

Authenticity and quality of spirit vinegar: Methods for detection of synthetic acetic acid addition

ADÉLA GRÉGROVÁ – HELENA ČÍŽKOVÁ – JIŘÍ MAZÁČ – MICHAL VOLDŘICH

Summary

A set of authentic spirit vinegars and mixtures of vinegars with synthetic acetic acid was analysed. Isotopic ratios, $^2\text{H}/^1\text{H}$ (SNIF-NMR; site-specific natural isotopic fractionation-nuclear magnetic resonance) and $^{13}\text{C}/^{12}\text{C}$ (IRMS; isotope ratio mass spectrometry), were determined and compared with the already published data. Gas chromatography analyses of volatiles and sensory analyses were applied to distinguish spirit vinegars, synthetic acetic acids and their mixtures. Concentrations of minor volatiles such as acetaldehyde and acetone were very low and too variable to be used as authenticity markers. The samples of authentic spirit vinegars contained ethanol and ethyl acetate, the indicators of the fermentation process, in concentrations ranging from $0.50\text{ g}\cdot\text{l}^{-1}$ to $3.40\text{ g}\cdot\text{l}^{-1}$ and from $0.10\text{ g}\cdot\text{l}^{-1}$ to $0.50\text{ g}\cdot\text{l}^{-1}$, respectively. Both the sensory assessment and the chromatographic method were found to be useful for fast screening of suspicious samples or for the detection of highly adulterated samples. However, these methods do not allow quantification of added synthetic acetic acid or confirmation of mixtures. In such cases, only the coupled SNIF-NMR and IRMS methods are able to authenticate the contents of the samples credibly with the estimated detection limit of 15% of synthetic acid addition.

Keywords

spirit vinegar; synthetic acetic acid; authenticity; SNIF-NMR; IRMS; GC-FID

Vinegar is extensively used as an acidifying and food-preserving agent. It regulates pH and counteracts growth of bacteria and yeasts. It is a traditional component of various food products (salads, dressings, mayonnaise, ketchup, sauces, pickles etc.) giving them characteristic aroma and taste. Vinegar is obtained by two-stage fermentation process, i.e. alcoholic fermentation (conversion of fermentable carbohydrates to ethanol) and acetous fermentation (oxidation of ethanol to acetic acid). Initial materials for vinegar production include white wine, red wine, apple, malt, yeast rice, whey, concentrated grape juice, alcohol etc. Refined alcohol produced from raw molasses, potatoes or grain alcohol are generally used for the production of spirit vinegars in Central Europe [1]. Vinegar is made exclusively by the biological process of alcohol fermentation and is enriched with nutrients with the help of vinegar bacteria. Spirit vinegar is made by fermentation of alcohol derived from starch or saccharides-containing raw materials.

Not only expensive and luxurious goods are adulterated, but also those that are cheap and common but sold in big quantities. The main problem in the vinegar industry lies in the difficult distinction between highly valued, high quality wine vinegars or aged balsamic vinegars and their cheaper alternatives derived from malt or alcohol and/or vinegar adulteration with diluted synthetic acid.

In Czech Republic, there are sometimes problems with availability of spirit vinegar on the market and/or the price varies according to the price and accessibility of spirit. The vinegar producers warned several times of the suspect vinegar in the distribution chain. The indications were mainly the price and also the higher concentration of acetic acid in vinegar, very often over the values that can be reached by fermentation. Subsequently, the differentiation between authentic and fully or partially synthetic “vinegar” is demanded. The addition of synthetic acid is not only an adulteration against the fair trade but it also could bring other dangers

Adéla Grégrová, Helena Čížková, Michal Voldřich, Department of Food Preservation, Institute of Chemical Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic.

Jiří Mazáč, Customs Technical Laboratory Prague, Budějovická 7, 140 96 Prague 4, Czech Republic.

Correspondence author:

Helena Čížková, e-mail: Helena.Cizkova@vscht.cz, tel.: +420 220443014, fax: +420 220444455

for the consumer, such as unexpected accompanying products from the synthetic production.

Synthetic acetic acid, which is not primarily intended for food purposes, is not permitted as a substitute for vinegar without clear designation. It is used in laboratories and chemical industry as an important solvent and it is applied in manufacturing of a variety of other organic compounds (vinyl acetate, acetone). Industrial production of acetic acid is based on the catalytic oxidation of acetaldehyde or butane and butene. Recently for the production of acetic acid, methanol carbonylation with carbon monoxide in the presence of cobalt iodide or possibly rhodium catalysis have been applied [2]. In accordance with the EU legislation [3], synthetic acetic acid, if properly labelled, may be contained in food products as an additive (E260).

The addition of synthetic acetic acid into the vinegars can be generally detected using the following methods or approaches:

- Stable isotopes analysis, which is the principle method for the determination of the origin of various substances mainly ethanol and relevant; the determination of $^2\text{H}/^1\text{H}$ ratios of acetic acid methyl groups using site-specific natural isotopic fractionation-nuclear magnetic resonance (SNIF-NMR) and $^{13}\text{C}/^{12}\text{C}$ ratios by isotope ratio mass spectrometry (IRMS), providing complementary information to characterize the botanical origin of acetic acid and to detect adulteration of vinegar by synthetic acetic acid [4–7].
- The synthetic acetic acid does not contain the volatiles that are typical for the raw materials used for the production, the volatiles produced during the fermentation process; the addition of synthetic acid into the authentic vinegar will decrease the usual levels of natural accompanying substances present in the product, the usually used markers being e. g. methanol, 1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol, ethyl propionate and acetoin [6, 8].
- The fermentation process can be also proved by the analyses of amino acids, D-alanine, D-aspartic acid and D-glutamic acid etc., which are produced during the fermentation process and may be present also in vinegars, sufficient concentrations being expectable in wine vinegars, apple vinegars and other products produced from fruit juices or wines [9].
- The macroelement and microelement profiles can be also used for characterization of vinegars; the concentration of macroelements and microelements is also affected by the plant source and soil geology as well as by processing

and leaching from storage containers, the synthetic acid profiles differing significantly from that of the authentic vinegar [10].

- To some extent, the quality of vinegar can be evaluated sensorially; the special flavour of vinegars depends on the synergic sensory effects of minor components from the raw materials and technological process, similarly the residues of the synthetic production can cause the specific flavour of the adulterated products [8].

Unfortunately, most of the methods mentioned above are suitable for the authentication of so called first grade vinegars (of wine or fruit origin). In the case of the spirit vinegar, which is produced from raw molasses, potatoes or grain alcohol, there is lack of the fermentation and raw material markers.

The objectives of this study were to extend the pool of already published isotope ratios with new data of the spirit vinegars produced by traditional technology and of synthetic acetic acids. The objective was also to evaluate the possibilities of simple analytical methods, gas chromatography with flame ionization detector (GC-FID) and gas chromatography–mass spectrometry (GC-MS) of volatiles, for rapid screening purposes and to evaluate the possibilities of distinguishing of spirit vinegar, synthetic acetic acid and their mixtures using the sensory analysis. The tests were focused to the vinegars and also to pickled cucumbers made using these vinegars.

MATERIALS AND METHODS

The set of samples (Tab. 1), which included eleven spirit vinegars purchased from the market (samples 1–11), three samples synthetic acetic acids diluted with water (8% w/w) (samples 12–14) and two model mixtures of authentic vinegar with addition of synthetic acetic acid (samples 15–20) were analysed by SNIF-NMR and IRMS, by GC-MS and/or GC-FID with direct injection and using the simple sensory assessment. At the beginning, the declared properties of the samples were confirmed using the isotopic analysis.

Samples

A set of twenty samples was analysed; details are given in Tab. 1. Seven samples (2 and 7–12) were used for the production of pickled cucumbers and the brines were subsequently analysed. Cucumbers were pickled in brine without spices; brine contained water, vinegar (8% acetic acid

Tab. 1. Analysed samples.

Sample number	Indication of sample		Country of origin	Purity [%]
1	Authentic	8%; authentic non-flavoured spirit vinegar	Czech Republic	
2	Authentic	8%; authentic flavoured spirit vinegar	Czech Republic	
3	Proved to be authentic	8%; spirit vinegar	Hungary	
4	Proved to be authentic	10%; spirit vinegar	Czech Republic	
5	Proved to be authentic	8%; spirit vinegar	Czech Republic	
6	Proved to be authentic	8%; spirit vinegar	Unknown	
7	Proved to be authentic	8%; spirit vinegar	Slovak Republic	
8	Proved to be authentic	8%; spirit vinegar	Slovak Republic	
9	Proved to be authentic	8%; spirit vinegar	Czech Republic	
10	Proved to be authentic	8%; spirit vinegar	Czech Republic	
11	Proved to be authentic	10%; spirit vinegar	Hungary	
12	Fully synthetic	Synthetic acetic acid p. a.	Czech Republic	min. 99.00
13	Fully synthetic	Synthetic acetic acid glacial	Germany	≥ 99.80
14	Fully synthetic	Synthetic acetic acid p. a.	Czech Republic	min. 99.80
15	Partially synthetic	Model sample; mixture of 1 and 12; dilution 1:9		
16	Partially synthetic	Model sample; mixture of 1 and 12; dilution 5:5		
17	Partially synthetic	Model sample; mixture of 1 and 12; dilution 9:1		
18	Partially synthetic	Model sample; mixture of 2 and 13; dilution 1:9		
19	Partially synthetic	Model sample; mixture of 2 and 13; dilution 5:5		
20	Partially synthetic	Model sample; mixture of 2 and 13; dilution 9:1		

concentration), salt (sodium chloride) and saccharose. Both pickled cucumbers and brine were used for sensory analysis.

Isotopic analysis

The isotopic analyses, the determination of the $^2\text{H}/^1\text{H}$ ratios (SNIF-NMR: Avance 400, Bruker BioSpin, Rheinstetten, Germany), $^{13}\text{C}/^{12}\text{C}$ (IRMS: Delta Plus XP + ConFlo III, Thermo Electron, Bremen, Germany; Elemental Analyser: EA/NA 1110, Fisons Instruments, Rodano, Italy) and $^{18}\text{O}/^{16}\text{O}$ ratios (IRMS: Delta Plus XP, Thermo Electron; Gas Bench II: ThermoFisher Scientific, Bremen, Germany) were carried out in the Customs Technical Laboratory Prague of the Czech Ministry of Finance (Prague, Czech Republic) according to THOMAS et al. [7].

Acetic acid was extracted from vinegar and purified in order to be analysed by isotopic techniques. The acetic acid from vinegar was first extracted with diethyl oxide. It was then purified by distillation (with a manual Cadiot column). The water content of the residue was determined by a Karl Fischer method [7].

Method for SNIF-NMR determination of acetic acid from vinegar was adapted from EC Regulation 2676/90 [11]. The deuterium contained in

acetic acid is distributed in isotopomers I and II of the molecule: (I) CH_2DCO_2 and (II) $\text{CH}_3\text{CO}_2\text{D}$. The second isotopomer is affected by water and atmospheric conditions whereas the first isotopomer is related to the botanical origin of vinegar. $^2\text{H}/^1\text{H}$ is the isotope ratio associated with molecule I. Only the parameter $^2\text{H}/^1\text{H}$ was used for authenticity testing. The parameter $^2\text{H}/^1\text{H}$ was determined by nuclear magnetic resonance of deuterium in the acetic acid from the vinegar. The principle was the same as used for wines (EC 2676/90) [7].

Method to determine of the isotopic ratio $^{13}\text{C}/^{12}\text{C}$ of acetic acid from vinegar was adapted from EC regulation 440/2003 [12]. The $^{13}\text{C}/^{12}\text{C}$ ratio of acetic acid from vinegar could be determined on CO_2 gas after complete combustion at high temperature. The isotopic ratio $^{13}\text{C}/^{12}\text{C}$ was determined by isotopic ratios mass spectrometry from ion currents m/z 45 ($^{13}\text{C}^{16}\text{O}_2$) and m/z 44 ($^{12}\text{C}^{16}\text{O}_2$) produced by carbon dioxide obtained after complete combustion in an elemental analyser. Corrections were made to delete the contribution of $^{12}\text{C}^{16}\text{O}^{17}\text{O}$ in current m/z 45 (Craig correction) [7].

Method to determine the isotopic ratio $^{18}\text{O}/^{16}\text{O}$ of water in vinegar was adapted from EC regulation 822/97 [13]. The $^{18}\text{O}/^{16}\text{O}$ ratio of

water from vinegar could be determined on CO₂ gas after equilibration of reference CO₂ gas with raw vinegar. The isotopic ratio ¹⁸O/¹⁶O was determined by isotopic ratios mass spectrometry from ion currents *m/z* 46 (¹²C¹⁶O¹⁸O) and *m/z* 44 (¹²C/¹⁶O₂) produced by carbon dioxide obtained after an exchange with water in wine according to the reaction: C¹⁶O₂ + H₂¹⁸O ↔ C¹⁶O¹⁸O + H₂¹⁶O. Carbon dioxide in the gaseous phase was used for analysis [7].

Volatile substances analysis

Two columns (DB-5 and DB-624; Agilent Technologies, Santa Clara, California, USA), two methods of isolation (headspace solid phase microextraction and direct injection) and two methods of detection (FID and MS detector) were tested on authentic beet spirit vinegars (samples 1 and 2); MS in first for the qualitative analysis.

The samples were analysed by GC-MS (Agilent Technologies 7890A/5975C, Agilent Technologies, Santa Clara, California, USA) and GC-FID (Agilent Technologies 6890N, Agilent Technologies) on a column DB-624 (Agilent Technologies) with direct injection of samples diluted with water to 8% (w/v) acetic acid. The conditions of method were modified from GUERRERO et al. and MEJÍAS et al. [14, 15].

Chromatographic conditions for GC-FID: column DB-624 60 m × 0.25 mm × 1.40 μm, temperature programme: initial temperature held at 40 °C for 5 min, then ramped at 10 °C·min⁻¹ to 260 °C, held at 260 °C for 2 min, the detector temperature 300 °C, the carrier gas was nitrogen at a flow rate of 1.70 ml·min⁻¹. Quantification was performed using the internal standard, 3-pentanol [16].

Chromatographic conditions for GC-MS were: column DB-624 60 m × 0.25 mm × 1.40 μm, temperature programme: initial temperature held at 40 °C for 5 min, then ramped at 10 °C·min⁻¹ to 260 °C, held at 260 °C for 2 min, the detector temperature 280 °C, the carrier gas was helium at a flow rate of 1.80 ml·min⁻¹. Quantification was performed using the internal standard, 3-pentanol [16].

Sensory analysis

Triangle and paired comparison tests were used; quality of the samples (taste, aroma and overall impression) was evaluated [17–19]. For sensory analysis, the samples of spirit vinegars and synthetic acetic acids were diluted to 1% acetic acid concentration and 1.50 g of sodium chloride per 100 ml was added [8].

The triangular test was used to determine the

differences between samples. The assessor evaluated the overall sensory properties (olfaction and taste). The paired comparison test was used to determine which sample, in respect of overall sensory properties, had a higher quality for the evaluator.

The sensory evaluation was performed by a total of 10 panelists from the Department of Food Preservation. The assessors were selected, trained and monitored according to the standard [20]. The performance was in agreement with the international standard [18].

RESULTS AND DISCUSSION

Isotopic analysis

The isotopic analysis of acetic acid extracted from vinegar by SNIF-NMR and IRMS enables the distinction of grape origin from other sources, such as beet, cane, malt, apple and synthesis. In addition, ¹⁸O/¹⁶O ratio of water in wine vinegar also allows differentiating wine vinegar from vinegars made from raisins [7]. Over the past twenty years, published data for the spirit vinegars represented a fifth of all published information on isotopic profiles of vinegars. However, none of the published articles was focused to the analysis of spirit vinegars or differentiation between spirit vinegar and the synthetic acetic acid-based alternative.

The origin of the acetic acid is “natural” if all its precursors (saccharide, saccharose and alcohol) are traceable back to the photosynthesis of a plant. As a rule of thumb, low (for C3 plants: 90–100 ppm; parts per million) to moderate (for C4 plants: about 110 ppm) values of the ²H/¹H ratio for this type of vinegars are expected, while the complete chemical synthesis of acetic acid results in a rather high value (130–140 ppm) [5]. Nevertheless, mixtures of synthetic and natural acetic acid cannot be revealed by ²H/¹H ratio alone.

The values of relative isotopic ratio δ¹³C range from –30‰ to –10‰ for fermented vinegar and from –40‰ to –30‰ for synthetic acetic acid and in particular the C3 plants-based authentic vinegar cannot be distinguished based on ¹³C ratios alone. From Fig. 1 it is obvious that it is necessary to use two-dimensional analysis (i.e. determination of both ²H/¹H and δ¹³C) for reliable discrimination between the sample origins [5].

From the perspective of the ²H/¹H ratio, differences between spirit vinegars and synthetic acetic acids diluted with water were proved. In agreement with the literature [4], the ratio of ²H/¹H for spirit vinegars ranged from 90.30 ppm to

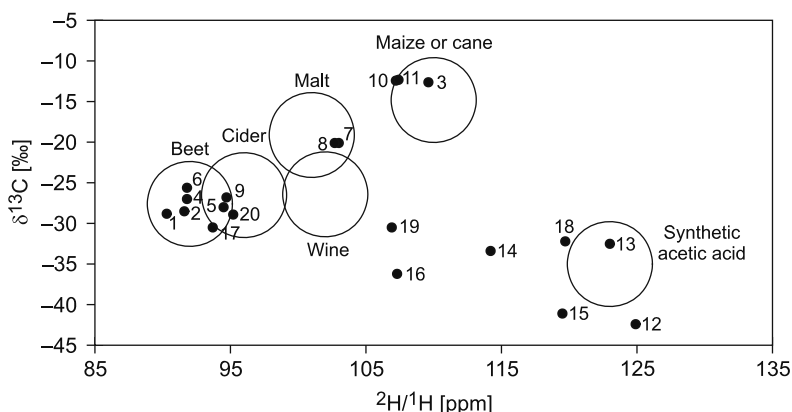


Fig. 1. Natural isotope ratios plot of the set of analysed samples.

Sample numbers correspond to Tab. 1. To illustrate better the distribution of the results, the zones of typical values for other sources of saccharides and/or vinegars from the literature are indicated by big illustrating circles in the plot [4].

109.60 ppm and for synthetic acetic acids it ranged from 114.20 ppm to 124.90 ppm; the results are presented in Tab. 2 and Fig. 1. The samples were further analysed with respect to their $^{13}\text{C}/^{12}\text{C}$ ratio, which ranged from -12.30‰ to -28.80‰ for spirit vinegars and from -32.50‰ to -42.40‰ for synthetic acetic acids. The $^{13}\text{C}/^{12}\text{C}$ ratios of samples 3, 10 and 11 showed that C4 spirit had been used for the vinegar production. The values -12.60‰ , -12.40‰ and -12.30‰ were out of the usual range of C3 plants including sugar beet, wine or malt. The declared origin of the samples was confirmed. According to the literature, the coupled SNIF-NMR and IRMS methods are able to reveal as low as 15% synthetic acid addition into the beet vinegar [4]. It is evident that the isotope analysis allows detecting synthetic acetic acid addition in vinegar, and it is also possible to prove the origin of saccharides or ethanol, which were used for the production of vinegar.

The $^{18}\text{O}/^{16}\text{O}$ ratio of water determined by IRMS allows differentiation of wine vinegar from vinegars made from dried grapes [7]. Regarding determination of synthetic acetic acid addition in spirit vinegars, this parameter does not appear to be valuable.

Determination of volatile substances; volatiles as markers for the vinegar authentication

Volatile substances or the profile of volatiles appear to be a useful tool for the authentication of the vinegar origin, raw materials and technology. The methods described in the methodology part were those optimal from the evaluated procedures. Subsequently, based on the preliminary results, as the most appropriate method for determining the profile of volatile compounds of

a set of vinegars there was indicated the DB-624 column with FID detector with direct injection. According to the expectation, the profiles were not too rich, the characteristic fermentation markers [6, 14] such as 3-hydroxy-2-butanone, butanediol, butanedioic acid diethyl ester and 5-acetoxyme-

Tab. 2. Results of natural isotope ratio analysis.

Sample number	$^2\text{H}/^1\text{H}$ for acetic acid [ppm]	$\delta^{13}\text{C}$ for acetic acid [‰]	$\delta^{18}\text{O}$ for water [‰]
1	90.3	-28.8	-9.6
2	91.6	-28.5	-9.6
3	109.6	-12.6	-11.2
4	91.8	-28.3	-8.3
5	94.5	-28.0	-9.1
6	91.8	-28.4	-9.9
7	103.0	-20.1	-10.0
8	102.7	-20.1	-11.0
9	94.7	-26.8	-9.5
10	107.2	-12.4	-10.4
11	107.4	-12.3	-10.8
12	124.9	-42.4	NM
13	123.0	-32.5	NM
14	114.2	-33.4	NM
15	119.5	-41.1	-14.3
16	107.3	-36.2	-12.3
17	93.7	-30.5	-10.5
18	119.7	-32.2	-15.2
19	106.9	-30.5	-13.5
20	95.2	-28.9	-12.1

NM – not measured.

Tab. 3. Validation parameters of the GC/FID method with direct injection (column DB-624).

	Ethanol	Ethyl acetate
Linearity [$\text{g}\cdot\text{l}^{-1}$]	0.0161–8.0742 ($R^2 = 0.9998$)	0.0180–9.0000 ($R^2 = 0.9986$)
Repeatability, <i>RSD</i> [%]	0.60	3.30
Detection limit [$\text{g}\cdot\text{l}^{-1}$]	0.0050	0.0050
Quantification limit [$\text{g}\cdot\text{l}^{-1}$]	0.0200	0.0200
Recovery rate [%]	110.90	95.50

RSD – relative standard deviation.

thyl-2-furfural as well as other aliphatic alcohols, diols were all absent. Only five components were present in all tested vinegars in concentrations high enough to be considered as the indicators of fermentation process: acetaldehyde, acetone, ethanol, ethyl acetate and acetic acid. Unfortunately, the concentrations of acetaldehyde and acetone were generally low and too variable to be successfully used as authenticity markers. Validation parameters (linearity, repeatability, detection

limit, quantification limit and recovery) for ethanol and ethyl acetate are given in Tab. 3.

The results of the analyses of the set of samples are summarized in Tab. 4. All samples contained 8% (w/v) of acetic acid, and ethanol and ethyl acetate concentrations ranged from $0.50\text{ g}\cdot\text{l}^{-1}$ to $3.40\text{ g}\cdot\text{l}^{-1}$ and $0.10\text{ g}\cdot\text{l}^{-1}$ to $0.50\text{ g}\cdot\text{l}^{-1}$, respectively. In the model samples (15–20), ethanol and ethyl acetate concentrations ranged from $0.15\text{ g}\cdot\text{l}^{-1}$ to $1.46\text{ g}\cdot\text{l}^{-1}$ and from $0.02\text{ g}\cdot\text{l}^{-1}$ to $0.22\text{ g}\cdot\text{l}^{-1}$, respectively. The results corresponded to the theoretical values. Similar values were found in the brines, the determined quantities corresponded to the dilution of the vinegar during the brine preparation.

Ethanol and ethyl acetate seem to be useful as authenticity markers. Ethanol is a natural residue from the fermentation medium, but its residual concentration depends on the process. Usually, the fermentation is stopped at about 0.30% volume residual ethanol level to avoid oxidation of acetic acid to carbon dioxide and water [21]. Ethyl acetate is formed during the fermentation by microbial esterases [22] and, in the vinegar, it can slowly hydrolyse due to the low pH, but its concen-

Tab. 4. Results of the analysis of volatile substances.

Sample number	Ethanol concentration [$\text{g}\cdot\text{l}^{-1}$]	Ethyl acetate concentration [$\text{g}\cdot\text{l}^{-1}$]	AA/E	AA/EA	Confirmation by isotopic analysis
1	1.20	0.20	67	400	Beet
2	1.60	0.20	50	400	Beet
3	2.50	0.40	32	200	Maize or cane
4	0.50	0.10	160	800	Beet
5	1.80	0.30	44	267	Beet
6	1.30	0.20	62	400	Beet
7	2.80	0.50	29	160	Malt
8	0.80	0.13	100	615	Malt
9	3.40	0.50	24	160	Beet
10	1.00	0.20	80	400	Maize or cane
11	2.00	0.30	40	267	Maize or cane
12	ND	ND	ND	ND	Synthetic acetic acid
13	ND	ND	ND	ND	Synthetic acetic acid
14	ND	ND	ND	ND	Synthetic acetic acid
15	0.15	0.02	552	3810	Model sample
16	0.63	0.10	127	808	Model sample
17	1.11	0.16	72	510	Model sample
18	0.16	0.02	494	3478	Model sample
19	0.79	0.12	101	667	Model sample
20	1.46	0.22	55	367	Model sample

AA/E – ratio of acetic acid ($80\text{ g}\cdot\text{l}^{-1}$) and ethanol; AA/EA – ratio of acetic acid ($80\text{ g}\cdot\text{l}^{-1}$) and ethyl acetate.

ND – not detected.

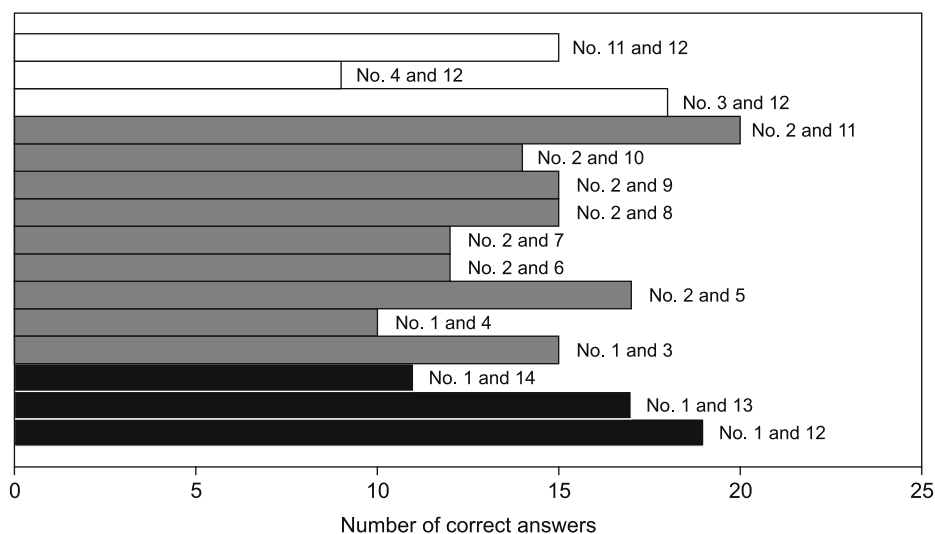


Fig. 2. Determination of differences between vinegars and synthetic acetic acids by triangular method.

$P = 0.95$; black – authentic versus fully synthetic; grey – authentic versus proved to be authentic; white – proved to be authentic versus fully synthetic.

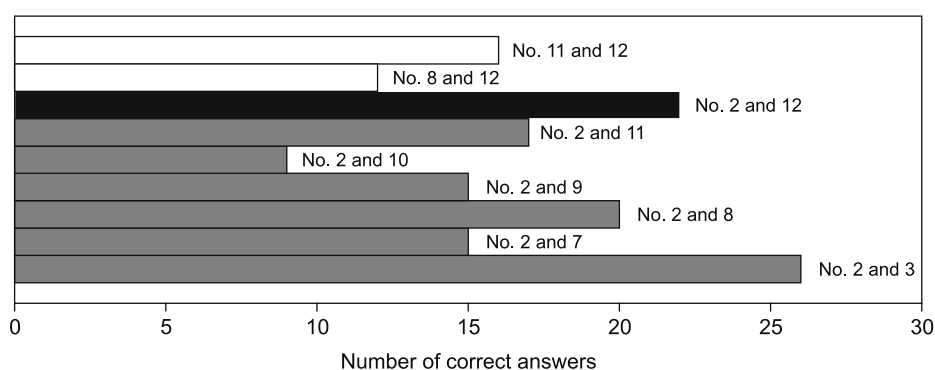


Fig. 3. Determination of differences between pickled cucumbers by triangular method.

$P = 0.95$; black – authentic versus fully synthetic; grey – authentic versus proved to be authentic; white – proved to be authentic versus fully synthetic.

tration is in equilibrium with ethanol. We tried to calculate the ratios of acetic acid/ethanol (AA/E) and acetic acid/ethyl acetate (AA/EA) as a tool for the authenticity evaluation. For vinegars, the AA/E and AA/EA ratios ranged from 24 to 160 and from 160 to 800, respectively (Tab. 4). However, the number of samples that were analysed was not high enough to define reliable limit values for these ratios, which could be used for identification of suspect vinegars. Estimated “detection limit” for the added synthetic acetic acid, using the limit values calculated from our data, was unacceptably high to be effectively used. The vinegars with higher residual ethanol levels could be spiked with synthetic acetic acid and it was not possible

to confirm the adulteration using this simple procedure only. However, there is no doubt that very low concentration or absence of ethanol and/or ethyl acetate can indicate the synthetic acetic acid in samples.

Sensory analysis

Sensory properties of vinegar are very important as they may modify the overall appreciation of a food or meal that contains it. Besides acetic acid, minor components also affect the overall perception of vinegar and allow distinguishing of samples according to their origin [8].

Triangular and paired comparison tests were used to distinguish the samples of vinegars and

also of the brines of the pickled cucumbers prepared with the vinegar samples according to both olfaction and taste.

The results of triangular tests in pairs of vinegar and synthetic acetic samples and for the brines are given in Fig. 2 and Fig. 3, respectively. The results were evaluated according to the tabulated values for a given number of evaluations [18].

From Fig. 2 it is evident, that 9 out of 15 pairs of the samples (vinegars and synthetic acetic acids) have shown statistically significant differences, majority of assessors having detected the sensory differences between the pairs of the samples of spirit vinegars and synthetic acetic acid solutions and between different spirit vinegars. The pairs of vinegars and vinegars spiked with synthetic acid were also analysed, but the sensory differences were not proven in any of the tested pairs of the samples. In the case of pickled cucumbers (Fig. 3) it is obvious that 7 out of 9 pairs of samples have shown statistically significant differences too (more than 15 correct answers mean a statistically significant difference between two samples). The paired comparison method was used to evaluate the preferences of assessors to various samples. The results of paired comparison tests were not statistically significant, which means that there were no differences in preferences between the spirit vinegars and synthetic acid solutions.

CONCLUSIONS

Taking into consideration the acceptable sensory properties of synthetic acetic acid solutions, the problem of adulteration of vinegar lies in adulteration of traditional food composition or in adulteration of traditional technological procedures.

Detection of added synthetic acid into spirit vinegar or into relevant products produced with the adulterated vinegar or synthetic acetic acid remains difficult. The sensory evaluation allows distinguishing between different samples of vinegars and also between vinegars and synthetic acetic acid solutions. The output of the sensory evaluation is not the confirmation of the synthetic acetic acid addition, but the differentiation between two samples. Simple analysis of volatile substances based on the AA/EA and AA/E ratios seems to be useful for fast screening of suspicious samples (prior to the sophisticated analysis) or for detection of very outlying samples, but it does not facilitate quantification of the potential addition of synthetic acetic acid in the sample. The isotopic analyses (SNIF-NMR and IRMS) are very useful tools, allowing the detection of above 15% of

synthetic acetic acid into spirit vinegars. The determined values of $^2\text{H}/^1\text{H}$ and $^{13}\text{C}/^{12}\text{C}$ extend and refine the available database for spirit vinegars. In order to reduce the detection limit of the method, it would be necessary to obtain additional data on spirit vinegars from specific species and geographic origins, as only a limited amount of data can be traced in the literature presently.

Acknowledgments

The research was supported by the Ministry of Education, Youth and Sports, projects 21/2012 and 6046137305, and by the Ministry of Agriculture, project QI91B283.

REFERENCES

1. Rychtera, M.: Výroba octa. In: Kadlec, P. – Melzoch, K. – Voldřich, M.: Co byste měli vědět o výrobě potravin? Technologie potravin. 1st ed. Ostrava: KEY Publishing, 2009, pp. 412–422.
2. Brown, W. H. – Foote, Ch. S. – Iverson B. L. – Anslyn E. V.: Organic Chemistry. 6th ed. Belmont: Brooks/Cole Cengage Learning, 2011, 1193 pp. ISBN-13: 978-0-8400-5498-2.
3. Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. Official Journal of the European Union, L 83, 2012, pp. 1–295.
4. Remaud, G. – Guillou, C. – Vallet, C. – Martin, G. J.: A coupled NMR and MS isotopic method for the authentication of natural vinegars. *Fresenius Journal of Analytical Chemistry*, 342, 1992, pp. 457–461.
5. Hermann, A.: Determination of D/H isotope ratio in acetic acid from vinegars and pickled products by ^2H -NMR-spectroscopy. *European Food Research and Technology*, 212, 2001, pp. 683–686.
6. Caligiani, A. – Acquotti, D. – Palla, G. – Bocchi, V.: Identification and quantification of the main organic components of vinegars by high resolution ^1H NMR spectroscopy. *Analytica Chimica Acta*, 585, 2007, pp. 110–119.
7. Thomas, F. – Jamin, E.: ^2H NMR and ^{13}C -IRMS analyses of acetic acid from vinegar, ^{18}O -IRMS analysis of water in vinegar: International collaborative study report. *Analytica Chimica Acta*, 649, 2009, pp. 98–105.
8. Tesfaye, W. – Morales, M. L. – García-Parrilla, M. C. – Troncoso, A. M.: Wine vinegar: technology, authenticity and quality evaluation. *Trends in Food Science & Technology*, 13, 2002, pp. 12–21.
9. Erbe, T. – Brückner, H.: Chiral amino acid analysis of vinegars using gas chromatography – selected ion monitoring mass spectrometry. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A*, 207, 1998, pp. 400–409.

10. Crews, C.: Authenticity of vinegars. Final Report. In: foodbase [online]. London: Food Standards Agency, April 2004 [cited 20 December 2010]; <http://www.foodbase.org.uk/admintools/report-documents/272-1-493_Vinegar_authenticity_draft_final_report.pdf>.
11. Commission Regulation (EEC) No 2676/90 of 17 September 1990 determining Community methods for the analysis of wines. Official Journal of the European Union, L 272, 1990, pp. 1–192.
12. Commission Regulation (EC) No 440/2003 of 10 March 2003 amending Regulation (EEC) No 2676/90 determining Community methods for the analysis of wines. Official Journal of the European Union, L 66, 2003, pp. 15–23.
13. Commission Regulation (EC) No 822/97 of 6 May 1997 amending Regulation (EEC) No 2676/90 determining Community methods for the analysis of wines. Official Journal of the European Union, L 117, 1997, pp. 10–12.
14. Mejías, R. C. – Marín, R. N. – Moreno, M. de V. G. – Barroso, C. G.: Optimisation of headspace solid-phase microextraction for analysis of aromatic compounds in vinegar. *Journal of Chromatography A*, 953, 2002, pp. 7–15.
15. Guerrero, E. D. – Marín, R. N. – Mejías, R. C. – Barroso, C. G.: Stir bar sorptive extraction of volatile compounds in vinegar: Validation study and comparison with solid phase microextraction. *Journal of Chromatography A*, 1167, 2007, pp. 18–26.
16. Blanch, G. P. – Tabera, J. – Sanz, J. – Herraiz, M. – Reglero, G.: Volatile composition of vinegars. Simultaneous distillation-extraction and gas chromatographic-mass spectrometric analysis. *Journal of Agricultural and Food Chemistry*, 40, 1992, pp. 1046–1049.
17. ISO 4120:2007. Sensory analysis – Methodology – Triangular test. Prague: Czech Office for Standards, Metrology and Testing, 2009. 20 pp.
18. ISO 6658:2005. Sensory analysis – Methodology – General Guidance. Prague: Czech Office for Standards, Metrology and Testing, 2009. 24 pp.
19. ISO 5495:2007. Sensory analysis – Methodology – Paired comparison test. Prague: Czech Office for Standards, Metrology and Testing, 2009. 24 pp.
20. ISO 8586-1:1993. Sensory analysis – General guidance for selection, training and monitoring of assessors – Part 1: Selected assessors. Prague: Czech Office for Standards, Metrology and Testing, 2002. 24 pp.
21. Belitz, H. D. – Grosch, W. – Schieberle, P.: Food chemistry. Berlin, Heidelberg: Springer, 2009. 1070 pp. ISBN 978-3-540-69933-0.
22. Kashima, Y. – Iijima, M. – Okamoto, A. – Koizumi, Y. – Udaka, S. – Yanagida, F.: Purification and characterization of intracellular esterases related to ethyl acetate formation in *Acetobacter pasteurianus*. *Journal of Fermentation and Bioengineering*, 85, 1998, pp. 584–588.

Received 13 February 2012; revised 17 April 2012; accepted 2 May 2012.