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PII: S0308-8146(98)00010-7

Food Chemistry, Vol. 63, No. 3, pp. 423–428, 1998
Published by Elsevier Science Ltd
Printed in Great Britain
0308-8146/98 $19.00 + 0.00
Analytical, Nutritional and Clinical Methods Section

Investigation into the use of carbon isotope ratios ($^{13}C/^{12}C$) of Scotch whisky congeners to establish brand authenticity using gas chromatography–combustion–isotope ratio mass spectrometry

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(Received 11 July 1997; revised version received and accepted 13 November 1997)

Volatile congeners of whisky have been analysed by gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS). Acetaldehyde, ethyl acetate, *n*-propanol, isobutanol and amyl alcohol from a popular blended whisky have been separated using established GC conditions and their $\delta^{13}C‰$ values determined. Eight samples of the whisky blend taken over the last 2 years of production have been analysed to allow the authentic range to be determined. A radar diagram has been used to graphically represent the $\delta^{13}C‰$ data. This was used as a simple means of comparing the carbon isotope profiles of whiskies. Two other whisky samples were analysed and shown to differ from the whisky blend on the basis of the $\delta^{13}C$ values. Published by Elsevier Science Ltd.

INTRODUCTION

Food adulteration describes the process of extending or completely replacing a premium food product with cheaper materials. This foodstuff is subsequently sold as the authentic product. Thus, not only is the quality of the food at issue but also the claims which are made about it on the label. As the knowledge of food composition has developed, the technology applied to food adulteration has become increasingly sophisticated. In consequence, regulatory authorities require continual improvements in analytical methodology in order to combat this type of fraud. Ensuring product authenticity in the UK is the responsibility of local authority Trading Standards departments as defined in the Trade Descriptions Act (1968) and sections 14 and 15 of the Food Safety Act (1990).

Scotch whisky is a premium spirit drink and, as such, economic incentives exist to mix, or completely substitute, one brand with another less expensive brand. This usually occurs in bars or restaurants (the 'on-trade') and therefore affects the consumer and places honest traders at a financial disadvantage. Scotch whiskies are produced by fermenting malted barley and other cereals such as wheat and maize. The fermented product is then distilled, blended and matured in oak casks for a minimum of 3 years (The Scotch Whisky Order, 1990). Water and spirit caramel may then be added prior to bottling the whisky at an alcoholic strength of at least 40% v/v in the European Union [Council Regulation, 1989 Council regulation (EEC) no. 1576/89, 1989; Commission Regulation, 1994 Commission regulation (EC) no. 2675/94, 1994].

The most useful method for establishing whisky brand authenticity is to determine the concentration profile of volatile organic compounds (congeners) resulting from the raw materials, the processing by fermentation, distillation and maturation of the whisky. Volatile congeners such as acetaldehyde, methanol, ethyl acetate, propanol, isobutanol, diethyl acetal and the amyl alcohols (2- and 3-methyl butanol) are measured using gas chromatography coupled with flame ionisation detection (GC–FID) (Singer, 1966; Simpkins, 1985). This technique is comparative and therefore relies on the availability of genuine whiskies to establish a database of reference values. It is also dependent on specific brands exhibiting relatively consistent congener ranges. However, the addition of neutral alcohol, (a spirit distilled at >94.8% alcohol and devoid of congeners) from such as cane molasses spirit, may go undetected by GC–FID if flavours have been added to restore the congener profile.

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Aylott *et al.* (1994) have published data on five unspecified brands of blended Scotch whisky and demonstrated that, for these brands, the congener profile is consistent over production batches. Simpkins and Rigby (1982) have used stable carbon isotope ratio analysis to determine the illicit extension of blended whisky by addition of grape spirit and molasses spirit. In this case, the isotopic composition of the whisky was measured by combusting the alcohol to generate carbon dioxide which was subsequently purified and measured using an isotope ratio mass spectrometer. Detection of adulteration was possible because of the significant differences between the $^{13}C/^{12}C$ isotope ratios of cane sugar molasses spirit and other spirit types.

The differences in isotope ratios, expressed as $\delta^{13}C‰$ ('delta carbon 13 per mil') are a result of the photosynthetic pathway used by a plant to fix atmospheric carbon dioxide. Most plants, including barley, grapes, oats and wheat, utilise the C3 photosynthetic path. Enzyme fractionation of the carbon isotopes during C3 photosynthesis results in constituents that are relatively depleted in ^{13}C. Spirits produced from C3 materials typically have global $\delta^{13}C‰$ values between -25 and $-30‰$. Maize and cane are two of a small number of plants which use the C4 photosynthetic cycle. This pathway is less discriminating against the ^{13}C isotope than the C3 route and hence spirits produced from C4 substrates have constituents which are relatively richer in the heavier carbon isotope. Authentic C4 spirits typically have global $\delta^{13}C‰$ values between -10 and -12. Therefore, adulteration of a spirit such as brandy, made from a single fermentable C3 substrate, with alcohol derived from cane molasses (C4) can be readily detected (Simpkins and Rigby, 1982).

Since blended Scotch whisky is comprised of different amounts of C3 and C4 spirit, it will have a carbon isotope ratio intermediate between the lower limit for authentic C3 plants and the upper limit for authentic C4 plants. The global carbon isotope ratio of the whole sample cannot be used to unequivocally determine whether small quantities of grape or cane molasses spirit have been fraudulently added unless the original unextended blend is available. This need for original samples or a database of authentic samples to determine an authentic range is a feature common to the majority of authenticity analyses.

It is also known that significant differences in concentration of specific congeners exist between malt and grain whiskies (Aylott, 1995). For instance, amyl alcohols and other high-boiling congeners are removed from the distillate during the continuous distillation used in the production of grain whiskies. Furthermore, the distillation process itself can be a fractionating process if the recovery of the distillate is not quantitative. This results in a depletion in the ^{13}C isotope in the collected fraction relative to the distillate residue. Consequently we considered that specific congeners, in a given brand of whisky, may exhibit unique $\delta^{13}C‰$ ratios which are representative of manufacturing effects.

In this paper we detail initial experiments using GC–combustion–isotope ratio mass spectrometry (GC–C–IRMS) of carbon isotopes for Scotch whisky brand identification and suggest a number of areas for further development.

MATERIALS AND METHODS

Authentic whisky samples

For the study, eight samples of a popular Scotch whisky blend representative of 2 years of production were used. Also analysed was a blended Scotch whisky prepared from Scotch malt and grain whiskies, with the grain portion of the blend derived from C4 grain spirit only and a product from India, made from Scotch whisky, Indian malt whisky and neutral alcohol.

Sample preparation

Whisky samples were transferred directly into autosampler vials. For the Scotch whisky blend, samples were prepared in duplicate in separate vials.

Gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS)

Gas chromatography

Established GC conditions for GC–FID congener profiling were used to separate the principal congeners of the whisky samples. The separation of the individual congeners was achieved using a Hewlett Packard 5890 Series II gas chromatograph fitted with a CP-WAX57CB (Chrompack) capillary column (50 m × 0.32 mm i.d.) coated with a 0.2 μm film. Helium was used as the carrier gas and injection (1 μl) was in the split mode with a split ratio of 20:1 using an A200S autosampler (Cueni, Switzerland). The following temperature programme was used for the GC oven: initial oven temperature, 40°C; initial isothermal period, 6 min; programme rate, 5°C min^{-1}; oven temperature, 120°C; isothermal period, 3 min; programme rate A, 15°C min^{-1}; final oven temperature, 190°C; final isothermal period, 10 min.

Isotope ratio mass spectrometry (IRMS)

The column effluent was split into two streams with a minor amount directed to a standard FID. The majority was directed into a combustion interface (GCII Finnigan-MAT, Bremen, Germany) at 940°C which quantitatively oxidised each of the congeners to carbon dioxide and water. The CO_2 was swept into the IRMS (Delta S, Finnigan MAT, Bremen, Germany) by a stream of helium carrier gas. The mass spectrometer measured the relative abundance of the m/z 44 ($^{12}CO_2$) and 45 ions ($^{13}CO_2$). Pulses of calibrated carbon dioxide

reference gas of known isotopic ratio were superimposed on the column effluent at predetermined times. This permitted the measurement of the carbon isotope ratio ($\delta^{13}C‰$) of the sample by comparison with the isotope ratio of the CO_2 reference gas. The reference gas was calibrated relative to the PDB scale using the carbonate standard reference material, NBS-19.

RESULTS AND DISCUSSION

Table 1 shows the congener concentrations and statistical data for the eight samples of the popular Scotch whisky blend, measured using an FID. These data show mean, standard deviation, minimum concentration, maximum concentration and the range of concentrations for acetaldehyde, ethyl acetate, *n*-propanol, isobutanol and the amyl alcohols. These data show that the congener profile is quite stable for this blend over the 2-year sampling period and, where reported, the ranges are comparable with those recorded by Aylott *et al.* (1994).

Figure 1 is a typical trace of the *m/z* 44 ion ($^{12}CO_2$) recorded for a blended Scotch whisky. The vertical axis is in V and the horizontal axis is time, in s, beginning at 100 s. The peaks of interest are labelled with peak names. The four parallel-sided peaks centred around 110, 200, 1310 and 1610 s and labelled, 'Reference peaks', are the pulses of CO_2 gas of accurately known $\delta^{13}C‰$ value. These are superimposed on the helium carrier gas stream from the combustion interface and are used to calculate the delta value of the individual components. The period of flat baseline between 450 and 600 s is the time when the ethanol peak elutes. Owing to the size of this peak it has been diverted away from the combustion interface to preserve the life of the combustion tube. As seen from Fig. 1, typical amplitudes of the acetaldehyde and ethyl acetate were less than 1 V whereas the *n*-propanol, isobutanol and amyl alcohols had amplitudes between 1 and 2 V. Full-scale is 10 V which is equivalent to a current of about 30 nA at the *m/z* 44 ion cup.

Table 1. Congener concentrations and statistical data for eight samples of a well known Scotch whisky representative of two years production, measured using GC–FID

Congener name	Concentration (g/100 l ethanol)
Acetaldehyde	
Mean	7.07
SD	1.47
Maximum	9.90
Minimum	6.00
Range	3.90
Ethyl acetate	
Mean	30.6
SD	1.06
Maximum	31.8
Minimum	29.1
Range	2.70
n-Propanol	
Mean	71.9
SD	6.06
Maximum	78.3
Minimum	64.4
Range	13.9
isoButanol	
Mean	65.9
SD	4.54
Maximum	70.4
Minimum	59.0
Range	11.4
Amyl alcohols	
Mean	70.5
SD	3.92
Maximum	76.9
Minimum	66.1
Range	10.8

Table 2 shows the carbon isotope data, measured by GC–C–IRMS, for the same samples of the popular Scotch whisky blend as in Table 1. Eight samples were measured in duplicate. Each pair was averaged to reduce the effect of instrument variation and then the mean of means calculated over all eight samples. The standard deviation of the means shows the sample-to-sample variation. The minimum and maximum values of the means for each component and the range between the minimum and maximum are also shown.

The data show that there is a wider variation for these less intense peaks. This is a combination of the genuine natural variation, possible fractionation due to the volatility of these components, and the decrease in the precision of measurement with decrease in peak amplitude. It was not possible to increase the amount of sample on the column as injections larger than 1 μl overloaded the stationary phase. The less volatile and later-eluting components show less variation in the carbon value.

Acetaldehyde, ethyl acetate and isobutanol exhibit $\delta^{13}C‰$ values that are in the typical range of ethanol fermented from C3 substrates (Simpkins and Rigby, 1982). For this blend, the amyl alcohols showed a delta value that was quite depleted in ^{13}C compared with the acetaldehyde, ethyl acetate and isobutanol. Amyl alcohols are indicative of the amount of malted barley in the whisky. As barley uses the C3 pathway to produce plant components, the depleted $\delta^{13}C‰$ value is consistent with the absence of a C4 grain spirit contribution to the amyl alcohols. *n*-Propanol, on the other hand, had a delta value that is considerably more enriched than the others and is between those values expected from components that come from C3 and C4 plant material.

The mean delta values from the five components of the blended whisky have been plotted on five axes of a radar diagram in Fig. 2. They are joined together with a solid line on the diagram to give an irregular pentagon. Two other pentagons are shown: these are the minimum $\delta^{13}C‰$ ratios for each component connected together (to indicate the most depleted value that was obtained) and the maximum $\delta^{13}C‰$ ratios to show the most enriched

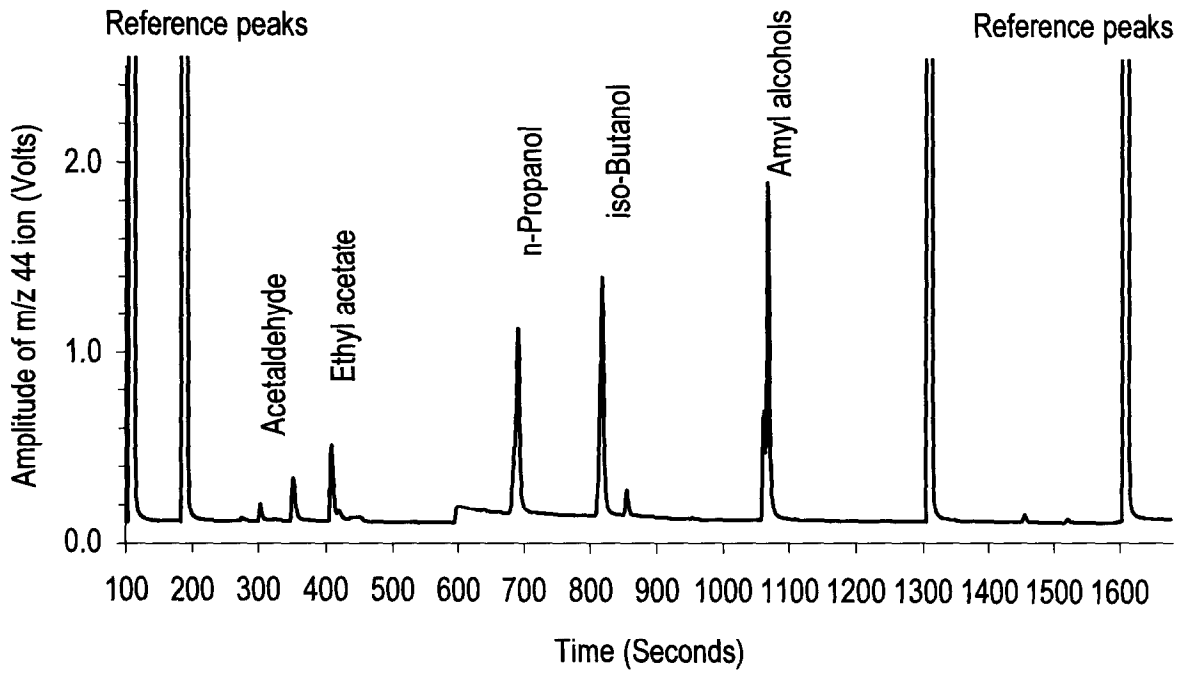

Fig. 1. 44 ion chromatogram of a well known Scotch whisky blend measured by GC–C–IRMS.

Table 2. Congener carbon isotope values and statistical data for eight samples of a well known Scotch whisky representative of two years production, measured using GC–C–IRMS

Congener name	Delta value (δ^{13}C‰)
Acetaldehyde	
Mean	−25.5
SD	1.65
Maximum	−21.7
Minimum	−26.9
Range	5.22
Ethyl acetate	
Mean	−26.5
SD	1.47
Maximum	−23.1
Minimum	−27.5
Range	4.34
n-Propanol	
Mean	−16.0
SD	1.01
Maximum	−14.4
Minimum	−17.1
Range	2.72
isoButanol	
Mean	−25.8
SD	0.45
Maximum	−25.3
Minimum	−26.5
Range	1.15
Amyl alcohols	
Mean	−29.2
SD	0.33
Maximum	−28.6
Minimum	−29.6
Range	0.97

values that were obtained. Together these plots give a simple graphical representation of the mean and the range of values for this whisky blend against which to compare other samples.

Two other whiskies were analysed to evaluate the potential of this method for determining generic authenticity. A blended Scotch whisky known to contain only malt whisky and grain whisky derived from maize was analysed. The data are shown in Table 3 giving GC–FID and GC–C–IRMS values. The carbon isotope data are plotted in Fig. 3. Comparison with Fig. 2 shows that the delta values were all more positive than those for the blended Scotch whisky and the radar plot had a distinctive shape that clearly separated it from the whisky blend. The more enriched

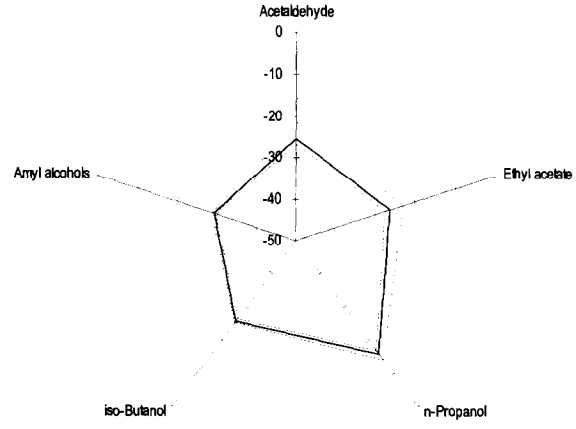

Fig. 2. Radar plot of δ^{13}C‰ ratios for a popular blended Scotch whisky. The dotted lines represent the maximum and minimum ranges over 2 years of production.

Table 3. Congener concentrations and carbon isotope values for a sample of a blended Scotch whisky prepared from Scotch malt whiskies and a maize-derived grain whisky measured using GC–FID and GC–C–IRMS

Congener name	Concentration (g/100 l ethanol) (by GC–FID)	Delta value (δ^{13}C‰) (by GC–C–IRMS)
Acetaldehyde	1.77	−23.6
Ethyl acetate	23.3	−21.9
n-Propanol	47.3	−8.79
isoButanol	49.9	−16.8
Amyl alcohols	55.0	−24.7

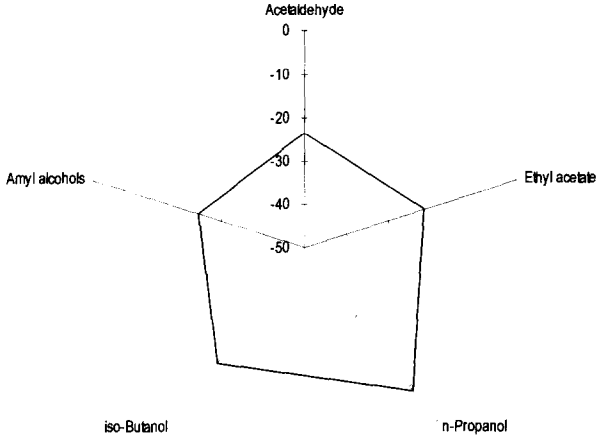

Fig. 3. Radar plot of δ^{13}C‰ ratios for a blended Scotch whisky prepared from Scotch malt whiskies and a maize-derived grain whisky.

Table 4. Congener concentrations and carbon isotope values for a blended Scotch whisky known to contain Indian malt whisky and neutral alcohol measured using GC–FID and GC–C–IRMS

Congener name	Concentration (g/100 l ethanol) (by GC–FID)	Delta value (δ^{13}C‰) (by GC–C–IRMS)
Acetaldehyde	7.88	−20.4
Ethyl acetate	2.26	−15.6
n-Propanol	11.9	−6.29
isoButanol	2.66	−28.4
Amyl alcohols	10.3	−27.5

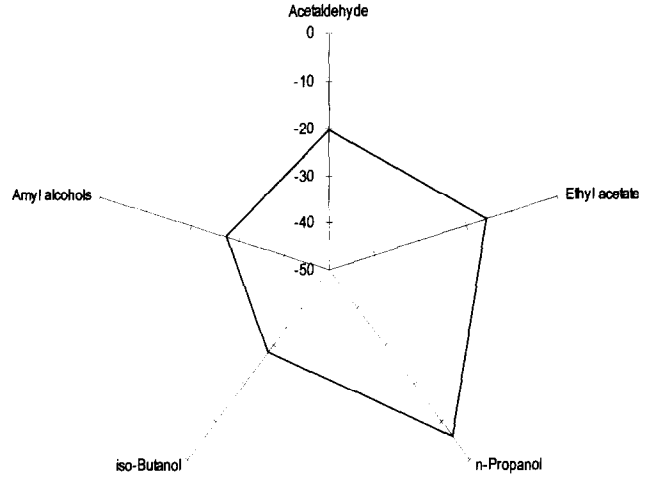

Fig. 4. Radar plot of δ^{13}C‰ ratios for a blended Scotch whisky known to contain Indian malt whisky.

delta values could possibly be due to a fractionating effect of the malt whisky during distillation. They are, however, more likely to be due to a considerable contribution from the C4 maize grain whisky. Without the original malt and grain whisky it is not possible to determine which of these is the case. Such analysis does also raise the possibility of using carbon data to confirm the relative mixes of C3- and C4-based spirits.

Similarly a mixture of a blended Scotch whisky known to contain an Indian malt whisky was analysed to determine whether a difference could be observed. The data are shown in Table 4 giving both GC–FID and GC–C–IRMS values. Figure 4 shows the carbon isotope data where it was again very clear that there was a significant difference between the popular whisky blend (Fig. 2) and this sample simply on the basis of the carbon isotope data.

It is clear that GC–C–IRMS has potential as a means of determining brand authenticity. Combined with GC–FID congener profiles, it will add a further parameter for each congener by which to classify and authenticate whisky. The combined techniques may allow differentiation between brands that currently can not be distinguished by GC–FID congener profiles alone (Aylott *et al.*, 1994). One of the advantages of this GC–C interface is that the FID continuously samples the column effluent and is recorded by the data system. By simply integrating the FID signal it would be possible to measure both FID and carbon data simultaneously, allowing the congener profile and individual carbon isotope values to be acquired with only one injection of sample. Use of an internal standard like that used for GC–FID congener analysis (e.g. 3-pentanol) but with a well-characterised delta value to which these data may be normalised, may also help in reducing instrument variation and narrowing the authentic range. Finally, by using a larger split ratio and small volume injection an assessment of the delta value of the ethanol itself may be made which would also characterise the spirit (Simpkins and Rigby, 1982).

CONCLUSION

The use of stable isotope analysis for the authenticity determination of whisky has already been demonstrated by Simpkins and Rigby (1982), by combusting the whole sample to give a measure of the delta value of the ethanol. Here, the use of GC–C–IRMS extends the application of this technique to allow the delta value of individual volatile congeners to be measured.

Measurements on samples of a popular whisky blend over a period of 2 years of production show that the congener profile by FID is constant and the $\delta^{13}C‰$ values for the individual congeners are also relatively constant. Comparison of the $\delta^{13}C‰$ values with other whisky brands shows that carbon isotope ratios can be used to differentiate between whiskies and therefore adds a further parameter by which to authenticate and characterise this premium product.

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