Próbka	Country of Origin Kraj pochodzenia	Package / volume Opakowanie / objętość	Description Opis
A 100% orange juice 100% sok pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, sugar and preservative free pasteryzowany, bez dodatku cukru i konserwantów
B 100% orange juice 100% sok pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, sugar and preservative free pasteryzowany, bez dodatku cukru i konserwantów
C 100% orange juice 100% sok pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, sugar and preservative free pasteryzowany, bez dodatku cukru i konserwantów
D 100% orange juice 100% sok pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, sugar and preservative free pasteryzowany, bez dodatku cukru i konserwantów
E 100% orange juice 100% sok pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, preservative free pasteryzowany, bez dodatku konserwantów
F 100% orange juice 100% sok pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, sugar and preservative free pasteryzowany, bez dodatku cukru i konserwantów
G 100% orange juice 100% sok pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, preservative free pasteryzowany, bez dodatku konserwantów
H 100% orange juice 100% sok pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, preservative free pasteryzowany, bez dodatku konserwantów
I 100% orange juice 100% sok pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, sugar and preservative free pasteryzowany, bez dodatku cukru i konserwantów
J 100% orange juice 100% sok pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, sugar and preservative free pasteryzowany, bez dodatku cukru i konserwantów
K 100% orange juice 100% sok pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, sugar and preservative free pasteryzowany, bez dodatku cukru i konserwantów
L 100% orange juice 100% sok pomarańczowy	Czech Republic Czechy	Tetra Pak / 1 dm^3	pasteurised, preservative free pasteryzowany, bez dodatku konserwantów
M 100% orange juice 100% sok pomarańczowy	Austria Austria	Tetra Pak / 1 dm^3	pasteurised, sugar free pasteryzowany, bez dodatku cukru
N 60% orange nectar 60% nektar pomarańczowy	Slovakia Słowacja	Tetra Pak / 1 dm^3	pasteurised, preservative free pasteryzowany, bez dodatku konserwantów
P 12% orange juice 12% sok pomarańczowy	Czech Republic Czechy	Al-PE / 0,25 dm^3	pasteurised, sugar and preservative free pasteryzowany, bez dodatku cukru i konserwantów

indicator after destilation with steam [15]. Chlorides were performed according to Mohr [15]. Glucose was determined by visual titration with $Na_2S_2O_3$ with solution of starch as indicator after oxidation with iodine [15]. L-ascorbic acid was determined by visual titration by 2,6-dichlorophenolindophenol [15]. Ammonia was determined by microdiffusion [15].

Measurements of organic acids were realised on the isotachophoretic analyser ZKI 01, Villa Labeco Spišská Nová Ves with conductivity detector. For determination of lactic acid, acetic acid and citric acid electrolytic system of following composition was applied [16, 17]: Leading electrolyte (LE): 10^{-2} mol/dm^3 hydrochloric acid (HCl), counter-ion ε–aminocaproic acid, additive methylhydroxyethylcellulose (MHEC), pH 4.25 and terminal electrolyte (TE): 5×10^{-3} mol/dm^3 caproic acid. The samples are analysed at the driving current of 300 μA in preseparation column. For determination of D-isocitric acid was applied electrolytic system with this composition [10, 18]: LE: 6×10^{-3} mol/dm^3 Histidine Chloride, 5×10^{-3} mol/dm^3 Histidine + 2×10^{-3} mol/dm^3 $CaCl_2$, additive hydroxyethylcellulose (HEC), pH 6 and TE: 10×10^{-3} mol/dm^3 citric acid. The current in preseparation column was 200 μA. Quantitative analysis was performed by calibration.

To evaluation results of chemical analysis was applied principal component analysis (PCA) - statistical program Statgraphics plus for Windows, Version 1.4. PCA is method for reducing the dimensionality of a set of variables by constructing uncorrelated linear combinations of them. The combinations are computed in a way that the first component accounts for the major part of the variance; that is, it is the major axis of the points in the p-dimensional space [19, 20].

Results and discussion

13 samples declared as 100% orange juices by producer and also 60% and 12% orange nectars with purpose of comparison were tested in this work. Products were obtained from domestic and furthermore from foreign producers. In all samples following parameters were analysed: titrable acids, glucose, chlorides, ammonia, volatile acids, L-ascorbic acid, lactic acid, citric acid, isocitric acid and ratio of citric and isocitric acid. Further pH value and content of citric acid was also determined.

First standards for fruit juices which were developed in Germany were denominated RSK values (Richtwerte und Schwankungsbreiten bestimmter Kennzahlen) [6, 13, 14]. Selected RSK values and standards according to Code of Practice set by AIJN (Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (EEC) [5, 14] for 100% orange juices are presented in Tab. 2.

Results of chemical analysis in evaluated samples of orange juices are summarised in Tab. 3 and 4.

T a b l e 2

Selected RSK values and values according to Code of Practice (CP) for orange juices.
Wybrane wartości RSK i CP dla soków pomarańczowych.

Parameter Parametr	RSK values Wartości RSK	Code of Practice Wartości CP
Titrable acids [g/dm^3] Kwasowość miareczkowa	8-12	5.8-15.4
Volatile acids [g/dm^3] Lotne kwasy	max. 0.4	max. 0.4
Lactic acid [g/dm^3] Kwas mlekowy	-	max. 0.5
Citric acid [g/dm^3] Kwas cytrynowy	7.6-11.5	6.3-17
D-isocitric acid [mg/dm^3] Kwas D-izocytrynowy	65-130	65-200
Citric/ D-isocitric acid ratio Stosunek kwas cytrynowy/kwas D-izocytrynowy	80-130	max. 130
L-ascorbic acid [mg/dm^3] Kwas L-askorbinowy	min. 200	min. 200
Glucose [g/dm^3] Glukoza	min. 20	20-50
Ammonia [mg/dm^3] Amoniak	max. 1.5 mmol/dm^3	max. 17
Chlorides [mg/dm^3] Chlorki	max. 60	max. 60

Titrable acidity of juices was from 6.0 g/dm^3 to 15.01 g/dm^3. All of samples including 60% and 12% orange nectars fulfilled the standard value for this parameter in Code of Practice, however in comparison with RSK value almost 50% samples had higher value of titrable acidity. The relative standard deviations at determination of titrable acidity were from 1.5% to 2.9%. Samples of 100% orange juices had pH value from 3.5 (F) to 4.2 (G). Sample of 60% orange nectar (N) had pH value 3.6 and sample of 12% orange nectar (P) marked out in the lowest pH value (3.0).

Content of glucose in the juices was from 20.18 g/dm^3 (G) to 92.24 g/dm^3 (12% orange nectar P). Samples of 100% orange juices A, B, E, G, H, I, L and M fit the requirements from Code of Practice. In the samples C and D were determined higher content of glucose by 0.44 g/dm^3 against standard in Code of Practice. In the sample of 60% orange nectar (N) (content of glucose was 61.97 g/dm^3) producer declared addition of sugars in the label of product. The relative standard deviations for determination of glucose varied from 0.3% to 3.5%.

T a b l e 3

Analytical parameters of orange juices
Analityczne parametry soków pomarańczowych

Sample Próbka	pH	Titrable acids Kwasowość miareczkowa [g/dm^3]	Glucose Glukoza [g/dm^3]	Chlorides Chlorki [mg/dm^3]	Ammonia Amoniak [mg/dm^3]	Volatile acids Lotne kwasy [g/dm^3]	L-ascorbic acid Kwas L-askorbinowy [mg/dm^3]
A	3.90	13.21	47.56	28.15	9.18	0.29	339.7
B	3.65	12.01	49.00	31.92	9.45	0.19	248.7
C	3.65	9.01	50.44	28.36	8.16	0.21	267.5
D	3,90	13.81	50.44	29.93	8.45	0.25	324.6
E	3.80	14.41	46.12	28.98	8.59	0.24	218.7
F	3.50	10.21	66.30	30.67	8.62	0.25	194.9
G	4.20	9.60	20.18	27.08	9.90	0.25	252.3
H	3.85	13.22	38.91	29.78	9.28	0.22	293.2
I	4.00	13.81	36.01	28.10	8.09	0.23	248.7
J	3.75	14.41	57.65	29.35	8.07	0.21	233.5
K	3.85	8.41	66.30	27.86	8.73	0.19	152.3
L	3.90	14.41	47.56	30.47	9.18	0.23	326.9
M	3.90	15.01	47.56	26.31	6.35	0.25	403.8
N	3.60	10.81	61.97	25.30	8.03	0.15	262.8
P	3.00	6.00	92.24	ND	3.56	0.07	216.7

Content of chlorides in the samples was from 25.30 mg/dm^3 to 31.92 mg/dm^3. In the sample of 12% orange nectar (P) the content of chlorides was under limit of quantification of applied method. The relative standard deviations for determination of chlorides were from 3.0% to 6.0%.

Samples of orange juices contained from 2.65 mg/dm^3 to 9.90 mg/dm^3 of ammonia and 0.07 mg/dm^3 to 0.21 mg/dm^3 of volatile acids. Content of chlorides, ammonia and volatile acids fulfilled the criterions in Code of Practice in all of analysed samples. The relative standard deviations at determination of ammonia and volatile acids ranged from 2.9% to 6.0% and from 0.9% to 2.5% respectively.

According to RSK values and standards in Code of Practice 100% orange juices have to contain minimally 200 mg/dm^3 of L-ascorbic acid. Samples F (194.90 mg/dm^3) and K (152.30 mg/dm^3) did not fill this requirement. Only in following three of analysed samples producer declared minimally content of L-ascorbic acid: D (300 mg/dm^3), L (270 mg/dm^3) and M (400 mg/dm^3). All of this samples suit values of declared contents of L-ascorbic acid. The relative standard deviations for determination of L-ascorbic acid varied from 0.9% to 3.3%.

T a b l e 4

Content of organic acids in orange juices (ND – not detectable).
Zawartość kwasów organicznych w sokach pomarańczowych (ND – poniżej poziomu wykrywalności).

Sample Próbka	Organic acids Kwasy organiczne				Citric/D-isocitric acid ratio kwas cytrynowy/kwas D - izocytrynowy
	Lactic mlekowy [g/dm^3]	Acetic octowy [g/dm^3]	Citric cytrynowy [g/dm^3]	D-isocitric D-izocytrynowy [mg/dm^3]	
A	ND	0.42	7.90	43.98	179.7
B	ND	0.24	6.73	25.13	267.8
C	ND	0.24	6.42	34.56	185.7
D	ND	0.32	9.83	19.89	195.5
E	ND	0.35	9.34	12.57	742.8
F	ND	0.32	5.61	28.28	198.3
G	ND	0.37	7.18	103.68	69.3
H	ND	0.35	7.85	37.70	208.3
I	ND	0.42	10.10	50.27	200.9
J	ND	0.32	7.76	34.56	224.7
K	ND	0.27	5.65	109.96	51.4
L	ND	0.29	4.85	40.84	118.6
M	ND	0.50	8.39	25.13	333.9
N	ND	0.27	6.73	28.28	238.1
P	ND	0.24	3.41	47.13	72.3

Measurements of lactic, acetic, citric and isocitric acids were realised by capillary isotachophoresis. The relative standard deviations for determination of organic acids were from 0.8% to 3.8%. Limit of determination for individual acids were: 2.0 mg/dm^3 for lactic acid, 1.62 mg/dm^3 for citric acid and 2.14 mg/dm^3 for isocitric acid. Content of lactic acid in all samples was under limit of quantification of used method. Content of acetic acid was from 0.24 mg/dm^3 (B, C and P) to 0.50 mg/dm^3 (M). Content of citric acid, isocitric acid and their ratio are significant parameters for authentication of orange juices. Analysed samples contained from 3.41 mg/dm^3 (12% orange nectar P) to 10.10 mg/dm^3 (I) of citric acid and from 12.57 mg/dm^3 (E) to 109,96 mg/dm^3 (K) of isocitric acid. Content of citric acid was under requirement in Code of Practice in samples F, K and L. The lowest content of isocitric acid was determined in 12% orange nectar P (3.41 mg/dm^3). Standard RSK – value for ratio of citric and isocitric acid for 100% orange juices represent from 65 to 130 and according to Code of Practice from 65 to 200. These requirements fulfilled only two of analysed samples (G and K). It is noteworthy that 12% orange nectar P contained higher content of isocitric acid (47.13

mg/dm^3) than majority of analysed 100% orange juices. A limit to 130 for ratio of citric to isocitric acid in the 100% orange juices which has been proposed by Code of Practice was not exceeded only in the samples G (69.3), K (51.4) and L (118.6). Ratio of citric and isocitric acid in 12% orange nectar P was 72.3 that remarkably fulfilled requirement on 100% orange juices.

Results of analytical determinations of orange juices (without variable lactic acid which content was under limit of quantification of used method in all analysed samples) were organised to data matrix type 15 x 11 and evaluated with principal component analysis. This method was used to reduction of initial variables.

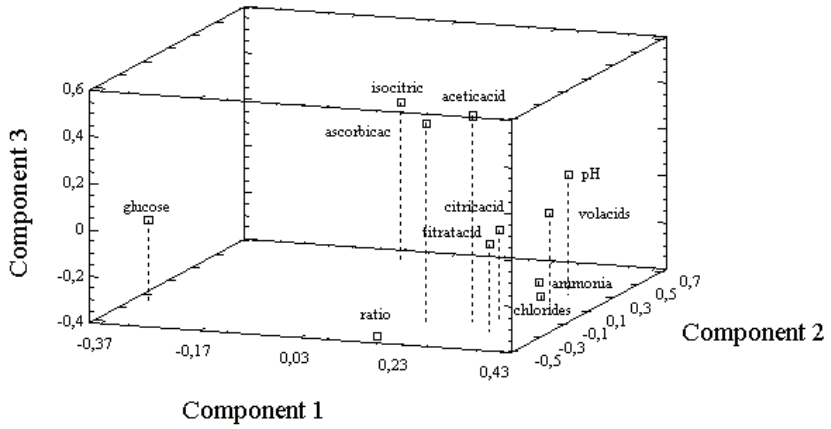

Fig. 1. Component weights of analytical parameters in axes of first three principal components.
Rys. 1. Wagi składników parametrów analitycznych w trójwymiarowej przestrzeni pierwszych trzech głównych składowych.

PCA reduced original 11 analytical parameters to 3 principal components (PC) that accounted 83.3% from total variance of data (PC1 51.2%, PC2 19.9% and PC3 12.2%). In the Fig. 1, the component weights of analytical parameters in the axes of first three components are presented. This figure shows that the PC1 the most described variables: volatile acids, pH, glucose, citric acid and total acidity, PC2 the most described content of D-isocitric acid and ratio of citric and D-isocitric acids and PC3 content of L-ascorbic acid. In the Fig. 2 are showed score of samples of orange juices in axes of first two components. From the Fig. 2 seen that PCA separated samples of orange juices to 5 groups (A – E):

group A – contained 12% orange nectar P; this sample had the lowest value of pH, the highest content of glucose and the lowest content of ammonia, volatile acids and citric acid;

group B – contained 100% orange juice K; this sample had the lowest content of L-ascorbic acid, the lowest ratio of citric and D-isocitric acid and the highest content of D-isocitric acid;

group C – contained 100% orange juice G; this sample had the lowest content of glucose;

group D – contained 100% orange juices M and E; those samples had the highest ratio of citric and D-isocitric acid and the lowest content of D-isocitric acid;

group E – contained orange juices which had average value of determined analytical parameters. It is interesting that 60% nectar N belong to this group.

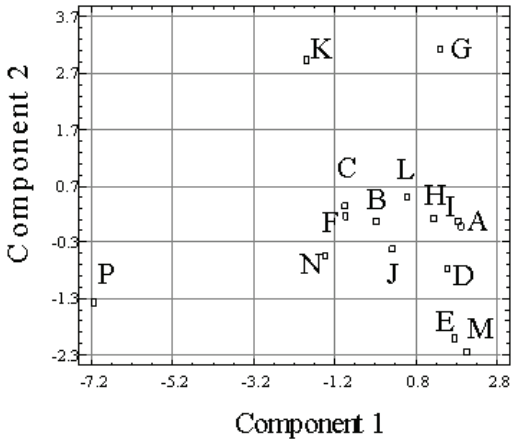

Fig. 2. Score of samples in axes of first two principal components.
Rys. 2. Rzut próbek na płaszczyznę pierwszych dwóch głównych składowych.

Conclusions

1. Chemical analysis denoted that content of glucose in the samples of 100% orange juices C, D, F, J and K and content of L-ascorbic acid in the samples F and K were under requirement limits according to Code of Practice.
2. Only two samples of orange juices (G and K) contained desiderative content of D-isocitric acid and samples G, K and L fulfilled criterion for ratio of citric and D-isocitric acid in Code of Practice.
3. Principal component analysis extracted variables which is important to authentication of 100% orange juices.

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OCENA AUTENTYCZNOŚCI SOKU POMARAŃCZOWEGO NA PODSTAWIE WYBRANYCH WYRÓŻNIKÓW

S t r e s z c z e n i e

Próbki soków pomarańczowych oceniano pod względem zgodności z kryteriami Code of Practice (CP) i porównywano z wartościami standardowymi RSK (Richtwerte und Schwankungsbreiten bestimmter Kennzahlen). We wszystkich 15 badanych sokach kwasowość ogólna, zawartość chlorków, lotnych kwasów i amoniaku nie przekraczała wartości podanych w CP. Stwierdzono, że próbki F i K zawierały więcej glukozy (odpowiednio 66.30 g/dm^3 i 57.65 g/dm^3) niż jest to dopuszczone przez CP, z kolei soki G i K osiągnęły najwyższe zawartości kwasu D-izocytrynowego (odpowiednio 103.68 g/dm^3 i 109.96 g/dm^3). Kryteria stosunku stężeń kwasów cytrynowego i D-izocytrynowego spełniały próbki soków 100% G, K i L oraz nektarów 12% P. Odpowiednie wartości wynosiły: 69.3; 118.6; 51.4 i 72.3.

Słowa kluczowe: sok pomarańczowy, zafałszowanie, autentyczność żywności, izotachoforeza