ELSEVIER

Food Chemistry 69 (2000) 215–220

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Detection of fruit juice authenticity using pyrolysis mass spectroscopy

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Received 12 July 1999; received in revised form 16 September 1999; accepted 16 September 1999

Abstract

Orange juices originating from seven countries, were successfully discriminated using pyrolysis–mass spectrometry coupled with multivariate statistical analysis. The method was demonstrated to be both rapid and sensitive. The mass spectral ions associated with the discrimination were identified. The discrimination of juices from Israel and Brazil was maintained over a range of juice concentrations and appeared to be a function of the chemical composition of the juices. Adulteration of these juices with 5% sucrose could be detected.

Keywords: Authenticity; Adulteration; Pyrolysis mass-spectrometry; Orange juice; Discriminant analysis

1. Introduction

The fruit juice industry is one of the fastest growing sectors of the world-wide beverage industry. Many different types of fruit are now being processed into juice but oranges still provide most of the fruit juices and other fruit products sold around the world. Consumers expect manufacturers and retailers to provide wholesome and authentic fruit juices. Retailers are concerned to maintain their reputation as well as to comply with labelling legislation. These factors have underlined the need for reliable techniques that can authenticate the purity of fruit juices.

Analysis of a single natural juice component would be inadequate to provide sufficient information to define the authenticity of a juice; therefore, multiple component chemical analyses are required to reliably evaluate the differences between adulterated and pure juices. This approach, however, is both time-consuming and expensive.

Pyrolysis–mass spectroscopy (Py–MS) has considerable potential as a rapid method for the assessment of food authenticity and the detection of adulteration. Compared with the use of a combination of chemical analyses of juice components, such as amino acids, sugars, organic acids, phenolics and metals, for determining the authenticity of juices and detecting adulteration, Py–MS involves shorter analysis time and less manpower. Other advantages of Py–MS are that it only requires a small amount of sample and involves very little sample preparation. This fast and simple method gives reproducible data, which are easily accessible to computer processing.

The application of Py–Ms to detect adulteration in food has received relatively little investigation. The Py–MS studies of foods have been mainly concerned with the characterisation and identification of food components such as carbohydrates (Baltes & Schmahl, 1978; Donelly, Voigt & Scallet, 1982), food thickeners (Sjöberg & Pyysalo, 1984), phenols (Heinrich & Baltes, 1985), proteins (Raghavan, Ho & Daun, 1986), polysaccharides (Schulten & Görtz, 1978) and caramels (Tschiersky & Baltes, 1988). Py–MS has also been used in the identification and/or differentiation of small taxonomic groups of microorganisms, where conventional identification techniques cannot be used (Gutteridge, Sweatman & Norris, 1984; Meuzeelar, Kistemaker & Posthumus, 1974; Shute, Gutteridge, Norris & Berkeley, 1984).

In 1984, Gutteridge et al. successfully characterised tea extracts according to their countries of origin. Ottley and Maddock (1986) used Py–MS to successfully characterise wines from Spain, Sicily and France according

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0308-8146/00/$ - see front matter
PII: S0308-8146(99)00274-5

to their origins and varieties. Raghaven (1986) used pyrolysis coupled with gas chromatography and mass spectrometry to characterise meat products. He was able to determine the protein content of a mixture and so measure the proportion of meat in a given sample.

Very little work has been reported on the use of Py–MS in the authentication of juices. Aries, Gutteridge and Ottley, 1986, used Py–MS to authenticate and characterise orange juices from Brazil and Israel. However, the discrimination between these countries of origin was not completely achieved due to poor replicate reproducibility of the discriminating mass spectral ions within the pyrolysis mass spectra.

More recently, Goodacre, Hammond and Kell, 1997 attempted to quantify the adulteration of orange juices with sucrose using Py–MS and chemometrics. The authors found that each of the chemometrics methods used could be utilised to provide calibration models, which gave excellent predictions for the level of sucrose adulteration of the juices. The authors claimed to detect sucrose adulteration down to less than 1.0%.

This present paper reports on the application of Py–MS in the discrimination of orange juices from seven different countries of origin. The effect of soluble solids content of juices has been investigated and the ability to detect adulteration by the addition of sucrose is reported.

2. Materials and methods

2.1. Materials

Orange juice concentrates used in this study were obtained from several packers in the UK. Prior to Py–MS analysis, all the orange juice concentrates had been analysed using Multiple Component Chemical Analyses in the laboratory of Reading Scientific Services and judged found to be authentic with the sole exception of a Spanish juice. The analysis indicated that this Spanish juice was probably of Brazilian origin.

Sucrose was purchased from Aldrich Chemicals Co. Ltd., UK. HPLC grade water (Rathburn Chemical Ltd., Scotland, UK) was used for all dilutions.

2.2. Preparation of samples

Analyses were carried out using samples of orange juice concentrates from Israel and Brazil. Four orange juice concentrates from Brazil and five orange juice concentrates from Israel were diluted 1 in 6 (w/w) with HPLC grade water to give single strength juices. Within this batch, two samples, each from Brazil and Israel, were adulterated with sucrose at 5 and 10% level, respectively. These adulterated samples were compared with all the other "pure" orange juice samples, in order to assess the sensitivity of the technique in detecting adulteration. The soluble solids content of the sucrose solution used for adulteration was the same as that of the juices to which it was added. Samples were adulterated at levels of 5 and 10% on a volume to volume basis prior to further preparation for Py–MS analysis.

The soluble solids content of Israel and Brazil juices can typically vary from 55 to 65° Brix. Therefore, simple dilution, 1 in 6 (w/w) to single strength, will give rise to variable brix content of the finished product. In order to eliminate the soluble solids content as a variable in the Israel and Brazil discrimination, samples were prepared at a constant soluble solids level. Ten orange juice concentrates, each from Brazil and Israel, were diluted to the same soluble solids content (11° Brix). The soluble solids contents of the diluted samples were measured using a Bellingham and Stanley RFM 90 refractometer.

In a further experiment, to assess the effect of solids content on the Israel/Brazil discrimination, one orange juice orange juice concentrate from Brazil and one orange juice concentrate from Israel were diluted to different strengths: 1:6; 1:8; 1:10; 1:13; and 1:20 with HPLC grade water.

To evaluate the effectiveness of Py-MS in discriminating orange juice samples by country of origin, juice concentrates from Florida, Cuba, Spain, Israel, Brazil, Cyprus, and South Africa were diluted 1 in 6 (w/w) with HPLC grade water to give single strength juices.

2.3. Pyrolysis mass spectrometry

The diluted juices, made up from the samples described above, were centrifuged for 15 min (1000 *g*) and the supernatant was used for Py–MS analysis. Four microlitres of the sample were pipetted onto a Curie-point foil and dried in a vacuum oven at 75°C for an hour prior to Py–MS analysis. Triplicates of each sample were analysed.

The analyses of the juices were carried out using a pyrolysis mass spectrometer (RAPYD-400 Horizon Instruments, Heathfield, UK) under the control of an IBM compatible PC. The full description of this system has been given elsewhere (Goodacre, 1994).

The connection of an auto-sampler to the mass spectrometer enabled the rapid processing of samples at the rate of 1.5 min per sample. The foils were of 50:50 Fe/Ni alloy composition with a Curie-point temperature of 530°C. The electron ionisation impact was set to 25 eV and the inlet temperature was set at 160°C. One hundred scans over the mass spectral range of m/z 50–300 at a rate of 10 μ s/scan were made for each sample.

2.4. Data analysis

A Genstat statistical package program (similar to that described by Aries, Gutteridge & Evans, 1986; Goodacre, 1994; Gutteridge et al. (1984); Windig, Haverkamp

& Kistemaker, 1983) was used to analyse the pyrolysate mass spectra. This Genstat program contains routines for Principal Component (PCA) and Canonical Variate Analysis (CVA).

Principal component analysis reduces data containing many variables down to two or three-dimensional sets of data, which can be interpreted visually. Variables, which do not show maximum variance, will be discarded. Those, which show maximum variance, are formed into new sets of uncorrelated variables that are called the principal components. Each variable (i.e. m/z ratio) is weighted according to its contribution towards the principal component. This weighting is called the loading. PCA determines the loading of each mass (m/z).

Canonical variate analysis emphasises the difference between the groups and, therefore, facilitates the separation of the samples into their respective groups. It assesses the group's structures and those of the sample replicates. The principal component generates the output data through canonical variate analysis. This means that the resulting canonical variates are related to the original m/z by a set of combined principal component canonical variate loadings. These loadings, therefore, reflect the extent to which each mass m/z discriminates within the given canonical variate. This can allow some chemical interpretation of the discrimination seen between samples.

3. Results and discussion

3.1. The effect of soluble solids in the discrimination of orange juices

Fig. 1 shows the pyrolysate mass spectra obtained from Brazilian and Israeli juices. The mass spectra of the juices from both countries appeared to be similar, but there were small differences between the two countries of origins. These were large enough to allow very clear discrimination using combined principal component canonical variate (PCCV) analysis.

Py–MS has previously been used to differentiate the country of origin of orange juices (Aries, Gutteridge & Evans, 1986), but the discrimination that these authors achieved was not complete. The authors postulated that the poor discrimination of juices being studied may have been caused by the incorrect labelling of the sources of the samples, or might be due to the lack of reproducible spectral ions produced by the pyrolysis.

The poor reproducibility of mass spectral ions could be due to the inherent limitations of the specific pyrolysis mass spectrometer that was used. The present results show that very clear discrimination of juices, according to country of origin, can be obtained from the first PCCVs (Fig. 2). The improved discrimination of the juices compared with the previous studies, was

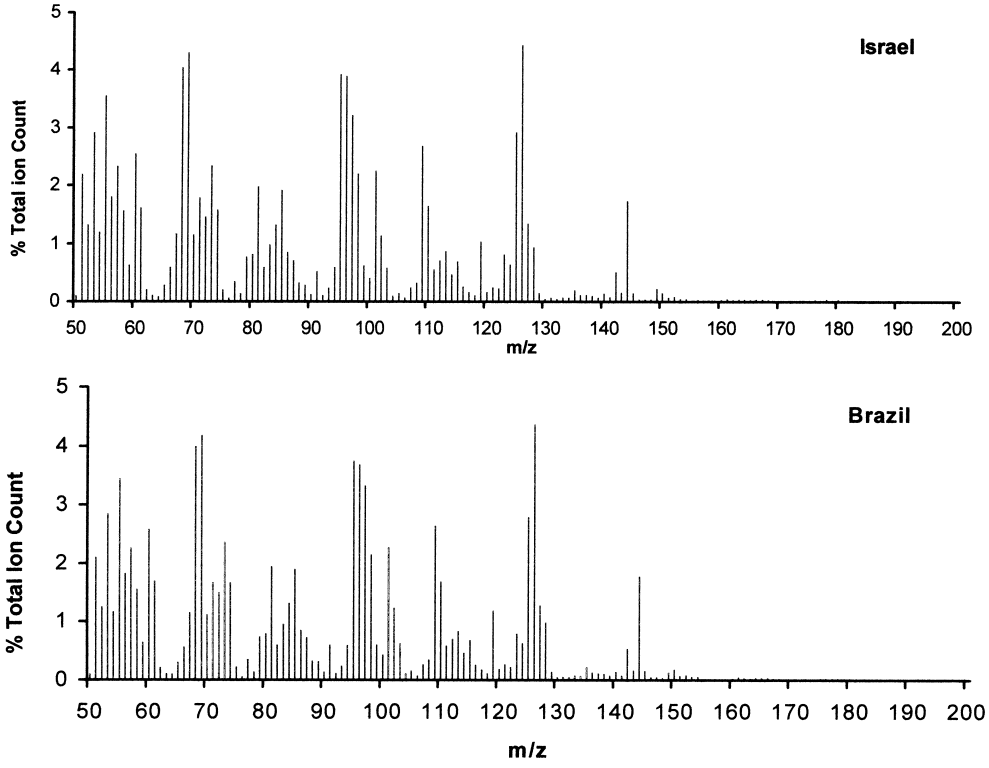

Fig. 1. Typical mass spectra of single strength orange juices from Israel and Brazil.

probably due to the use of a more sophisticated pyrolysis mass spectrometer.

One of the differences between Brazilian and Israeli juices is the extent to which they are concentrated. Normally, Brazilian concentrates have Brix values in the range of 60–66°, whereas Israeli concentrates typically have values in the range of 55–65°. In this study, the samples were diluted 1:6 to give "single" strength juice. Therefore, the discrimination found between these two countries of origin could have been due to the difference in the soluble solids contents of the samples. However, when the samples were diluted to the same soluble solids content the discrimination was still clearly discernible (Fig. 3).

To further investigate the effect of soluble solids content on the Brazil/Israel discrimination, samples from these two countries were diluted to different solids contents (Fig. 4). The discrimination between these two countries was still clearly evident at all dilution levels. Analyses of juices that had been diluted to a level containing only 10% of the original juice solids, still showed a very clear discrimination between samples from Israel and Brazil. This showed that the soluble solids content of the reconstituted concentrates did not have an overriding influence on the discrimination observed between Israel/Brazil, and that the discrimination was due to the fundamental composition of the juice.

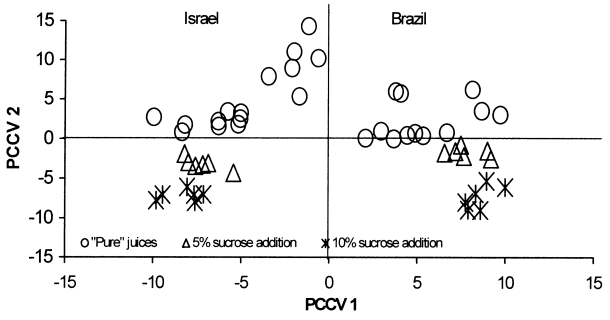

Fig. 2. The first two principal component canonical variate analyses of the Py–MS data of Israel and Brazil single strength orange juices.

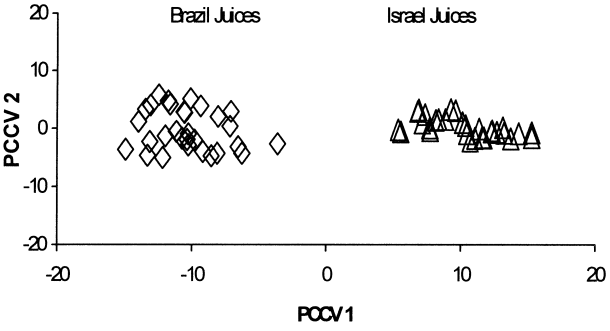

Fig. 3. PCCV plot of Py–MS data of orange juice concentrates from Israel and Brazil diluted to contain the same soluble solids.

3.2. Detection of adulteration by sucrose addition

Included in the sample set of the experiment shown in Fig. 2, were a number of samples (8) which were spiked with sucrose to investigate the lowest level of adulteration that could be detected. The samples adulterated at the 10% level were clearly discriminated from the pure juices. The 5% sucrose-adulterated samples were also separated from their unadulterated equivalents. As can be seen in Fig. 2, PCCV 1 contained most of the variance due to country of origin, whereas the discrimination seen in the second PCCV is mainly due to adulteration with sucrose. This is highly significant as it suggests that the second PCCV could be used as a method to detect adulteration of sucrose. This can be seen clearly in the plot of the second PCCV against the third (Fig. 5). The limit of detection of adulteration was estimated to be about 5%.

Inspection of the mass spectral ion loadings plot (Fig. 6) showed which mass spectral ions were responsible for the discrimination of "pure" as well as adulterated juices. Mass spectral ions m/z 57, 64, 75, 91, 109, 119 and 142 were important in discriminating "pure" Brazilian juices, while m/z 68, 101 and 110 were associated with "pure" Israel juices. Mass spectral ions m/z 113 mainly characterised adulterated Israeli samples. However, the ions which had the largest negative scores in the PCCV 2 of the loadings scores plot (Fig. 6) were responsible

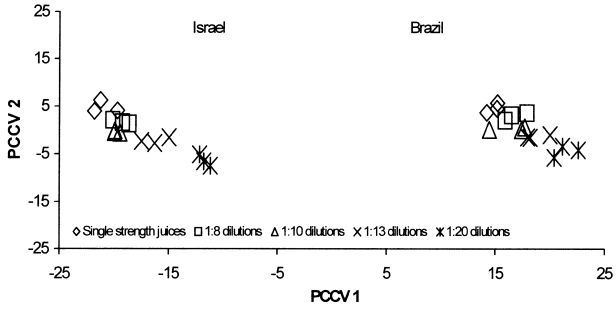

Fig. 4. PCCV plot of the Py–MS data for orange juices from Brazil and Israel diluted to differing degrees of concentration.

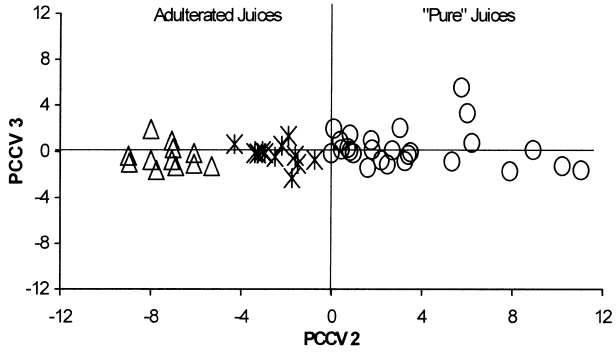

Fig. 5. Second and third principal component canonical variate analysis of "pure" and adulterated single strength orange juices.

for the discrimination of "pure" juices from adulterated ones. These mass spectral ions are m/z 58, 59, 60, 61, 62, 70, 72, 95, 96, 113, 125 and 127.

The sucrose solution that was used to adulterate the samples was also analysed in the Py–MS. The pyrolysate showed m/z 60 as the base peak with a large ion at m/z 61. Other major peaks were m/z 55, 56, 62, 72, 73, 97 and 126 (Fig. 7). The sucrose pyrolysis products that were associated amongst the mass spectral ions with adulterated Brazil juices were, m/z 60, 62, 72 and 73 whereas m/z 61, 56 and 126 were amongst the mass spectral ions that were associated with adulterated Israel juices.

Due to the diversity of the concentrations of orange juices parameters, the origin of the mass spectral ions in the pyrolysis spectra is difficult to determine. Many of these mass spectral ions are likely to have multiple origins and therefore it is not possible to define their exact chemical origins. However, this should not detract from the fact that Py–MS, together with PCCV analysis, was able to classify the samples as authentic and/or adulterated.

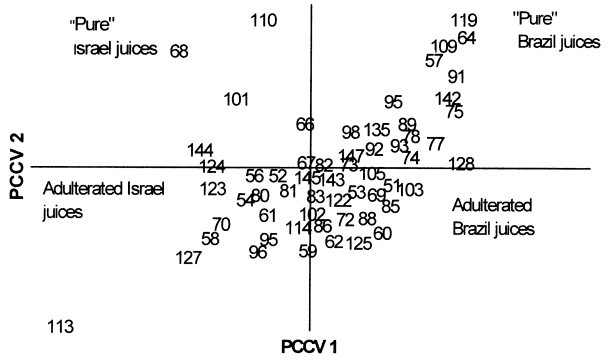

Fig. 6. Plot of the combined PCCV loadings scores for the first two canonical variates of "pure" and adulterated juices from Israel and Brazil.

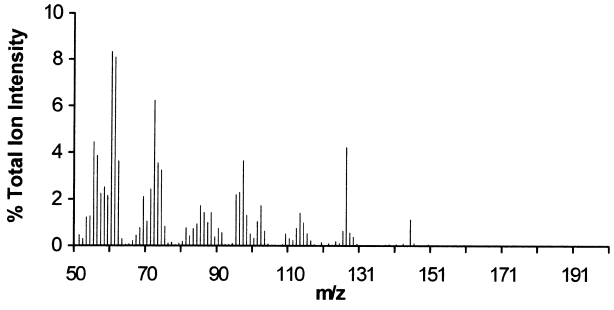

Fig. 7. Mass spectra of sucrose pyrolysate.

3.3. Discrimination of juices originating from various countries of origin

A set of 36 samples from a wider range of countries was also examined by Py–MS. These samples of juices were found to cluster into two groups (Fig. 8). One group was comprised of juices from Florida, Cuba and Brazil. The other group was comprised of juices from Cyprus, Israel, and South Africa. The difference in the group clusters may be a response to a number of different factors. One of the factors may be the effect of climate. Israel, Cyprus and South Africa have similar climates, characterised as warm temperate rainy with a dry summer sometimes called Mediterranean type. Brazil, Cuba and Florida tend to have a much more tropical climate; i.e. humid, hot and rainy in the summer and dry in the winter.

The varieties of the oranges used for these juices were unknown, but this factor may also contribute to the differences observed. The variety Valencia is grown in all the above countries. However, Florida also grows

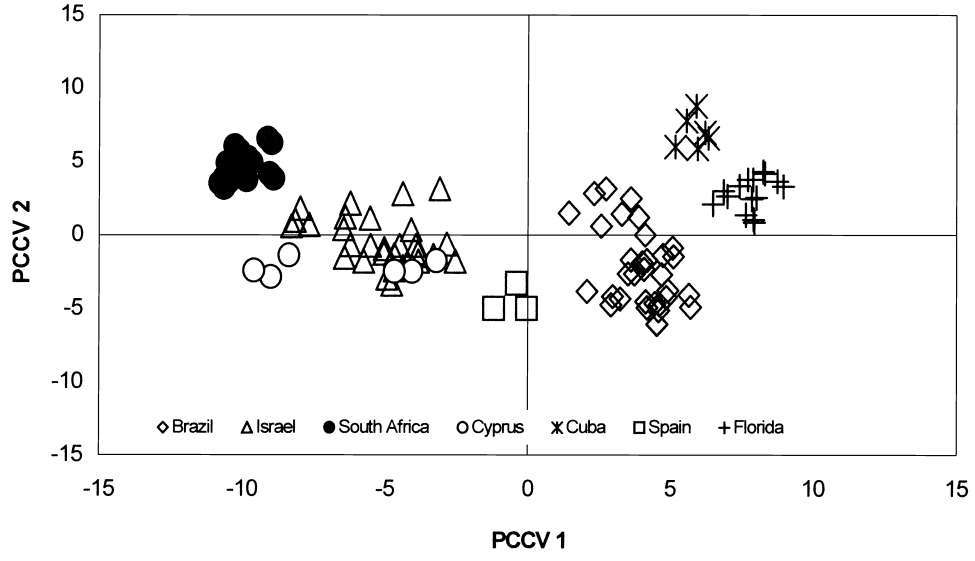

Fig. 8. Plot of the first two PCCVs of the Py–MS data of single strength juices from various countries of origin.

seasonal varieties such as Hamlin, Parson Brown (sweet variety) and Pineapple (Chen, Shaw & Parish, 1992). Brazil also grows seasonal varieties, such as Hamlin and Pineapple, Natal, Pera, Westin and Rubi. Israel also prevalently grows the variety Shamouti.

Soil type may be another factor that contributes to the characterisation of the juices in this study. The different types of soil on which the oranges are grown will affect the total amounts of nutrients available and also the rates at which those nutrients are absorbed. Unfortunately there are no data available on the soils for the samples studied.

The juice from Spain appears to have an anomalous position. The PCCV plot showed it to be similar in characteristics to those of Israel juices. However, the multiple component chemical analysis authenticity tests of the sample revealed it to be more similar to juices from Brazil. It is possible that the assumed origin, based on the processor's receipt, was inaccurate.

4. Conclusion

The data reported in this paper show that Py–MS, coupled with multivariate analysis, has considerable potential for determining the origin of commercial juices and for detecting adulteration by sucrose. Detection of adulteration of sucrose could be estimated at about 5%. The Py–MS method was far simpler and quicker than traditional methods using multi component chemical analysis.

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