

Assessment of the Authenticity of Fruit Spirits by Gas Chromatography and Stable Isotope Ratio Analyses

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Abstract

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The gas chromatographic (GC) determination of volatile constituents and the determination of $^{13}\text{C}/^{12}\text{C}$ isotope ratios by isotope ratio mass spectrometry – IRMS analysis as well as SNIF–NMR analysis of (D/H)I and (D/H)II ratios in ethanol are prospective analytical methods which can be used for checking the authenticity of fruit spirits and for detecting their adulteration. Different concentrations of volatile compounds such as acetaldehyde, ethyl acetate, diethyl acetal, methanol, 1-butanol, 2-butanol, 1-propanol, 2-methyl-1-propanol, 2- and 3-methyl-1-butanol, volatile fatty acids and isotopic data were demonstrated using discriminant analysis. The results show that the determination of isotope ratios can be used especially for distinguishing between fruit spirits and others spirits, i.e. those made from beet sugar, maize, cane sugar, grain, potato, or synthetic alcohol. Gas chromatography also makes it possible to discriminate between respective spirits derived from one kind of fruit such as sweet cherry brandy, sour cherry brandy, pear brandy, apple brandy, apricot brandy, or plum brandy.

Keywords: authenticity; fruit spirits; gas chromatography; stable isotope ratio analysis; IRMS; SNIF–NMR; linear discriminant analysis

The requirements for quality food products have been increasing in recent years and the interest in the quality and purity of fruit spirits has grown in this connection as well. The everyday practice of market supervision reveals that high-quality distillates are often blended with cheaper raw materials of lower quality. Sugar is sometimes added during fermentation of fruits to obtain a higher yield of spirit, at other times ethanol made from cheaper raw materials (beet sugar, maize, cane sugar, grain, potato) or synthetic alcohol is added.

One of the possibilities of preventing the adulteration of fruit spirits is an advanced analytical control. A study of proving the authenticity and

identification of respective kinds of fruit spirits was therefore started. This study includes the creation of a statistical file of analytical data.

Methods based on the determination of fruit spirit components were developed for these purposes. These methods include gas chromatography – GC (BAUER-CHRISTOPH *et al.* 1997; KELLY *et al.* 1999; Council Regulation EEC No. 2870/2000), the determination of stable isotope ratio using nuclear magnetic resonance – ^2H -NMR, and $^{13}\text{C}/^{12}\text{C}$ isotope ratio using mass spectrometry – IRMS (Council Regulation EEC No. 2676/90; BAUER-CHRISTOPH *et al.* 1997, 2003).

The assessment of the $^{13}\text{C}/^{12}\text{C}$ carbon isotope ratio reliably reveals the adulteration of fruit spirits

with sugar. The determination of deuterium/hydrogen (D/H)I and (D/H)II ratios in the ethanol molecule by ^2H -NMR serves to detect the source of non-fruit ethanol.

Gas chromatography is a suitable method for the identification and specification of respective kinds of fruit spirits. The use of gas chromatography can determine major as well as minor components of fruit spirits (KELLY *et al.* 1999; BAUER-CHRISTOPH *et al.* 1997). The contents of volatile compounds, especially aroma components, in the finalised spirits can verify the use of single fruit materials for their production.

The requirements for determining the authenticity of fruit spirits are described in the Council Regulation EEC No. 2870/2000. The authentic fruit spirits cannot contain ethanol other than that of fruit origin.

MATERIALS AND METHODS

Materials. A total of 153 samples of fruit spirits (from years 2003–2006) made from different kinds of fruit (plum brandy, pear brandy, apple brandy, apricot brandy, sweet-cherry brandy, sour-cherry brandy) were analysed and the results were processed statistically.

The samples were provided by three producers located in the Czech Republic, who had guaranteed the authenticity of spirits.

Gas chromatography

Methods. Volatile components such as acetaldehyde, ethyl acetate, methanol, and higher alcohols (1-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol) were analysed by gas chromatography (Council Regulation EEC No. 2870/2000), using a FID detector and a Hewlett Packard gas chromatograph with split injection (20:1). The injector temperature was 150°C; the detector temperature was 250°C. The capillary column CP-WAX 57CB (length 50 m, I.D. 0.32 mm, film thickness 0.2 μm) was used.

The oven temperatures were programmed as follows: starting at 40°C with 17 min isothermal period, then increasing to 70°C at the rate of 12°C/min, with the final 5 min thermal persistence. The carrier gas was helium at the flow of 2.7 ml/minute. The standards from Fluka and Aldrich companies were used for qualitative and quantitative calibrations. All determinations were executed

by the internal standard method. Pentane-3-ol was used as the internal standard substance. The components contents were expressed in mg/l of pure ethanol (p.e.).

Volatile components such as ethyl esters of fatty acids, benzaldehyde, and flavour compounds were analysed by gas chromatography using mass selective detection (MSD) (WARDENCKI 2003; PINO 2002; NG 2002; GOMEZ 2005; PAWLISZYN 2000; SOUFLEROS 2004). The apparatus used was a Finnigan gas chromatograph. The SPME (Solid Phase Micro Extraction) method was chosen for extracting these substances. This technique is suitable for the organic components concerned. It is based on the adsorption of substances from the sample to the surface of a siliceous fibre covered with the appropriate stationary phase. The substances adsorbed to the fibre were desorbed in the injector of the gas chromatograph. A 75 μm CARTM/PDMS fibre was used for SPME extraction.

SPME extraction conditions: sample temperature –25°C; time of extraction 15 min; desorption in injector 3 min; injector temperature 280°C (splitless).

A GC/MSD capillary column DB-WAX (length 30 m, I.D. 0.25 mm, film thickness 0.25 μm) was used for the analysis by gas chromatography. The oven temperatures were programmed in two steps. In the first step, the temperature increased from 55°C (isothermal 3 min) to 150°C (isothermal 5 min) at 10°C/min, in the second step it ramped from 150°C to 200°C (isothermal 1 min) at 10°C per minute. The carrier gas used was helium at the flow of 2.0 ml per minute. The internal standard method (heptanoic acid) was used for the determinations.

^2H -NMR analysis

The D/H ratios of ethanol in the samples were determined according to the official analytical method for wine analysis by quantitative deuterium NMR spectroscopy, as described in the Council Regulation EEC 2676/90, method No. 8. A 70 ml subsample was distilled using the Cadiot spinning band column. To prevent the isotopic fractionation, the minimal distillation yield of 95% was acquired. To determine the water content in the distillate, a Mettler DL18 Karl Fischer titrator was used.

The Bruker AVANCE DPX 400 spectrometer equipped with a 10 mm dual deuterium probehead (fluorine lock) and a BACS-60 automatic sample changer was used for ^2H -NMR measurement. The

NMR tubes were prepared as follows: 2.3 ml of distillate were placed into a pre-weighed bottle and weighed nearest to 0.1 mg; 1.3 ml of the internal standard (tetramethylurea with known value of D/H) was then added and weighed nearest to 0.1 mg; finally, 150 µl of the lock substance (10:1 mixture of C₆F₆ and trifluoroacetic acid) was added and weighed nearest to 0.1 mg; the blend obtained was then filtered into the NMR tube. For each tube, 10 NMR spectra were recorded at a frequency of 61.4 MHz with the acquisition time 6.2 s, 90° pulse, and 200 scans at 30°C. The processing of the FIDs and the calculation of D/H of ethanol were performed using the EUROSPEC software.

Carbon isotope analysis by IRMS

The determination of $\delta^{13}\text{C}$ ratio of ethanol contained in the samples was carried out according to the official analytical method for wine analysis by EA-IRMS spectroscopy, as described in the Council Regulation EEC 2676/90, method No. 45. Approximately 1 µl of the distillate was injected into the EA 1110 CHN (Fisons Instruments) using a liquid autosampler CTC-AS200S. The CO₂ obtained by the combustion of the distillate was introduced into the Thermo Finnigan DELTA Plus Advantage IRMS spectrometer using the ConFlo interface. CO₂ calibrated by certified reference materials was used as the reference gas.

RESULTS AND DISCUSSION

Volatile components, esters and aroma components suitable for the specification of fruit spirits, analysed by GC/FID and GC/MSD

Tables 1 and 2 summarise the minima and maxima of the volatile compound contents in the authentic samples of individual sorts of fruit spirits. These volatile compounds are important for characterising alcoholic distillates and fruit spirits.

The amounts of methanol in the samples varied from 932 to 12 053 mg/l p.e. Methanol is a constituent arising from the enzymatic degradation of pectin contained in fruits. Generally, its quantity is related to the amount of pectin present in fruits used for fermentation. The methanol concentration is suitable for proving the authenticity of fruit spirits.

Limits are posed by the Council Regulation EEC No. 1576/89 on the methanol content in many

spirits. Its determination is part of the quality control of spirit drinks.

Following this regulation, the authentic fruit spirits should meet the maximum limit approved for the methanol concentration, i.e. 10 000 mg/l p.e. This limit was exceeded in 9 samples (4 apricot brandies, 1 pear brandy, 2 sweet cherry brandies, 2 plum brandies).

Fruit spirits typically had high content of methanol and 1-propanol, whereas spirits made from grain contained significantly less of these. This compares well with the data by BAUER-CHRISTOPH *et al.* (1997), who found that the grain spirits mostly contained only 100 mg/l p.e. of methanol and 1-propanol. In the fruit spirits, the concentrations of higher alcohols were significantly lower than those of methanol. The contents of higher alcohols fluctuated over a wide range of values.

Higher alcohols are characteristic components which are metabolised from amino acids by yeasts during alcoholic fermentation of fruits and other raw materials. The amounts of these compounds depend on the quantity of amino acids in fruits.

The higher alcohols most frequently found in low concentrations were 1-butanol and 2-butanol. The lowest values measured (5–31 mg/l p.e.) were those of 1-butanol in sweet cherry and sour cherry brandies. WENCKER *et al.* (1981) showed that 1-butanol is a strongly discriminating parameter for the fruit spirits.

Table 2 shows the values of the aroma components that were present in concentrations significantly lower than those of higher alcohols (Table 1).

The concentrations of esters and aroma components were mostly lower than 1 mg/l p.e., in some cases 1–50 mg/l p.e., and only sporadically higher than 50 mg/l p.e.

The lowest concentrations were found of β -citronellol (below 0.3 mg/l p.e.) in all sorts of fruit spirits. Low contents of β -linalool, α -terpineol, and eugenol (0–15.3 mg/l p.e.) were also observed. Although the aroma compounds were only found in smaller amounts, they should also contribute to the verification of fruit spirit authenticity.

In all fruit spirits, the contents of volatile components were probably correlated with technological parameters, such as the activity of yeasts during fermentation or the conditions of fermentation, and with the distillation process, i. e. the separation of particular fractions. The contents of the respective components may also depend on fruit ripeness and storage.

Table 1. Concentrations (minimum–maximum) of volatile components of fruit spirits, determined by GC/FID (mg/l of pure ethanol; n = number of samples)

	Pear brandy (n = 44)	Apple brandy (n = 12)	Sweet cherry brandy (n = 31)	Plum brandy (n = 29)	Sour cherry brandy (n = 21)	Apricot brandy (n = 16)
Acetaldehyde	13–562	30–260	16–355	26–385	13–597	25–320
Ethyl acetate	76–2937	125–2334	270–6921	563–2359	199–6565	279–3394
Diethyl acetal	20–375	63–778	17–254	18–321	19–361	42–203
Methanol	932–10 809	1794–9168	4520–10 695	2877–11 414	4376–8784	6723–12 053
2-Butanol	6–733	8–323	15–1531	13–195	5–176	7–1715
1-Propanol	141–7068	121–2290	244–3758	356–3084	129–1562	292–2869
2-Methyl-1-propanol	341–1116	392–968	178–1366	222–1361	113–1955	511–1776
1-Butanol	16–228	80–205	5–31	21–126	7–31	27–516
2-Methyl-1-butanol	201–753	333–705	110–618	149–735	128–726	254–818
3-Methyl-1-butanol	900–3998	1705–4225	589–3017	591–2649	674–3120	799–2878

Analysis of stable isotope ratios for determining the authenticity of fruit spirits

The amount of stable isotopes in raw materials is influenced by the growing location and the growth conditions of the plant from which ethanol has been made.

The ^2H -NMR analysis is based on the measurement of the deuterium to hydrogen (D/H) ratio of the methyl (D/H)I and methylene (D/H)II groups in the ethanol molecule.

Isotopic parameters (D/H)I, (D/H)II, and $\delta^{13}\text{C}$ of ethanol from the fruit spirits are summarised in Table 5. It should be noted that the parameters

Table 2. Concentrations (minimum–maximum) of esters, aroma components of fruit spirits, determined by GC/MSD (mg/l of pure ethanol; n = number of samples)

	Pear brandy (n = 44)	Apple brandy (n = 12)	Sweet cherry brandy (n = 31)	Sour cherry brandy (n = 21)	Plum brandy (n = 29)	Apricot brandy (n = 16)
Ethyl caprylate	0.3–39.1	14.2–106.7	1.1–46.6	1.4–52.7	2.9–107.6	0.6–49.1
Benzaldehyde	< 0.1–5.9	0.8–73.8	1.3–47.0	0.7–195.0	0.3–31.2	0.3–46.7
β -Linalool	< 0.9	< 0.9	< 0.9–2.8	< 0.9–1.7	< 0.9–1.8	< 0.9–85.5
Methyl caprylate	< 0.3–1.6	< 0.3–5.1	< 0.3–2.4	< 0.3–1.8	< 0.3–3.3	< 0.3–1.3
Ethyl caprylate	0.8–167.1	13.4–360.0	1.3–136.2	3.1–176.8	6.0–306.0	2.1–144.2
α -Terpineol	< 0.5–0.8	< 0.5–2.3	< 0.5–1.4	< 0.5–3.1	< 0.5–1.2	0.7–63.2
β -Citronellol	< 0.3	< 0.3	< 0.3	< 0.3–3.2	< 0.3	< 0.3–3.5
Ethyl laurate	< 0.1–134.9	< 0.1–254.2	1.4–157.5	1.1–186.6	3.6–226.1	2.4–206.0
Ethyl myristate	0.9–43.1	4.2–72.7	0.4–55.8	< 0.4–69.5	< 0.4–47.1	2.2–54.6
Eugenol	< 0.7–2.7	< 0.7–1.9	< 0.7–2.3	< 0.7–15.3	< 0.7–9.4	< 0.7–13.2
Methyl palmitate	< 0.2–8.0	< 0.2–4.1	< 0.2–4.0	< 0.2–10.8	< 0.2–9.8	0.4–13.2
Ethyl palmitate	1.0–192.8	22.1–156.4	1.0–179.3	0.4–229.1	< 0.2–490.7	13.2–289.7
Phenylethyl oktanoate	< 0.4–93.3	< 0.4–147.0	< 0.4–5.1	0.6–269.2	< 0.4–11.8	< 0.4–10.4

Table 3. Stable isotope concentrations (minimum and maximum) in ethanol from fruit spirits (n = number of samples)

Fruit spirit	(D/H)I (ppm)	(D/H)II (ppm)	$\delta^{13}\text{C}$ (‰)
Pear brandy ($n = 44$)	94.46 to 98.95	118.92 to 127.41	–28.17 to –25.27
Apple brandy ($n = 12$)	94.40 to 96.09	119.88 to 124.73	–28.80 to –26.52
Sweet cherry brandy ($n = 31$)	94.17 to 100.46	120.68 to 140.13	–28.44 to –25.42
Sour cherry brandy ($n = 21$)	95.50 to 98.68	121.06 to 131.40	–27.21 to –25.74
Plum brandy ($n = 29$)	95.67 to 99.51	120.60 to 126.27	–27.50 to –24.30
Apricot brandy ($n = 16$)	95.08 to 99.80	121.78 to 127.20	–27.27 to –23.30

(D/H)I, (D/H)II, and $\delta^{13}\text{C}$ of all kinds of the fruit spirits tested had similar values.

The typical values for ethanol of non-fruit origin are shown in Table 6 (BAUER-CHRISTOPH *et al.* 1997). Tables 6 and 5 present the values of isotope parameters found in the samples of commercial spirits originating from various raw materials.

The values displayed show that isotopic parameters (D/H)I of the spirits from cane sugar or maize and especially of those made from synthetic alcohol are significantly higher than isotopic parameters (D/H)I of the fruit spirits. On the other hand, the spirits from beet sugar have isotopic parameters (D/H)I lower than the fruit spirits. The spirits from cane sugar and maize have the isotopic parameters $\delta^{13}\text{C}$ markedly lower than the fruit spirits.

It is not possible to distinguish between the fruit spirits of different origins using solely their isotopic parameters because the variation ranges of these parameters overlap too much. On the other hand, the isotopic parameters enable the recognition of the fruit spirits containing ethanol of non-fruit (such as beet sugar, cane sugar or maize) origin. The only exception is ethanol from grain, which fits to the isotopic parameters otherwise typical for

the fruit spirits. There is also slight overlap of the ranges of isotopic parameters of ethanol from the fruit spirits and ethanol from potatoes (Figure 1). The graph demonstrates the differences in the position of stable parameters of the fruit spirits and of other materials (alcohol from beet sugar, cane sugar, maize, potato, and synthetic alcohol).

Statistical evaluation

MISSELHORN and GRAFAHREND (1990) were the first to use linear discriminant analysis (LDA) in conjunction with the isotope parameters of ethanol in order to differentiate between highly rectified ethyl alcohols made from diverse raw materials.

If the separation potential of LDA is efficient, the resulting discriminant variables can be used as a means of assigning an unknown sample to one of the groups considered.

A total of 153 samples of fruit spirits were statistically evaluated using discriminant analysis (MELOUN & MILITSKÝ 2002). The purpose of the discriminant analysis was to find new variables. These variables should sufficiently distinguish between the samples of particular spirit types made from fruits such as plumes, sweet cherries,

Table 4. Stable isotope concentrations (minimum and maximum) in ethanol derived from various raw materials (BAUER-CHRISTOPH *et al.* 1997)

Raw material	(D/H)I (ppm)	(D/H)II (ppm)	$\delta^{13}\text{C}$ (‰)
Beet sugar	91 to 93	116 to 120	–28 to –26
Cane sugar, maize	108 to 110	127 to 130	–13 to –11
Grain	96 to 99	121 to 124	–26 to –24
Potato	93 to 95	124 to 126	–28 to –25
Synthetic alcohol	123 to 124	138 to 139	–32 to –25

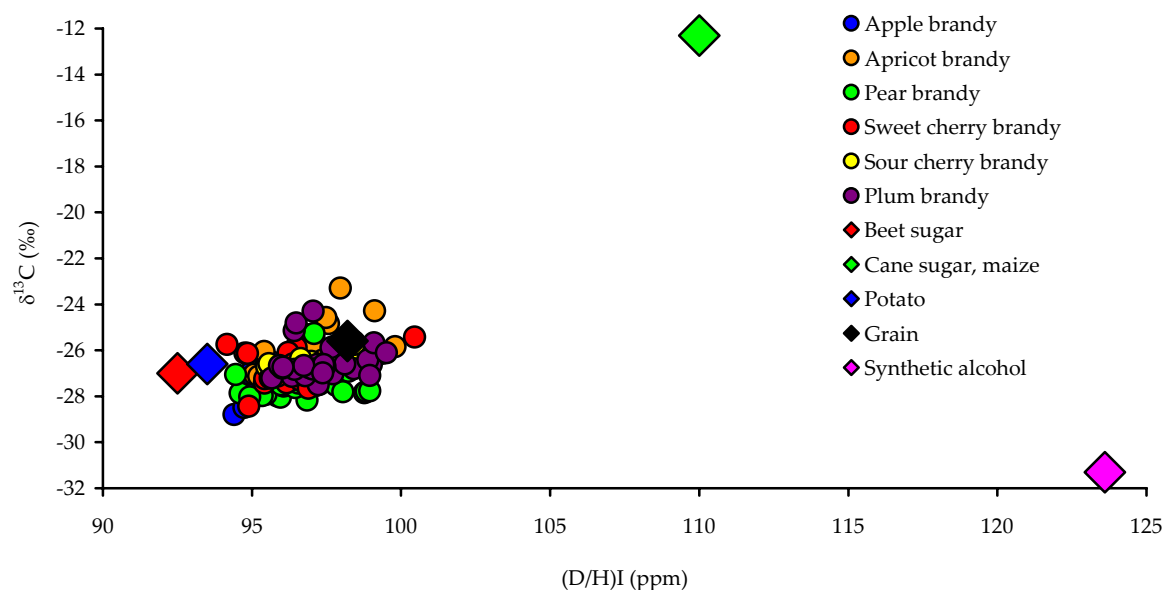


Figure 1. Correlation between the means of (D/H)I and $\delta^{13}\text{C}$ isotope ratios in ethanol from fruit spirits, beet sugar, cane sugar, potato, maize, grain, and synthetic alcohol

sour cherries, pears, apples and apricots. In addition, the discriminant analysis with different ranges of parameters was carried out in order to recognise the sole influence of particular groups of parameters including:

- all 26 parameters (data from GC-FID and GC-MSD with isotopic parameters used);
- 10 parameters (only data from GC-FID used);
- 3 parameters (only data from isotopic analysis used);

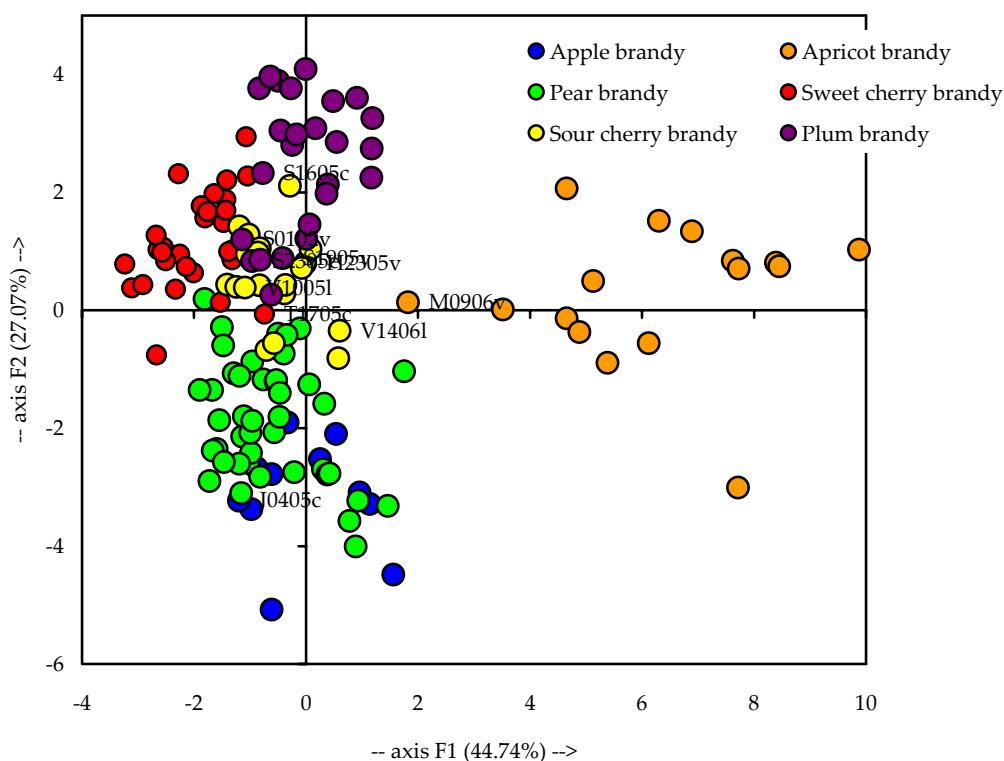


Figure 2. Plot of data from all samples set along first and second new canonical axes

- 13 parameters (only data from GC-MSD used);
- 23 parameters (data from GC-FID and GC-MSD without isotopic parameters used).

The results of the discriminant analysis of the respective data sets with different data sizes showed that the discriminant success rate between the individual types of spirits reached 93% and was influenced by the production date. Figure 2 displays the distribution of the data for all samples, set along the first and second new canonical axes. The similarity of spirit pairs such as apple–pear brandy, sour cherry–sweet cherry brandy is shown. Contrariwise, the conspicuous dissimilarity between the apricot spirits and other spirits is obvious. By employing a test against an independent data set, the discrimination success rate was found to be from 73 to 93% (if covered by the discrimination model) or 45% (not covered by the discrimination model).

Furthermore, it was possible to identify the important parameters for discrimination, also with respect to the sufficient distinction between the ranges of the values of individual analytical parameters. However, the evaluation of the data sets as well as the practical point of view revealed the suitability of using all parameters from GC-FID and GC-MS analyses. The results of the isotopic analyses showed to be very appropriate for the identification of outliers (suspected of containing components of different botanical origin), but not for distinguishing between the individual types of spirits.

CONCLUSIONS

The results of this work and the statistical processing of the data showed the possibilities of authenticity detection of fruit spirits based on stable isotope determination by using nuclear magnetic resonance – ^2H -NMR and mass spectrometry – IRMS (Figure 1). This paper also describes the way of identification of the individual kinds of fruit spirits based on the determination of higher alcohols and aroma components using gas chromatography.

Figure 2 shows the similarity of spirit pairs such as apple – pear brandy, sour cherry – sweet cherry, and the dissimilarity of apricot brandy to other spirits.

The data obtained show that the combined use of the volatile compounds and isotope parameters in LDA provide an efficient tool for detecting the adulteration of fruit spirits.

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